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I am pleased to present our Scientific Annual Report that contains an overview of the scientific achievements of the Netherlands Cancer Institute in 2015. More background information on our research programs and principle investigators can be found on our website (www.nki.nl) or in our Scientific Brochure that is available for download on our website.

The Netherlands Cancer Institute is a Comprehensive Cancer Center, combining a dedicated cancer hospital and cancer research institute in a single organization. Our hospital underwent a significant expansion in 2015. These new facilities are complemented by a large radiotherapy department and an extensive infrastructure for clinical research that includes clinical data management and a large array of diagnostic facilities. Over the years the hospital has built a large repository of patient data and a large collection of tumor and normal tissues. Clinical research spans across medical, surgical and diagnostic oncology, radiotherapy, pharmacology, epidemiology and psychosocial oncology and research into cost effectiveness of health care and efficiency of planning and organization. Our hospital has seen steady growth in patient numbers over the last years, with an average annual growth of 5%. As a result of this growth, we have registered ~10,000 new unique patients at our hospital in 2015. To continue to accommodate this growth, we will need to plan additional expansions of our clinical capacity, and foresee that new building activities (in addition to the ones that are currently ongoing) will be required from 2018 onwards. We are currently nearing completion of our new unit for rehabilitation and the expansion of our pathology department, which will become operational during the course of 2016. Also, we have established joint clinical programs for urology, neuro-endocrine and head-and-neck tumors together with the UMC Utrecht, and aim to expand this alliance to encompass pathology during the course of 2016. At the end of 2015 Wim van Harten stepped down from our Board of Directors, to become the chairman of the Board of Directors at Rijnstate hospital in Arnhem. Wim was our financial director for almost 15 years. We are very thankful for everything Wim has achieved in those years, and very pleased that as of March 1st, 2016, Marien van der Meer joined our board as the new Financial Director.

At the end of 2015 we closed down our former laboratory animal facility and at the same time, our mouse cancer clinic became fully operational. This new facility incorporates a transgenic unit, an intervention unit and an imaging unit equipped with Bioluminescence, SPECT, MRI and a linear beam irradiation set-up for the treatment of small laboratory animals. The mouse cancer clinic is equipped with most of the clinical modalities that we use in our regular clinic, so that we can use all of these for preclinical testing of new treatment strategies that are identified in our preclinical research program. Our former animal facility will be broken down in 2016 to make space for the construction of our new hospital pharmacy to create our own production units for experimental drugs and clinical grade biologicals preparations.

We managed to end 2015 with a profit for the hospital and finalized the much needed expansion of our clinical facilities. But the sustained growth in numbers of patients that come to our hospital continues to put a strain on our system. Our excellent reputation is attracting more patients than we can possibly treat due to limitations in physical space and personnel. This, combined with tighter budgets from health insurance companies, are putting an increasing strain on all of our activities. Our progress is severely hampered by limitations in the time that clinicians can dedicate to research during their training. An important challenge for our institute is to accommodate sufficient growth to be able to provide the required clinical care for the growing numbers of cancer patients and at the same time be able to continue to develop better treatments. We strive to improve treatment options for all types of cancer, and given the development of personalized medicine this requires higher number of patients to provide the evidence that is necessary
to make these new treatment options available to patients throughout the Netherlands. The financial challenges that we face at the national level to be able to achieve the desired innovations to improve the outlook for our patients are daunting, and we currently lack the resources to take benefit from all of the new opportunities that have presented themselves in the most recent years. We see this as a serious and nationwide shortcoming. Despite the consistent growth of our research budget over the last years (table 1), the growth does not parallel the rate of growth of the financial demands that our research program brings forward. We need to invest in large programs such as immunotherapy and image-guided interventions, but maintaining an international competitive program requires continuous investments in infrastructure which remains a challenge. Also, we run many projects that produce very large datasets, but the long-term maintenance of these valuable resources requires extensive management and storage costs. These challenges are not unique to the Netherlands Cancer Institute, but they are more difficult for us to overcome due to the fact that our research program is in the largest part financed from project (short-term) funding. That ratio has steadily shifted towards external grants, donations and short-term research agreements with third parties. Currently ~65% of our total research budget comes from such sources, making it challenging to maintain sufficient manpower in the underlying infrastructure. On top of that, the complexity of current day cancer research also requires that we adopt a team-science approach. We are very excited that in 2015, the Dutch Cancer Society (KWF) and the NKI have renewed their collaboration, adding a 5-year extension to our collaboration that has been active for over 65 years. The goal of this collaboration is to decrease the incidence of cancer, to improve treatment and the improve quality of life for cancer patients. Under this new 5-year agreement, KWF has committed 14% of its annual revenues (in 2015: 16 million euros), as an institutional subsidy to the NKI. This is in line with the vision of KWF to support a national institute for cancer research that takes a leading role in dedicated and most promising areas of cancer research. As part of this new contract, KWF will monitor and evaluate the planning, results and impact of the research program with respect to the mission of KWF. With this new mode of interaction, we aim to better visualize the impact on our research on the knowledge of cancer and the development of new treatment strategies. In addition, it will help KWF to improve its role as the facilitator of cancer research. In addition to this core funding, our Antoni van Leeuwenhoek Foundation, that we have established together with the Dutch Cancer Society, totaled over 1.5 million euro’s in 2015. We are very grateful for these generous donations, as they make it possible for us to quickly seize new opportunities in cancer research.

### Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Dutch Cancer Society</th>
<th>Ministry of Health, Welfare &amp; Sport*</th>
<th>Total</th>
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<td>20</td>
<td>55</td>
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<tr>
<td>2015</td>
<td>40</td>
<td>25</td>
<td>65</td>
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* Excluded are the reimbursement for interest and depreciation of buildings.
HIGHLIGHTS

It is impossible to provide a complete overview of the total impact generated by our Institute in 2015 in this introduction. Many of the highlights can be found in the reports of the individual group leaders further on in this annual report or on our website. I have chosen to mention just a few highlights here.

The Agami lab devised a novel genetic approach to screen for cancerous enhancers using the genome-editing CRISPR-Cas9 tool. This approach yielded novel insights in enhancers with potential tumor suppressor and oncogenic roles. Following the initial functional annotation of the identified enhancers, the group setup experiments to investigate local mutations that cause resistance to cancer therapy. This information will be used for cancer diagnosis and treatment. The lab of Thijn Brummelkamp identified all of the essential genes in the human genome, and characterized novel synthetic lethal interactions, providing very useful information for the development of novel combination therapies. The lab of Emile Voest unraveled a fatty acid signaling pathway that confers chemoresistance and started personalized medicine trials based on genomics and tumor organoids.

In collaboration with his clinical colleagues John Haanen and Christian Blank, Daniel Peeper’s group showed that in melanoma, distinct mechanisms of drug-resistance are operational in different metastatic lesions from a single patient. Several of the genetic alterations responsible for the resistance could already be identified in the pre-treatment melanoma. This indicates that drug-resistant tumor clones may be pre-existing, causing heterogeneous treatment responses and thereby limiting durable clinical benefit.

The group of René Bernards identified a vulnerability that BRAF mutant melanomas acquire when they become resistant to MAP kinase pathway inhibitors. His group found that treatment of these drug resistant cells with histone deacetylases (HDAC) inhibitors results in persistently increased MEK-ERK signaling and a more lasting proliferation arrest. Their data indicate that sequential treatment of BRAF inhibitor resistant melanomas with HDACi leads to a prolonged state of proliferation arrest and cell death, which ultimately leads to regression of the BRAF-inhibitor resistant tumor in vivo. A clinical study with HDAC inhibitors in drug-resistant melanoma will start in 2016.

Nienke de Vries and colleagues in the Van Lohuizen lab showed that in high grade Glioma, long term exposure to Ezh2 inhibition resulted in a tumor identity switch to a more aggressive stem cell like subtype. This finding is highly relevant to the application of Ezh2 inhibitors in Glioma, a concept based on the observation that short term Polycomb inhibition can be beneficial. This shows that dosing and exposure time to Ezh2 inhibitors matters and that long term Ezh2 inhibition can unleash an unsuspected tumor suppressive role in Glioma. In collaboration with the the groups of Wim Vermeulen and Jurgen Martejn at the Erasmus MC in Rotterdam, the Medema group identified the core spliceosome as a target of the DNA damage-activated kinase ATM, thus demonstrating that transcription-blocking lesions play an important role in the DNA damage response of non-replicating cells.

Bas van Steensel and colleagues developed a method to map the contacts of the genome with the nuclear lamina in single human cells. This enabled them to obtain a detailed view of the spatial organization of DNA inside the cell nucleus, and how this varies from cell to cell. Hundreds of genomic regions were identified that are anchored to the lamina in nearly every cell; these regions may help to package the genome inside the nucleus. It will be interesting to investigate how genome rearrangements, which frequently occur in cancer, affect this spatial organization.

Hanna van Waart in the group of Neil Aaronson showed that various physical exercise interventions resulted in significantly less decline in cardiorespiratory fitness and better physical functioning, compared to usual care. A high intensity, supervised exercise program also resulted in significantly less necessity for reduction in chemotherapy doses. This is the first clinical trial to demonstrate that women who exercise during their breast cancer treatment are significantly more likely to complete their chemotherapy regimen without dose adjustments.

Michael Schaapveld in Floria van Leeuwen’s group reported that the risk of second solid cancers had not changed appreciably among patients with Hodgkin lymphoma who were treated during the 1990s, as compared with those who were treated during earlier decades. The results suggest that reduction of the incidence of second cancers can best be achieved by more substantial reductions in the radiation exposure of healthy organs.
and tissues and by avoidance of high-dose procarbazine. In addition, the Van Leeuwen group also reported on substantially increased risk of several cardiovascular diseases after treatment for Hodgkin lymphoma and identified a linear radiation dose response curve for the association between mean heart dose and risk of coronary heart disease. These results provide a solid basis for future research that can help us to develop novel anti-cancer therapies. In recent years, our research program has become very successful in clinical translation of the therapeutic concepts that stem from our basic and translational research program. Several clinical trials were already ongoing at the Netherlands Cancer Institute at the start of 2015 that are the direct result of basic and translational research that was performed in our Institute (table 2). During the course of 2015, a number of new trials that are based on our own (pre-)clinical research were added to this list (table 3). We are very pleased with the fact that we have managed to significantly shorten the time between discovery and clinical application in the last few years, with examples of clinical studies that were opened before the original discovery was published.

### Table 2

**Therapeutic Concepts that are the Product of Fundamental and Translational Research Performed at the Netherlands Cancer Institute, and Currently in Clinical Development in Our Institute**

<table>
<thead>
<tr>
<th>Reference Number**</th>
<th>ClinicalTrials.gov</th>
<th>AVL Code</th>
<th>Novel Treatment</th>
<th>Tumor Type</th>
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<td>NCT01719380</td>
<td>M12LGX</td>
<td>EGFRi + BRAFi ± PI3Ki</td>
<td>Mutant BRaf Colorectal Cancer</td>
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<td>1</td>
<td>NCT01750918</td>
<td>M13DPT</td>
<td>EGFRi + BRAFi ± Meki</td>
<td>Mutant BRaf Colorectal Cancer</td>
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<td>Pan-HERi + Meki</td>
<td>Mutant KRas Colorectal Cancer</td>
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<td>2</td>
<td>NCT02230553</td>
<td>M14LTX</td>
<td>Pan-HERi + Meki</td>
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<tr>
<td>3-5</td>
<td>Registration pending</td>
<td></td>
<td>Carboplatin + PARPi</td>
<td>Advanced Breast Cancer with BRCA mutation</td>
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<tr>
<td>6,7</td>
<td>NCT02285179</td>
<td>M14PDS</td>
<td>Tamoxifen + PI3Ki</td>
<td>ER/PR+ and HER2+ Breast Cancer</td>
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<tr>
<td>8-10</td>
<td>NCT01057069</td>
<td>M09TNM</td>
<td>Neo-adjuvant Chemo</td>
<td>Triple-Negative Breast Cancer</td>
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<tr>
<td>8-15</td>
<td>NCT01898117</td>
<td>M13TNB</td>
<td>Paclitaxel + VEGFi</td>
<td>BRCA1-like Breast Cancer</td>
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<td>16,17</td>
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<td>TIL vs. Ipilimumab</td>
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<td>Chemoradiotherapy + surgery</td>
<td>Resectable Gastric Cancer</td>
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<td>N11ORL</td>
<td>Radiotherapy + Cisplatin + PARPi</td>
<td>Laryngeal and HPV-Negative Oropharyngeal SCC</td>
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<td>21</td>
<td>NCT02227082</td>
<td>N13ORB</td>
<td>Radiotherapy + PARPi</td>
<td>Locally Advanced NSCLC</td>
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<td>22</td>
<td>NCT01504815</td>
<td>M11ART</td>
<td>Cisplatin + Adaptive High Dose Radiotherapy</td>
<td>Locally Advanced Triple-Negative Breast Cancer</td>
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<td>23</td>
<td>*NCT01024829</td>
<td>M09PBO</td>
<td>FGd-PET-based Boosting RT</td>
<td>Inoperable NSCLC</td>
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<td>NCT01780675</td>
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<td>Hippocampus Avoidance PCI</td>
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<td>Combined Stereotactic and Conventional Fractionated RT</td>
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<td>Breast &amp; Colon Cancer</td>
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* Therapeutic Concept not solely, but primarily developed at the Netherlands Cancer Institute
** See reference on the next page
1. Prahalad A et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:105-3


The quality of our research can be monitored in several ways. First of all, objective bibliometric parameters (citations and impact of scientific articles published by NKI staff) demonstrate that our scientific productivity, as measured in numbers of citations, is significantly increasing over time (table 4). Our position at the international forefront of cancer research was confirmed by the institutional site visit that took place in 2015, the full report of this site visit can be found on pages 10-20. Every five years this institutional site visit is executed under supervision of the Royal Netherlands Academy of Science and Arts (KNAW), the Dutch Cancer Society (KWF) and the Ministry of Health, Welfare and Sport (VWS). In consultation with the KNAW, KWF and VWS, we select a site visit committee that comprises world-leading experts in cancer research. In addition, reflecting our strong interest in performing translational research we include a patient advocate. For this edition of the institutional site visit we were very honored that Prof. Dr. Harold Varmus (Nobel Prize winner and former director of the National Institutes of Health and the National Cancer Institute was willing to chair this committee. In addition, the committee consisted of: Dr. Margaret Frame (Director of Research of the Edinburgh Cancer Research Centre), Prof. Dr. David Livingston (Emil Frei Professor, Harvard Medical School and chair of the research committee of the Dana-Farber Cancer Institute), Prof. Dr. Simon Powell (Enid A. Haupt Professor and chair of the department of Radiotherapy, Memorial Sloan-Kettering Cancer Center), Prof. Dr. Charles Sawyers (HHMI Investigator and chair of the department of Medical Oncology, Memorial Sloan-Kettering Cancer Center), Dr. Daniel Speiser (Chair of the Clinical Immunotherapy Development and Trial Program at the Ludwig Cancer Research Center, Lausanne), Dr. Thomas Würdinger (representative for KWF and staff researcher at the department of Neuro-Oncology, VUMC, Amsterdam), Prof. Dr. Rick Grobbee (representative from the Royal Dutch Academy of Sciences (KNAW) and University Professor Clinical Epidemiology at the UMC Utrecht) and Dr. Jurgen Seppen (Patient advocate, Chair of the Stichting Lynch Polyposis and staff researcher at the AMC). We were very proud to receive excellent marks from this committee of international top-class scientists. The general conclusion of the committee was that the NKI manages “to perform at an exceptionally high level and remains one of the world’s leading institutions

### TABLE 3
STUDIES STARTED IN 2015, BASED ON (PRE-)CLINICAL RESEARCH PERFORMED AT THE NETHERLANDS CANCER INSTITUTE

<table>
<thead>
<tr>
<th>CLINICALTRIALS.GOV</th>
<th>AVL CODE</th>
<th>NOVEL TREATMENT</th>
<th>TUMOR TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02375066</td>
<td>M13PA5</td>
<td>Panopanib + RT</td>
<td>Non-metastatic Sarcoma</td>
</tr>
<tr>
<td>NCT024500556</td>
<td>M14AFS</td>
<td>Afatinib + Selumetinib</td>
<td>Advanced Mutant Kras and PIK3CA wildtype Colorectal, NSCLC or Pancreatic Cancer</td>
</tr>
<tr>
<td>NCT02278133</td>
<td>M14WLC</td>
<td>WNT574 + LGX81B + Cetuximab</td>
<td>Colorectal Cancer with Mutant BRAF and Wnt Pathway mutations</td>
</tr>
<tr>
<td>NCT02418624</td>
<td>M14REV</td>
<td>Carboplatin + PARPI</td>
<td>Advanced Breast Cancer with BRCA mutation</td>
</tr>
<tr>
<td>NCT02337279</td>
<td>N14PC</td>
<td>Ipilimumab + Nivolumab</td>
<td>Melanoma</td>
</tr>
<tr>
<td>NCT02316197</td>
<td>M15M9R</td>
<td>DNA-PKI + Radiotherapy</td>
<td>Advanced Solid Tumors</td>
</tr>
<tr>
<td>NCT02324452</td>
<td>M14PD7</td>
<td>Genotype-directed dosing of Fluoropyrimidines</td>
<td>Various Neoplasms</td>
</tr>
<tr>
<td>NCT02572661</td>
<td>N14US</td>
<td>Sentinel node mapping using SPECT</td>
<td>Head and Neck Cancer</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NTR CODE</th>
<th>AVL CODE</th>
<th>NOVEL TREATMENT</th>
<th>TUMOR TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR5219</td>
<td>N14LMN</td>
<td>Lymphatic mapping of the neck with ICG-nanocolloid</td>
<td>Oral Cavity Malignancies</td>
</tr>
<tr>
<td>NTR5220</td>
<td>M14HSN</td>
<td>Sentinel node mapping with ICG-99mTc-nanocolloid</td>
<td>Bladder Cancer</td>
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<tr>
<td>pilot</td>
<td>N14IMR</td>
<td>Immunological effects of (chemo) radiotherapy</td>
<td>HPV positive Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>pilot</td>
<td>N15M9L</td>
<td>Magnetic Marker Localization</td>
<td>Non-palpable Breast Cancer</td>
</tr>
<tr>
<td>+</td>
<td>M14SEA</td>
<td>Strengthening exercises using the Swallowing Exercise Aid</td>
<td>Head and Neck Cancer</td>
</tr>
</tbody>
</table>
The committee recommended that we further enforce our research in the areas of quality of life and image-guided interventions. The committee felt it is essential that we somehow expand the amount of time that is allocated to clinicians that have a highly successful research program. The committee was particularly impressed with our contributions to precision medicine and immunotherapy, concluding that “several examples of discoveries from NKI laboratories with direct implications for precision medicine, some of which have led to clinical trials conducted at AVL Hospital that could result in changes in clinical practice.” and “NKI’s growing strength in the field of cancer immunology positions the NKI as a world-leader in this field and contributes significantly to the Institute’s overall status as a center of excellence in the cancer arena, especially now that immunotherapy has become a major component of cancer medicine.”

The committee was concerned that the limitations in financial resources will make it very difficult to maintain this extraordinary high level of quality. They concluded that “the promise offered by a continuous investment in basic cancer research performed by collaborating NKI group leaders is paying extraordinary dividends. It has led to a deeper appreciation of how normal and cancer-related biological processes work and the successful conversion of such insights into a major advance in cancer treatment. This remarkable record of accomplishment revalidates the Institute’s commitment to the support of research in mechanism-based cancer science.” Clearly, the NKI will need to uncover new ways of financing its excellent research program to be able to deliver on all of the promising leads that are produced from preclinical research these days.

### Honors and Appointments

The NKI-AVL cannot award university degrees, but many of our staff members hold special part-time chairs at Dutch Universities. This allows them to award PhD degrees to graduate students who receive their training at the Netherlands Cancer Institute. Currently, 35 staff members have professorships at one of the Dutch Universities. Secondly, our prominent international standing in cancer research is reflected by the frequency with which our staff members are invited to present at international meetings and in the prestigious appointments, awards and grants that they obtain. We continue to score high on all of these accounts. In 2015, Kees Jalink, has been appointed as an affiliate professor in High

### Table 4

<table>
<thead>
<tr>
<th>Year</th>
<th>Publications</th>
<th>Citations</th>
<th>Citations/ Publications</th>
<th>Impact</th>
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<td>2002</td>
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<td>2455</td>
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<tr>
<td>2003</td>
<td>366</td>
<td>5094</td>
<td>13.9</td>
<td>2122</td>
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<tr>
<td>2004</td>
<td>348</td>
<td>5267</td>
<td>15.1</td>
<td>1882</td>
</tr>
<tr>
<td>2005</td>
<td>405</td>
<td>6350</td>
<td>15.7</td>
<td>2461</td>
</tr>
<tr>
<td>2006</td>
<td>435</td>
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<td>2007</td>
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<td>2009</td>
<td>511</td>
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<td>3074</td>
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<tr>
<td>2010</td>
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</tr>
<tr>
<td>2011</td>
<td>459</td>
<td>8651</td>
<td>18.8</td>
<td>3110</td>
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<tr>
<td>2012</td>
<td>573</td>
<td>9268</td>
<td>16.2</td>
<td>3333</td>
</tr>
<tr>
<td>2013</td>
<td>512</td>
<td>8989</td>
<td>17.6</td>
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</tr>
<tr>
<td>2014</td>
<td>568</td>
<td>3734</td>
<td>6.5</td>
<td>2817</td>
</tr>
<tr>
<td>2015</td>
<td>627**</td>
<td>4681**</td>
<td>7.5</td>
<td>4681**</td>
</tr>
</tbody>
</table>

* In 2015 a new standard was used to perform the citation and impact factor analyses. Consequently the numbers can differ from the previous years.

** Analysis was performed in February 2016. Data can be subject to change.
Resolution Microscopy at the University of Amsterdam, Simon Horenblas was appointed professor in Urology at the University Medical Center Utrecht and Uulke van der Heijden was appointed as affiliate professor in Radiotherapy at the Leiden University Medical Center. Anton Berns was appointed fellow of the American Association for Cancer Research, Jacques Neefjes was appointed member of the Royal National academy of Sciences and Arts (KNAW), and appointed van Loghem Laureaat 2015. Harry Bartelink received the ECCO Lifetime Achievement Award 2015, Thijn Brummelkamp from the division of Biochemistry received the Ammodo Award, and Pia Kvistborg (division of Immunology) and Denise Hilling (departments of Surgery and Radiology) both received a Bas Mulder award. In addition to these awards, several NKI-postdocs have received competitive grants from national and international organizations. Staff of the NKI-AVL fulfilled numerous functions in national and international organizations, on boards of scientific journals, as members of study sections, site visit committees and as organizers or co-organizers of scientific meetings, workshops and conferences.

OUTLOOK AND ACKNOWLEDGEMENTS

For the last decennia our Institute has been at the international forefront in cancer research and innovative cancer treatments. It has demonstrated to be able to maintain that position, despite the difficult economic situation of the last few years. We have been very successful in obtaining external grants for our research and I am convinced that we will continue to do so. Provided that we can match this with a healthy ratio of core funding, I am convinced that the Netherlands Cancer Institute can continue to deliver important breakthroughs that will prove beneficial in the treatment of cancer. Particularly in a time when our ever growing molecular understanding of cancer meets up with a new generation of anti-cancer drugs that target well-defined nodal points in the cancer cell. This calls for a more individualized treatment of cancer, in which molecular pathology in the form of a genetic and/or immunological fingerprint of the tumor is extensively used in making clinical decisions how to treat the individual patient. Success in this area will critically depend on a close collaboration between basic and clinical research; where basic research can provide the concepts for new drug combinations that can be taken to the clinic, and vice-versa, where response failure of a genetically and immunologically defined tumor in the clinic can be taken to the lab to identify alternative strategies. Success in this area requires that we further optimize the links that exist between research and clinic. The fact that the Netherlands Cancer Institute has integrated research and clinic in a single Comprehensive Cancer Center provides us with the ideal setting to facilitate this collaboration, and the examples of therapeutic concepts that we have brought to the clinic (tables 2 and 3) provide solid proof of the added advantage of this integral model. We are actively recruiting new principal investigators with highly creative and innovative research programs aimed at groundbreaking research to continuously improve ourselves for the benefit of our patients. To uncover new insights in cancer biology, develop new tools to study cancer, and to develop novel therapeutic strategies that can benefit patients.

I want to end by thanking all of our employees and everyone that supported us. Ever since our creation in 1913 our organization has received enormous support from our highly motivated employees, volunteers and sponsors. Ever since its creation in 1948, the Dutch Cancer Society (KWF) has been a very significant sponsor of our research; the Ministry of Health Welfare and Sport that provides a substantial core grant to our Institute and has provided the funds to renovate our research facilities; and all of those individuals that provided us with financial, moral and practical support. Their support is making it possible for us to continue to strive for better treatments to improve the outlook of cancer patients. And last but not least, I would like to extend my sincere gratitude to all of our patients willing to participate in our clinical studies; they are vital to the progress that we can make.

René Medema
Director of Research
SUMMARY

Over the course of two days, April 28 and 29, 2015, our committee had an opportunity to evaluate the status of one of the world’s leading cancer research institutions, the Netherlands Cancer Institute (NKI). Our charge was to determine whether the NKI was continuing to fulfill its mission in research and training; to assess the changes that had occurred since the previous institute-wide review in 2009; and to make recommendations about the management of the Institute with the intention of confronting perceived problems. The site visit was aimed to evaluate the NKI’s performance of only those activities supported by funds provided by the Dutch Cancer Society and the Ministry of Health, Welfare and Sport. The program of the site visit was therefore arranged in a way that excluded the research activities of clinicians who are fully employed on the hospital budget, without part-time appointment on the NKI’s research budget. As a consequence, we could not evaluate the full breadth and impact of the clinical research program of the NKI.

To help us pursue this charge, the NKI provided printed materials that included a detailed summary of recent events at the NKI; facts about the organization’s governance, staff, and budgets; biographies and publication lists for its staff members; and reports from recent evaluations of departments. During the two days of our visit, we met with the Board of Directors and heard reports from Rene Medema, who has been the Director of the NKI since 2012, and from Emile Voest, who became the Director of the affiliated cancer hospital, Antoni van Leeuwenhoek (AVL), last year; heard scientific presentations from representative scientists working on the five featured research themes; discussed training, management, and research issues with a group of trainees (graduate students and post-doctoral fellows) and with the leaders of most research departments; and had additional time for informal discussions with staff and for private conversations among the committee members. (Despite all the information and views received, we recognize our limited understanding the complexities of the NKI and offer our opinions and recommendations knowing that they are based on incomplete information.)

Our overall impression is that the NKI continues to perform at an exceptionally high level and remains one of the world’s leading institutions for cancer research. We also came to understand that the NKI now has to confront issues that reflect several important developments over the past five to ten years. Powerful new technologies and a deeper knowledge of cancer are driving an expansion of more applied and generally more expensive forms of research, both translational and clinical, at all cancer research institutions - including those, like the NKI, that have traditionally featured great strength in fundamental research. At the same time, the economic recession that has affected most of the world, including The Netherlands, has restricted the growth of funding for all forms of science, including the health-related sciences, at a time when opportunities for progress are greater than ever. In addition, the number of well-trained scientists has grown, and the costs of doing research have expanded.

Under these circumstances, it is difficult for the NKI to enhance its clinical and translational research programs without encroaching on the funds needed to sustain its reputation as a leader in basic cancer research. Moreover, the opportunities to raise additional revenues for research are limited by several factors mentioned in the Recommendations section. This situation requires careful consideration of several options that our committee discussed extensively: new avenues for raising more revenue for the NKI; careful prioritization of research programs; reconsideration of the
relationship between the research arm (NKI) and the hospital (AVL) at the cancer center; and the nurturing of relationships between the NKI and other educational and research institutions in The Netherlands and throughout Europe. These options are summarized at the end of our report.

The difficult questions raised by the current scientific and budgetary situation are now being tackled by the relatively new leaders of the NKI and the AVL. Both Drs. Medema and Voest have been employed elsewhere for most of their careers and were recruited to their posts from the University of Utrecht, not from within the NKI. Their status as "outsiders" may offer special benefits under these circumstances, since they may be less wedded to long-standing practices and willing to make substantial changes that might be difficult for long-time members of the NKI staff to entertain.

**RESEARCH THEMES**

**Introduction**

To facilitate presentation of research accomplishments and plans, the committee heard from speakers who represented five major "research themes" selected by Dr. Medema and his colleagues, rather than from speakers representing the traditional departments. This approach had a number of advantages. It allowed us to review the science believed by NKI leadership to be the most important and promising, and it reflected a laudable attribute of the NKI: the extraordinarily high level of interaction and collegiality among the faculty, allowing investigators from different departments to join efforts in pursuit of major research objectives. Everyone - from graduate students to senior faculty - mentioned the importance of the interactions among the components of the NKI, and it was clear from the presentations that departmental boundaries do not hinder the formation of important relationships among NKI faculty. There are, however, concerns about the relationship of NKI to AVL faculty that are addressed in the recommendations below.

Despite the virtues of the thematic organization of the program, the committee did not have uniform levels of enthusiasm for the five chosen themes. In part, the responses may reflect the breadth and level of effort that characterize each area, but our views are also influenced by the perception that the NKI is truly a leader in some domains but simply doing significant, competent work in others.

**Molecular Oncology: Genomics**

During the past 5 years, NKI has burnished its long-standing reputation for remarkable discovery in basic cancer research by converting new mechanistic insights into cancer cell 'behavior' into clinical progress. Extraordinary developments in the analysis of chromatin biology and approaches to understanding mammalian gene function have emerged in parallel.

For example, Thijn Brummelkamp’s development of powerful, new cultured cell approaches to haploid genetic analyses in somatic cells represents a major advance in understanding how key genes, e.g. some dedicated to the control of infection by highly pathogenic human viruses, operate in human cells. Similarly, Bas van Steensel’s elegant approaches to defining the effects of chromatin fine structure on the control of local gene expression have the potential to spawn major advances in the understanding of this highly complex process.

Rene Bernards has cleverly used hairpin RNA-based depletion methods and functional screens to reveal unexpected, clinically relevant, synthetic lethality mechanisms in a common human cancer. With the invaluable collaboration of Jan Schellens in the clinical translation of these findings, this team has advanced the mechanism-based treatment of B-RAF mutant colon cancer from an ineffective state to an effective one, as discussed further in a later section.
Thus, the promise offered by a continuous investment in basic cancer research performed by collaborating NKI group leaders is paying extraordinary dividends. It has led to a deeper appreciation of how normal and cancer-related biological processes work and the successful conversion of such insights into a major advance in cancer treatment. This remarkable record of accomplishment revalidates the Institute’s commitment to the support of research in mechanism-based cancer science.

The imprint of mechanism-driven discovery of new therapeutic strategies at NKI is growing. However, the cadre of scientifically trained oncologists at NKI with the high order skills needed to translate these findings to the clinic is relatively small and the time they can spend on translational research is limited due to their other clinical duties. Thus, we sense a strong need for the recruitment of at least one or two outstanding, young clinician-scientists whose skills lie in formulating and performing proof of concept therapeutic trials. The number of readily available physicians with these skills is not large, especially in Europe. Therefore, we recommend that NKI search widely for suitable candidates, including in the US and Canada, where the training of such clinician-scientists is more widely practiced.

**Molecular Oncology: Chemical and Cellular Biology**

This theme is focused on basic science (cellular and chemical biology) that strives to promote deeper understanding of cancer biology, with particular emphasis on research into genomic instability in cancer, mechanisms of regulation of gene expression, epigenetics, cellular signaling, understanding host mechanisms and integrin/cytoskeletal structures. These are important topics for which NKI scientists are internationally renowned. The mechanistic information and biological knowledge they are providing is contributing to the understanding of important clinically-relevant translational problems, for example discovering therapeutic targets, treatment responses and failures (mechanisms of inherent and acquired drug resistance), and tumor evolution and heterogeneity.

The long-term NKI strengths in technological innovation have been excellent drivers of novel biological research in the past, and this continues. This theme encompasses several key aspects of fundamental cancer biology, and the local expertise should ensure continuing success. Important strengths include the genomics services (including DNA sequencing), the development of innovative genomics tools, and research infrastructure for protein production and proteomics. There is also excellent focus on techniques that allow single cell-based analyses and linear tracing technologies, including expert use of barcoding genome-wide systems and screens, and ‘haploid’ screening as described in the previous section.

Importantly, the links of such analyses to biological phenotypes are strong. Use of state-of-the-art technologies such as si/shRNA screening tools (with associated robotics) and CRISPR/Cas9 genome editing is also evident, and the latter will gain increasing importance in future years for both intervention experiments in mouse models of cancer and in discovery science. There are a number of other core facilities, with dedicated posts, that facilitate distinct projects within the Molecular Oncology theme - for example, a genomics core facility, screening facility with robotics, flow sorting facility and microscopy. The core functions, provided at minimal costs to individual NKI investigators, provide extraordinary opportunities for all PIs to perform excellent research. The data analyses associated with projects in this theme make use of a core bioinformatics service and/or the work of individual group bioinformaticians and postdoctoral fellows who are receiving some fundamental informatics training. How this works for different groups is not uniform, and not quite clear, but the panel noted that Dr. Wessels is an outstanding recruit.

In the chemical biology field, in-house chemical and analytical tools have been developed - for example smart FRET sensors, ubiquitin variants with useful chemical substitutions, and tool compounds for inhibiting molecular targets. A particular ambition is the desire to link tumor analyses and tumor cell biology to clinically-relevant questions and applications, providing strong opportunities for collaboration within the NKI and between the NKI and AVL. The PIs and their teams are well placed to maintain the NKI’s prominent position in studying fundamental mechanisms in cancer biology, in part fueled by their development of innovative technologies and approaches.
### Precision Medicine

The precision medicine theme at NKI is represented by 19 laboratories with a breadth of expertise including cancer biology, mouse modeling of cancer, drug development and bioinformatics/big data. Several are also part of the molecular oncology theme, which represents complementary interests in chromatin biology, functional genomics and chemical biology. The review panel heard presentations from investigators in the areas of innovative therapies, big data and mouse models.

### Innovative therapies

Rene Bernards presented several examples of discoveries from NKI laboratories with direct implications for precision medicine, some of which have led to clinical trials conducted at AVL Hospital that could result in changes in clinical practice. In an shRNA screen of colon cancer cell lines, the Bernards’ laboratory identified the EGFR tyrosine kinase receptor as a potential drug target in tumors with classic driver mutations in the BRAF kinase. This discovery suggested that inhibitors of EGFR (e.g. antibodies that are currently used to treat colon cancers lacking KRAS mutations) might also be useful in colon cancers bearing a BRAF mutation, in combination with small molecule kinase inhibitors targeting BRAF or the downstream kinase, MEK. Dr. Jan Schellens presented supportive phase I/II clinical data from trials conducted at AVL showing partial responses in patients with BRAF mutant colorectal cancer using inhibitors obtained from Novartis and from GlaxoSmithKline. An additional colorectal cancer example emerged from transcriptome analysis revealing a “BRAF-like” signature in a subset of non BRAF-mutant cancers and the observation that the kinase core protein, RANBP2, is specifically required in these cells for their viability. In keeping with these findings, clinical investigators at NKI identified a colorectal cancer patient whose tumor was positive for the “BRAF-like” signature and revealed an extraordinary response to the vinca alkaloid, venorelbine. Based on these findings, a clinical trial of venorelbine in “BRAF-like” colon cancer has been initiated.

Additional innovative therapies have been developed in breast cancer. Building on the initial success of the MammaPrint RNA signature in identifying women at low risk of relapse following surgery, the NKI group has now found a subgroup at ultra-high risk of relapse who may benefit from additional therapy with the HER kinase inhibitor, neratinib. In another application of transcriptome profiling to guide treatment selection, NKI investigators are evaluating the role of high dose chemotherapy in women with “BRCA1-like” breast cancer, based on results pointing to superior results in genetically engineered mouse models of breast cancer initiated by BRCA1 mutations.

### Mouse models for cancer

For decades, NKI has been an international leader in developing mouse models for cancer led by work from the laboratories of Anton Berns, Maarten van Lohuizen, Jos Jonkers and others. Dr. Jonkers reviewed recent investments in a new mouse facility, built with a 14.5 million Euro subsidy. It will house 20,000 cages together with state-of-the-art small animal imaging and radiotherapy devices, as well as an intervention unit for administering various treatment protocols by personnel hired specifically for mouse work. The group has assembled an impressive toolbox of genetically engineered embryonic stem cells with common cancer mutations, enabling the rapid generation of chimeraic mice with multiple mutant alleles, without the need for multiple generations of breeding.

In addition to characterizing the mechanisms by which these genes contribute to the cancer phenotype, these mice have been useful for the study of treatment responses and mechanisms of drug resistance. Dr. Jonkers discussed one example of acquired resistance to PARP inhibitors caused by increased expression of the drug efflux pump, Mdr1, in a BRCA1 breast cancer model. After deletion of the Mdr1 gene, he detected additional mechanisms of acquired resistance arising from defects in the DNA damage response, caused by reduced expression of p53BP1 or REV7. The clinical relevance of these mechanisms is now being addressed in tumor samples from patients treated with PARP inhibitors.
Dr. Daniel Peeper reported on the use of patient-derived xenograft (PDX) models and highlighted recent work in melanoma. PDX models derived from one patient with BRAF mutant melanoma revealed 3 distinct mechanisms of acquired resistance (BRAF gene amplification, MEK mutation, synthesis of a BRAF splicing variant) in mice treated with a BRAF inhibitor, each of which was subsequently documented in that patient’s tumor cells. Dr. Peeper also presented results from a pooled shRNA library screen conducted in mice that implicated three DNA damage response genes (CHK1, CHK2, ATM) as potential drug targets for combination therapy with BRAF inhibitors. 

The examples provided by Drs Jonkers and Peeper underscore the unique leadership position of NKI in the field of mouse cancer modeling and the opportunity to extend these efforts into a precision medicine approach to drug development for mice and then patients. Their mouse clinic truly allows a planned clinical trial to be executed in the mouse clinic as part of the protocol development.

Big data
Drs. Lodewyk Wessels and Gerrit Meijer discussed the growing investment by NKI and AVL Hospital in bioinformatics and in health information technology. Over the past five years, a strategic decision was made to embed bioinformatics expertise in the laboratories of NKI investigators rather than build core infrastructure devoted to providing that service. In addition, several investigators, including Dr. Wessels, whose primary research focus is computational biology, have been recruited. These labs serve as key collaborators for many of the more biologically-focused groups and provide an intellectual center for innovation in computational and bioinformatic approaches.

In the hospital, Dr. Meijer is leading a new effort to integrate the electronic health record, image analysis, biobank databases and clinical genomic data generated in molecular pathology into an IT infrastructure that can be leveraged by laboratory-based scientists and the clinical research community. Since Dr. Meijer only recently joined the NKI, this ambitious and important undertaking is still in the early stages. That said, it has the potential for exerting major impact on Precision Medicine research.

Immunology and cancer
Dr. Ton Schumacher presented results of mutational analyses in melanoma. He and his team are pioneers in the field of mutated tumor “neo-antigens”, and they have identified and characterized tumor-responsive T cells in cancer patients. The group has developed a highly multiplexed tetramer assay to identify tumor antigen-specific T cells recognizing MHC binding peptides from tumor RNAseq analysis. As part of a multi-institutional effort, Schumacher et al. have provided some of the first evidence that tumors with high mutational rates are more likely to respond to immunotherapy. This brilliant work stands out as an example of how fundamental immunology can reach into the clinic.

Two other investigators are also making strong contributions to the development of immunotherapy at the NKI. Dr. Karin de Visser presented excellent basic science focused on the novel mechanisms by which innate immune cells help to shape the tumor microenvironment and promote tumor growth through inflammation. And Dr. John Haanen plays a key role in translational research through transfer of autologous T cells for the treatment of melanoma and other disorders.

The NKI’s growing strength in the field of cancer immunology positions the NKI as a world-leader in this field and contributes significantly to the Institute’s overall status as a center of excellence in the cancer arena, especially now that immunotherapy has become a major component of cancer medicine. Internationally, including at the NKI, immunotherapy is an excellent example of the importance of fundamental research, because recent medical progress would never have been possible without the investments in basic immunology research made since the late 1960s. The outstanding performance of immunology at the NKI is built on at least three pillars: excellent basic research; the development and application of advanced immunologic methodologies; and a series of outstanding contributions to clinical research and development.
The success of the immunology program at the NKI is even more remarkable when one considers the fact that it represents only a small part of the overall NKI science effort and budget. Because of its success, the remarkably high quality of its immunology group, and the high potential for it to deepen its imprint in cancer therapeutics, we believe that NKI immunology deserves to be strengthened. We recommend (i) assigning greater priority and resources to basic immunology, and (ii) amplifying clinical cancer immunology research, as will be discussed later in the report.

Future development of basic immunology should be aimed at reaching a critical mass, to allow the program to address such cancer-related topics as inflammation and other immune mechanisms that operate in the tumor microenvironment and immune-related aspects of tumor biology. The NKI could exert significantly greater influence on immunologically-driven clinical outcomes by creating closer and better coordinated links to the work of the AVL hospital than currently exist---for instance, by scaling up immune mechanism-based protocols at the hospital. Such efforts would have to be coordinated with expansion of clinical investigation, biobanking, and training programs.

In summary, the further development of the immunology program provides great promise at multiple levels, including in the acceleration of basic, translational, and clinical research at NKI/AVL.

**Image-Guided Intervention**

During the presentation of this theme, two major topics were discussed:

1. Image-guided radiotherapy, including the use of intentional dose gradients based on imaging and adaptive radiotherapy
2. Image-guided surgery, including the use of optical reflectance and absorbance for defining tumor location and three-dimensional representation of tumor sites to guide surgical removal

The image-guided radiotherapy program is well known internationally and received strong comments at both the last institutional site visit and the divisional site visit. The presentation from Jan-Jakob Sonke revealed excellent multi-parametric imaging methods being used to define radiation therapy target volumes, taking into account tumor motion, adaptive shrinkage of target volumes as the tumor responds to therapy, and dose-painting based on imaging characteristics. The latter concept was the basis of a randomized clinical trial with 571 patients, in which "conventional" 77 Gy was given evenly to the entire target volume or doses up to 95 Gy were given to the areas with the greatest tumor cell density (based on imaging and validated by histology in some cases). This is a novel trial and potentially important (depending on the findings).

There are plans for developing a proton therapy facility, as a consortium in Amsterdam, in which adaptive therapy is deemed to be even more important. Proton therapy is dependent on minimizing the integral dose, in hopes of reducing late complications of treatment, such as second cancers and heart disease. If adaptive therapy is followed during the course of proton therapy, then the integral dose is further improved.

An additional consortium effort for developing an MR in-room imaging capacity co-incident with the treatment by a linear accelerator is planned. The prototype is being developed in Utrecht, and the potential for defining tumors with a greater degree of accuracy is the presumed improvement in treatment planning.

Despite the departure of Marcel Van Herk, the NKI’s long-standing head of medical physics, the Institute is in a good position to stay at the forefront of this area of research. However, there are also some missed opportunities, future capture of which would make the research program even stronger. Multi-parametric imaging could include novel imaging characteristics (radiomics) that can be linked to tumor phenotype, ultimately predicting the response to therapy. The trajectory of response could also be predicted by early response imaging, using novel parameters rather than just volumetric changes. A more significant array of functional imaging, including the use of novel biological probes linked to a detector system (such as PET) could be developed.
to make the program stronger and more cutting edge. However, there were clearly limitations in the capacity to develop new molecular imaging systems.

There is a program in radiosensitization (Marcel Verheij), which was not presented this time and would add potentially useful combination therapies. The opportunity to link these novel combinations to particular molecularly defined tumor types seemed like another lost opportunity. In particular, response predictions to radiation and molecularly targeted therapies would link this program to other themes in precision medicine at NKI. The use of radiotherapy as a means to enhance tumor neo-antigen presentation for incorporation into novel immunotherapies was mentioned, but the scope of the research plan was not clear. That would be another opportunity to make the research in this theme feel less isolated from the rest of the institution.

The presentation by Theo Ruers on optical imaging to guide tumor resection also seemed isolated from the rest of the institute’s research plan. He made the case that a surgeon wants to know how much tissue to remove for successful surgical treatment of a tumor, while minimizing the volume of resection. Defining margins in the operating room has been a challenge for surgeons for a long time. An improvement (reduction) in the volume of tissue removed and in the likelihood of obtaining tumor-free resection margins would improve the overall results of surgery. However, the research plan was very narrow. There are many options for defining the location of a tumor (and therefore its margins) by imaging, and the reasons that optical imaging was used seem to be driven by available technology rather than a systematic approach to comparing different technologies. A much broader approach to imaging seems to be warranted, including the use of molecular imaging probes to help define whether tumor cells are present or absent.

Importantly, the image-guided intervention theme did not include a clearly defined role for radiology or nuclear medicine, which makes this theme seem somewhat imbalanced. Similarly, for both topics that were described, greater integration into other themes in the Institute would help. The micro-imaging suite and micro-radiotherapy unit, although not unique, could be further developed, with special benefits to be gained from NKI’s strength in mouse models, both GEMMs and PDXs. It may have been partly due to the time limitation for presenting details at the site visit, but this research theme seemed significantly isolated from other efforts in the Institute. There are significant strengths but also missed opportunities.

Survivorship
The committee recognizes the importance of studying long-term outcomes of patients who have been treated for cancer. Such patients, even those who appear to be entirely free of disease, are at risk for recurrence or for the appearance of second tumors, and are subject to the physical and psychosocial complications of treatment. Moreover, the numbers of individuals who are cancer survivors, especially in economically advanced countries, are increasing with the continued aging of the population and the increasing success of cancer therapies.

The NKI has made a modest investment in several studies of various facets of the survivorship theme, and a few of those were presented by Sanne Schagen and Flora van Leeuwen. While the committee viewed each of these projects favorably and believes that the work is being done in accord with international standards, it was not apparent whether the Institute’s leadership wishes to develop the overall theme into a major initiative or whether the program is being sustained for other reasons, such as institutional history, an obligation to funding organizations, or a contribution to national cancer care programs.

From any of these perspectives, the program seems somewhat unlinked to the most prominent and successful themes of the NKI - in precision medicine, genomics, cell biology and immunology - and yet could be brought together with them, for example, by efforts to evaluate the consequences of novel therapies or by plans to use molecular profiling, both somatic and germ-line, to assess the probability of recurrence or second cancers. The work of Dr. Marjanka Schmidt, a junior group leader active in this program,
is a good example how this could lead to successful links between epidemiology and genomics. In addition, the survivorship research initiatives could be used as another means to bring the AVL and the NKI more closely together.

**OBSERVATIONS AND RECOMMENDATIONS**

Our committee believes that NKI has done very well over the past five years and remains one of the outstanding centers for cancer research in the world. In general, we agree with the “SWOT Analysis” presented by Rene Medema, which lists many strengths and some current weaknesses and concerns.

Overall, we are concerned about three clouds on the horizon:

(i) **The need for growth and its possible detrimental consequences.** The collegial, collaborative spirit of the Institute is supported by its small size, but it now seems necessary to pursue limited growth to bolster the clinical research components and to ensure continued rejuvenation of the research staff.

(ii) **The need for increased revenues.** The budget situation - limited growth of revenues, with increases in project costs that are not matched by increased resources for infrastructure and core facilities - is creating a dilemma that is difficult to resolve without novel approaches to fundraising.

(iii) **The need for stronger links to clinical research.** The Institute is not optimally positioned to take advantage of the clinical research opportunities created by recent advances in basic and translational cancer research, despite its close ties to an esteemed cancer hospital (AVL).

**The growth problem**

With just over 50 principal investigators, one of the strengths of the NKI is that it more closely resembles a small research institute than an academic medical center. The relatively small size offers several advantages - access to faculty for trainees, promotion of collaborative work among laboratories, sharing of core facilities, development of team efforts, and strong institutional loyalty.

Yet, at least some further growth seems necessary to achieve at least three long-term objectives: intellectual vitality, clinical application, and international prominence.

(a) **Maintaining intellectual vitality.** To remain current with new technologies and fresh ideas, it generally helps to continue to recruit new faculty, so we support the idea of adding at least one or two new scientists to the faculty each year, even if space and resources are not provided by retirements (some are scheduled to happen soon) or by departures (which are not frequent among tenured scientists at the NKI). The resulting growth, even on a limited scale, will continue to increase the demand for financial support and could become unsupportable, if new sources of funding are not found.

(b) **Enhancing the clinical dimension.** Because of advances in basic and translational cancer research, at the NKI and elsewhere, there are now many reasons for cancer research institutions, including those that emphasize basic research, to form strong relationships with a cancer hospital and to have a substantial and seamless interface with highly skilled and knowledgeable clinical scientists. Yet, as discussed further below, the relationship between the NKI and the AVL, despite their adjacent locations, is limited by the number of clinician-scientists who can devote sufficient time to research; as a result, clinician-scientists do not seem to have produced an optimal interface with other investigators at the NKI. There are currently 11 clinical-scientists who are able to devote 50% of their time to research, but they are competing with full-time scientists for research grants. Therefore, their role is likely to be limited to enhancing specific research programs, rather than enhancing the entire interface between the research institute and the clinic. Consideration of alternative clinical investigator appointments to help initiate new programs in clinical and translational research
seems imperative for future success. Therefore, we believe that a closer organizational relationship between the two entities, recruitment of additional patient-oriented investigators, and closer attention to the study of new options for diagnosis and treatment of cancers would strengthen both institutions.

(c) Ensuring international status. Concerns were expressed by the new leadership about the predominance of Dutch citizens among the faculty members at the NKI (and the AVL): only about six percent of the NKI faculty are non-Dutch. That does seem unusual for a European research institution with such high credentials. But the difficulties claimed to impede the recruitment of non-Dutch faculty - non-competitive salaries and benefits, the trajectory of research funding in The Netherlands, and even Dutch weather and behavior - seem real and problematic. We recommend that the NKI continues to pursue the recruitment of non-Dutch scientists, but only within the more general goal of ensuring that offers be made to the best candidates. Since it will be difficult to make the NKI, with its limited hiring opportunities, truly international in a short period of time, the major efforts should be directed at maintaining scientific standards at a high level, keeping the NKI engaged in international consortia (like Cancer Core Europe), and continuing to encourage staff to meet and collaborate with leading scientists elsewhere.

(d) NKI and AVL: Because the future of NKI and AVL as world class cancer research institutions will depend, increasingly, upon a seamless connection between NKI’s basic and translational research and the clinical research spawned by it and translated into trials designed and performed at AVL, the research relationships between NKI and AVL must be well understood. Only then will it be possible to propose overarching recommendations aimed at maximizing the value of their combined research efforts and, therefore, of the entire cancer center.

In our review, the nature of NKI-AVL interactions and of AVL research was, in general, difficult to assess. This was in large part due to the fact that the evaluation was limited to the research activities carried out within the NKI, rather than the complete NKI-AVL research program in which additional clinical scientists, besides the 11 clinical scientists mentioned above, are active. Therefore, as a means of understanding the state of the combined research agendas of these institutions, we recommend that a dedicated analysis of this subject be performed in the not too distant future, either internally or, preferably, by an outside panel. The reason for this is that the results of such a review will add considerable dimension and detail to our current appreciation of where things stand. Such an outcome will, in all likelihood, lead to a more incisive and comprehensive set of recommendations aimed at maximizing the power of combined NKI/AVL science. As a formula for long term success in cancer research in the Netherlands and beyond, we foresee a vigorous, two-way flow of ideas and results between NKI and AVL as a very important component of the center’s activities.

(ii) The funding problem
Despite the NKI’s success and the rising costs of research (due especially to the expensive technologies for which the NKI is best known), the Institute’s budget has grown only slightly over the past few years. Moreover, as repeatedly stressed by the NKI’s scientific leaders, increases in funds available for new projects have not been matched by increased funds for the core facilities and other infrastructure. This situation will require action on some of the following options.

The most appealing option, and the one recommended by the committee, is to develop means for raising additional funds, but it is difficult for us to decide which means to pursue. We would urge consideration of the following possibilities:

- Renegotiate the current arrangement with the Dutch Cancer Society that appears to preclude active fund-raising by the NKI.
- Create a development plan and increase fund-raising staff at the NKI to solicit donations from Dutch industry and financial institutions, foundations, advocacy groups, wealthy Dutch people, and patients treated at the AVL and their families.
- Make additional efforts to increase government spending at the NKI and the AVL by the Ministries of Health or of Science and Education.
- Build a stronger communications team to make the work of the NKI better known in The Netherlands.

Another option that would provide additional support for the essential core facilities is to increase the charge back rates for those investigators who use those facilities. At present, the rates are very low by any standard; indeed that is a very attractive aspect of conducting research at the NKI and an inducement to pursue technically complex studies, so they should be changed only gradually and with ample warning. This is not an ideal option by any means, but it would allow some expansion of research activities when funds are secured for new projects, without jeopardizing the continuation of core services.

A third option, the least attractive, would be to place an institutional tax on laboratories when they receive funding from external grants to provide the resources needed to maintain core facilities and other infrastructure.

(iii) The clinical research problem

Throughout this assessment, we have commented on the need for more physician-scientists and clinical investigators at the NKI to carry the scientific advances that have therapeutic implications into patient care - for example, by conducting novel clinical trials and other studies with targeted drugs and manipulations of the immune system. Yet there seem to be a number of impediments to increasing the number and quality of such investigators and their integration into the work of the NKI. The committee feels that these impediments need to be forcefully addressed, but we do not know which strategies are most likely to improve the situation. We list below some changes that we believe should be carefully considered and pursued if they are viewed by the NKI’s leaders and governing bodies to be possible and promising:

- **Create a new structure for the relationship between the NKI and the AVL.**
  There are many ways in which the NKI and the AVL interact. About a dozen clinicians at the AVL are also members of the NKI staff and devote about half of their time to research (and half to patient care). A number of important projects, especially in the realms of molecular oncology and precision medicine, are jointly conducted by hospital and research institute staff. There is a strong historical link between the two institutions: the NKI and the AVL were both founded in 1913 and constituted as a Comprehensive Cancer Center, and during our discussions, reference was occasionally made to the comprehensive center; consistent with that notion, the small Board of Directors includes the Medical Director, Dr. Voest, as well as Dr. Medema and the director of management for the center, Dr. van Harten. Yet, the budgets for research (NKI) and clinical care (AVL) are strictly separated, making the Institute appear to stand apart from the hospital with respect to research commitment. As a consequence the entire complex does not seem to have achieved the coordinated effects that are found, for example, at US cancer centers with the “comprehensive” designation. Our committee believes that a higher level of coordination between the NKI and the AVL is possible, but we are not sufficiently well-informed about the legal restraints to make specific recommendations about how to achieve more integration (see (i) The Growth Problem subsection (d) above).

- **Allow more time for clinically credentialed faculty to engage in clinical research.**
  Based on observations at academic health centers, the committee believes that it is very difficult for clinicians to maintain a major role in research, if they are required to devote half of their time to patient care. To build a more effective cohort of clinicians deeply engaged in modern cancer research, we believe it would be useful to explore options for changing that requirement so that some clinically skilled faculty devote as much as 80% of their time to research. Perhaps a redefinition of patient care, so that it includes the conduct of patient-oriented research, such as oversight of clinical trials, would be possible without fundamental changes in the regulations.
- Change the methods for recruitment of clinically oriented investigators. If more clinicians are to be added to the NKI, it may prove necessary to cast a broader net for promising candidates, searching actively among physician-scientists trained outside The Netherlands, in places such as the US and Canada, where such investigators are more common. To do this successfully, it will likely be necessary to increase the salaries for such faculty and to allow them to devote the majority of their time to research activities.

- Improve the numbers of physician-scientists trained in The Netherlands and likely to be interested in appointments at the NKI. In the long run, efforts to strengthen the representation of clinically-trained scientists at the NKI will likely require a larger pool of local candidates. One way to do this would involve building stronger connections with the best academic medical centers in The Netherlands and elsewhere in Europe. Offering laboratory opportunities to students in MD-PhD or MD training programs at such institutions and building formal relationships there would be among the possible means to make the NKI’s interest in and need for physician-scientists more apparent.

**CONCLUDING COMMENTS**

Overall, the committee applauds the NKI and its leadership for the job it is doing with a relatively small staff and a modest budget. The tradition of scientific accomplishment is strong, and the levels of internal collaboration and institutional loyalty are high. But, as we have outlined in the preceding sections, there are at least three major concerns - clearly identified by Dr. Medema and his colleagues and also recognized by our committee - that need careful attention if the NKI is going to remain in a leadership position in cancer research internationally.

We also want to express our appreciation for the efficient and thoughtful manner in which our review activities were designed and conducted. We received helpful materials in advance, had a chance to hear from major staff members who represented the major research themes and from leaders of departments as well as the institute and hospital. And we received frank answers to our questions.

We would, however, recommend that future review committees have an opportunity to meet with - and perhaps discuss our recommendations with - the NKI Governing Board. We also suggest that this five year review be conducted before, not after, the next funding decisions are to be made by the Dutch Cancer Society (as well as the relevant government agencies) so that our report can help to influence those decisions.

Respectfully submitted May 28, 2015,

Harold Varmus MD (chair)
Margaret Frame PhD
David Livingston MD
Simon Powell MD, PhD
Charles Sawyers MD, PhD
Daniel Speiser MD
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Behavioral interventions in clinical oncology and health-related quality of life assessment

This research line has two primary foci: (1) development and testing of behavioral and psychosocial interventions to reduce symptom burden and improve the HRQL of patients with cancer; and (2) development of methods and applications of health-related quality of life (HRQL) assessment in clinical research and clinical practice.

Characteristics of women with breast cancer who choose (not) to participate in research on physical exercise during chemotherapy

Only between 25% and 50% of patients invited to participate in clinical trial-based physical exercise programs during cancer treatment agree to do so. The purpose of this study was to identify factors associated significantly with the decision (not) to participate in a randomized controlled trial of physical exercise during adjuvant chemotherapy for breast cancer. Based on questionnaire data, we compared trial participants and non-participants on a range of sociodemographic, clinical health-related, practical, behavioral and attitudinal variables. 230 of 524 patients agreed to participate in the trial (44%). The 294 (56%) non-participants indicated that they wanted to exercise on their own or that they did not wish to exercise in the context of a trial. Those who preferred to exercise on their own were relatively similar to trial participants, but were more likely to be in the maintenance exercise stage. Those non-participants who did not wish to exercise had a significantly lower level of education, were less likely to be working, reported more fatigue and lower health-related quality of life, had lower sense of self-efficacy, more negative attitudes towards exercise, less social support, and perceived fewer benefits and more barriers to exercising during treatment than trial participants. Minimizing practical barriers to participation, providing educational materials on the benefit of exercise and giving adequate professional and social network encouragement may increase the number of patients willing to exercise during treatment and to participate in such studies.

Levels of psychological distress in newly diagnosed breast cancer patients who know that they are at risk of hereditary cancer

Little is known about levels of psychological distress in newly diagnosed breast cancer (BC) patients who know that they are at increased risk of carrying a BRCA1/2 mutation, as compared with distress levels in BC patients without (knowledge of) such increased risk (labeled HRBC versus BC, respectively). We administered the Hospital Anxiety and Depression Scale to a sample of 238 Dutch HRBC patients and 165 Norwegian BC patients within 3 weeks of BC diagnosis and prior to primary surgery. We used analysis of covariance and logistic regression analysis to compare levels of distress between these two groups. After adjusting for age at diagnosis, marital and employment status and parity, the HRBC patients reported, on
average, significantly more symptoms of depression than BC patients (adjusted means (CI) of 5.4 (4.8-5.9) and 3.7 (3.0-4.3), respectively; p<0.001, effect size=0.40). The percentage of women with a suspected (HADS score > 8) and probable (HADS score > 11) diagnosis of depression was 29% versus 12% (p<0.001) and 15% versus 5% (p<0.01) for the HRBC and BC groups, respectively. There were no significant group differences in HADS anxiety scores. These results suggest that caregivers should be particularly alert for symptoms of depression in high-risk BC patients.

Internet-based cognitive behavioral therapy for problems with sexuality and intimacy in women treated for breast cancer

In this multicenter RCT we are evaluating the efficacy and cost-effectiveness of an internet-based cognitive behavioral therapy program for problems with sexuality and intimacy in women treated for breast cancer. Participants were recruited via 10 hospitals in the broader Amsterdam region. One hundred sixty-nine women have been recruited into the study and randomized to an intervention (n=84) or control group (n=85). Primary study endpoints include problems with sexuality and intimacy. Secondary outcomes include body image, menopausal symptoms, marital functioning, psychological distress and health-related quality of life. Questionnaires are administered at baseline, mid-treatment, and immediate post-treatment (or equivalent times for the control group). The intervention group completes additional follow-up questionnaires three and nine months post-treatment. Patient follow-up will continue to September 2016.

Online cognitive behavioral therapy (CBT) for climacteric symptoms in breast cancer patients experiencing treatment-induced menopause

In this randomized controlled study we are evaluating the (cost-) effectiveness of an internet-based cognitive behavioral therapy (CBT) program for climacteric problems in women treated for breast cancer (EVA-Online). 264 women will be recruited from at least 10 hospitals in the Netherlands and randomized to one of 3 study arms: (1) guided internet-based CBT; (2) self-managed internet-based CBT; or (3) a waiting list control group. Primary outcomes are climacteric symptoms. Secondary outcomes include psychological distress, sexuality problems, sleep quality and health related quality of life. Questionnaires are administered at baseline, post-intervention (or equivalent time for the control group) and at 6 months follow up. Patient recruitment began in September 2015 and will last approximately 1 year.

Establishing thresholds for clinical importance for four key domains of the EORTC QLQ-C30

The aim of our study was to identify thresholds for clinical importance for four EORTC QLQ-C30 scales: Physical Functioning (PF), Emotional Functioning (EF), Pain (PA) and Fatigue (FA). We recruited adult cancer patients from Austria, the Netherlands, Poland and the UK. No restrictions were placed on diagnosis or type or stage of treatment. Patients completed the QLQ-C30 and three anchor items reflecting potential attributes of clinically important levels of PF, EF, PA and FA. We merged the anchor items assessing perceived burden, limitations in daily activities and need for help into a dichotomous external criterion to estimate thresholds for clinical importance using Receiver

Operator Characteristic (ROC) analysis. In our sample of 548 cancer patients (mean age 60.6 years; 54% female), the QLQ-C30 scales showed high diagnostic accuracy in identifying patients reporting burden, limitations and/or need for help related to PF, EF, PA and FA. All areas under the curve were above 0.86. We were able to estimate thresholds for clinical importance for four QLQ-C30 scales. When used in daily clinical practice, these thresholds can help to identify patients with clinically important problems requiring further exploration and possibly intervention by health care professionals.

Kuijpers W, Gieslinger JM, Zabernigg A, Young T, Friend E, Tomaszewska JM,Aaronson NK, Holzner B. Patients’ and health professionals’ understanding of and preferences for graphical presentation styles for individual level EORTC QLQ-C30 scores. Qual Life Res 2015


Identifying and characterizing novel vulnerabilities of cancer

Our main research objective is to identify novel cellular vulnerabilities that can be exploited for cancer therapies. For this purpose we develop innovative genomic and genetic tools. Key targets are non-coding RNAs, mRNA translation, and enhancers. In particular, we employ novel unbiased functional genetic screening approaches, perform mechanistic studies to understand their connection with the cancerous phenotype, and use this information for the development of innovative cancer therapeutic approaches.

Functional genetic screens for enhancer elements

Only about 2% of our genome encodes genes for proteins. The rest 98% contains regulatory regions that control with high precision gene expression in time and space. In the past decade, whole genome sequencing efforts uncovered hundreds of thousands of mutations related to disease and cancer. While the minority of these mutations occurs in the coding genome and their potential causal role can be relatively easily deciphered, the majority of the mutations occurs in noncoding regions. The lack of detailed functional understanding of the noncoding genome hampers utilization of mutational information for diagnosis and therapy of cancer.

A prominent part of the noncoding genome is enhancers. Enhancers are genomic domains that regulate transcription of distantly located genes through chromatin looping. It is estimated that the human genome harbors close to million enhancers. Enhancers function as binding platforms for transcription factors and are characterized by specific chromatin signatures of histone methylation and acetylation. Only a small subset of all enhancers is active at a given space and time during development, indicating a tight regulation of enhancer activity to control gene expression. Intriguingly, recent studies have indicated that single nucleotide polymorphisms, large-scale genomic rearrangements and somatic mutations can affect enhancer activity and contribute to tumorigenesis. Moreover, the relevance of enhancers in cancer biology is highlighted by the fact that enhancer-associated factors are frequently mutated in tumors, and targeting these factors by small molecule inhibitors yields great therapeutic potential.

To date, however, systematic identification of enhancer activity to control gene expression is hampered by the lack of tools to perform unbiased functional genetic screens. We therefore devised a novel approach for this purpose by utilizing the recently developed genome editing CRISPR-Cas9 tool. We presented two distinct genetic screens to identify and characterize functional enhancers in their native environment. As a result, we identified several functional enhancer elements that are required for either inhibition or stimulation of proliferation by the tumor suppressor p53- and mitogen ERa, respectively. Finally, we showed that precise mapping of functional elements within enhancers is possible using a genomic CRISPR-Cas9 tiling screening approach. Altogether, our results demonstrated for the first time the expansion of the utility...
of functional genetic screens to the noncoding genome under normal and pathological conditions. Currently, we perform experiments to identify and characterize cancer-associated causal somatic mutations and risk nucleotide polymorphisms that deregulate our identified enhancers. This knowledge would be used for cancer diagnosis and for predicting treatment-resistance response.

**p53, long non-coding RNAs and cancer**

p53 – the major tumor suppressor gene in our genome – is a transcription factor that binds enhancers to regulate key target genes. Beyond the transcriptional regulation of protein-coding genes, p53 has the capacity to regulate long intergenic non-coding RNA genes (lincRNAs). However, the importance of lincRNAs to the p53 tumor suppressive function is poorly characterized. We hypothesized that lincRNAs contribute to p53-tumor suppressor function.

In our first study, we globally mapped p53-regulated enhancers by looking at enhancer RNA (eRNA) production and screened for responsive target lincRNAs. We identified a p53-target lincRNA (linc-86) and demonstrate that its expression is required for p53 tumor suppressive function. Chromatin-binding and eRNA expression analyses show that linc-86 associates with and activates strong enhancers. We therefore named linc-86 LED (for LincRNA activator of Enhancer Domain). One prominent target of LED was located at an enhancer region within CDKN1A gene, a potent p53-responsive cell cycle inhibitor. Knockdown reduces CDKN1A enhancer induction and activity, and cell cycle arrest following p53 activation. Finally, promoter-associated hypermethylation analysis shows silencing of LED in human tumors. Thus, our study identifies a new layer of complexity in the p53 pathway and suggests its dysregulation in cancer.

In a second related study, we identified and characterized a novel p53-bound intronic enhancer that controls the expression of its host linc-475 gene. We demonstrated the requirement of linc-475 for the proper induction of a p53-dependent cell cycle inhibitory response. Our data suggest a direct role of p53-bound enhancer domains in the activation of lincRNAs that are required for an efficient p53 transcriptional response. Altogether, our experiments demonstrate an essential role of noncoding RNAs in p53 function. This information opens up new opportunities for cancer diagnosis and treatment.

**Myc coordinates transcription and translation processes to enhance tumorigenicity and suppress invasiveness**

c-Myc (Myc) is a major human proto-oncogenes often associated with tumor aggression and poor clinical outcome. Paradoxically, Myc was also reported as a suppressor of cell motility, invasiveness, and metastasis. Myc is known to activate transcription, but it is largely unknown whether it also affects mRNA translation. We therefore measured global changes in gene expression following Myc induction at the transcription, translation, and protein levels. This way we uncovered a broad coordination between transcription and translation, and demonstrated the connection of these responses to mTOR signaling to enhance oncogenic transformation and to the TGFβ pathway to modulate cell migration and invasiveness. Our results elucidate novel facets of Myc-induced cellular responses and provide a more comprehensive view of the consequences of its activation in cancer cells.
Unraveling signaling networks in cancer

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Publications


Kulijpers AMJ. Tailored treatment of metastatic colorectal cancer. Thesis University of Amsterdam 2015


Kulijpers AMJ. Tailored treatment of metastatic colorectal cancer. Thesis University of Amsterdam 2015


Precision medicine: the right target in context
The development and selection of novel cancer therapies is largely driven by the characterization and understanding of the genomic alterations present in individual cancers. However, the genomic complexity and heterogeneity of tumors still poses a challenge for the identification and selection of effective cancer therapies. Genomic alterations occurring in human tumors frequently affect components of signaling networks thereby contributing to cancerous phenotypes. As a result, components of these signaling networks represent potential targets for cancer therapy. However, the complex structure of these networks including unanticipated feedback control and the extensive crosstalk between pathways complicates the selection of the right targets in the context of several other alterations in individual patients.

Our research continues to evolve around the characterization of cancer specific signaling networks with the goal to identify critical components of affected signaling networks that can be explored as drug targets in precision therapy. To achieve these goals, we use functional genomic technologies including large scale RNAi screening, CRISPR based gene-editing, CRISPR based transcriptional activation and repression and high throughput technologies to study pathway activation in large panels of cancer cell lines and primary tumor samples established as 3D organoid cultures. This integrated approach allows for the identification of specific dependencies in the context of tumor- and patient-specific alterations that can be explored for cancer therapy and lead to the development of predictive models and biomarkers for therapy response to pathway-targeted therapeutics in cancer.

Understanding sensitivity to mTOR inhibitors in breast cancer
The understanding of the complex dynamic circuitry of signaling pathways in the context of targeted inhibition is highly valuable not only to identify novel targets but also for identifying biomarkers to stratify patients, to understand resistance and to enable the identification of more effective combination therapies.

We have characterized a panel of 30 human breast cancer lines for their genomic characteristics including copy number variation, mutations, gene expression profiles and determined their sensitivity to a large panel of pathway targeted drugs. We have studied the individual components of signaling networks, using reverse (phospho)protein arrays. In collaboration with the group of Lodewyk Wessels, we performed integration of the different datasets to construct models of drug response in, among others, triple negative breast cancer (TNBC). In TNBC, alterations in the PI3-K pathway are frequently observed, either through mutation or amplification of PIK3CA, the catalytic component of the PI3K enzyme, or the loss of the negative regulator PTEN. Inhibitors of this pathway,
including inhibitors of mTOR, the downstream target of the PI3K pathway, have been explored in the clinic albeit with little success. It has been found that mutational activation of PI3K represents an important determinant of sensitivity to mTOR inhibitors. Nevertheless, a number of PI3K wild-type TNBC cell lines do show sensitivity to these inhibitors. Based on the data integration and modeling, we found that the protein expression levels of 4E-BP1, a downstream target of mTOR involved in regulating cap-dependent protein synthesis, correlated with response. In poorly responding cell lines expressing low levels of 4E-BP1, overexpression of the protein increased sensitivity. Conversely, in sensitive cell lines expressing high levels of 4E-BP1, knockdown of 4E-BP1 led to reduced sensitivity to mTOR inhibition. These results indicate that effective inhibition of protein synthesis downstream of PI3K/mTOR is an important determinant of mTOR inhibitor activity. Given that approximately 20% of breast cancer tumors show an amplification of the 4E-BP1 locus, such patients would likely benefit from mTOR inhibitor treatment.

Enhancers for response to targeted therapeutics in TNBC

Complex interactions, extensive cross talk, feedback loops and adaptive mechanisms within or between signaling pathways frequently underlie the limited response to pathway-targeted therapeutics. In this project we have applied large scale RNAi screens to identify genes that upon knockdown either increase response or revert acquired resistance to specific inhibitors in the PI3K pathway. By using our pooled shRNA screening technology in combination with a TNBC model for PI3K inhibitor (GDC0941) response, we have found that knockdown of the insulin-like growth factor-1 receptor (IGF1R) expression potently increases sensitivity to GDC-0941. Pharmacological inhibition of IGF1R using OSI-906 (Linsitinib) shows a strong synergy with PI3K inhibition. The combination of GDC-0941 and OSI-906 is synergistic in about half of the TNBC cell lines tested indicating that this characteristic is not shared between all TNBC. RNA expression analysis indicates that the expression levels of IGF2BP3 can be used as a potential predictor for sensitivity to the PI3K/IGF1R inhibitor combination. Interestingly, inactivation of IGF1R signaling can also restore sensitivity in a TNBC cell line that acquired resistance to PI3K inhibition. Together, these findings provide rationale for a novel combination therapy consisting of PI3K and IGF1R inhibitors in a biomarker-stratified subset of triple-negative breast cancers.

Patient derived Cancer Organoid Cultures

Cancer cell line models are not ideal to represent the growth characteristics and heterogeneity of patient’s tumors. To improve on these models to more accurately determine the molecular determinants of therapy response and resistance, we have over the last years established a large number of patient derived primary colorectal cancer and metastatic cancer organoid cultures. Phenotypic comparison of the primary tissue and the organoids using immunohistochemistry with markers specific for colorectal cancer, showed a very high degree of similarity. Genomic analyses of the organoids showed a preservation of the genomic alterations with only a limited number of additional mutations in the organoids. In contrast, using gene expression analysis, we found that expression profiles of organoids strongly deviate from their respective tumor tissues. As consequence organoids do not always accurately classify as the same subtype, in particular for the mesenchymal, stromal – and stem cell subtype. It is of interest to understand these differences in particular with respect to therapy response. We will use the organoid models to develop a platform to study resistance to chemotherapy, including approaches to address intrinsic resistance due to heterogeneity. The availability of primary and metastatic cancer organoid cultures allows us to study intrinsic differences with respect to treatment response between primary tumors and their metastasis and the site of metastasis.
Pharmaceutical research: drug manufacturing – bioanalysis – pharmacokinetics

The research programmes of the Pharmacy department deal with different themes but share the same ultimate aim which is optimization of cancer treatment with medicines including cellular therapeutics. We have both preclinical and clinical projects ongoing. We work closely with the Schellens, Schinkel and Van Tellingen groups. Some projects are initiated by us, others receive our support and are executed in collaboration with other groups in or outside the Institute. The Pharmacy department holds governmental GMP (Good Manufacturing Practice) and GLP (Good Laboratory Practice) licenses. The GMP license allows us to manufacture (investigational) cytotoxic drugs and cellular products for clinical testing and we can claim a GLP standard for our bioanalytical laboratory work. A GDP (Good Distribution Practice) certificate has been awarded for worldwide distribution of medicinal products for multicentre clinical trials.

I. Bioanalytical method development + implementation in pharmacokinetic (PK) studies

Several mass balance studies with 14C-labelled drugs were supported this year. Mass balance studies investigate the absorption, distribution, metabolism and excretion (ADME) of a drug. Using radiolabeled drugs, the drug disposition, the major metabolic pathways, the exposure to the parent drug and the metabolites and the rate and route of elimination can be determined. Omacetaxine mespessuccinate (hereafter called omacetaxine) is a protein translation inhibitor approved by the FDA for adult patients with chronic myeloid leukemia (CML) with resistance and/or intolerance to two or more tyrosine kinase inhibitors (TKIs). Total radioactivity measurements revealed that omacetaxine was excreted equally through urine and faeces. High-performance liquid chromatography (HPLC) mass spectrometry (MS) (high resolution) in combination with off-line radioactivity detection was used for metabolite identification and (semi) quantitative analysis of these metabolites. In total six metabolites of omacetaxine were detected. Found conversions were mespessuccinate ester hydrolysis, methyl ester hydrolysis, pyrocatechol conversion from the 1,3-dioxole ring and combinations of these conversions. In plasma, unchanged omacetaxine was the most abundant compound. Unchanged omacetaxine together with its desmethyl metabolite 4’-DMHHT were mainly excreted in urine within the first 24 hours after administration. In faeces the pyrocatechol metabolite of omacetaxine, M534, and 4’-DMHHT were the most abundant metabolites.

A mass balance study with 14C-labelled vosaroxin, a first-in-class quinoline derivative that is currently in development in combination with cytarabine for the treatment of acute myeloid leukemia (AML), showed that 28% of the administered radioactive dose was recovered in urine and 53% in feces. Furthermore mass balance studies (also with 14C-labelled compounds) are ongoing with niraparib and plitidepsin.

For the optimization of the treatment of tyrosine kinase...
III. Pharmacokinetic and Pharmacodynamic (PK/PD) modeling and simulation

Research on population PK/PD modelling and simulation was focused on the optimization of trials with novel agents and improvement of the use of approved agents. A modelling framework that relates PK, toxicity, tumor size response, survival and cost-effectiveness for metastatic prostate cancer was developed informed by data for a phase II trial with eribulin. In this model PSA dynamics in response to eribulin was related to survival measures. Using this integrated model it is possible to optimize phase III design for novel agents in this disease and get an early estimate of cost-effectiveness.

Many approved oral anticancer agents have poor absorption characteristics. We have developed semi-mechanistic PK models for some of these oral anticancer agents (docetaxel and paclitaxel in combination with ritonavir and the tyrosine kinase inhibitor pazopanib) describing these complex absorption profiles. Within the oral taxane program, modelling and simulation was used to estimate the impact of different oral formulations on the PK of these agents in combination with ritonavir. We have shown that the novel solid dispersion tablets of both paclitaxel and docetaxel had superior absorption characteristics compared to the capsule formulation and had an almost similar profile as the drinking solution. These models are currently used to aid selection of the appropriate dosing regimen.

II. Drug manufacturing

Last year we have manufactured several oral pharmaceutical products (e.g. docetaxel, paclitaxel, elacridar) for clinical trials. Some years ago, with collaborators of the Immunology Department (Ton Schumacher and John Haanen) we founded The Amsterdam BioTherapeutics Unit (AmBTU; www.ambtu.nl). This is an independent biotech facility located in the Antoni van Leeuwenhoek Hospital which develops and produces several products for clinical trials. Currently, Tumor Infiltrating Lymphocytes (TIL) infusions are produced for melanoma patients enrolled in the first multi-center phase III trial with TIL therapy in the world. In 2015, this treatment was selected to receive temporary re-imbursement by the ministry of Health. In addition to the TIL products, AmBTU has manufactured two MART-1 T cell receptor modified T cell infusions in 2015. We have observed high on-target reactivity of these cells in melanoma patients, indicating that the developed production protocol results in highly reactive T cells. Besides the T cell products, AmBTU is currently producing DNA vaccines for HPV induced malignancies, in the context of the FP7 RAIDs program. For the second clinical trial in this consortium, in which plasmids containing a minimal helper cassette and subcellular localization sequence will be tested, Master Cell Banks and bulk plasmid DNA have been produced. In addition, the AmBTU has joined the FP7 TargetAMD consortium for which we will produce clinical grade plasmid DNA for the ex vivo transfection of retina cells.

In parallel, AmBTU is preparing itself for future clinical trials. We are aiming to apply TIL therapy in other malignancies. In addition, we would like to develop new T cell therapies directed against other tumor (neo)-antigens.
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The role of the microenvironment in prostate carcinogenesis

There is emerging evidence that stromal cells and immune cells recruited to the microenvironment play a crucial role in prostate cancer initiation and development of castration-resistant prostate cancer, which represents late stage disease with high morbidity and mortality. In contrast to the tumor cells, the stroma and infiltrated immune cells in the tumor microenvironment consists of normally regulated cells and might hold promise for clinical valuable biomarkers. The aim of our research is to address the role of the microenvironment in prostate cancer initiation, development, of CRPC and drug sensitivity.

Exploring differential gene expression between localized and metastasized human prostate cancer

In this study we aim to evaluate differential gene expression between localized prostate cancer and prostate cancer metastasized to the regional lymph nodes, with emphasis on the tumor and the tumor microenvironment. Prostatectomy specimens are selected for co-occurrence of lymph node metastases and, tumor size, initial PSA and Gleason matched control prostatectomy specimen without lymph node involvement are identified. Biopsies are taken from the area containing the tumor and from stroma from the opposite site of the specimen, at a maximal distance from the tumor. Also biopsy are taken from the lymph node metastases. Biopsies are included in a Tissue Micro Array (TMA) or DNA and RNA are isolated. RNA isolated from the lymph node metastases. Biopsies are included in a Tissue Micro Array (TMA) or DNA and RNA are isolated. RNA isolated from the different tissue compartments is sequenced. Differentially expressed genes will be found between metastasized and non-metastasized prostate cancers. Expression of differentially expressed genes will be correlated with clinical determinants including biochemical and distant recurrences. The ultimate goal is to define a signature with predictive properties for metastastic cancers.

Androgen receptor genomics and responsiveness to anti androgen drugs

Chip-seq is employed to identify Androgen Receptor (AR) responsive genes with classifier properties for sensitivity of human prostate cancer to antiandrogen drugs. We have shown that AR chromatin binding patterns can separate treatment-sensitive prostate cancers from resistant disease. A gene set with predictive properties is selected and its prospective value is currently investigated. Moreover, in a clinical trial biopsies are taken from a metastatic lesion and submitted for AR Chip-seq. Patients are treated with the antiandrogen enzalutamide and AR chromatin binding patterns are correlated with treatment outcome.

Myeloid cell populations in human prostate cancer

Macrophages are among the most abundant non-cancerous cells in the tumor microenvironment and relatively recent studies...
introduced the concept of different subtypes of macrophages that are able to influence tumor progression. The overall aim of this project is to assess the phenotype of the myeloid cells compartment and their secreted factors in the tumor microenvironment of human prostate cancer. Myeloid cell populations are quantified in human prostate cancer specimen. Moreover, macrophages are isolated from biopsies from the cancer affected peripheral zone of human prostates and phenotypically characterized by single cell sequencing (figure 1).

**Androgen receptor signaling in differentiation of prostate cancer associated macrophages**

Multiple macrophage differentiations have been described, including inflammation associated M1 and cancer promoting M2 macrophages. The amount and differentiation of infiltrating macrophages proved to be prognostic factors for prostate cancer development. Prostate cancer cells express the AR and testosterone is the main driver of prostate cancer cell growth. Moreover, CD163+ and CD206+ (M2) macrophages, in vitro generated from the peripheral blood lymphocyte fraction also expressed AR. Since macrophages differentiation might dictate prostate cancer development, we assessed the occurrence of AR expression in native human prostate cancer associated macrophages and the role of AR in differentiation of macrophages. Immunohistochemical double staining for the pan-macrophage marker CD68 and AR of paraffin embedded human prostate cancer specimen showed co-localization and mRNA sequencing of myeloid (CD14+) cells isolated from human prostate cancer biopsies showed AR expression. Both results suggest AR expression in native human prostate cancer associated macrophages. In in vitro stimulated M1 macrophages, AR translocated to the nucleus (figure 2) and CD163 and CD206 were expressed upon testosterone exposure, suggesting a direct involvement of AR in the differentiation of the macrophages. Simultaneous exposure to testosterone and the androgen receptor inhibitor RD162 restored the initial M1 phenotype (figure 3). Maintaining macrophages in M1 differentiation might be a novel mechanism of action of androgen receptor inhibitors.

**Functionality of Androgen receptor expression in human prostate cancer associated fibroblasts**

Not only normal and malignant epithelial prostate cells express the AR, but also fibroblasts. Fibroblasts are thought to contribute to prostate cancer development. Therefore is the functionality of the AR in these cells of interest. Fibroblasts are isolated from biopsies of cancer affected areas in prostatectomies and cultured in vitro. The isolated cells express various fibroblasts markers and the AR. The AR binds to the chromatin upon testosterone, which suggests transcriptional activity. AR Chip-seq showed regulation of AR of distinct gene sets that might be related to regulation of prostate cancer development.
Functional Genomics

My group uses genome-wide functional genetic approaches to identify powerful drug combinations, new drug targets and mechanisms of resistance to cancer drugs. In the past year, we have transitioned from the use of shRNA-based to CRISPR/Cas9-based vectors to perform loss of function genetic screens.

Cancer therapies based on synthetic lethality

We use synthetic lethality genetic screens for two major purposes. The first is to identify genes whose inactivation is particularly toxic in combination with a targeted cancer drug. Such experiments may reveal particularly powerful drug combinations. The second is the identification of "genotype-specific lethality", i.e. genes whose loss is lethal only in a specific genetic context. We focus these genetic screens on druggable gene families. We work closely with Jan Schellens of the Division of Clinical Pharmacology to test the drug combinations we identify in the clinic. There are currently five clinical trials active at our hospital that are based on our genetic screens and we look forward to the start of four more studies in 2016.

In a first genetic screen we took advantage of our earlier finding that \textit{BRAF(V600E)} mutant colon cancers have a characteristic gene expression signature. Interestingly, this same gene signature is also found in a subset of colon cancer that lacks a \textit{BRAF} mutation. Collectively, we refer to tumours having the \textit{BRAF} mutation gene expression signature as "\textit{BRAF-like}" tumours. \textit{BRAF-like} tumours represent some 20% of colon cancers, whereas only some 10% are \textit{BRAF} mutant. We used a shRNA-based genetic screen focused on genes upregulated in these \textit{BRAF-like} colon cancers to identify vulnerabilities of this tumour subtype that might be exploited therapeutically. We identified \textit{RANBP2} (also known as NUP358) as essential for \textit{BRAF}-like colon cancers to identify vulnerabilities of this tumour subtype that might be exploited therapeutically. We identified \textit{RANBP2} (also known as NUP358) as essential for \textit{BRAF}-like colon cancers to identify vulnerabilities of this tumour subtype that might be exploited therapeutically.

In a second synthetic lethality screen we searched for phosphatases whose suppression is synthetic lethal with \textit{BRAF} inhibition in \textit{BRAF} mutant colon cancer. We found that loss of \textit{PTPN11} is synthetic lethal with \textit{BRAF} inhibitors in \textit{BRAF} mutant colon cancers. In \textit{KRAS} mutant tumours of colon, lung and pancreas, inactivation of \textit{PTPN11} resulted in sensitivity to MEK inhibitors in mitogen-rich culture media. However, in low serum
conditions and in vivo, KRAS mutant lung cancer cell lines failed to proliferate, due to a reduced level of RAS GTP loading. It is not clear at present why PTPN12 loss would cause a reduction in RAS GTP loading, considering that these cells carry an oncogenic KRAS(G12V) allele.

We found earlier that inhibition of the MEK kinase in KRAS mutant tumours of colon and lung is strongly synthetic lethal with suppression of the ERN1 stress kinase in human cancer cell lines. To our surprise we found that small molecule inhibitors of ERN1 kinase activity failed to replicate the effects of ERN1 protein suppression, suggesting that a scaffold function of the ERN1 protein is important in the observed synthetic lethality between inhibition of the ERN1 and MEK kinases. We are currently investigating the signalling pathway downstream of ERN1 kinase that is responsible for the insensitivity of KRAS mutant colon cancer cells to MEK inhibition. Elucidation of this pathway may uncover novel synthetic lethal interactions of MEK inhibitors in KRAS mutant cancers.

Finding mechanisms and biomarkers of drug resistance

Both intrinsic and acquired unresponsiveness to therapy is a recurring problem in the treatment of cancer. A key difference between resistance to chemotherapies and resistance to targeted therapies is that the latter are more predictable in terms of the mechanism used to induce drug resistance. As one example, resistance of melanomas to small molecule BRAF inhibitors is almost invariably associated with re-activation of signaling through the MAP kinase pathway. We have shown recently that drug withdrawal in such BRAF inhibitor-resistant melanomas leads to a growth arrest that is the result of hyperactivation of signaling through the MAP kinase pathway. This inspired us to search for vulnerabilities of BRAF inhibitor resistant melanomas that have hyperactivated MAP kinase signaling during the development of drug resistance that we might be able to exploit therapeutically. We found that treatment of therapy-naïve melanoma cells with Histone Deacetylases inhibitors (HDACi) results in increased MEK-ERK signaling. This finding predicted that treatment of BRAF inhibitor-resistant melanomas (which already have hyperactivated MAP kinase signaling) with an HDACi would result in a further activation of this pathway, which may become lethal to the drug resistant cells. Our data indicate that indeed sequential treatment of BRAF inhibitor resistant melanomas with HDACi leads to a prolonged state of proliferation arrest and cell death, which ultimately leads to regression of the BRAF inhibitor resistant tumour in vivo. Mechanistically, we find that the hyperactivation of the MAP kinase pathway, which is induced by the sequential treatment with BRAF and HDAC inhibitors, results in the generation of high levels of Reactive Oxygen Species, which is detrimental to the cancer cells. Our data indicate that simultaneous treatment of BRAF mutant melanoma with a combination of BRAF and HDAC inhibitors is not beneficial, but that a sequential treatment with BRAF inhibitors, followed by a switch to HDAC inhibitor once resistance to BRAF inhibitors has developed, results in durable responses in BRAF inhibitor-resistant melanomas. As such, our findings identify a novel vulnerability of drug resistant melanoma cells that can be exploited in the clinic. We expect to start a clinical study with HDACi in BRAF-resistant melanoma in the course of 2016.

More sophisticated models to study cancer

To date, our genetic screens to identify novel cancer-relevant genes were performed in conventional cancer cell lines grown in 2-dimensional culture. This places limits on the biological aspects of cancer that can be studied. We have therefore spent considerable time over the past year to develop more sophisticated cancer models that can be used to perform genetic screens. These models include colon cancer organoid cultures and an ex-vivo brain slice culture model to study glioblastoma invasion. Finally, we have set up a cancer model of virally transformed cancer cells that are rejected in syngeneic immunocompetent mice by T cell immunity with the aim to find genes whose inactivation mediates escape from T cell killing.

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A vulnerability of BRAF-like colon cancer cells. Genes upregulated in BRAF-like colon cancer cells were selected to perform a loss of function screen to find genes whose suppression is only lethal to BRAF-like colon cancers. RANBP2 was found to be essential in BRAF-like colon cancer to form microtubules that connect the kinetochores to the spindle poles. Consequently, such cells have an exquisite sensitivity to microtubule poisons of the vinca alkaloid family.

Tursz T, Bernards R. Hurdles on the road to personalized medicine. Mol Oncol. 2015;9:335-9


Mouse models for cancer

We use the mouse as a model organism for establishing the role of oncogenes and tumor suppressor genes in tumor development. By utilizing recombination-mediated switching and taking advantage of somatic gene transfer methods we can alter the expression of multiple oncogenes and tumor suppressor genes in a tissue-specific and temporal fashion permitting accurate modeling of tumorigenesis as it is observed in man. In this way we can study genotype-phenotype correlations and determine the synergistic activity of proto-oncogenes and tumor suppressor genes in tumor development and metastatic spread. Furthermore, we exploit these models for testing new intervention strategies.

Functional analysis of oncogenes and tumor suppressor genes

Our emphasis is on lung cancer and mesotheliomas. We use Adenovirus or Lentivirus-mediated somatic gene transfer to introduce new genes, to inactivate genes by Crisp/Cas9 or to switch conditional oncogenes and tumor suppressor genes on or off at a defined time in the appropriate target cell. Subsequently, tumor initiation and progression is monitored over time using a variety of in vivo imaging techniques.

When Rb and p53 are inactivated specifically in lung, small cell lung cancer (SCLC) ensues in almost all mice. These tumors closely resemble human SCLC. SCLC often consists of two different cell types, one showing neuroendocrine markers whereas the other has mesenchymal features. These cells show paracrine interactions that promote metastasis. FGF2 signaling resulting in MAPK pathway activation and PEA3 expression appeared critical for the metastatic spread. Earlier we have shown that overexpression of L-Myc, a gene often found amplified in SCLC, strongly accelerates SCLC development. Nfib, which is also frequently amplified, strongly accelerates SCLC development. Nfib overexpression primarily promotes mesenchymal-metastasis transition in non-small cell lung cancer Cancer Cell. 2015 (in press)

Cell of origin of SCLC and NSCLC

The cell-of-origin likely has influence on tumor characteristics. We have developed a series of Adeno-Cre viruses with cell-type specific promoters driving Cre expression. These vectors enable us to switch conditional alleles specifically in Club, Alveolar type II, Neuroendocrine and Basal epithelial cells. Whereas distinct cell types appear the preferred cell-of-origin of either SCLC or
NSCLC, we observe substantial plasticity with regard to the cells that can give rise to these tumors. We have performed an insertional mutagenesis screen to enhance SCLC development from different cell types in lung. This screen did not show accelerate tumor development. However we could identify new candidate cancer genes and confirm others that were earlier found in human SCLC. The analysis is still ongoing.

Role of p53 mutations in SCLC
We have generated an allelic series of p53 mutant SCLC tumors by shuttling frequently occurring p53 mutants into the ColA1 locus of Rbfl/fl;p53fl/fl mice. No obvious differences were found in tumor latency, tumor characteristics, and response to chemotherapy. An initial high throughput screen performed at the WTSI with mouse SCLC-derived cell lines did not reveal consistent differences in drug response. We are however pursuing a few differences we did find and are testing these in matched cell lines with and without the DN p53 allele.

Squamous cell carcinoma of lung
We have worked on generating a mouse model for squamous cell carcinoma (SCC) of lung. Various combinations of tumor suppressor loci such as Cdkn2ab (harboring Cdkn2a, Cdkn2b, p19Arf) and Pten as well as the oncogenes Sox2 and Fgfr1 were tested. The mutations introduced belong to the most frequent genetic lesions found in human SCC. These genetic lesions were conditionally activated in tracheobronchial basal cells. Cdkn2ab and Pten inactivation with or without mutant Fgfr1 overexpression gave rise to a range of tumors, some with SCC features. However when Sox2 overexpression was combined with Cdkn2ab and Pten inactivation, we observed a high incidence of exclusively SCC indicating that Sox2 overexpression forces squamous differentiation. These tumors carry markers characteristic for SCC. We are currently combining all 4 lesions in order to see whether we can accelerate further SCC development.

In generating the Cdkn2ab null mice we noted a high incidence of skin SCC. However upon breeding we lost this feature. We now have found why this phenotype was lost. We have mapped a “normal” locus in the 129P2 ES cell background that very strongly predisposes to SCC but only when Cdkn2ab is deleted. We are currently zooming in on the responsible gene(s) and will study their interaction with Cdkn2ab. This interaction is of particular interest since the Cdkn2ab tumor suppressor cluster is frequently deleted in human SCC. This might allow us to dissect the nature of the interaction with this seemingly normal locus that jointly facilitates SCC development.

Mesotheliomas
Previously we have generated mouse models for mesotheliomas by the inactivation of Nf2, p53 and Cdkn2a in the intra-thoracic mesothelium of mice. We have now also tested whether Bap1 loss can contribute to mesothelioma development in mice as BAP1 appears very frequently inactivated in human mesotheliomas. Whereas Bap1 loss by itself does not predispose to mesotheliomas, in combination with Nf2 and Cdkn2ab deletion Bap1 loss dramatically accelerates mesothelioma development making it a good model for testing interventions. We have also shown that the different mesothelioma subtypes originate from different cell types. To show this we have generated single cell clones from the mesothelial lining. Upon inactivation of Nf2, Cdkn2a, and p53 some give exclusively rise to the epithelial mesothelioma subtype. Upon introduction of the same lesions others only gave rise to sarcomatoid tumors or exclusively to mixed tumors. Each of these tumor cell clones showed a distinct marker protein expression pattern. This indicates that the cells from which the various mesothelioma subtypes arise do differ. The distinct tumor phenotype, characteristic aggressiveness and differential response to drugs present a clear example of how mesothelioma heterogeneity can be imposed by the features of the cell that undergo transformation.

Parallel to these experiments we are propagating human mesotheliomas in mice (PDX). These tumors appear to retain their mutational landscape. We have now collected a decent set of mesothelioma PDX to start further analysis (WGS and drug screens in collaboration with the WTSI).
Combining targeted therapy and immunotherapy

We aim to identify mechanisms of tumor immune escape and to develop therapeutic protocols to combine cancer immunotherapy with targeted and other therapies. Tumor immune escape mechanisms include inhibitory molecules on tumor cells or on antigen presenting cells and immune regulatory cells in the tumor environment. The functional characterization of inhibitory molecules, exploration of their inhibition and the examination of possible synergy with small molecule-based targeted and other therapies may help in designing novel approaches to improve cancer immunotherapy.

Alteration of immune infiltrates upon combined targeted therapies

Targeted therapy does not only alter tumor signaling pathways, but also the tumor environment. Thus it is crucial to simulate targeted therapies in immune-competent spontaneous mouse models for cancer. We have crossed mice that inductively express in melanocytes BRAFV600E and loose PTEN. In addition, we have a transplantable model of BRAF/PTEN melanoma for high throughput analyses. In both models, we have tested combined targeting of the MAPK and the PI3K pathways (selective BRAF, MEK, PI3K and mTOR inhibitors). We have found that the combination of BRAF and MEK inhibition was superior to single BRAF inhibition. Triple or quadruple targeted therapy further increased tumor control. Analyzing the tumor immune infiltrates, we found that the combination of BRAF and MEK inhibition induced the most favourable immune modulatory effect of BRAF/PTEN melanoma, as defined by high lymphocyte infiltration and high CD8+/Treg ratios.

Analysis of patient material in collaboration with Georgina Long, Melanoma Institute Australia, Sydney revealed that this favorable immune modulatory effect of BRAF+MEK inhibition is transient and disappears beyond 4-6 weeks of treatment. Our pre-clinical analyses have led to the setup of the ImpPemBra study, an investigator initiated phase 2 study (PI Christian Blank), testing different schedules of short term BRAF+MEK inhibition in combination with PD-1 blockade.

Biomarker identification for personalized immunotherapy

Immunotherapies like CTLA-4 or PD-1/PD-L1 blockade have revolutionized the treatment of late stage melanoma. In addition, cellular therapies, like TIL or gene-modified T cell therapies have shown clinical activity. This raises the question, which therapy would benefit which patient long-term. We have developed a concept of different biomarker groups to characterize the patient’s tumor, immune status, and tumor microenvironment. In collaboration with other groups we could show that LDH, lymphocyte count, inflammation, and mutational load are strong prognosticators for outcome upon CTLA-4 and/or PD-1 blockade. To verify these biomarkers and analyze their individual contribution within all markers, we have set up a melanoma


Christian Blank MD PhD Group leader Marcel Dekker MSc PhD student Jules Gadiot Technical staff

Publications

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patient cohort of 160 ipilimumab-treated patients analyzed for all these markers, and are currently setting up similar cohorts of patients treated either with anti-PD-1 or with the combination of PD-1 and CTLA-4 blockade. We aim to develop from these data algorithms for personalized immunotherapy.

**Sunitinib as combination partner for immunotherapy in renal cell carcinoma**

Sunitinib is a widely used targeted therapy in renal cell carcinoma (RCC). While *in vitro* data argue for an immune inhibitory effect of sunitinib, analysis of peripheral blood from sunitinib-treated patients indicates the contrary. We therefore compared the expansion of tumor infiltrating lymphocytes (TIL) from RCC tissues of patients that had or had not been treated with sunitinib. We found that sunitinib pretreatment improved TIL expansion due to a reduction of myeloid-derived suppressor cells (MDSC) in the tumor. This makes sunitinib a promising combination partner for immunotherapy, like checkpoint inhibition or adoptive cell therapy.
Psychosocial oncology in clinical genetics and supportive care

This psychosocial oncology group is concentrating on research to improve the quality of life and quality of care of individuals diagnosed with cancer and those at high risk of developing cancer. Three related research topics can be distinguished: 1) Studies investigating the uptake and psychosocial impact of genetic counseling and testing for specific hereditary cancer syndromes, 2) Studies investigating cancer-risk reducing behavior (primary and secondary cancer prevention) among at-risk individuals, and the sociodemographic, clinical, and psychosocial variables that may be associated with (non)compliance with prevention recommendations, and 3) Studies aimed at understanding and improving health care for individuals at high risk of developing cancer.

Communication of inconclusive genetic test results to first-degree relatives

In this study we assess the long-term psychosocial and medical impact of genetic counselling offered soon after diagnosis of breast cancer. The study, which is funded by Pink Ribbon, is performed in collaboration with the department of Medical Genetics of the UMCU (Ausems). In total, 112 breast cancer patients who had been actively offered genetic counseling and testing during radiotherapy between 1997 and 2004 participated in our study. One of our aims was to explore communication of inconclusive breast cancer genetic counselling results with daughters and sisters over a long period of time. Breast cancer patients who had received an inconclusive DNA test result 7-14 years earlier, completed a self-report questionnaire. Additionally, in-depth interviews were conducted and analysed thematically. Of the 93 respondents with a daughter or sister, 85 (91%) considered themselves responsible for communicating genetic test results to relatives. In-depth interviews (n=14) showed, that counselees wanted ‘to hand over’ their responsibilities to communicate the test results and screening recommendations to their sisters. Once the duty of informing is completed, little is spoken about genetic test results and screening recommendations. We recommend that, similar to the procedure for BRCA1/2-mutation carriers, a separate letter for first-degree relatives of patients with an inconclusive test result should be provided. Information about risks and screening recommendations can then be verified by family members years after genetic testing has been completed.

A second study about family communication, funded by the KWF, was initiated in the Antoni van Leeuwenhoek hospital in 2015. This study aims to develop, test, and implement a new way to facilitate communication of genetic test results to first and second degree relatives of counselled individuals. Currently, a digital portal is being developed. The extent to which tested counselees inform their relatives will be monitored. Data collection will start in 2016.
**The burden of repeated pancreatic surveillance in high-risk individuals**

The aim of this ongoing, prospective study is to evaluate the psychological burden of undergoing repeated pancreatic surveillance, including MRI and endoscopic ultrasound (EUS), offered to individuals at genetically high risk to develop pancreatic cancer (PC). In this multicenter study, individuals are invited to complete a questionnaire each year to assess motivations to participate in surveillance, experiences with MRI and EUS, perceived PC risk, topics of concern, and psychological distress. Questionnaires are sent after intake for participation in the surveillance-study but before the first MRI and EUS (T1), after the first MRI and EUS (T2), and each year after the next MRI and EUS. To date, 140 out of 152 individuals returned one or more questionnaires (response 92%). In total, 477 questionnaires were analyzed. The most frequently reported motivation to participate in PC surveillance was the possible early detection of a (precursor stage of) cancer (95-100%). Only a minority of respondents experienced MRI and EUS as uncomfortable (10% and 11%, respectively) and respondents dreaded their next EUS-investigation less as surveillance progressed. Respondents’ cancer worries decreased significantly over time. Best predictor of a higher level of cancer worries in the 2nd year of follow-up is ‘having a family member affected by PC ≤50 years of age’. The data suggest that the psychological burden of pancreatic surveillance is low at all assessments. From a psychological point of view, participation of high-risk individuals in an annual pancreatic surveillance program seems feasible. Attention should be paid to the worries of those participants who have a family member affected by pancreatic cancer <50 years.

**Facilitating patients’ decision making in breast surgery and reconstruction**

In 2015, funding was received from Alpe d’HuZes/KWF for a five-year study that aims to develop and implement an interactive, online, decision aid (Breast RECONstruction Decision Aid: BRECONDA-NL) for the Dutch population of women who have to decide on breast surgery and reconstruction. The ultimate aim of the study is to facilitate women’s decision making on breast surgery and reconstruction, and, by doing this, decrease levels of decisional conflict in relation to the choice of surgery and reconstruction, lower levels of post-decisional regret, and improve satisfaction, and ultimately quality of life. This study is coordinated by the Netherlands Cancer Institute in Amsterdam and performed in close collaboration with the UMCU and Slotervaart Hospital and the Australian BRECONDA study. The website will be unique in that it focuses on both cognitive and affective representations of all treatment options, providing a balanced view that neither favors nor opposes specific types of treatment. The study will follow 3 phases: I. Development and pilot testing of the BRECONDA-NL Internet decision aid (first year), II: Testing of the decision aid using a randomized controlled trial (second and third year), and III: implementation and testing of the aid in Dutch clinics (fourth and fifth year). Currently, we are at the first phase of the development of the Decision Aid.

**Identifying psychosocial and physical problems in dermato-oncology patients**

The aim of this study is to investigate which physical, psychosocial and functional problems are most prevalent in patients visiting a Skin and Melanoma Centre, and which tool is best in identifying these problems. All new patients who visited the Skin and Melanoma Centre of the NKI during a six-months period were invited to participate in the study, by completing questionnaires one week after their visit. The questionnaires included the distress thermometer (DT) and its checklist, the Skindex-29 (assessing skin related symptoms, emotions, and functioning). In total 210 patients (57% response) completed the questionnaires (mean age= 59, SD= 14 years; 44% men). Most prevalent complaints assessed by the problem checklist of the DT were: feeling tense (41%), fatigue (37%), and dry, itchy skin (37%). With a cut-off of >4 for the DT, 100 (48%) were identified as being distressed. One patient with a severe skin related problems (Skindex-29) was missed by the DT. Significant correlations were found between scores on the DT and the overall score of the Skindex-29 (r = 0.65; p<0.001). It seems that the DT can be adequately used as a first screening instrument to identify the presence of physical, emotional, social, and practical problems. The Skindex-29 can be offered subsequently to those patients scoring >4 on the DT to identify skin-specific problems in more detail. This step-wise procedure minimizes patients and physicians measurement burden while improving patient-centred care.
Molecular mechanisms that govern the T-cell response

Our work is inspired by the desire to improve immunotherapy of cancer. Understanding the mechanisms that govern the T-cell response will enable us to improve cytotoxic T lymphocyte (CTL)-based anti-tumor immunity. We focus on T cells and dendritic cells (DCs), costimulatory and coinhibitory receptors and their downstream signaling pathways. In the context of radiotherapy, we explore the concept of “immunogenic cell death”, combining our immunological expertise with our expertise on cell death signaling. Our work is carried out in mouse models and in matching mouse and human cellular systems in vitro.

Optimizing CTL responses to cancer

There are three key bottlenecks in the T cell response to cancer: 1) The cancer may not offer (sufficient) antigens that can be recognized by T cells; 2) DCs are not sufficiently activated to kick-start tumor-specific T cells; 3) Effector T cells are suppressed in the tumor micro-environment. We use therapeutic vaccination and radiotherapy in mouse models systems to delineate bottlenecks in T cell responsiveness and to validate means to overrule these. A large part of our work is concerned with DC activation. When DCs are not activated, they promote T cell tolerance, by offering ligands for T cell coinhibitory receptors. When DCs are activated, they promote T cell immunity, by offering ligands for costimulatory receptors. While pathogens are good at activating DCs, tumors are not. From our work, the costimulatory TNF receptor CD27 has emerged as an important target in cancer immunotherapy, since it overcomes this limitation. In collaboration with the biotech company BioNovion, we examine the activity of agonistic antibodies to CD27 with the aim to apply them clinically.

Therapeutic vaccination

CD4 T cells can also activate DCs. Therefore, therapeutic vaccines, aimed at eliciting CTL responses, contain both MHC Class I and MHC Class II epitopes. We are delineating the molecular mechanism of CD4 T-cell help for the CTL response to enable the rational optimization of anti-cancer vaccination. We use two versions of a DNA vaccine that contain a human papilloma virus (HPV)-derived CD8 T-cell epitope alone or in combination with CD4 T cell epitopes. This intra-epidermal vaccination strategy optimally reveals the impact of CD4 T cell help on CD8 T cell priming, the generation of effector and memory CTLs. We have found that CD27-CD70 costimulation is the key effector pathway of CD4 T-cell help for the CTL response. We have also revealed that antibody-based stimulation of CD27, inhibition of CTLA-4 or PD-1 all support the CTL response to cancer in distinct and complementary ways. Importantly, we have delineated by deep-sequencing the molecular signature of CD4 T-cell help in newly primed CTL effector cells. This will deliver new markers and mediators of CTL response optimization. In this model, we have found that antigen delivery to DCs is not a limiting factor in T cell priming.
However, many DCs that carry antigen, have not received an activation signal. This is a bottleneck in T cell priming and explains why CD4 T-cell help or CD27 agonism improves T cell responsiveness. We are now interested in the “danger” signals that play a role in DC activation in this model system and ways to enhance these. We explore novel modes of cell death and their consequences in this model, as well as in vitro.

Radio-immunotherapy
Radiotherapy is aimed at local tumor control with curative or palliative intent. In our radio-immunotherapy approach, spearheaded by associate staff scientist Dr I. Verbrugge, local radiotherapy is combined with systemic antibody-based immunomodulation with the aim to achieve a systemic anti-tumor immune response that eliminates both the irradiated tumor and distant metastases. In a mouse model of s.c. or orthotopically implanted breast cancer cells (AT-3), combining radiotherapy with PD-1 blockade and CD137 agonism is highly efficacious in local tumor control. The work is currently extrapolated to genetic mouse models of spontaneous breast cancer and melanoma, in collaboration with the De Visser and Blank groups. A tumor implanted outside the field of radiotherapy is not eliminated in the current experimental setting. We therefore perform mechanistic studies in the AT-3 model to delineate the cellular and molecular bottlenecks that impair systemic immunity. Next, we will rationally test approaches that alleviate these bottlenecks. For optimization of the T cell read outs a key antigen recognized by CD8 T cells on the AT-3 tumor has been identified and a TCR specific for this tumor antigen has been captured from the T cells. Our ultimate aim is to define strategies to achieve improve systemic immune responsiveness that can be implemented in radio-immunotherapy trials in the clinic.

Generating primary DCs and related cell types from their precursors
Associate staff scientist Dr Y. Xiao has developed a research line on the homeostatic development of mouse and human DCs from hematopoietic precursors. By cell surface markers and in vitro differentiation assays, she has defined in the mouse bone marrow a common precursor of monocytes/macrophages, osteoclasts and DCs (MODP) and a downstream common precursor for monocytes/macrophages and osteoclasts (MOP). She has also identified the MODP in human bone marrow and cord blood and proved that it lies downstream of what was known as the granulocyte/macrophage progenitor, but should be redefined as granulocyte-, macrophage-, osteoclast and OC progenitor (GMODP). mRNA deep sequencing has confirmed the identity of and relationship between these progenitors in mouse and human. Y. Xiao and coworkers have developed protocols for the generation of DC subsets from the human progenitors and have proven that these subsets can crosspresent antigen and prime T cells. Having the ability to generate functional human DC subpopulations at will, we can characterize them and optimize them for T-cell priming. We have thus a system in hand to study “danger” signals. We are also able to study osteoclast development, which is important for diagnostics and interventions in human cancers that display preferred metastasis to bone. In mouse studies, we perform in vivo tracing of lineage specification from the progenitors to confirm their developmental potential in the relevant cellular niches.

Understanding regulatory T cells
Regulatory T cells (Tregs) are hallmarked by the expression of the Foxp3 transcription factor that installs all their unique characteristics. Tregs are an important target in cancer immunotherapy, since they impede anti-tumor immune responses. However, simply eliminating Tregs can be dangerous, since they have an important role in preventing auto-immunity. We have recently started a large project, carried out in collaboration with the groups of Drs. D. Amsen at Sanquin and C. Berkers at the University of Utrecht to delineate key differences between conventional CD4 T cells and Tregs. This work is supported by grants from ZonMW and the Institute of Chemical Immunology and involves global analyses by transcriptomics, proteomics and metabolomics. The idea is to define unique vulnerabilities of Tregs that will be validated by genetic intervention and can addressed by (novel) chemical drugs.
Drug resistance and transporters

Our studies on mechanisms of drug resistance in cancer cells have diverged into three sub-lines:
- Characterization of a channel-promoting uptake of cisplatin/carboplatin by cells.
- The physiological function of ATP-binding cassette (ABC)-transporters, initially identified as possible contributors to multi-drug resistance.

Resistance against PARP inhibition
We have screened for genes that affect resistance to inhibitors of Poly-ADPribose Polymerase (PARP) in a mouse breast cancer model induced by deletion of the Brca1 and p53 genes in the mammary gland. Two new genes were found, Rev7 and HelB. Both counteract the resection of double-strand DNA breaks and thereby promote Non-Homologous End Joining DNA repair. The absence of both genes results in increased break resection and in a partial restoration of homologous recombination and, hence, resistance to PARP inhibitors. This project is led by Prof. Sven Rottenberg, a former post-doc in my group, now working in Bern (Switzerland). I act as advisor.

VRAC is a cisplatin/carboplatin channel
It was long known that uptake of cisplatin/carboplatin by mammalian cells is complex. About half of the drug enters by passive diffusion; the other half via a protein component, possibly a channel. We now identified this channel as VRAC, the Volume-Regulated Anion Channel. In collaboration with Thijn Brummelkamp, Sven Rottenberg et al., we used Thijn’s gene disruption procedure in haploid (KBM7) cells to search for genes involved in carboplatin resistance and found the A- and D-sub-units of VRAC. The involvement of VRAC in resistance was characterized in detail in collaboration with Thomas Jentsch in Berlin, who cloned the genes of VRAC sub-units. Although we have an indication that VRAC could affect the response of ovary cancer patients to cisplatin/carboplatin, this remains to be verified in prospective studies.

MRP5 (ABCC5) and glutamyl-conjugates
To study the physiological function of ABC transporters, we have inactivated genes for several drug transporters by targeted gene disruption in mice. We are mainly studying the Multidrug Resistance Protein (ABCC) family members ABCC5 and 6. In a systematic search for endogenous substrates of ABCC5, a transporter that is present in the basolateral membrane of most mammalian tissues, we identified N-lactoyl-amino acids, a new class of mammalian metabolites, and glutamyl-conjugates (figure 1). Further research is required to determine the full consequences of the altered disposition of these substrates in the KO mice.
**MRP6 (ABCC6) and Pseudoxanthoma elasticum**

Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease characterized by progressive ectopic mineralization of the skin, eyes and arteries, for which no effective treatment exists. PXE is caused by inactivating mutations in the gene encoding ABCC6 (MRP6), an ABC transporter that is mainly present in the liver. Abcc6−/− mice have been instrumental in the demonstration that PXE is a metabolic disease caused by the absence of an unknown factor in the circulation, the presence of which depends on ABCC6 in the liver. Why absence of this factor results in PXE remained a mystery. In the past 2 years we have shown by untargeted metabolomics that HEK293 cells expressing ABCC6 secrete large amounts of nucleoside triphosphates. Extracellularly, ectonucleotidases hydrolyzed the excreted nucleoside triphosphates to nucleoside monophosphates and inorganic pyrophosphate (PPi), a strong inhibitor of mineralization that plays a pivotal role in several mineralization disorders similar to PXE. Abcc6−/− mice and patients with PXE have a strongly reduced plasma PPi, explaining their mineralization disorder. Extensive inhibitor studies indicate that ABCC6 either acts as an ATP transporter or as an ATP channel, but we still cannot exclude that ABCC6 stimulates ATP export by affecting another channel/transporter.

**DNA BASE J**

Base J (β-glucosyl-hydroxymethyluracil), which we discovered in African trypanosomes in 1993 (Gommers-Ampt et al, Cell 1993;75:1129-1136), is a DNA-base present in kinetoplastid flagellates and in *Euglena*. It replaces 1% of thymine in nuclear DNA and we have shown that it acts as an indispensable transcription termination signal for RNA polymerase II in *Leishmania* (Van Luenen et al, Cell 2012;150:909-921). We have previously shown that the initial step of J biosynthesis, the hydroxylation of selected T-residues in DNA to yield hydroxyethyluracil, is catalyzed by 2 enzymes, J-binding Protein (JBP) 1 (which also binds to J-DNA) or JBP2 (which does not bind to J-DNA). To determine the DNA sequences recognized by JBP1/2, we used SMRT sequencing of DNA segments inserted into plasmids grown in *Leishmania tarentolae*. This has shown that JBP2 hydroxylates selected T’s in G-rich segments, potentially able to make G-quadruplexes. JBP1 then binds to J and hydroxylates another T-residue 13 bp downstream (but not upstream) on the complementary strand, allowing JBP1 to maintain existing J following DNA replication. This explains why in the telomeric sequence (GGGTTA)n only the second T is replaced by J as the only T on the complementary strand (CCCAAT)n is then exactly 13 nucleotides downstream. Figure 2 shows how we now think that JBP1/2 mediate J insertion into unique positions in DNA. Our experiments in this project were terminated in Amsterdam and are being continued by Peter Myler (Seattle).
We use and develop experimental genetic approaches to study gene function in human cells.

**Saturation-level mutagenesis of the human genome**

Basic research carried out over the last forty years in the field of molecular biology has dramatically increased our understanding of cell biology. Unfortunately, even with today's knowledge it is rarely possible to predict key players in networks related to human disease. An unbiased way to gain insight into complex systems is to randomly damage components and monitor the effects. Unfortunately, genome mutagenesis has been applied to biological processes operating in human cells to a limited extend. To study the human genome in the most simple and direct manner we use a genetic model system in which human genes are present in a single copy due to genetic haploidy. Here, deep mutagenesis can be combined with next-generation sequencing to link genes to phenotypes. We use this yeast-like system to identify genes that play a role in human disease.

**The core genes essential for life of cultured haploid cells**

Genome mutagenesis has been used in genetic model organisms to identify the genes that are required for viability. Most single-cell organisms can tolerate loss-of-function mutations in the majority of their genes. In yeast, about 1,000 of the total of 6,000 genes are essential for viability. Due to technical restraints the landscape of essential genes in human cells remained unclear. Complex human cells could require proportionally more essential genes, or alternatively, if humans evolved increased genetic redundancy and robustness, they may require relatively fewer genes that are essential for cell fitness.

We have mapped -60 million gene-trap mutations scattered around the genome of viable haploid cells. We identified approximately 2,000 human genes that are required for optimal fitness and therefore depleted for disruptive integrations (figure 1). When compared to the complete protein-coding genome, essential genes are overrepresented in functional modules such as transcription, DNA replication and protein synthesis while depleted from categories like signaling. This survey also identified numerous previously uncharacterized essential genes. This led to the identification of TMEM258 as a new component of the oligosaccharyltransferase (OST) complex which plays a central role in protein glycosylation.

**Genetic interactions between human genes**

Although genome mutagenesis indicated that ~10% of human genes are needed for fitness, the majority of genes are not required for cell survival when mutated individually. Gene loss is often buffered by other genes and only simultaneous disruption results in lethality. The frequency of such synthetic lethal
interactions between human genes is a matter of debate and it has been challenging to address this experimentally. To study the principles of synthetic lethality in human cells we apply an approach that for the first time enables systematic synthetic lethal screens in human cells solely based on paired genetic mutations. Focusing on the secretory pathway we observe that human genes frequently engage in synthetic lethal interactions (figure 2), that these can be readily identified using an approach that combines genetic knockout alleles and that they are highly ‘compartmentalized’, i.e. the genes that are synthetic lethal with a query gene often function in the same compartment. Finally, we used this network to link the uncharacterized factors TSSC1, C10orf76 and PTAR1 to Golgi biology.

**Viral entry tactics**

Our group studies viral families that cause the most deadly human infections (Filovirus [e.g. Ebola virus], Arenavirus [e.g. Lassa virus], Bunyavirus [e.g. Hanta virus] or the most frequent human infections (Picornavirus [e.g rhinovirus]). We use haploid genetics to gain insight into the entry routes of these pathogens into human cells.

Haploid genetic screens for Ebola and Lassa virus entry revealed that our previous understanding of virus entry was incomplete. In the classic model receptors are recognized at the cell surface and then mediate every step of virus delivery into the cytoplasm. This model does not seem to apply to Ebola and Lassa viruses. Instead, entry of these pathogens requires an additional step that involves a ‘receptor switch’ to an intracellular transmembrane protein, recognized deep in the endosomal compartment. For Ebola virus this intracellular receptor is NPC1, the lysosomal cholesterol transporter. We have addressed in detail how NPC1 contributes to virus entry. We have shown that a single loop of this 13-pass transmembrane protein interacts with the Ebola glycoprotein. This interaction is required for fusion of the viral membrane with cellular membrane and delivery of the viral genome into the cytoplasm. Moreover, NPC1 fulfills a cardinal property of viral receptors: it dictates species tropism. Single amino acid changes in NPC1 influence the cellular host range of Ebola virus. Our work suggests that NPC1 functions as the long-sought intracellular entry receptor for Ebola virus.

We also studied Lassa virus entry into human cells. For this virus it was known that the entry receptor at the cell surface is a glycosylated form of α-dystroglycan. Indeed, our haploid screen identified α-dystroglycan as a critical host factor for virus infection. In addition the screen pointed out that entry into human cells involved a pH-dependent switch to a new receptor: the intracellular lysosome-resident protein Lamp1. Binding to Lamp1 enables membrane fusion and further haploid screens showed that the sialyl-transferase St3gal4 prepares Lamp1 for interaction with the virus glycoprotein. A single glycosylated residue (N76), present in susceptible species but absent in birds is essential for this interaction. The resistance of Lamp1-deficient mice to virus propagation emphasized the importance of this receptor switch in vivo. The shared requirement for receptors encountered in the late endocytic compartment – despite the evolutionary, morphologic, and functional differences between Ebola and Lassa viruses – suggests that additional viruses might also utilize internal receptors that are yet to be discovered.
Impact of the immune system on metastatic breast cancer and therapy response

Metastasis formation and unresponsiveness to conventional therapies are the challenges in cancer therapy that most urgently need solutions. We focus on the immune system and its influence on breast cancer metastasis and therapy responsiveness. Through mechanistic understanding of the crosstalk between the immune system and cancer cells, we aim to contribute to the design of novel immunomodulatory strategies to fight metastatic breast cancer and to increase the efficacy of anti-cancer therapies.

**Dissecting the impact of the immune system on breast cancer metastasis**

Metastasis accounts for over 90% of breast cancer-related deaths. Despite its devastating effects, metastatic disease is still poorly understood and incurable. Emerging evidence indicates that immune cells and their mediators modulate cancer dissemination. However, the precise contribution of immune cells to metastasis is unknown. One major focus of our lab is to dissect the impact of the immune system on the different steps of the metastatic cascade.

Based on the strong association between accumulation of neutrophils in blood of cancer patients and metastasis formation, we set out to study the consequences of breast cancer-induced systemic inflammation. Utilizing mouse models of spontaneous breast cancer metastasis that mimic the disease course in humans, we discovered that tumors elicit a systemic inflammatory cascade to dampen anti-tumor T cells and maximize metastasis formation. We demonstrated that tumor-derived CCL2 and IL1β elicit IL17 expression from γδ T cells, resulting in systemic, G-CSF-dependent expansion and polarization of neutrophils in mammary tumor-bearing mice. Tumor-induced neutrophils had the ability to suppress CD8+ T lymphocytes, which limited the establishment of metastases. Neutralization of CCL2, IL17 or G-CSF and absence of γδ T cells prevented neutrophil accumulation and down-regulated the T cell-suppressive phenotype of neutrophils. Moreover, the absence of gd T cells or neutrophils profoundly reduced pulmonary and lymph node metastases without influencing primary tumor progression (Coffelt et al. Nature 2015). These findings provide novel mechanistic insights into the thus far poorly understood metastatic cascade. Moreover, our data indicate that targeting this novel cancer cell-initiated γδ T cell-IL17-neutrophil axis represents a new strategy to inhibit metastatic disease.

We are currently validating our findings in human breast cancer patients. In addition, we are performing mechanistic studies to understand how the genetic make-up of tumors influences this systemic inflammatory cascade. In collaboration with the lab of Prof. Jos Jonkers, we have screened twelve genetically engineered mouse models of spontaneous mammary cancer,
each carrying different genetic drivers, for cytokine abundance
in the serum and neutrophil expansion in the circulation. We
found that tumors deficient in p53 have increased serum levels
of IL1b, IL17 and G-CSF. In accordance with this observation,
mice bearing p53 null tumors displayed a greater proportion
of circulating neutrophils than p53-proficient tumors. Current
efforts are underway to determine how loss of p53 function
regulates the gd T cell – IL17 – neutrophil axis. In parallel, we
are assessing the tumor- and metastasis-modulating effects
of other inflammatory mediators and myeloid cells. Together,
these mechanistic data provide further insights into how breast
cancer metastasis occurs and uncover potential new targets.
The ultimate goal of these studies is to contribute to the rational
design of novel strategies that target inflammatory pathways to
fight metastasis.

**Elucidating the impact of the immune system on the efficacy of anti-cancer therapies**

One of the major impediments to effective cancer therapy is
the acquisition of therapy resistance. Using the K14cre:EcdF/
F::p53F/F mouse mammary tumor model, we study the ability
of the immune system to modulate the anti-cancer efficacy
of chemotherapy and immunotherapy. We have observed that
different types of chemotherapeutic drugs have a differential
effect on influx of immune cells into the tumor. This raises
the question whether the altered inflammatory response in
chemotherapy-exposed tumors is just a bystander effect, or
whether it actually influences chemoresponsiveness. Against
the prevailing dogma, we have demonstrated that adaptive
immune cells do not affect the outcome of chemotherapy against
established spontaneous mammary tumors in our mouse models.
Instead, components of the innate immune system were found to
counteract the anti-cancer efficacy of chemotherapy. Targeting
macrophages by CSF-1R blockade markedly improved the anti-
cancer efficacy of cisplatin, but not of docetaxel. We discovered
that depletion of macrophages through CSF-1R blockade
together with cisplatin treatment evoked a compensatory
neutrophil response limiting the synergistic anti-cancer effect.
These data highlight the importance for optimally matching
chemotherapeutics with immunomodulatory compounds and
indicate that the inherent flexibility and redundancy of the
immune system lends itself to deleterious feedback mechanisms
in which the function of a depleted population is reinstated
by another population. The goal of our current projects is to
understand the mechanisms by which myeloid cells counteract
the efficacy of conventional anti-cancer therapies, and to
dissect the resistance pathways arising from within the immune
system upon treatment with immunomodulatory therapies.

In parallel, we are dissecting cancer-induced
immunosuppressive mechanisms driving escape of primary
breast tumors and metastases from immunotherapeutic
strategies. We found that K14cre:EcdF/F::p53F/F mice with
established mammary tumors do not respond to therapy with
the immune checkpoint inhibitors anti-PD-1 and anti-CTLA-4
when applied as a single treatment modality; however, synergy
is observed when combined with platinum-based chemotherapy
and this is dependent on CD8+ T cells. In line with these results,
we found an increase in the number of tumor-infiltrating CD8+ T
cells in K14cre:EcdF/F::p53F/F mice treated with the combination
therapy as compared to chemotherapy only. We are currently
expanding these studies to the breast cancer metastasis
model. We are also testing whether reprogramming of the
immunosuppressive microenvironment increases success of
cancer immunotherapy. Besides providing mechanistic insights,
this research line has clear relevance for the clinic, and we
foresee that findings from this project may contribute to the
rational design of combinatorial strategies aimed at maximizing
clinical success of immunotherapy for metastatic breast cancer
patients.
Immunotherapy, immunomonitoring and production facility

This research is aimed at developing novel T-cell based immunotherapies that can be applied in cancer patients. The focus is on patients with solid tumors, especially melanoma, renal cell carcinoma, and HPV-associated cancers. These immunotherapies comprise DNA based vaccines and T-cell products, including TILs and genetically modified peripheral blood T cells. GMP production of these therapeutic agents takes place in the Amsterdam Biotherapeutics Unit (AmbTU), situated in the hospital pharmacy. A second objective concerns immunomonitoring, primarily to evaluate the effects of novel immunotherapies. These studies are conducted together with the Schumacher and Blank lab at the NKI-AVL and with national and international collaborators.

DNA vaccination for the treatment of high-risk HPV-associated cancers

HPV infection (serotypes 16 and 18) is associated with development of squamous cell cancer of the cervix, penis, vulva, anus and oropharynx. HPV proteins E6 and E7 are foreign antigens and are required for carcinogenesis. Therefore, they are exquisite targets for immunotherapy, as already demonstrated by therapeutic vaccination in patients with vulval lesions.

In 2015, in collaboration with Prof Gemma Kenter, gynaecologic oncologist, and co-workers we are performing a phase I study in patients with HPV 16-positive Vulvar Intraepithelial Neoplasia Grade III (VIN III) using a novel and potent intradermal DNA vaccination strategy. In preclinical studies, we have developed highly immunogenic and safe HPV 16 E6- and E7-containing DNA vaccines for which we have started GMP production in 2010. The E7 DNA vaccine has been prepared and has been tested in the phase I dose-escalating clinical trial. Immunomonitoring is being performed at the NKI (see below) and in collaboration with Prof S. van den Burg at the LUMC.

TIL therapy

Adoptive therapy with TIL is based on results from the NIH, Bethesda, USA and the Sheba Medical Center, Tel Aviv, Israel, showing a 50% objective response rate in heavily pretreated stage IV melanoma patients. This treatment combines the ex vivo culture of melanoma-reactive T cells isolated from metastases with non-myeloablative chemotherapy and high dose bolus IL-2. Our goals are: 1) to show that this treatment can be given safely at the NKI-AVL, 2) to demonstrate in a randomized controlled phase III trial that this treatment improves progression-free survival compared to standard treatment and 3) to perform a comprehensive analysis of the T-cell specificities of the melanoma-reactive TIL prior to and after adoptive transfer. We have started a pilot study and enrolled ten patients. Five patients had an objective response (3 PRs and 2 CRs). One patient with CR is now free of disease for over 4 years and the other patient for more than 2.5 years. The median overall...
survival in this small study is 16 months. The safety of the TIL treatment was as expected and side effects could be attributed to the chemotherapy and high dose IL-2. In collaboration with Sanquin and two European cancer centers, we have initiated an international, randomized controlled phase III trial in stage IV melanoma patients, comparing TIL with standard of care for second line treatment. Enrollment of patients started in October 2014. Up to date 21 patients have been randomized. Materials are collected for translational research.

Together with the biotech company Miltenyi, we explore TIL production using a novel integrated cell-processing device. We will compare the final infusion product obtained by standard TIL culture methods with that grown in the integrated device, examining cell viability, total number, phenotype and function. The device may in the future (partially) replace cell production in large GMP facilities.

**TCR gene therapy**

In collaboration with the Schumacher lab, we have selected a highly avid TCR specific for melanocyte differentiation antigen MART-126-35. This TCR, called ID3, has been produced by a German GMP manufacturer, by expression from a retroviral vector (MP-71). The construct prevents mispairing with endogenous TCR chains to prevent unwarranted cross-reactivities of transduced T cells. Clinical grade culturing and transduction of peripheral T cells with the ID3-MP-71 retrovirus has been validated step-by-step in our GMP facility. A phase I/II clinical study was started in melanoma patients in 2012. The first patient had lethal multi-organ failure, probably induced by a cytokine release syndrome. The study was immediately halted and was reopened in 2013 as a dose escalation study. A second patient was treated with 100-fold fewer TCR gene transduced T cells. In 2014, two more patients were enrolled. The treatment was without complications except a skin rash, which was biopsied. Pathology showed infiltration of activated CD8+ T cells in the epidermis and loss of MART-1 expressing melanocytes. In 2015, two patients were treated with 5 times higher T cell dose (2.5 x 10^6 transduced T cells). For the first time apart In 2015, two patients were treated with 5 times higher T cell dose (2.5 x 10^6 transduced T cells). For the first time apart

**Immunomonitoring of melanoma patients treated with immunotherapy**

We aim to map the effect of immunotherapies by dissecting cancer-specific T-cell responses in peripheral blood and tumor tissues of cancer patients. The results are correlated with clinical outcome to improve our understanding of the mechanisms underlying the anti-tumor effects of the therapies. In recent years, we have obtained tumor tissues and peripheral blood from stage III and IV melanoma patients treated with ipilimumab (anti-CTLA-4), TIL, or TCR gene therapy. We expect to add to this material from patients treated with anti-PD1 and other immune checkpoint inhibitors in the near future. In collaboration with the Schumacher lab, dr Pia Kvistborg, who heads the immunomonitoring group, has set-up flow cytometric analysis of TCR specificities by combinatorial coding using MHC peptide exchange technology. This elegant approach identified T-cells with defined specificities for melanoma antigens in peripheral blood and metastases of melanoma or lung cancer patients treated with ipilimumab, anti-PD1 or TIL. T-cell responses were directed against several shared tumor antigens and also against mutated self-antigens. The data indicate that in ipilimumab-, anti-PD1- and TIL treated patients neo-antigen-specific T cells can be readily detected sometimes in very high frequencies, which point towards an important role of these T cells in the clinical response to immunotherapy.

**Tumor grafting and resistance to targeted agents**

In collaboration with Prof Daniel Peeper (Division Molecular Oncology), we have developed a mouse model to study drug resistance of human melanomas, such as to the BRAF inhibitor vemurafenib, dabrafenib or the combination of a BRAF and MEK inhibitor. Biopsies or resected melanoma metastasis tissue are subcutaneously grafted in immunodeficient (NSG) mice. The tumor take in these animals is highly successful and hence these animals can subsequently be treated with targeted drugs to study resistance. Tumor tissues from these animals are subsequently subjected genomic, transcriptomic and proteomic analysis. This work is performed under supervision of Prof Peeper.

**References**


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**Detailed References**

The group investigates the health effects of low dose radiation exposure from medical sources, particularly cancer risk following computed tomography scans, and addresses various statistical challenges in radiation epidemiology. Moreover, the group provides statistical expertise and training to investigators from the hospital and the research laboratories on topics from basic to clinical and epidemiological research.

**Publication**

- **Biostatistics**
  - Exposure to ionizing radiation from pediatric computerized tomography scans and subsequent cancer risk – The Dutch CT study
  - Computed tomography (CT) delivers substantially higher radiation doses than most other diagnostic imaging techniques, such as a simple X-ray, and its use has increased dramatically during the past 10-15 years. We collected data on 162,886 patients who received 258,297 pediatric CT scans during 1979-2014 in one of 42 participating Dutch hospitals. After extrapolation, the estimated annual number of pediatric CT scans in the Netherlands increased from 7,731 in 1990 to 26,023 in 2012. More than 70% of all scans were of the head and neck. During the last decade, substantial increases of more than 5% per year were observed in general hospitals with less than 500 beds and among children of 10-18 years of age (Meulepas et al, submitted). As children are more sensitive to radiation-induced cancer, we linked our data with the Netherlands Cancer Registry to determine cancer incidence. The augmented data will be used to evaluate the association between radiation exposure from pediatric CT scans and risk of leukemia and brain tumors. The data will be contributed to the EPI-CT consortium for a pooled analysis of several European studies, in order to investigate risk for rarer cancers (Bosch de Basea et al., J Radiol Prot 2015). The group is leading the statistical analysis of the combined European consortium data, which is expected to include about one million children. We also studied bias of studies on CT studies scans and cancer risk by unobserved cancer susceptibility syndromes (CSS), using a theoretical approach. CSS might be confounders since they are associated with increased cancer risk and may increase uptake of pediatric CT scans. We showed that associations with leukemia reported in previous studies are unlikely to be substantially confounded, whereas brain tumor risks might have been overestimated due to confounding by tuberous sclerosis complex (Meulepas et al., Cancer Epidemiol Biomarkers Prev, 2016).

- **Radiation dose-response and second primary cancers of the gastrointestinal tract: an international study of cancer survivors**
  - Testicular cancer (TC) survivors are at increased risk for second gastrointestinal cancers. However, the roles of specific treatments are unclear. We performed two case-control studies nested in an international cohort of 23,982 5-year survivors of TC diagnosed during 1947-1991 to evaluate treatment-related stomach and pancreatic cancer risk. Doses from radiotherapy to
the stomach were estimated for 92 stomach cancer cases, and
doses to the pancreas for 80 pancreatic cancer cases. Cases
were matched to 2-3 controls. Among TC survivors, stomach
cancer risk increased with stomach dose (p-trend=0.001), with
odds ratios (OR) of 2.0 (95% CI 0.5-8.7) for 10-19.9 Gy, 2.5 (0.8-
7.9) for 20.0-29.9 Gy, 7.2 (2.1-24.9) for 30.0-39.9 Gy, 6.7 (1.7-
27.1) for 40.0-49.9 Gy, and 20.5 (3.7-114.3) for ≥50.0 compared
with doses <10 Gy (Hauptmann et al., Br J Cancer 2015). For
pancreatic cancer, TC survivors who received radiotherapy (90%
cases, 80% controls) had a 2.9-fold (95% CI 1.0-7.8) increased
risk. The OR increased linearly by 0.12 per Gy to the pancreas
(p-trend<0.001), with an OR of 4.6 (95% CI 1.9-11.0) for >25 Gy
compared with <25 Gy (Hauptmann et al, submitted). Radiation-
related risks remained elevated >20 years after TC diagnosis
(p=0.020). A dose-response relationship therefore exists
between radiation exposure to the stomach and the pancreas and
subsequent cancer risk, and persists for over 20 years. Although
second cancers of the stomach or the pancreas are a rare
complication of TC therapy, pancreatic cancer is highly fatal. The
results may also be applicable to patients with cancers at other
sites in whom similar abdominal regions may be irradiated today.

**Biostatistics**

Several members of the group provide state-of-the-art statistical
expertise to researchers and doctors from the laboratories and
the hospital. This involves developing and implementing diverse
statistical approaches to cover a wide range of topics including
the design and analysis of epidemiologic studies and clinical trials,
the identification of prognostic and predictive biomarkers, sample
size calculations, risk prediction, as well as design and analysis of
animal and in vitro experiments. We routinely employ parametric
and non-parametric statistical methods, regression models
for binary, continuous and time-to-event outcomes, including
general models such as additive, multiplicative, and geometric
mixture models for absolute and relative risks, multilevel and
mixed modelling, missing data imputation techniques, competing
risk modelling, exact methods for small sample sizes, simulation
studies, cross-validation, and receiver operating characteristic
methodology.

An important area of research is the development of markers
indicating which treatment will be of benefit to a particular
patient (“personalized medicine”). We collaborated on the
early validation of several candidates for such predictive
markers, mostly for breast cancer (Schouten et al., Mol Cancer
Therapeutics, 2016; and Schouten et al., Clin Cancer Res
2015). Treatment-related adverse effects are another focus of
involvement. For head and neck cancer patients, we developed
a statistical model to predict hearing loss due to cisplatin
chemoradiotherapy (Theunissen et al., JAMA Otolaryngol
Head Neck Surg 2015). For prostate cancer patients, we
 demonstrated that acute toxicity levels were reduced after
image-guided intensity modulated radiation therapy compared
to 3D conformal radiation therapy (Wortel et al., Int J Radiat
Oncol Biol Phys 2015) and evaluated acute toxicity after
fractionated radiotherapy in the HYPRO trial (Aluwini et al.,
Lancet Oncol, in press). With regard to the late cardiovascular
effects of radiotherapy among Hodgkin lymphoma survivors,
we established dose-response relationships for coronary heart
disease (Van Nimwegen et al., J Clin Oncol, 2016) and valvular
heart disease (Cutter et al., J Natl Cancer Inst 2015).
Genes involved in breast cancer

We have previously identified a series of candidate cancer genes involved in breast cancer by using insertional mutagenesis in various mouse models for human breast cancer. We have validated several of these genes as genuine oncogenes and of a selection of these genes we are characterizing the function and molecular pathways they are acting in. We mainly focus on Eras, Irs4 and R-spondin genes. This knowledge can lead to novel drug targets and personalized treatment options for patients with breast cancer.

R-Spondins and their receptors LGR5 and LGR6

The R-spondin (Rspo) gene family consists of four members of which we found Rspo2 and Rspo3 most frequently tagged in our insertional mutagenesis screens. RSPOs are secreted proteins that act in the Wnt-signaling pathway. WNT and RSPO proteins bind to different receptors that strongly synergize in activation of canonical Wnt signaling. The Rspo receptors LGR5 and LGR6 are markers for tissue stem cells and likely also for tumor initiating cells. We are investigating Rspo3 as a paradigm for oncogenic R-spondin signaling in more detail. To this end, we created a mouse strain with a CAAGS-Rspo3 cassette in the antisense orientation flanked by two dissimilar lox sites in the Rosa locus designated Rspo3inv. The orientation of the Lox sites is such that upon Cre expression the Rspo3-ORF inverts to the sense orientation and remains locked. All female MMTV-Cre;Rspo3inv mice that ectopically express Rspo3 develop mammary tumors after a median latency of 349 days, validating Rspo3 as a genuine oncogene. The Rspo3 induced mammary tumors are predominantly adeno-squamous invasive carcinomas showing regions with epithelial to mesenchymal differentiation. In addition, we raised Lgr6-GFP-CreERt2;Rspo3inv mice in which we expect autocrine stimulation of LGR6 cells. These mice develop mammary gland hyperplasias but rarely mammary tumors. Interestingly, the histological phenotype of the mammary gland is very similar to that of mammary glands from pregnant mice. FACS analysis of the mammary epithelial cells revealed that Rspo3 expression caused expansion of the LGR6 positive cell population. The latter cell population was also expanded during pregnancy, in line with the notion that stimulation of LGR6 positive epithelial cells leads to a lactational phenotype. These results suggest that R-spondins play a role in mammary gland development during pregnancy by stimulating LGR6 positive cells.

In the small intestine of MMTV-Cre;Rspo3inv mice Rspo3 expression also leads to intraepithelial hyperplasia in older mice. To further study the effect of Rspo3 expression in the intestines, we have generated Lgr5-GFP-CreERT2;Rspo3inv mice, in which autocrine stimulation of Lgr5 positive stem cells causes a dramatic hyperplastic phenotype in the small as well as large intestines with expansion of the Lgr5 positive cells and surprisingly also the paneth cells. Both cell types were no longer limited to the crypt.
bottom but localized also high up in extended crypt-like structures. The hyperplasias developed in some instances to adenomas and carcinomas. We are currently dissecting the underlying mechanisms that lead to the hyperplastic growth.

**Eras and Irs4**

Irs4 is a gene encoding insulin receptor substrate 4 and we found that it confers tumorigenic properties to mammary epithelial cells by hyperactivation of the PI3K/AKT-pathway. We have shown that IRS4, in contrast to the other IRS family members is a constitutive active IRS and is regulated at the transcriptional level. Eras encodes a constitutive active Ras-like gene, which is normally only expressed in embryonic stem cells. We have shown that ERAS also strongly activates the PI3K pathway, whereas it does not activate the MAPK pathway in a series of different cell lines. Moreover, expression of Eras in immortalized mammary epithelial cell lines renders these cells tumorigenic when injected s.c. in the flanks of nude mice.

An MMTV insertional mutagenesis screen in ErbB2 transgenic mice and the FVB parental mouse strain showed that Eras was significantly more frequently tagged in tumors from MMTV infected ErbB2 transgenic mice than in tumors from MMTV infected wild-type mice indicating that Eras and ErbB2 collaborate in tumorigenesis. We have shown that Eras as well as Irs4 strongly synergize with ErbB2 in cell proliferation and anchorage independent growth in vitro and in tumorigenesis. It is well established that ERBB2 preferentially forms a heterodimeric complex with ERBB3 wherein ERBB2 predominantly activates the MAPK pathway and ERBB3 the PI3K pathway. Thus activation of the PI3K-pathway in cells overexpressing ErbB2 via ERAS or IRS4 may replace activation of this pathway through ERBB3. Indeed, we found that Eras expression is negatively correlated with ErbB3 expression in tumors that develop in MMTV infected ErbB2 transgenic mice.

Although Irs4 has been implicated in a small subset of T-ALL and some other cancers, to date neither Irs4 nor Eras have been implicated in human breast cancer. However, we found that either of these genes are expressed in subsets of ErbB2 positive and triple negative human breast carcinomas using gene expression array data sets and subsequently confirmed this finding by RT-PCR. Since we showed that ERAS and IRS4 proteins are constitutively active, the mere expression of the ERAS and IRS4 genes implicates activation of the PI3 kinase pathway and thus direct involvement in human breast carcinogenesis. The importance of IRS4 for human breast cancer is further stressed by our finding that expression of Irs4 in various cell lines with ErbB2 overexpression greatly reduced the sensitivity in vitro to therapeutic agents targeting ERBB2. We found that ERAS and IRS4-expression could also be attained in naive ERBB2-overexpressing breast cancer cell lines by culturing the cells for several passages in medium with increasing concentrations of Trastuzumab or Lapatinib. ERAS as well as IRS4-expression increased gradually with every passage, indicating selection for ERAS and IRS4 expressing cells by the increasing drug concentrations while untreated control cells did not show any increase in expression of these genes. These experiments indicate that Trastuzumab or Lapatinib resistance may be acquired during treatment by overexpression of ERAS or IRS4 and both genes may play a role in Trastuzumab or Lapatinib resistant relapse in breast cancer patients.
Cells move during the development of our body and to maintain it healthy. If uncontrolled, cell motility can cause many diseases and cancer metastasis. Our aim is to understand how cells move to combat the metastatic dissemination of cancer.

As movement requires force generated by the assembly of actin into filaments, we study the molecular mechanisms dictating actin polymerization within normal and malignant cells. To this end, we exploit cellular and animal models and deploy a multidisciplinary approach based on biochemistry, cell biology, molecular genetics and advanced imaging.

**Regulation of actin-based protrusions and cell migration**

Cellular actin cannot form filaments without actin nucleators, proteins that catalyze the polymerization of short actin filaments. When these filaments grow and push towards the plasma membrane, different types of cell protrusions arise. Lamellipodia and ruffles are veil-shaped protrusions of the plasma membrane consisting of a dense meshwork of branched actin filaments generated by actin nucleator Arp2/3 complex. Importantly, lamellipodia play an essential role in the movement of mesenchymal cells. Filopodia are finger-like and highly dynamic extensions of the cell surface composed of a bundle of linear actin filaments nucleated by Formins. Filopodia may act as antennas that sample the extracellular environment to guide the migration of cells. Invadopodia represent a different type of finger-like protrusions with an internal core consisting of both linear and branched actin filaments assembled by Formins and the Arp2/3 complex, respectively. Invadopodia promote cancer-cell migration in complex 3D environments by mediating local proteolysis of the extracellular matrix. Notably, normal and cancer cells adapt their motility program to overcome obstacles during their journey inside the body by interchanging actin-based protrusions. This plasticity ensures robustness to developmental and homeostatic programs relying on cell migration, but it also favours cancer dissemination and enables cancer cells to escape anti-metastatic therapies.

**Mechanism of initiation of lamellipodia and ruffles and role of the Arp2/3 complex in cancer metastasis**

In the past, we showed that WAVE complex activates the Arp2/3 complex and found that this signalling axis is required for the formation of lamellipodia and ruffles, as well as mesenchymal cell migration. Yet, how actin polymerization by the Arp2/3 complex is initiated within nascent lamellipodia and ruffles remains poorly understood. We have recently discovered that Formin mDia1 is an actin nucleator involved in membrane ruffling (Figure 1). At the molecular level, we observed mDia1 polymerizes linear actin filaments, activating the Arp2/3 complex, and localizes within nascent and mature membrane ruffles. We
Endogenous mDia1 localizes within EGF-induced ruffles. Control (Control KD) and mDia1 knockdown (mDia1 KD) HeLa cells were serum-starved and then stimulated with EGF for 7 minutes. Fixed cells were stained with anti-mDia1 antibodies (mDia1) and TRITC-phalloidin (F-actin). Representative central confocal sections are shown. Bar, 10 mm.

also showed that mDia1 cooperates with the Arp2/3 complex in initiating lamellipodia and ruffles. Consistently, genetic and pharmacological interference with this cooperation impaired ruffling and cell migration. Thus, our results demonstrate that the lamellipodium/ruffle-initiating machinery consists of two actin nucleators that act sequentially to regulate membrane protrusion and cell migration. Surprisingly, the Arp2/3 complex has been attributed both pro- and anti-metastatic functions depending on the origin of the tumour. To determine whether and how the Arp2/3 complex contributes to onset and progression of different cancer types, we are exploiting conditional Arp2/3-complex knockout mice and genetically engineered mouse models of cancer.

Role of Formins in cancer cell migration
In the past, we demonstrated that mDia2 plays a crucial role in the making of filopodia. As mDia2 has also been shown to regulate the life cycle of invadopodia, it is not surprising that mDia2 expression and activity are often dysregulated in metastatic cancer.

However, how mDia2 controls the formation of different types of cell protrusions and what specifies its action remain unclear. To tackle these questions, we have compiled the interactome of mDia2. Using this information, we discovered that mDia2 regulates p53 and apoptosis in an actin-independent way and how this unexpected new role of mDia2 is functionally specified. Taking advantage of the mDia2-interactome, current efforts aim at understanding how mDia2 regulates the formation of invadopodia and coordinates filopodia when cells move in complex environments.

Although mDia2 is crucial for the formation of both filopodia and invadopodia, we previously published that it is dispensable for that of lamellipodia and ruffles. Given that the opposite holds true for mDia1, our data indicate that different Formins exert distinct roles in actin-based protrusion and cell migration. In line with this notion, our characterization of general Formin inhibitor SMIFH2 revealed complex phenotypic effects on the cytoskeleton. Our ongoing work focuses on unexpected new ways whereby Formins regulate membrane protrusion and cell migration.
Lymphocytes and their precursors are licensed to transiently activate specific mutation pathways that enable efficient remodeling of antigen-receptor coding genes. To generate the enormous diversity of clonotypic antigen receptors, specific DNA lesions are generated and resolved in an error-prone fashion at defined developmental stages. These lymphocyte-specific characteristics provide an ideal model system to study the role of DNA damage response (DDR) and DNA damage tolerance (DDT) pathways in resolving specific DNA lesions to shape the immunoglobulin (Ig) repertoire and in the maintenance of genome stability.

Our research activities are focused on three subjects:
(i) DNA damage tolerance in physiology and pharmacology
(ii) Regulation of allelic exclusion
(iii) Origin of chromosomal translocations

Exploring DNA-damage tolerance as a drug-target for chemosensitization
Platinums and alkylating agents belong to the most successful chemotherapeutic drugs. Platinums induce intra- and interstrand cross-links (ICLs) and are widely applied in cancer therapy. To augment the clinical utility of these agents and enlarge the therapeutic window, we need to selectively increase their toxicity for tumor cells. DDT is a natural and effective survival strategy in response to ICL and provides an essential first line of defense conferring a survival advantage in response to genotoxic ICLs. Effective DDT depends on damage-induced, site-specific ubiquitination of proliferating cell nuclear antigen at lysine residue 164 (PCNA K164). Monoubiquitinated PCNA (PCNA-Ub) recruits damage-tolerant translesion synthesis (TLS) polymerases capable of extending the nascent DNA strand directly across a lesion, such as a non-instructive, unhooked ICL. Since DDT depends on site-specific PCNA ubiquitination, we are employing advanced genetic and biochemical methods to prohibit PCNA ubiquitination and explore the chemosensitizing potential of a DDT-blockade. Chemosensitization is determined by measuring tumor cell survival, toxicity and therapeutic outcome.

Besides these translational aspects we are interested in understanding the physiological role of DDT pathways in the DDR network. Given the relevance of PCNA-Ub in TLS activation, special focus is drawn to the relevance of PCNA-Ub in response to specific DNA lesions, the choice of specific TLS polymerases, and the identification and characterization of back-up modes.

Roles of PCNA ubiquitination and TLS polymerases κ and η in the bypass of methyl methanesulfonate-induced DNA damage
Translesion synthesis (TLS) provides a highly conserved mechanism that enables DNA synthesis on a damaged template. TLS is performed by specialized DNA polymerases of which...
polymerase (Pol) κ is important for the cellular response to DNA damage induced by benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), ultraviolet (UV) light and the alkylating agent methyl methanesulfonate (MMS). As TLS polymerases are intrinsically error-prone, tight regulation of their activity is required. One level of control is provided by ubiquitination of the homotrimeric DNA clamp PCNA at lysine residue 164 (PCNA-Ub). Our studies indicate that Polκ can function independently of PCNA modification and that Polη can function as a back up during TLS of MMS-induced lesions. Compared to cell lines deficient for PCNA modification (PcnaK164R) or Polκ, double mutant cell lines display hypersensitivity to MMS but not to BPDE or UV-C. Double mutant cells also displayed delayed post-replicative TLS, accumulate higher levels of replication stress and delayed S-phase progression. Furthermore, we show that Polη and Polκ are redundant in the DNA damage bypass of MMS-induced DNA damage. Taken together, we provide evidence for PCNA-Ub-independent activation of Polκ and establish Polη as an important back-up polymerase in the absence of Polκ in response to MMS-induced DNA damage. The reverse situation holds for UV induced DNA damage, where Polκ back-ups the absence of Polη. Our data indicate the existence of two distinct pathways of TLS polymerase activation: A major, PCNA-Ub-dependent and a minor, PCNA-Ub-independent pathway, both of which appear to depend on PCNA interaction. Furthermore, our studies reveal a lesion-specific preference in recruiting a specific TLS polymerase. How this selectivity is established at the molecular level is subject of further investigation.

**Anti-mutagenic activity of PrimPol**
During replication, lesions in the DNA template can stall replicative polymerases. The stalled replication fork can be rescued by direct replication across the lesion (translesion synthesis, TLS) or by repriming behind the lesion. PrimPol has recently been identified as a novel, ubiquitously expressed, and unique DNA polymerase, which in vitro displays both TLS and primase activities. These unique molecular properties led us to generate PrimPol deficient mice and study the role of PrimPol in DDT and the DDR network in general. Employing the CRISPR/Cas9 technology in zygotes we successfully inactivated PrimPol directly in the mouse germ line. Our in vivo investigations indicate critical anti-mutagenic activities of PrimPol.

**Establishing allelic exclusion of Ig genes**
Though potentially each B cell can express two different IgH and six different IgL chains (2κ and 4 λ) and hence twelve different antigen specificities, Ig allelic exclusion ensures that B cells are monospecific and antibody responses antigen-specific. How this critical ‘one B cell – one antibody rule’ is established at the molecular level is still enigmatic, but is controlled at three main levels: lineage specificity (e.g., completed rearrangements of B cell loci occur only in B cells and not T cells), coordinated rearrangement within a given lineage (e.g., the heavy chain locus in B cells rearranges before the light chain loci), and allelic exclusion (cell surface expression of only one allele). Present data are best compatible with the regulated model of allelic exclusion, where the product of the first productively rearranged allele prohibits further rearrangement of the second. Our studies aim to determine the contribution of the Ig transcripts and protein in establishing allelic exclusion.

**Origin of chromosomal translocations**
The field of chromosomal translocations is characterized by a number of controversies, with indiscrète borders between correlation and causality. To identify and distinguish between correlative and causal translocation risk determinants we are generating and integrating big NGS-based genome-wide data. New analytical tools are developed and applied to identify key denominators and relevant molecular and biophysical risk determinants of chromosomal translocations. These novel insights are critically and comprehensively integrated with current models. The final goal is to resolve the controversies regarding the origin of chromosomal translocations and delineate the predominant mechanism(s) of this frequent cancer-specific and cancer-initiating genetic lesion.
Telomere damage

Our genome is continuously challenged with different kinds of DNA damage. To cope with this and maintain genome integrity, cells have complex DNA damage response and repair mechanisms. To prevent repair events from acting at natural chromosome ends and causing genomic instability, natural chromosome ends are shielded by specialized nucleoprotein structures, known as telomeres. Telomeres in human somatic cells progressively shorten with every cell division, eventually causing telomere deprotection. This activates a DNA damage response that causes cell death or permanent growth arrest. By doing so this response contributes both to organismal aging and to suppression of tumor development. However, deprotected chromosome ends are also processed by DNA repair factors, causing chromosome end-to-end fusions. If cells with fused telomeres manage to divide, this results in complex, unbalanced chromosome rearrangements that promote the development of cancer. On a mechanistic level, the cellular responses to telomere dysfunction are poorly understood. Therefore, our laboratory undertakes both candidate-driven and unbiased approaches to identify, and mechanistically study, the genes and activities that play important roles in the telomere damage response and telomere-driven genomic instability. In addition, we investigate the activity of these factors in the responses to DNA double-strand breaks (DSBs). By doing so we aim to significantly increase our understanding of how DNA damage responses at telomeres, and elsewhere in the genome, affect genome stability, cancer development and aging. These new mechanistic insights will facilitate the development of novel therapeutic and diagnostic strategies for cancer and (premature) aging-related disease.

Identifying critical factors controlling DNA damage responses at telomeres

To identify novel factors that contribute to telomere-driven genomic instability we developed a loss-of-function genetic screen that we call 'TIGIR' screen for telomere-induced genomic instability regulators. The TIGIR screen relies on telomere uncapping via inactivation of the telomeric protein TRF2, using a temperature-sensitive mutant allele (TRF2ts). In this system genomic instability, caused by fusion of uncapped telomeres by the non-homologous end-joining (NHEJ) DNA repair machinery, becomes so severe that cells arrest or die. However, interference with the efficiency of telomere fusion enhances survival. Indeed, small-scale TIGIR screens identified multiple factors that, when inhibited with RNAi, allow cells to survive the lethal consequences of severe telomere uncapping. Multiple TIGIR screen hits are involved in different forms of post-translational modification. This brought us to investigate how histone methylation by specific histone methyltransferases, as well as ubiquitylation by specific ubiquitin E3 ligases and E2 conjugating enzymes, impact on DNA repair activity at telomeres and thereby on telomere-driven genomic instability.
Along these lines we previously studied the potential telomere damage response activity of the ubiquitin E3 ligase RNF8. We found that RNF8 promotes processing of uncapped telomeres and telomere-induced genomic instability by facilitating the ubiquitylation of telomeric histones H2A and H2AX and thereby the accumulation of both 53BP1 and p-ATM at uncapped telomeres. We continued addressing the role of ubiquitylation in DNA damage responses at telomeres by investigating the mechanisms by which an E2 ubiquitin-conjugating enzyme, identified in our TIGIR screens, promotes NHEJ at uncapped telomeres. We identified multiple alterations caused by depletion of this enzyme that could explain how it impacts on telomere NHEJ and that we are characterizing in more detail.

Much remains unknown about the precise requirements for DNA damage response activation and, even more so, about the mechanisms that terminate these responses. In addition to the TIGIR screen, we have therefore set up a different, but complimentary RNAi screening approach that uses the well-controllable properties of the TRF2ts system to specifically identify activities that contribute to the initiation of a DNA damage response at telomeres, or to its shutdown. We have further optimized this approach and will shortly initiate the first screens.

**Mechanisms of DNA repair pathway control at telomeres and DNA DSBs**

The main mechanisms by which cells repair DSBs are NHEJ and homology-directed repair (HDR). While HDR is a mostly error-free mechanism of DNA repair, NHEJ is intrinsically error-prone. The appropriate choice for engaging these repair pathways is critical for genome stability and is regulated at the level of DNA end-resection. In TIGIR screens we identified MAD2L2, a.k.a. REV7 or MAD2B. MAD2L2 has a well-established role in translesion synthesis (TLS) and acts in several additional cellular processes unrelated to telomeres or NHEJ. We found that MAD2L2 accumulates at uncapped telomeres and promotes their fusion by NHEJ. While MAD2L2 depletion does not impair recognition of uncapped telomeres as damaged DNA, it causes elongated 3’ telomeric overhangs, implying that MAD2L2 inhibits 5’ DNA end-resection. End-resection strongly inhibits NHEJ while committing to HDR, and is under control of 53BP1 and its interaction partners RIF1 and PTIP. In line with MAD2L2 promoting telomere NHEJ by inhibiting 5’ end-resection, knockdown of the end-resection nucleases CTIP or EXOI partially restores telomere-driven genomic instability in MAD2L2-depleted cells. Control of DNA repair by MAD2L2 is not limited to telomeres. We found that MAD2L2 also accumulates and inhibits end-resection at irradiation-induced DSBs and promotes end-joining of DSBs in multiple settings, including during immunoglobulin class switch recombination (CSR). Our additional analyses revealed MAD2L2 as a critical regulator of DNA repair activity that promotes NHEJ by inhibiting 5’ end-resection downstream of 53BP1 and RIF1. This activity of MAD2L2 appears independent of REV1 and REV3, which act with MAD2L2 in TLS, indicating that MAD2L2 controls DNA repair at telomeres and DSBs in a manner separate from its activity in TLS. This newly discovered role of MAD2L2 in DNA repair suggests that aberrant MAD2L2 expression could have pathological consequences by compromising genome integrity due to inappropriate DNA repair pathway activity at telomeres or DNA lesions. Furthermore, we finding that MAD2L2 is essential for CSR identifies MAD2L2 as a potential disease-susceptibility gene for human primary immunodeficiencies. We now use these important new insights to further investigate the mechanisms underlying control of DNA repair pathway activity at telomeres and DNA DSBs. Our efforts focus on understanding how MAD2L2 interacts with other end-resection factors and acts to inhibit 5’ DNA end-resection to block HR and facilitate NHEJ. In addition we are exploring potential additional activities of MAD2L2 at telomeres.
Biophysics of Cell signaling

Our group employs advanced microscopy and spectroscopy techniques to study cell signaling events and cytoskeletal dynamics with high spatial and temporal resolution. Electrophysiological (e.g. patch clamping) and advanced imaging (e.g. Fluorescence Resonance Energy Transfer (FRET), Fluorescence Lifetime Imaging (FLIM), Fluorescence Cross Correlation Spectroscopy (FCCS) and photorelease of caged compounds) are used in research projects in our group as well as in collaborations within and outside our institute. We also develop FRET hard- and software and biosensors for various intracellular messengers, and we develop and apply ‘super-resolution’ light microscopy.

Migration and invasion of melanoma cells

Invadopodia, the cell adhesion structures that are involved in cell invasion through degradation of the extracellular matrix, can self-organize into higher-order rosette-like structures at the basal plasma membrane. We found that G-protein coupled receptor (GPCR) agonists including the bioactive lipid lysophosphatidic acid (LPA), endothelin and thrombin can induce spectacular and highly dynamic invadopodia in human melanoma cells. Agonist-induced rosette formation causes increased breakdown of gelatin and correlates with increased invasion into the extracellular matrix.

In collaboration with Dr Wouter Moolenaar in our department, we identified mechanism and intracellular signals involved in agonist-induced rosette formation. Receptor activation appears to trigger the Rho-family GTPase Cdc42 and its downstream effector N-WASP in a Gi-dependent manner. PI3K activity is also essential, whereas the Raf-MEK-MAPK pathway and classic second messengers such as Ca²⁺, cyclic Adenosine MonoPhosphate (cAMP) and DiAcylGlycerol/Protein Kinase C are not. We hypothesize that highly motile invadopodia rosettes help cells finding suitable spots to penetrate into the matrix and thereby play a role in LPA-induced tumor cell dissemination. LPA signaling promotes metastasis in mice, while the LPA-producing enzyme autotaxin is often found overexpressed in tumor cells. LPA is also effectively degraded by melanoma cells and these thereby play a role in LPA-induced tumor cell dissemination. LPA signaling promotes metastasis in mice, while the LPA-producing enzyme autotaxin is often found overexpressed in tumor cells.

Starting to address this issue, we collaborated with Dr Carsten Schultz (EMBL, Heidelberg) to synthesize and characterize a coumarin-caged LPA derivative. Upon local illumination with UV light, this compound disintegrates into coumarin and bioactive LPA, yielding an effective tool to challenge cells with an LPA gradient in a spatiotemporally very well defined manner. Indeed, melanoma cells reacted to such gradients of LPA by developing invadopodia rosettes preferentially in the direction of the gradient (figure 1).
Super resolution imaging

Super-resolution (SR) Microscopy has become a very hot topic in the last few years (Nobel prize 2014 for Hell, Betzig and Moerner). SR microscopy employs a ‘trick’ to circumvent the fundamental limit in achievable resolution in light microscopy and obtains ~10 nm localization precision. In collaboration with Leica (Wetzlar, Germany) we have implemented the GSDIM (Ground-State Depletion Imaging) method in our lab. Over the past few years, preparation techniques and acquisition for GSDIM were much refined and problems related to the extreme sensitivity of the technique (notably, nanometer-scale drift and chromatic aberration) were solved in our lab. We organized and hosted the international workshop on SR in June.

We have now shifted towards application of GSDIM microscopy. Collaborations with several groups within and outside the NKI have led to publications on topics varying from invadopodia, intermediate filaments and microtubules to receptor distribution and clustering at the cell surface. It has become apparent that SR images also demand novel quantitative analysis algorithms, because conventional analysis methods like quantification of colocalization no longer suffice (figure 2).

siFLIM: single-image Fluorescence Lifetime Imaging Microscopy

FLIM is used to record the fluorescence lifetime $t$ of a fluorophore, i.e. the average time that a fluorophore remains in the excited state following excitation. This intrinsically quantitative technique is used to detect the physicochemical properties of the molecular environment of the dye (e.g. pH, ionic strength, radical stress, oxygen levels and more) and to determine FRET efficiency in a robust manner. The conventional FLIM detection approach, Frequency Domain analysis, suffers from drawbacks in that it is slow, requires several (typically 12) individual fluorescent images to be taken in rapid succession, and it tends to produce artifacts when the preparation changes during acquisition (as is the case for living cells).

Using a novel camera chip (MEM-FLIM design) that is capable of recording two images that are phase-shifted by 180 degrees simultaneously, we have devised and tested an innovative manner to obtain FD-FLIM images based on just a single image acquisition. siFLIM, as we call our new technique, is equally quantitative, but it is significantly faster, causes less photodamage to the cells and less bleaching to the dye, and it is completely devoid of above-mentioned artifacts. This project is carried out in collaboration with prof. Ian T. Young (Delft University of Technology) and his team. siFLIM holds the promise to replace competing techniques for fast and quantitative FRET imaging in live cells, making it the method of choice for high-throughput screening. siFLIM also holds the promise that results can be expressed directly in terms of populations of molecules (e.g., for quantification of the fraction of interacting proteins) which will make siFLIM much more suitable for a broad range of biologist users.
Mouse models of breast cancer

Research in our group is focused on the genetic dissection of human breast cancer through the use of genetically engineered mouse models (GEMMs) and patient-derived tumor xenograft (PDX) models. To this end, we have developed various models for BRCA1/2-associated hereditary breast cancer and E-cadherin-mutated invasive lobular carcinoma (ILC). We are using these models to (1) investigate genotype-phenotype relations and identify genetic changes underlying breast tumorigenesis; (2) study the role of stromal fibroblasts in mammary tumorigenesis; (3) develop prophylactic therapies for prevention of breast tumors in BRCA1-mutation carriers; (4) develop combination therapies targeting BRCA-deficient mammary tumors; (5) study mechanisms of acquired resistance to targeted therapeutics such as PARP inhibitors, FGFR inhibitors and PI3K pathway inhibitors.

Generation of novel germline and non-germline GEMMs

To speed up the generation of novel GEMMs of human breast cancer, we have developed together with the Berns group a procedure for RMCE-mediated introduction of additional mutations in embryonic stem cells (ESCs) derived from established GEMMs. The resulting GEMM-ESCs can be used to generate experimental cohorts of chimeric mice via blastocyst injections. We have successfully used this strategy to test the effects of Cre-inducible mutant alleles of Pik3ca and Akt in our WAPcre;EcadF/F mammary tumor model, and of conditional Myc overexpression in our WAPcre;Brca1−/−;p53−/− model (see below).

For rapid in vivo validation of candidate drivers in ILC, we have developed non-germline models based on intraductal injection of sgRNA encoding lentiviruses in mice with mammary gland-specific loss of E-cadherin and expression of Cas9. We have previously generated GEMMs for BRCA1- and BRCA2-associated hereditary breast cancer. Cross-species comparison of recurrent DNA copy number aberrations in human and mouse BRCA1-deficient mammary tumors revealed frequent RB loss and MYC amplification in tumors from both species. Indeed, conditional Myc overexpression in our WAPcre;Brca1−/−;p53−/− model caused dramatic acceleration of tumor development, highlighting MYC as a potential therapeutic target.

Mouse models for BRCA-associated breast cancer

We have previously generated GEMMs for BRCA1- and BRCA2-associated hereditary breast cancer. Cross-species comparison of recurrent DNA copy number aberrations in human and mouse BRCA1-deficient mammary tumors revealed frequent RB loss and MYC amplification in tumors from both species. Indeed, conditional Myc overexpression in our WAPcre;Brca1−/−;p53−/− model caused dramatic acceleration of tumor development, highlighting MYC as a potential therapeutic target.

BRCA1/2-deficient cancers are defective in DNA double-strand break (DSB) repair via homologous recombination
(HR) and therefore hypersensitive to DNA-damaging agents, including platinum drugs and poly(ADP-ribose) polymerase (PARP) inhibitors. However, these treatments do not result in tumor eradication and eventually resistance develops. To study therapy response and resistance to PARP inhibition in a realistic in vivo setting, we have performed preclinical tumor intervention studies in our GEMMs of BRCA-deficient breast cancer. Repeated treatment of these mice with the clinical PARP inhibitor olaparib resulted in induction of drug resistance due to upregulation of the P-glycoprotein drug efflux transporter or inactivation of S3BP1 or REV7. In collaboration with Sven Rottenberg and Connie Jimenez (VUmc), we are currently analyzing genomics and proteomics data from matched pairs of olaparib-sensitive and -resistant tumors to identify additional resistance mechanisms. In parallel, we are performing in vitro olaparib resistance screens in BRCA1/-2-deficient tumor cell lines, using genome-wide and DDR-focused shRNA and CRISPR/CAS9 libraries.

To study the effects of specific BRCA1 mutations on tumorigenesis and therapy response, we have generated mouse mutants mimicking defined BRCA1 founder mutations (185delAG, 5382insC and C61G) and introduced these alleles into our BRCA1 mammary tumor model. All three mutants fail to suppress mammary tumor formation, but show different activities following treatment of tumors with platinum drugs or PARP inhibitors. Whereas BRCA1-null and BRCA1-5382insC tumors never develop resistance to cisplatin, the BRCA1-185delAG and BRCA1-C61G tumors readily become resistant due to expression of a RING-less BRCA1 protein, suggesting that BRCA1 RING function is required for tumor suppression but dispensable for therapy resistance.

In collaboration with Jelle Wesseling, we have established a panel of PDX models of BRCA1-deficient and -proficient TNBC. We have also used these models to investigate response and acquired resistance to cisplatin and olaparib. Interestingly, BRCA1-methylated PDX tumors develop resistance via loss of BRCA1 promoter hypermethylation or via BRCA1 gene fusions.

**Mouse models for E-cadherin-mutated invasive lobular breast cancer**

ILC accounts for 10-15% of all breast cancers and shows frequent mutations in E-cadherin and components of the PI3K pathway. To study cooperativity between E-cadherin loss and PI3K pathway activation in ILC formation, we have generated mice with mammary-specific loss of E-cadherin and loss of PTEN or expression of mutant PI3K or AKT. The mammary tumors in these mouse models resemble human classical ILC and develop mammary tumors after a very short latency period of 110 days, demonstrating strong synergism between and E-cadherin mutation and PI3K pathway activation. To study the role of PI3K pathway activation in maintenance of established ILCs, we are performing intervention studies with PI3K pathway inhibitors.

Since the Ecad-/Pten- mammary tumors display strong stromal infiltration, we are also conducting experiments to assess the role of cancer-associated fibroblasts (CAFs) in ILC development and progression. Specifically, we will test whether genetic ablation of CAFs will attenuate tumor development or inhibit growth of established tumors.

To identify additional cancer genes that collaborate with E-cadherin loss in ILC development, we have performed Sleeping Beauty (SB) transposon mutagenesis screens in our mammary-specific E-cadherin knockout mice. We identified recurrent inactivating SB insertions in several candidate tumor suppressor genes, which we are currently characterizing in more detail. In addition, we observed truncating mutations in Fgfr2 leading to constitutively active FGFR2 in more than 50% of all tumors.

We made use of the constitutive activity of the SB transposon system to screen for genes conferring resistance to FGFR inhibitors in ILC. To this end, we performed orthotopic transplantsations with SB-induced tumors with Fgfr2 overexpression and treated the tumor-bearing mice with the FGFR inhibitor AZD4547. All tumors regressed completely but eventually acquired resistance to AZD4547. Several therapy-resistant tumors contained additional SB insertions in candidate resistance genes, which are currently being characterized.


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Molecular dissection of cancer by differential drug sensitivity

In the clinic, we mainly use anticancer drugs based on outcomes of clinical trials that have been carried out in the general breast and ovarian cancer population, whereas little is known about the molecular mechanisms underlying differential drug sensitivity. The focus of our research line is to unravel these molecular mechanisms in order to develop tests that may guide treatment decisions in the clinic and ultimately improve survival. For this purpose we use several genome-wide approaches and molecular techniques, in order to dissect the mechanisms that divide clinically well-defined cohorts of breast and ovarian cancer patients into resistant and sensitive to a particular drug. We have a close collaboration with the group of Jos Jonkers to study differential chemosensitivity in a controlled fashion. Our collaboration with Wilbert Zwart’s group focusses particularly on molecular mechanisms underlying endocrine therapy resistance. A second research line focuses on the impact of prognostic molecular classifiers on adjuvant systemic treatment advice in breast cancer.

Development of a predictive test for tamoxifen resistance in breast cancer

Previously we have identified several candidate intrinsic tamoxifen resistance biomarkers, including high expression of several phosphorylated proteins indicative of an activated PI3K and/or MAPK pathway in tumor cells. Currently, we are evaluating a number of these candidate biomarkers in the context of a preoperative window trial (NCT00738777), where hormone receptor-positive breast cancer patients are randomized between 4 ± 2 weeks of tamoxifen, anastrozole or fulvestrant before surgery. Pre- and post treatment tumor material is being collected, as well as serum samples. Analyses of paired samples, before and after anticancer agent exposure, are ongoing in collaboration with the Zwart lab. Percentage change in Ki67 score has been replaced by a more robust surrogate endpoint, based on gene expression. We demonstrated that neo-adjuvant tamoxifen treatment synchronizes ERα expression. We demonstrated that neo-adjuvant tamoxifen treatment synchronizes ERα expression. This is reflected in the Ki67 index, which is reduced by approximately 50% after treatment.

Molecular mechanisms underlying sensitivity to high dose alkylating agents

The inability of breast cancer cells deficient in homologous recombination, such as BRCA1/-2-mutated cells, to repair DNA double strand breaks (DSBs) appears to offer a target for DSB-inducing therapies, such as platinum agents, intensified alkylating therapy, and poly(ADP)ribose polymerase inhibitors. Our group previously employed array Comparative Genomic Hybridization (aCGH) to assess characteristic DNA copy number aberrations (CNAs) of BRCA1- and BRCA2-mutated breast
cancers. We called these aCGH profiles BRCA-like profiles that can be derived from any platform assessing DNA copy number aberrations. BRCA1-like tumors comprise ±7.5% of all breast cancers. In a retrospective analysis the BRCA1-like test appeared a promising companion diagnostic for adjuvant intensified alkylating (IA) chemotherapy (CT) with stem cell rescue in stage III breast cancer patients. In BRCA1-like patients the 7-year recurrence-free survival improved from 30% with standard CT to 78% with IA CT (adjusted hazard rate (HR) 0.12; p=0.001), while no benefit was observed in non-BRCA1-like patients. These striking data asked for further exploration, and were confirmed by two other retrospective studies (adjusted HR 0.15; p=0.03 and adjusted HR 0.18; p=0.003). Recently, we failed to demonstrate similar benefit with a non-myeloablative, intensified, dose-dense regimen in triple-negative, BRCA1-like patients when compared to a standard dose-dense regimen incorporating capecitabine (collaboration with the German Breast Group; GAIN study). All together, these results will now be followed by a prospective validation, called the SUBITO study. The SUBITO study is an international, randomized, controlled trial in stage III, BRCA1-like patients of 59 years or younger. The standard arm will consist of 4 x dose-dense doxorubicin (60 mg/m2)-cyclophosphamide (600 mg/m2)(AC), followed by 12 x paclitaxel 80 mg/m2 q1w with 4 x Carboplatin AUC 6 q3w. The experimental arm will consist of 4 x dose-dense AC, followed by 2 x intensified cyclophosphamide (3000 mg/m2)-thiotepa (250 mg/m2)-carboplatin (800 mg/m2) q3-q4 wks with autologous stem cell rescue. Final results are expected in 2021.

Earlier, in collaboration with the Borst, Rottenberg and Jonkers groups, we identified high XIST gene expression levels, as well as low 53BP1 protein levels as biomarkers for platinum resistance. In a follow-up study, we found indications that these markers may also serve to identify BRCA1-like patients as resistant to high-dose, alkylating chemotherapy (figures 1 and 2).

Netherlands Breast Cancer Project (NBCP)

In collaboration with the Dutch Cancer Registry and UMCU we have initiated a project to find answers for clinical and translational research questions that will never be answered anymore through prospective clinical trials. For this, we make use of the Dutch Cancer Registry, where data of over 150,000 breast cancer patients has been stored with clinical follow-up. The ultimate aim is to combine clinical data with molecular data of tumor material that has been traced back through PALGA, the Dutch nationwide surgical pathology registry. Currently, we have recollected tumor blocks of 2,500 patients aged < 40 years at diagnosis, who did not receive any form of adjuvant systemic therapy after surgery and have at least 10 years of follow-up available (see table 1 for patient characteristics). Patients had been diagnosed between 1989-2000, in an era when only node-positive patients were advised to undergo adjuvant systemic therapy. Digital images of ± 16,000 slides from ± 2,500 patients are available in a password-protected online system. Seventeen breast pathologists from several European countries have revised ± 1,400 cases so far according to an electronic, standardized case report form. First aims are to validate the MammaPrint prognostic test for breast cancer patients aged < 40 years at diagnosis and to develop a novel molecular prognostic test for triple negative young breast cancer patients. Data analysis is ongoing.


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The research in the Medema group aims to gain a better understanding of the cellular response to classic anti-cancer drugs that damage the DNA or perturb mitotic spindle assembly. His group uses the knowledge that is generated to define and experimentally test new anti-cancer strategies.

The cellular response to DNA damaging insults

In 2015 our work on the DNA damage response was primarily focused on the cellular response in the later stages of the cell cycle. In addition, we have set-up a novel system for the induction of DNA damage using inducible CRISPR-Cas9 that we intend to use to study the effect of location on the outcome of the DNA damage response. In addition, we collaborated with the laboratory of Dr. Eric Lam (Imperial College London) to identify OTUB1 as a novel FOXM1-interacting protein. Expression of OTUB1 is downregulated in response to DNA damage, but not in epirubicin-resistant cells. OTUB1 positively regulates FOXM1 expression by limiting ubiquitination and degradation of FOXM1. Clonogenic assays showed that OTUB1 can enhance the proliferative rate and epirubicin resistance through FOXM1. Collectively, these data suggest that OTUB1 limits the in breast cancer and has a key role in genotoxic agent resistance.

In collaboration with the groups of Jurgen Marteijn and Wim Vermeulen (Erasmus MC Rotterdam) we found that DNA damage influences splicing. We showed that transcription-blocking DNA lesions promote chromatin displacement of late-stage spliceosomes and initiate a positive feedback loop centred on the signalling kinase ATM. Based on these data we proposed that initial spliceosome displacement and subsequent R-loop formation is triggered by pausing of RNA polymerase at DNA lesions. In turn, R-loops activate ATM, which signals to impede spliceosome organization further and augment ultraviolet-irradiation-triggered alternative splicing at the genome-wide level. These findings define R-loop-dependent ATM activation by transcription-blocking lesions as an important event in the DNA damage response of non-replicating cells, and highlight a key role for spliceosome displacement in this process.

Exploiting chromosome instability in cancer treatment

The other aim of the lab is to unravel the mechanisms underlying bipolar spindle assembly and proper chromosome segregation, with the intention to enable us to exploit chromosome segregation errors as a means to selectively target the fitness of cancer cells. In this research line, we have focused our attention on Mps1/TTK. Mps1 is a dual-specificity kinase, with an essential role in mitotic checkpoint signalling, which has emerged as a potential target in cancer therapy. To explore the potential of Mps1 inhibition as a targeted therapy in the treatment of cancer, we have collaborated with the Netherlands Translational Research Center (NTRC) and developed a highly potent and selective small molecule inhibitor of Mps1, NTRC.
We have subsequently shown that NTRC 0066-0 inhibits tumor growth in MDA-MB-231 xenografts as a single agent after oral application. To address the effect of the inhibitor in breast cancer, we used a well-defined mouse model developed in the lab of Jos Jonkers (NKI) that spontaneously develops breast tumors that share key morphologic and molecular features with human triple negative breast cancer (TNBC). Our studies show that combination of NTRC 0066-0 with a therapeutic dose of docetaxel resulted in doubling of mouse survival and extended tumor remission, without toxicity. Furthermore, we observed that treatment efficacy is only achieved upon co-administration of the two compounds, which suggests a synergistic in vivo effect. Therefore, we propose TTK inhibition as a novel therapeutic target for neoadjuvant therapy in TNBC.

In parallel, we have identified four point mutations in the catalytic domain of Mps1 that give rise to resistance to Mps1 inhibitors. Interestingly, cross-resistance of the identified mutations to other Mps1 inhibitors is limited, suggesting that combinations of inhibitors could be used to prevent acquisition of drug resistance.

**Locking together the sister chromatids**

Cohesin stably holds together the sister chromatids from S phase until mitosis. To do so, cohesin must be protected against its cellular antagonist Wapl. Eco1 acetylates cohesin’s Smc3 subunit, which locks together the sister DNAs. We used yeast genetics to dissect how Wapl drives cohesin from chromatin and identified mutants of cohesin that are impaired in ATPase activity but remarkably confer robust cohesion that bypasses the need for the cohesin protectors Eco1 in yeast and Sororin in human cells. We uncover an unexpected functional asymmetry within the heart of cohesin’s highly conserved ABC-like ATPase machinery and show that an activity associated with one of cohesin’s two ATPase sites drives DNA release from cohesin rings. This key mechanism is conserved from yeast to humans. We propose that Eco1 locks cohesin rings around the sister chromatids by counteracting an asymmetric cohesin-associated ATPase activity.

A model for asymmetric ATPase-driven DNA release by cohesin

DNA release is dependent on ATPase activity conferred by one of cohesin’s ATPase sites. We propose that acetylation of Smc3 at the nearby K112 and K113 blocks this step and thereby locks cohesin around the sister DNAs. Both ATPase sites appear to be equally important for DNA entrapment.
Disease phenotype, including clinical outcome, is driven by underlying biological mechanisms. Translating disease biology into new diagnostic applications holds great promise for improving outcome for patients. We characterize gastrointestinal pre-malignant and malignant lesions at DNA, RNA, and protein level by tumor profiling using -omics techniques, in order to stratify patient groups and arrive at individually tailored therapies, as well as for biomarker development to improve colorectal cancer screening. Disease biology is studied using pre-clinical model systems such as organoid cultures. Clinical validation is performed by making use of large series of patient sample collections derived from screening programs and multi-center clinical trials. To facilitate the logistics that are needed for these validation studies we are involved in several international research infrastructure programs.

Early detection of colorectal cancer
Patients can be cured from colorectal cancer (CRC) when the tumor is detected and removed at an early stage. CRC as a disease lends itself perfectly for screening since it has a high prevalence, and it has a well-defined precursor lesion (adenoma) with a long dwell time providing an excellent window of opportunity for a variety of treatment options. Current immunochemical fecal occult blood test (FIT) based screening can reduce CRC mortality, but still approximately 30% of carcinomas and 70% of pre-malignant lesions remain undetected. The main objectives of this research line are unraveling the biology of adenoma to carcinoma progression, and identification and clinical validation of new biomarker based tests.

Biology of adenoma to carcinoma progression
We have extensively explored the genomics of colorectal adenoma to carcinoma progression and identified AURKA and TPX2 as major candidates driving 20q gain-associated colorectal adenoma to carcinoma progression. We have established organoid cultures derived from adenomas and carcinomas, which are currently being used to study the biology of AURKA and TPX2 in tumor progression. These studies comprise analysis of signaling pathways by (phospho)proteomics, studies that are performed as part of a longstanding collaboration with dr. Connie Jimenez (OncoProteomics Laboratory, Vumc, Amsterdam).

Identification and clinical validation of new biomarker based tests
Based on several tumor-profiling studies of adenomas and carcinomas we identified candidate biomarkers for early detection, including promoter hypermethylation markers, miRNAs and proteins. These biomarkers were subsequently validated in large collections of clinically well-characterized tumor and stool samples. New diagnostic applications holds great promise for improving outcome for patients.
and control samples. A total of 29 protein candidates were significantly upregulated in CRC. We established marker panels consisting of four complementary proteins that outperform hemoglobin as a single marker for the detection of CRC. We are currently in the process of developing antibody-based assays for further validation of these markers that ultimately will be tested in a prospective screening trial in the SU2C MEDOCC project.

**Patient stratification**
Cancer is a heterogeneous disease caused by genomic alterations that affect tumor biology and clinical behavior. Therefore, a better understanding of disease biology can help in determining who to treat, and how to treat. By DNA-, RNA-, and protein-profiling of tumor tissue it becomes feasible to stratify patients according to their molecular tumor profile, and to optimize treatment for individual patients. Next to tissue samples, also the minute amounts of tumor material in so-called liquid biopsies (e.g., blood samples) can be obtained more easily than tissue samples, are amenable to these assays. We previously collected tumor material from a series of 386 stage II and III colon cancer patients, and a series of 507 CRC patients with liver metastases. SLC2A1 and VEGFA were identified by immunohistochemistry as protein biomarkers with prognostic value for patients with metastatic disease. A prognostic classifier was established in which evaluation of AURKA expression was the first step in the classification tree. To investigate whether AURKA expression can be visualized in patients using a PET tracer, radiolabelled alisertib (MLN8237) was synthesized and evaluated preclinically. Somatic DNA alterations include gene point mutations, DNA copy number aberrations (CNA) and structural variants (SVs). The impact of SVs on carcinogenesis and patient outcome is poorly understood. We investigated recurrent CNA-associated chromosomal breakpoints in the genomes of 352 CRCs obtained from the CAIRO and CAIRO II phase III clinical trials, and identified 170 genes that were recurrently affected by chromosomal breaks in more than 3% of CRCs. Patient stratification based on recurrent breakpoint genes and point mutations revealed one CRC subtype with very poor prognosis, implying that these SVs are clinically relevant. Further studies are currently ongoing to validate these findings.

**Translational research infrastructure**
Standard hospital workflows collect clinical, medical imaging, and pathology information from cancer patients. Translational research is performed by integrating pseudonymized patient information with (molecular) experimental data, crucial for e.g. validating new biomarkers in clinical series. The CTMM TraIT (Translational Research IT) consortium is a Dutch national initiative that aims to facilitate this process more effectively by organizing, deploying, and managing a multi-partner IT infrastructure for data and workflow management targeted specifically at biomarker research. Initiatives in TraIT are closely linked to (inter)national knowledge and data management initiatives such as IMI eTRIKS, the tranSMART Foundation, Open PHACTS, BioMedBridges, BBMRI-NL, IKNL, ELIXIR and AACR GENIE. The tranSMART translational research workspace tool enables integration and querying of information across clinical, imaging, biobanking and experimental (any-omics) domains. We are currently populating tranSMART with multidimensional data sets of multiple TGO studies in the context of FAIR data stewardship. Biobanking of tissue, feces, and blood samples along with detailed phenotype annotations requires excellent logistics and infrastructure for data aggregation, data retrieval, and sample ordering. We utilize the biobank catalogue procedures as operational within the NKI CFMPB as well as (inter)nationally within BBMRI-NL, CTMM TraIT, BBMRI ERIC, BioMedBridges and Bioshare context.

Figure 1: Receiver operator characteristic (ROC) analysis of a new protein marker panel versus hemoglobin in stool samples from carcinoma patients versus controls (A) and from advanced neoplasia versus controls (B).

Figure 2: (A) DNA-based classification of CRC patients by Network Based Stratification revealed four CRC subtypes. (B) Kaplan-Meier plots indicate significant differences in overall survival among the four CRC subtypes. (C) Kaplan-Meier plots for overall survival of CRC subtype 3 patients with poorest prognosis versus patients in other CRC subtypes.
Lipid growth factor signaling

The major focus of our research is on the lipid growth factor lysophosphatidic acid (LPA), its signaling properties, biosynthesis and role in cancer. LPA signals through six specific G protein-coupled receptors, showing both unique and overlapping signaling properties. LPA receptor signaling stimulates cell proliferation, migration, survival and other functions in many cell types, both normal and malignant. LPA is produced by a secreted lysophospholipase D, named autotaxin (ATX), originally identified as an autocrine motility factor for melanoma cells. ATX-LPA signaling is vital for embryonic development and, when hyperactive, promotes tumor formation and metastasis. Therefore, the ATX-LPA receptor signaling axis qualifies as an attractive target for therapy.

Our current research focuses on ATX structure-function relationships as well as novel LPA receptor signaling pathways relevant to cancer, including the unique responses of tumor infiltrating lymphocytes (TILs) to LPA stimulation. We closely collaborate with the groups of Anastassis Perrakis (Division of Biochemistry), Kees Jalink (Division of Cell Biology-I) and Ton Schumacher (Division of Immunology). These studies should lead to novel ways of interfering with LPA receptor signaling and with undue ATX-mediated LPA production in the tumor microenvironment.

A second line of research focuses on the function of two related transmembrane glycerophosphodiester phosphodiesterases, notably GDE2 and GDE3, and their possible role in tumor suppression.

Autotaxin structure-function analysis

The LPA-generating activity of ATX has been well characterized, but the molecular basis of substrate recognition and catalysis by ATX, and how it interacts with target cells has long been elusive. Close collaboration with the group of Anastassis Perrakis (Division of Biochemistry) and others has led to the elucidation of the crystal structure of ATX, alone and in complex with a small-molecule inhibitor. The ATX structure reveals a unique hydrophobic lipid-binding pocket and a nearby open tunnel. Furthermore, ATX was found to interact with cell-surface integrins via its N-terminal somatomedin-B-like domains through an atypical mechanism. In close collaboration with the Perrakis group, current research focuses on the function of the enigmatic tunnel in ATX, especially on the characterization of certain steroids (bile acids) that bind to the tunnel and thereby modulate ATX activity and LPA signaling.

LPA receptor signaling

LPA receptors activate Rho family GTPases to regulate the actin cytoskeleton and promote cell migration and invasion. In close collaboration with the Jalink group, we find that LPA triggers the rapid remodeling of invadosomes, actin-rich membrane

Publications

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protrusions that degrade the extracellular matrix to drive tumor cell invasion. LPA, acting through the LPA₁ receptor, triggers the reassembly of invadosomes into dynamic rosette-like, matrix-degrading superstructures through activation of a G₁-linked Cdc42 pathway. Rosette formation is suppressed by the RhoA-ROCK signalling pathway. Given the abundance of ATX and LPA in tumor microenvironments, interfering with the LPA₁-G₁-Cdc42 signaling axis may help to suppress tumor cell invasion and metastasis.

Another area of renewed interest is the regulatory role of LPA in the immune system. LPA is known to stimulate the invasion of T-lymphoma cells and the motility of naïve T-cells and, hence, may act as an immunomodulator. In collaboration with the groups of Ton Schumacher and John Haanen (Division of Immunology), we have begun to explore how LPA receptor signaling affects the behavior and gene expression profile of tumor infiltrating lymphocytes (TILs) derived from melanoma patients. We find that LPA acts through the LPA₆ receptor to regulate a unique set of genes encoding membrane receptors and ligands involved in T-cell activation. Although preliminary, these results reveal LPA as a new player in the regulation of TILs. We are currently examining how the LPA-regulated transcriptome translates into altered TIL behaviour.

**GDEs, transmembrane glycerophosphodiester phosphodiesterases**

The glycerophosphodiester phosphodiesterases (GDEs or GDPDs) are a novel class of transmembrane ecto-enzymes implicated in phospholipid metabolism. However, their biochemical and biological functions remain poorly understood. GDE2 and GDE3 are of particular interest because of their ability to release glycosylphosphatidyl (GPI)-anchored proteins from the cell surface. We find that, interestingly, high GDE2 expression correlates with favorable clinical outcome in neuroblastoma patients, while GDE3 overexpression is a favorable prognostic marker in breast cancer. We are currently exploring the biochemical activities of GDE2 and GDE3 towards select GPI-anchored proteins, and how their activities affect gene expression and the phenotype of neuroblastoma and breast carcinoma cells, respectively. Collectively, these studies should reveal how selective GPI-anchor hydrolysis at the cell surface initiates new signaling pathways of direct relevance to cancer, which we hope may open new therapeutic possibilities.
Chemical cell biology of immune responses, cancer, and anticancer drugs

The Neefjes lab integrates cell biology, immunology and chemical biology to study antigen presentation to the immune system. In addition, these studies also include the address of fundamental questions in cell biology of organelles and solutes during mitosis. These include studies on the role of bacterial and other pathogen infections in tumor formation and a new approach to studying the cell biology of frequently used anti-cancer drugs. The Neefjes lab has a series of strategic collaborations with chemists and oncologists for realizing these ambitions.

MHC class I antigen presentation of peptides

MHC class I molecules are expressed on all cells and function to present a fragment of a protein to the immune system. These proteins include viral or mutated proteins and MHC class I molecules are therefore central in allowing recognition of tumors in various immunotherapy trials. We have shown how MHC class I molecules select peptides from a large pool of incorrect ones. We have combined crystal structures with biophysical experiments and cell-based assays to show that MHC class I molecules first binds many different peptides. They then undergo a conformational change that removes incorrect peptides. Peptide selection is then the result of multiple cycles of binding and testing peptides until low-rate peptides are captured.

MHC class II antigen presentation and the way the endosomal system works

MHC class II molecules resemble MHC class I in function and structure. However, MHC class II is mainly expressed on immune cells and acquire peptides in endosomes. The understanding of transport and fusion in the endosomal system has broad implications: first of all for MHC class II functioning but also for signaling by transmembrane receptors, mTOR signaling, autophagy and bacterial and viral infections. We have identified a full program controlling the positioning of the entire endosomal pathway. This appears to be controlled by an E3-ligase located in the ER that builds a ubiquitin-based connection with various adaptor proteins involved in endosome fusion and cell death. We have shown the implications of these endosomes from a perinuclear location. A DUB protein (USP15) is then required for release of vesicles in the cell periphery where they move by kinesin and dynein motors. We have now identified the mechanism driving the switch in direction with a major role of cholesterol in the control of these processes. We have expanded this by studying autophagosomes and their fusion with the endosomal pathway. This appears to be controlled by cholesterol as well. Our work forms a basis for the molecular control of endosomal motility and fusion with wide consequences for major fields in biology and disease.
**Pathogens as initiators of cell transformation**

Intracellular bacteria such as Salmonella reside in phagosomes and manipulate the host cells for uptake and intracellular survival. We have shown that Salmonella can transform cells that have TP53 mutations and MYC amplification as a minimal additional requirement. This strongly correlates to observations that we made in tissue sections of gallbladder carcinoma patients with a history of a chronic Salmonella Typhi infection. This work is the first description of a direct link between bacterial infections and cancer formation. We showed that cancer in these cases is the consequence of the bacterial manipulation of host cells for its infection cycle. Gallbladder carcinoma should then be considered collateral damage of this infection cycle.

We are now expanding this work by studying a potential role of Chlamydia and Brucella. We have also observed in our studies that Salmonella typhimurium (causing food poisoning) can induce colon carcinoma in pre-transformed mice. We are now testing whether this connection can also be found back in the Dutch population, as patients with a serious Salmonella infection as a result of food poisoning then can be motivated to attend the National Program Colon Carcinoma Screening. Finally, we are investigating whether Soluble Egg Antigens from Schistosoma pathogens can induce transformation, as this is strongly correlated to bladder carcinoma in the Third World.

**Segregation of proteins in nuclear and cytosolic subcompartments during mitosis**

MHC class I molecules sample fragments of proteins that are mainly located in the cytosol and nuclear sub-compartments. These compartments are connected by the nuclear pore that allows small proteins to pass by diffusion. Large proteins such as the proteasome require complex import machineries to move from the cytosol into the nucleus. The nuclear-cyttoplasmic distinction is lost during mitosis when the nuclear envelope is fragmented. This is then reassembled post-mitosis and in principle can capture cytosolic proteins. The team studied how cells resolve this issue. They showed that cytosolic proteins are excluded from a newly formed nucleus by physical forces. This shifted the problem to the re-introduction of nuclear proteins post-mitosis. Proteins with a nuclear localization signal are simply reintroduced within minutes after the nucleus is reformed. The proteasome is a complex without a defined nuclear import signal but a fraction of these is also swiftly reintroduced in the nucleus post-mitosis. This is a new concept in cell biology and the team is currently working on the mechanisms that drive nuclear import of proteasomes during the first minutes after mitosis. Manipulating this process likely affects cell viability and may yield new ways to control tumor cell growth.

**Chemical biology of old anti-cancer drugs and new applications**

Doxorubicin is a cornerstone in cancer treatment for over four decades. It generates DNA double stranded breaks and cancer cells are supposed to be more sensitive to this than normal cells. We have identified a new activity of doxorubicin and its family members: eviction of histones from chromatin. This delays DNA repair and alters the epigenetics. We have shown that different doxorubicin variants act at different sites in the genome and thus have different effects on tumor cells. Further structure-activity relationship studies allowed dissociation of the two effects of these drugs and we are further employing these to generate new variants with new activities. We have already developed one doxorubicin variant that appears more potent as an anti-cancer drug for particular tumors such as AML and mesothelioma. This variant drug also lacks some of the treatment limiting side effects such as cardiotoxicity and sterility. This drug is now prepared for introduction in the clinic.


Cellular protein degradation and many other cellular processes are controlled by post-translational modification of proteins with ubiquitin, a small protein that consists of 76 amino acids. Ubiquitination is a tightly controlled process. Approximately 1,000 ubiquitin ligases and 100 deubiquitinating enzymes (DUBs) are encoded in the human genome. Malfunction of several components that control ubiquitination frequently are associated with cancer. When specific ubiquitin modifications target for protein destruction, the proteasome can recognize these signals and destroy the ubiquitin-tagged protein. In this manner misfolded or redundant proteins are removed. Accumulation of such proteins can lead to diseases such as cancer and infection. These protein fragments are loaded onto major histocompatibility class I complexes that present these antigens to cytotoxic T-cells, which can subsequently eliminate infected and tumor cells. The Ovaa lab studies the biochemistry of ubiquitin modification, proteasomal proteolysis and antigen presentation using chemical approaches. It is our aim to develop novel methods and reagents that allow us to study these processes in unprecedented detail and to develop - where possible - novel small molecules to intervene with these processes to study potential new drug targets. Our research is carried out in close collaboration with various NKI investigators.

Three topics are central in the lab:
1. Ubiquitin chemistry, biology & proteomics
2. Proteasomal action
3. MHC class I antigen presentation

1. Ubiquitin chemistry, biology and ubiquitin proteomics

We study the ubiquitin system with chemical approaches by taking advantage of organic synthesis. We have recently developed methods to synthesize ubiquitin and virtually any ubiquitin conjugate. With these chemical techniques we can create practically any reagent that is needed for our research. For example, we recently synthesized all seven isopeptide-linked diubiquitin topoisomers. These conjugates are currently used to study how different ubiquitin linkage topologies interact with deubiquitinating enzymes. All ubiquitin chain topoisomers can be found in cells, but the role of only few chains is relatively well understood. This is understandable since they cannot be generated by biochemical means. With our chemical methods we are now well positioned to study the role of different ubiquitin chains in cellular signal transduction events and we can now generate the reagents required to detect interacting proteins with specific linkage specificities using proteomics approaches. Our synthetic strategy also allowed us to develop novel activity assay reagents to monitor DUB activity. The incorporation of
a native isopeptide bond makes these reagents physiologically more relevant compared to conventional assay reagents. We successfully applied them in high-throughput screens to find small molecule inhibitors of DUBs to modulate deubiquitination activity \textit{in vivo}.

2. Proteasome action
Over the last years we have developed reagents that allow monitoring of proteasomal activity. These reagents have been very instrumental in studying the specificity of inhibitors that are currently in use in the clinic or that are currently being evaluated in clinical trials. Such reagents were needed as the proteasome has six distinct active sites with different specificities. With our proteasome probes we are able to distinguish all these activities while overall proteasome activity can also be monitored in live cells. We are currently investigating how proteasome activity is regulated, as this is obviously a tightly regulated process. The tight regulation of proteasome activity is an understudied topic. The proteasome is one of the most abundant and stable complexes and its activity is likely tightly controlled.

3. MHC class I antigen presentation
Over the last few years we have developed technologies for the parallel loading of MHC complexes with either physical or chemical triggers in collaboration with the Schumacher lab. As a result high throughput measurements of MHC affinity are now routine, allowing high throughput searches for novel antigens or vaccine candidates. With this technology we have synthesized and tested over 10,000 peptides for MHC affinity with the goal of identifying novel epitopes and to find molecules that may act as immunomodulators.

Figure 1: Tool development to access the reactivity of enzymes in living cells. Cells ectopically expressing either free GFP or USE1-GFP (green) treated with Cy5-probe (red). Overlays with DAPI (blue) are shown. Pixel intensity plots (right panels) for the corresponding pairs of Cy5 (red, y-axis) and GFP material (green, x-axis) are shown.

Figure 2: Design and selectivity of diubiquitin activity based probes. The Ovaa group reported the development of a native chemical ligation handle that also functions as a masked electrophile that can be liberated during synthesis when required. This handle can be used for the synthesis of complex activity based probes with substrate context. This is a powerful tool to trap enzyme reactions and therefore extremely useful for future studies of enzymatic function and studies of protease specificity.
Functional oncogenomics for cancer drug target discovery

The Peeper laboratory develops and uses function-based approaches to ask clinically relevant questions, aiming to dissect tumor-driving genetic networks for drug target and biomarker discovery. For example, we are exploring ways to enhance the activity, or avoid resistance, of currently used targeted clinical compounds. Recently, we have also begun building experimental systems to develop combinatorial targeted and immunotherapeutic therapies.

To reach these goals, we develop and utilize in vitro and in vivo genome-wide functional perturbation systems. We are doing this for several cancer types including melanoma, lung and breast cancer. Candidate genes are validated in a clinically relevant context, for example, patient-derived tumor xenografts (PDx).

Given the advances made for immunotherapy, we have also begun, in collaboration with the laboratory of Ton Schumacher, to establish in vitro and in vivo systems allowing for including immunological factors. Candidate genes are analyzed first by bioinformaticians in the lab and then functionally to dissect the signaling networks that they operate in, uncovering pathways amenable to pharmacologic intervention as well as predictive biomarkers.

Melanoma progression

Previously, in a longstanding collaboration with prof. Mooi (VUMc), we were the first to discover that melanocytic nevi (moles) undergo oncogene-induced senescence (OIS) in vivo. We also obtained in situ evidence that reduction of PTEN levels causes abrogation of OIS and thereby contributes to melanoma progression. Numerous phenotypic changes occur during OIS, both in the cytoplasm and in the nucleus. These include the activation of autophagy, a catabolic process operating in the cytoplasm and downregulation of lamin B1, a component of the nuclear envelope. We discovered recently that cells entering BRAFV600E- or H-RASG12V-induced senescence downregulate not only lamin B1 but also lamin A, as well as several other nuclear envelope (NE) proteins, resulting in an altered NE morphology.

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Screening for novel therapeutic targets and predictive biomarkers

Targeting cancer cell metabolism

We have previously discovered by metabolic profiling (in collaboration with prof. Eyal Gottlieb, Glasgow) and functional perturbations that the mitochondrial gatekeeper pyruvate dehydrogenase (PDH) is a crucial mediator of senescence.
induced by BRAF<sup>V600E</sup>. While the activation of PDH enhanced the use of pyruvate in the tricarboxylic acid cycle, causing increased respiration and redox stress, abrogation of DIS coincided with reversion of these processes. Enforced normalization of either PDK1 or PDP2 expression levels inhibited PDH and abrogated DIS, thereby licensing BRAF<sup>V600E</sup>-driven melanoma development. Depletion of PDK1 eradicated melanoma subpopulations resistant to targeted BRAF inhibition and caused regression of established melanomas. These results revealed a mechanistic relationship between BRAF DIS and identified a key metabolic signaling axis that may be exploited therapeutically, which we are currently exploring.

**Developing an in vivo preclinical testing platform**

In recent years, the BRAF kinase inhibitor vemurafenib has generated tremendous excitement, following unprecedented responses observed in clinical trials of patients with metastatic melanoma carrying the BRAF<sup>V600E</sup> mutation. Despite such early promise, however, patients commonly relapse with drug-resistant tumours. Therefore, there is an urgent need to find drugs that can be used in combination with vemurafenib to reduce the risk of resistance. This is best done in a physiologically relevant setting. Therefore, we have established, in collaboration with our clinical colleagues prof. John Haanen, prof. Christian Blank and prof. Ton Schumacher, a xenograft platform for human melanoma. Matched patient samples, taken prior to therapy and after the patient has relapsed, are taken and placed into immunocompromised mice, which engraft melanomas highly efficiently. We have established some 100 PDX, showing cellular and genetic profiles that are similar to the patients’ melanomas. These PDX are an excellent platform drug resistance studies in a preclinical setting. Indeed, we recently discovered a new BRAF mutant protein that accounts for resistance to vemurafenib in approximately 10% of melanoma patients, and which is sensitive to a new generation BRAF inhibitor.

**A novel in vivo screening system**

We have developed a new type of cancer drug target discovery screens to identify therapeutic targets for melanoma and breast cancer in vivo, using large-scale RNAi libraries and massive parallel sequencing. To identify factors preferentially necessary for driving tumor expansion, we performed parallel in vitro and in vivo negative-selection shRNA screens. Melanoma cells harboring shRNAs targeting several DNA damage response (DDR) kinases had a greater selective disadvantage in vivo than in vitro, indicating an essential contribution of these factors during tumor expansion. In growing tumors, DDR kinases were activated following hypoxia. Correspondingly, depletion or pharmacologic inhibition of DDR kinases was toxic to melanoma cells, including those that were resistant to BRAF inhibitor, and this could be enhanced by angiogenesis blockade. These results reveal that hypoxia sensitizes melanomas to targeted inhibition of the DDR and illustrate the utility of in vivo shRNA dropout screens for the identification of pharmacologically tractable targets. In a similar in vivo screen in breast cancer we recently identified a synthetic lethal relationship between two factors, which may be explored therapeutically.

**Overcoming targeted drug resistance**

Increased expression of the key melanocytic Microphthalmia-associated transcription factor MITF is known to contribute to melanoma progression and resistance to BRAF pathway inhibition. We found that, in contrast, the lack of MITF is associated with more severe resistance to a range of inhibitors, while its presence is required for robust drug responses. Both in primary and acquired resistance, MITF levels inversely correlate with the expression of several activated receptor tyrosine kinases, most frequently AXL. The MITF-low/AXL-high drug-resistance phenotype is common among mutant BRAF and NRAS melanoma cell lines. The dichotomous behaviour of MITF in drug response is corroborated in vemurafenib-resistant biopsies, including MITF-high and -low clones in a relapsed patient. Furthermore, drug cocktails containing AXL inhibitor enhance melanoma cell elimination by BRAF or ERK inhibition. Our results demonstrate that a low MITF/AXL ratio predicts early resistance to multiple targeted drugs, and warrant clinical validation of AXL inhibitors to combat resistance of BRAF and NRAS mutant MITF-low melanomas. We are currently collaborating with pharma to explore clinical translation of these findings.

**Breast cancer metastasis: mechanism and drug target identification**

We have previously discovered a novel critical mediator of breast cancer metastasis, the Fra-1 transcription factor. Fra-1 depletion reduced metastatic potential by >3 orders of magnitude. Function-based mining of the prognostic Fra-1 signature revealed several additional factors amenable to targeted inhibition. For one of these, we have begun collaborating with MRC Technology (London) to develop a drug-screening program; the first series of inhibitors has been generated, which we are currently testing. Our recent observations suggest that the Fra-1 prognostic classifier harbors several breast cancer-driving factors amenable to targeted inhibition. This suggests the existence of a transcriptional network driving metastatic breast cancer.

**Figure 1:** Immunofluorescence microscopy showing profound loss and malformation of lamin B1 organization in BRAF<sup>wt</sup>-expressing cells. Lamins form a filamentous network lining the inner nuclear membrane and play an important role in the three-dimensional organization of the nucleus.

**Figure 2:** Somatic mutations present in several melanoma metastases in a single patient, revealing that mutations were either shared by the pre-treatment tumor and all metastases (dark grey bar), by some metastases (grey bar), or only by single metastases (light grey bar).
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Publications


Structural biology

Our research aims to provide molecular insight to macro-molecular interactions, and to understand how these regulate specific biological activities in space and in time. Many proteins have a specific biochemical activity that needs to be brought to a specific place in the cell. Typically, additional regulatory domains form interactions with other proteins, DNA, polysaccharides, or lipids, and bring the activity in the correct spatiotemporal context. Understanding these interactions can offer additional options for developing drugs that could inhibit the function of these proteins for therapeutic intervention, hopefully in a more subtle and specific manner.

In parallel to studying specific biological questions in the above context, we maintain an active interest in improving the methods that help us to understand these questions. Namely, we have a long-standing interest in providing new concepts, algorithms, and software for building optimal macromolecular models based on crystallographic data.

Structural studies of Autotaxin

ATX (or ecto-nucleotide phosphodiesterase 2, ENPP2), is capable of both lysoPLD and nucleotide pyrophosphatase activities. ATX produces the signaling phospholipid lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC); LPA and ATX have been shown by numerous studies to be involved in cancer metastasis and other pathogenic situations, such as chronic inflammation. After determining the structure of ATX (Hausmann et al, Nature Structural and Molecular Biology, 2011), we focused first in understanding the interaction of ATX with cell-surface integrins and heparan sulphate proteoglycans, suggesting mechanisms for the delivery of LPA close to the cell surface (Houben et al, JBC, 2013). We also concluded our study of the catalytic mechanism of ATX, determining crystal structures of ATX with orthovanadate and with a phosphorylated nucleophile Thr209. These structures mimic reaction steps of the substrate hydrolysis by ATX, supporting a concept of an associative two-step in-line displacement mechanism for ATX.

This year we determined the crystal structures of ATX bound to 7α-hydroxycholesterol and the bile salt tauroursodeoxycholate (TUDCA), showing how a tunnel near the active site of ATX selectively binds steroids. We also determined the crystal structure of ATX bound to TUDCA and LPA at the same time (Figure 1), which together with kinetic analysis establish that bile salts act as partial non-competitive inhibitors of ATX, thereby attenuating LPA receptor activation. This unexpected interplay between ATX-LPA signalling and select steroids (natural bile salts) may explain the emerging association of ATX with disorders associated with increased circulating levels of bile salts. Furthermore, our findings suggest potential clinical implications in the use of steroid drugs.
Structural studies of proteins involved in mitotic progression

The Spindle Assembly Checkpoint (SAC) is a protein network that ensures that the cell does not proceed with separating the sister chromatids in mitosis before all chromosomes have been aligned and attached to the spindle machinery. We previously showed that a module in the N-terminus of Mps1, which we call NTE-TPR, is important for localization of Mps1 to the kinetochores (Nijenhuis et al, J Cell Biology, 2013).

This year we showed by NMR and microscale thermophoresis that the NTE-TPR interacts with the HEC1 protein of the NDC80 complex in the outer kinetochores directly. Importantly, the strength of the interaction is enhanced upon phosphorylation of the NTE. Furthermore, we showed that microtubules compete with Mps1 for this NDC80 interaction. Validating this interaction by a series of Hec1 mutants, both in vitro and in cells, showed that the competition is most likely noncompetitive, with the NTE-TRP occupying a site nearby but not identical to the tubulin interaction locus.

This establishes for the first time a physical interaction of the spindle assembly checkpoint and the microtubule-binding end of kinetochores. This suggests a mechanism through which the inhibitory effect of the SAC is alleviated when microtubules occupy all kinetochore sites and exclude Mps1 (Figure 2).

Structural studies of JBP1

The JBP1 protein, originally discovered by Piet Borst and colleagues, binds to DNA that contains base J (β-D-glucosyl-hydroxymethyluracil) and is also a thymidine hydroxylase. Almost twenty years following the discovery of base J, Piet Borst and colleagues, last year found a function of base J in non-telomeric regions (van Luenen et al, Cell, 2013), being responsible for terminating transcription. We have previously shown that JBP1 recognizes J-containing DNA through a single aspartate residue in a small DNA Binding Domain (DBD), with ten thousand-fold preference over normal DNA (Heidebrecht et al, NAR, 2011). We have also demonstrated that full-length JBP1 binding to DNA takes place in two distinct steps, and suggested that the two-step mechanism represents a conformational change that might be important for hydroxylation (Heidebrecht et al, JACS, 2012).

Recent epigenomic sequencing data, allowed for the first time to locate J with single base resolution, suggest a specific pattern for the presence of J, could be explained by this structural hypothesis.

There are two steps in J-base biosynthesis. In the first step, a T base is hydroxylated to hmU, by JBP1. In the second step, hmU is converted to J, by the addition of a glucose moiety, by a glucosyl transferase. We have expressed and purified JBP1 and the glucosyl transferase responsible for making J, and we are creating enzymatically J-oligos and protein constructs suitable for crystallographic studies. Our aim remains to elucidate the structure and function of these two proteins that orchestrate the biosynthesis of J.

Methods for X-ray crystallography

We bring up-to-date the crystallographic models already in the Protein Data Bank (PDB), that were deposited there over several decades, and were created using the methods and software available at the time.

We are focusing on making better crystallographic structure models that are already Protein Data Bank, and we make these available through the PDB_REDO data bank. Concurrently, we make available to the academic community a web-server that allows practicing crystallographers to take full advantage of the PDB_REDO procedure without having to install complicated software.

We have designed and over the years we have enhanced and fully automated PDB_REDO, incorporating two decades of knowledge and algorithms that were used to build the ARP/wARP software. PDB_REDO is now developing into a decision-making system that makes rational decisions for the best crystallographic model optimization protocols; our latest endeavor is to treat metal sites (e.g. Zinc) and most importantly to use structural homology as a tool to create better structures, especially at low resolution.

We will continue developing PDB_REDO in that direction while maintaining our practice of updating the models in the PDB.
Therapy escape of cancer

We are studying drug resistance mechanisms in “spontaneous” mammary tumors arising in genetically engineered mice. In particular, we are using mammary tumors with conditional defects of the Brca1, Brca2, and p53 genes. In these models we are focusing on (1) mechanisms of secondary drug resistance, (2) the characterization of drug tolerant tumor cells, and (3) the identification of markers that are useful to predict therapy response. These projects are carried out in close collaboration with the group of Jos Jonkers and with Piet Borst (NKI).

Using our mouse models, we have also started to investigate the escape from local radiotherapy control (4). For this project we are collaborating with the NKI-AVL radiotherapists Gerben Borst, Marcel Verheij and Jan-Jacob Sonke.

Mechanisms of secondary drug resistance

Error-free repair of DNA double-strand breaks (DSB) is achieved by homologous recombination (HR), and BRCA1 is an important factor for this repair pathway. In the absence of BRCA1-mediated HR, administration of PARP inhibitors induces synthetic lethality of tumor cells of patients with breast or ovarian cancers. Despite the benefit of this tailored therapy, drug resistance can occur by HR restoration. Little is known about BRCA1-independent restoration of HR, however. We found that loss of REV7/MADEL2 re-establishes CtIP-dependent end resection of DSBs in BRCA1-deficient cells, leading to HR restoration and PARP inhibitor (PARPi) resistance. Moreover, we observed that REV7 is aberrantly reduced or lost in a subset of human breast carcinomas. In particular, we established that REV7 blocks DSB resection to promote non-homologous end-joining (NHEJ) during immunoglobulin class switch recombination. These data are corroborated by the work of the group of Jacqueline Jacobs (NKI), who independently discovered REV7 as a DNA end-joining factor involved in the fusion of unprotected telomeres. Despite the increasing knowledge regarding the decision to initiate resection, we know only little about mechanisms by which cells regulate the ongoing formation of ssDNA tails. Here, our loss-of-function screens for PARPi resistance in BRCA1-deficient cells yielded an interesting hit: helicase B (HELB). In collaboration with the group of Dan Durocher (Toronto), we found that HELB underpins a feedback inhibition mechanism that curtails resection. HELB is recruited to ssDNA by interacting with RPA, and employs its 5′-3′ ssDNA translocase activity to inhibit EXO1 and BLM-DNA2, the nucleases catalyzing resection. HELB acts independently of 53BP1 and is exported from the nucleus as cells progress through S phase, concomitant with the up-regulation of resection.

In 2015 we also made some progress in employing new technologies that are useful to study drug resistance mechanisms in vivo. In collaboration with Norman Sachs (group of Hans Clevers, Hubrecht Institute, Utrecht), we established the generation of 3D cancer organoids from our mammary
tumor models. As shown for 53BP1 as an example in figure 1, we can now introduce genetic modifications using the CRISPR/Cas9 technology in these organoid cultures. The transplantation of these organoids allows a rapid in vivo validation of drug resistance mechanisms. We think that this technology paves the way for larger genomic in vivo screens to identify mechanisms of drug resistance under more realistic treatment conditions.

Drug tolerance

Despite their high sensitivity to platinum drugs, we have found that mammary tumors arising in our mouse model for BRCA1-deficient breast cancer are usually not eradicated, not even by a frequent dosing schedule. Although relapse-free survival is tumor size-dependent, tumors of only 2-3 mm in diameter still contain enough cisplatin-tolerant tumor cells to escape elimination. We found that the resistance of “remnants” is not due to specific biochemical defense mechanisms of putative tumor-initiating cells, but due to the ability of a sub-fraction of the cells to stall in their cell cycle progression until the drug is gone and the DNA damage has been repaired. After treatment of tumors with cisplatin, most tumor cells initially became giant multi-nuclear cells; relapse comes, however, from cells avoiding entry into S phase. Slowly cycling cells are present within the drug-naive tumor population and are enriched in tumor remnants. High dose platinum therapy was not tolerated in mice, but we found that nimustine eradicates all tumors. Complete eradication is dose-dependent and lowering the nimustine dose by 50% results in relapse of all tumors. In contrast to platinum drugs, nimustine reduces the number of G0 cells, which appears to cause disease relapse in our model.

Identification of markers to predict therapy response

In collaboration with Thijn Brummelkamp (NKI), we are also employing haploid screens in KBM7 and HAP1 cells to identify mechanisms of drug influx that may be down-regulated in resistant tumors. With this approach we found that the loss of the subunits LRRC8A and LRRC8D of the heteromeric LRRC8 volume-regulated anion channels (VRACs) increased resistance to clinically relevant cisplatin/carboplatin concentrations. In particular, we observed that about 50% of the cisplatin/carboplatin uptake depended on the presence of LRRC8A/D-containing channels. Cis- and carboplatin are frequently used in the clinic to treat ovarian cancer patients. Using publicly available data sets of ovarian cancer patients, Daniel Vis (group of Lodewyk Wessels, NKI) found that a low expression of the LRRC8D gene in the tumors correlated with reduced survival. In addition, our collaborators of the group of Thomas Jentsch (FMP, Berlin) discovered that incorporation of the LRRC8D subunit into VRAC substantially increased its permeability (FMP, Berlin) discovered that incorporation of the LRRC8D subunit into VRAC substantially increased its permeability.

Escape from local radiotherapy control

More than 50% of cancer patients undergo irradiation as part of their treatment. Despite the frequent benefit of this treatment, local tumor recurrence is a major clinical handicap and accompanied by poor prognosis. To address this, we have started irradiating our genetically engineered mouse mammary tumors with clinically relevant doses using a high precision cone beam micro-irradiator. Despite their high sensitivity, BRCA1-deficient carcinomas were not easily eradicated, and eventually relapsed. Although most recurrent tumors responded again to radiation treatment, the response durations were shortened and some recurrent tumors could no longer be controlled. As an alternative way to escape local radiotherapy, we observed the occurrence of metastasis. Hence, basic mechanisms underlying the escape from local radiotherapy control can now be studied in this model.
Cognitive function in cancer patients

The projects constituting our lines of research center around the investigation of the incidence, pattern, course, cause, and risk of cognitive impairment associated with cancer and its treatments, and at the development of strategies to diminish cognitive symptoms.

Effects of tamoxifen and exemestane on cognitive functioning of postmenopausal patients with early breast cancer: updated results from the TEAM trial neuropsychological side study

As estrogens play an important role in certain cognitive functions it is plausible that endocrine therapy (ET) applied in the treatment of breast cancer can affect cognition. We evaluated the effects of tamoxifen and exemestane on cognitive functioning in the realm of a randomized trial (TEAM-trial). Patients were randomly allocated to 5 years of adjuvant exemestane (EXE;n=114), or to 2.5 years of tamoxifen followed by 2.5 years of exemestane (TAM/EXE;n=92). Cognitive performance was tested using 15 cognitive subtests subsumed under 7 domains before start of ET (T0), at one year (short-term) and at 5 years of ET use (long-term). A no-cancer control group (n=120) was also assessed. We used mixed effect models to model baseline to follow-up changes in cognitive performance between groups, adjusting for age, IQ, and drop-out patterns. Compared to controls, TAM/EXE pts had significantly greater short-term (using tamoxifen) and long-term (using exemestane) decline in performance on Verbal memory and Executive functioning. For the EXE pts, we only found a long-term decrease on Trailmaking B (Executive functioning subtest) compared to controls. Additionally, we found that TAM/EXE pts had a significantly greater short-term decline in performance on Trailmaking A (Information processing speed subtest) compared to EXE pts. The above results were stronger for TAM/EXE pts aged<65 yr compared to TAM/EXE pts ≥65 yr. Results show that TAM/EXE pts perform worse on several cognitive functions compared to controls, suggesting long-term effects of tamoxifen even though patients switched to exemestane halfway through the treatment. This is less so for EXE pts. Since current guidelines permit the use of both regimens, our observation stresses the necessity of including neuropsychological examinations in prospective safety studies examining the effect of ET.

Irradiation profoundly affects neurogenesis but does not affect learning behavior in mice

The aim of this study was to explore the effect of whole brain irradiation on several neurobiological markers and behavior in mice. This project is a first step in a larger project with the aim to develop an intervention strategy to prevent/ diminish radiotherapy-associated cognitive decline. Under isoflurane anesthesia, male C57Bl/6j mice received fractionated whole brain irradiation in a dosage of 4 Gy once a day for 5 days. In

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Publications


a first group of animals, analysis of Ki-67 and doublecortin positive cells 4 weeks later indicated that irradiation profoundly decreased neurogenesis. Also a shift in microglia activation (IBA positive) was observed in irradiated animals compared with control animals. No effect was seen in blood vessel density following Glut-1 staining. In a second group of mice, the early effects on behavior starting from 3 weeks onwards were explored. Irradiation did not affect learning behavior in the novel object recognition task, novel location recognition task, Barnes maze, and fear conditioning. Also no effect in spontaneous behavior of anxiety was observed at this early time point. In a third group, late effects in behavior were assessed from 10 weeks onwards, and indicated a subtle but significant learning deficit specific to the Barnes maze. A fourth, control, group that did not receive irradiation, but was subjected to repeated anesthesia showed a significant deficit in novel fear. In our animal model, even a robust decrease in neurogenesis did not lead to cognitive deficits. These data show that the relationship between neurogenesis and cognition might be more complex than often assumed. The study also shows the importance of the choice of animal model, behavioral tasks, neurobiological parameters, and experimental set-up when studying radiotherapy-induced cognitive impairment.

**Functional hyperconnectivity in resting state networks of testicular cancer survivors 14 years after exposure to BEP chemotherapy**

Chemotherapy (CT) for testicular cancer is associated with late impairment of cognitive functioning. Alterations in functional brain networks due to neurotoxicity of chemotherapy might (partly) underlie these effects. We acquired resting-state fMRI in 25 TC survivors who had been exposed to BEP chemotherapy (CT group) and in 22 TC survivors not exposed to chemotherapy (S(surgery) group, completion of treatment on average 14.4 y prior). Within the FSL software package, data were temporally concatenated and decomposed with ICA. Components of interest were selected by visual inspection based on previous literature. A voxel-wise comparison was carried out on the selected components using a dual-regression approach. Nonparametric permutation tests at a corrected cluster level of p<.05 were used to detect statistically significant differences for each component between the CT and S-only group, correcting for age. The group ICA estimated 27 components, of which 13 components were found to represent functional resting state networks. Significant hyperconnectivity of the CT group relative to the S group was observed in 4 networks: 1) the precuneus, 2) the sensory and motor function, 3) the executive control and 4) the ventral stream network. In the CT group these networks encompassed brain regions not conventionally considered part of the affected network.

Fourteen years after completion of BEP chemotherapy, TC survivors show hyperconnectivity in functional resting state networks relevant for cognitive functioning. Resting state hyperconnectivity might reflect functional and compensatory over-recruitment of brain regions in response to decrements in neural integrity as a result of late neurotoxicity of BEP chemotherapy. Resting state hyperconnectivity might be a sensitive neuromarker for late neurotoxicity of BEP chemotherapy.

**Cognitive impairment in a subset of breast cancer patients following systemic therapy – results from a longitudinal study**

We investigated the effects of systemic treatment on cognitive performance in breast cancer (BC) patients. Participants were BC patients scheduled to receive systemic treatment (chemotherapy +/- endocrine treatment) (BC+SYST; n=31), or no systemic treatment (BC; n=24) and no-cancer controls (NC; n=33). Neuropsychological assessment took place before adjuvant treatment (T1) and six months after chemotherapy (T2), or at similar intervals. Cognitive performance was assessed at the group level at T2 adjusting for T1 as well as by multivariate normative comparison, a method that compares the scores of each participant against the distribution of the scores of controls, taking the covariance between scores into account. MNC showed 16% of BC+SYST to be cognitively impaired at T2, compared to 4% in BC and 8% in NC. Although group differences in neuropsychological performance did not reach statistical significance, moderate effect sizes were found for worse performance in the BC+SYST group compared to NC for Flanker congruent (ES=0.44) and stimulus incongruent (ES=0.44) and compared to BC for CDOWAT (ES=0.47), Digit Span (ES=0.41), and HVLT immediate (ES=0.71) and delayed recall (ES=0.65). Overall differences in cognitive performance were not related to psychosocial factors. However, cognitively impaired patients had a significantly lower IQ, worse physical and social functioning, and more symptoms of distress at T2 compared to unimpaired patients. Our findings indicate that cognitive impairment after systemic treatment occurs in a subset of BC patients. The predictive value of demographic and psychosocial factors in cognitive impairment should be further investigated in a larger sample of impaired patients.

**Ongoing initiatives include:**

- A multicenter neuropsychological and brain MRI studies in small cell lung cancer patients after prophylactic cranial irradiation: the effect of hippocampal avoidance, together with University Hospitals Leuven and KU Leuven.
- An online testing approach to assess cognitive problems, together with the University of Amsterdam and the VU University.
- The influence of informing patients about the association between cognitive problems and chemotherapy on cognitive problems, together with the Radboud University.
- Risk factors for cognitive problems in breast cancer patients: the role of brain white matter, together with the Academic Medical Center.
- Trajectories of cognitive decline in survivors of non-CNS cancers: from pre-cancer diagnosis to late life after cancer, together with the Erasmus MC.
- Implementation of routine neuropsychological assessment and advanced MRI in brain tumor patients.
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Publications


Phase I dose-escalation study with R06895882 in solid CEAC-positive tumours
We are investigating the biodistribution of radiolabeled R06895882 (CEA-IL2v) using 89-Zirconium-PET and 18F-FDG-PET imaging techniques. Results reveal tumor uptake and pharmacological proof of concept. Relevant tumor stabilizations and regressions are promising, however a threat is the development of high titters of ADAs (anti-drug antibodies).

New phase I studies with immunotherapeutics
We initiated new phase I studies with CEA-TCB targeting CEA and CD3 T-cell receptor, a combination trial of CEA-IL2v and MPDL3280A (atezolizumab). OX40 and OX40 with an anti-PD1 avelumab and a phase I study with PDR001 (anti-PD1 MoAb).

Proof of concept study to prevent formation of anti-drug antibodies
In the ongoing phase I trial with CEA-IL-2v, anti-drug antibodies (ADAs) were formed against the study drug. We investigate a novel approach to prevent anti-drug antibody formation by pretreatment with an anti-COD20 antibody.

Phase IB Study of an FGF-trap in mesothelioma
We investigate first-line treatment of mesothelioma with the FGF trapping molecule GSK3052230 combined with pemetrexed and cisplatinum. Promising clinical improvement and tumor regressions have been observed. Toxicities were mainly creatinine elevation and infusion related reactions.

Study with the monoclonal antibody (MoAb) against HER2 and HER3
We initiated another phase I study with MCLA-128 a full-length bispecific IgG1 anti-HER2-HER3 MoAb.

Pharmacodynamics of anticancer drugs

Combined targeted therapy for the treatment of patients with BRAF mutant colorectal cancer
Randomized phase II study of the combination of encorafenib-cetuximab with or without alpelisib confirmed high activity and good tolerance in advanced CRC as established in our phase I study with this in our institute (Prahallad et al. Nature 2012) developed concept.

Combination treatment strategy for patients with KRAS mutant tumors
Based on previous work (Sun et al. Cell 2014) we developed multiple phase I/II studies to evaluate tolerability and efficacy of MEK inhibitors combined with dual EGFR/HER2 inhibitors. Thus far, 35 patients were treated at different dose-levels. Anti-tumor activity was seen in some patients, but seems to be limited due to toxicity at higher dose-levels.
Diagnostic and therapeutic studies of blood brain barrier (BBB) Diagnostic studies.

In total 66 patients have been included in the diagnostic study of patients clinically suspected for leptomeningeal metastases (LM). In 5 of 13 patients with LM circulating tumor cells (CTC) were detected whereas cerebrospinal fluid (CSF) cytology was negative in these patients. Examples of flow cytometry plots are shown in the figure.

Phase I studies
The first-in-man phase I study with the DNA-dependent protein kinase inhibitor MSC2490484A monotherapy is ongoing. Doses up to BID 400 mg are well tolerated. In the multicenter combination study of MSC2490484A with radiotherapy our institute recruited the first patient worldwide.

Phase II randomized study (REVIVAL) of carboplatin and olaparib in BRCA1/2 carriers with advanced breast cancer
The monocenter 3+3 dose escalation phase I trial with olaparib (PARP-inhibitor) BID plus carboplatin followed by olaparib monotherapy (REVIVAL trial) is ongoing of two cycles olaparib and carboplatin followed by olaparib monotherapy. To date five patients have been included in this trial, currently we are including in the second cohort. Diaparin is being well tolerated and no dose limiting toxicities have occurred at the first dose level and at the second dose level so far. In total three partial responses have been reported.

Assay for uracil and dihydouracil in plasma
Quantification of uracil (U) and (DHU) in plasma could allow for more sensitive and selective identification of dihydropyrimidine dehydrogenase (DPD) deficiency in patients. We developed and validated an ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) assay for quantification of U and DHU. Validated concentration ranges for U and DHU were from 1 to 100 ng/mL and 10 to 1000 ng/mL. The validated assay parameters were acceptable according to FDA criteria. Sample pre-treatment only consists of protein precipitation, which together with short chromatographic run time of five minutes, enables fast sample analysis.

Safety, feasibility and cost-effectiveness of genotype-directed individualized dosing of fluoropyrimidines (M14DPD)
In April 2015 a multicenter study has started, initiated by the NKI-AVL, to determine whether upfront genotyping of the enzyme dihydropyrimidine dehydrogenase (DPD) can increase safety of fluoropyrimidines. Seventeen centers in the Netherlands have confirmed to participate, of which 7 hospitals are currently open. 226 Patients have been enrolled, of a total of 1250. Patients identified carrying polymorphisms resulting in DPD deficiency are treated with a reduced starting dose of the fluoropyrimidine.

Phase I pharmacological study of chronomodulated capecitabine therapy
Capecitabine is an oral prodrug of 5-fluorouracil (5-FU), which is catabolized by the enzyme dihydropyrimidine dehydrogenase (DPD). Observations from healthy volunteers showed a circadian rhythm in DPD activity, with peak activity during the night.

Based on these results we currently perform a clinical phase I study to determine the maximum tolerated dose (MTD), safety, chronopharmacokinetics and -dynamics in patients that are treated with chronomodulated capecitabine, for which the capecitabine evening dose in proportion to the morning dose is 5:3. Thus far, 10 patients were enrolled in this study and no dose-limiting toxicities have been observed yet.

OCTC
The NKI is currently part of a special collaboration that GlaxoSmithKline (GSK) has with 6 oncology research centers around the world, called the Oncology Clinical & Translational Collaboration (OCTC). The goal of OCTC is to create a network of oncology research centers that will collaborate with GSK on preclinical, translational an clinical oncology programs. Several preclinical and clinical studies resulting from OCTC are currently underway at the NKI.

Cancer Core Europe (CCE)
The clinical pharmacology team also plays a major part in the development and realization of Cancer Core Europe (CCE), a collaboration between six of the leading oncology research centers in Europe. The goal is to represent a critical mass through sharing of data, performing multi-centered (pre-) clinical trials and ultimately creating a virtual European e-hospital. CCE is currently still in the early development stages, but is already discussing setting up two trials.


Our research focuses on genes and proteins that cause drug resistance or drug susceptibility in tumors, or influence the pharmacological and toxicological behavior of anticancer and many other drugs and toxins, including carcinogens. Insight into these systems may: i) improve chemotherapy and more generally pharmacotherapy approaches for cancer and other diseases; ii) increase insights into factors determining susceptibility to toxins and carcinogens, and; iii) allow elucidation of physiological functions. To study the physiological, pharmacological and toxicological roles of the proteins involved, and their interactions, we generate and analyze knockout or transgenic mice lacking or overexpressing the relevant genes. Below we describe a few recent studies that illustrate our approach.

**P-glycoprotein, CYP3A, and plasma carboxylesterase determine brain disposition and oral availability of the taxane cabazitaxel**

We aimed to clarify the roles of the multidrug-detoxifying proteins ABCB1 (P-glycoprotein), ABCG2, ABCC2, and CYP3A in oral availability and brain accumulation of cabazitaxel, a taxane developed for improved therapy of docetaxel-resistant prostate cancer. Cabazitaxel pharmacokinetics were studied in Abcb1a/1b, Abcg2, Abcc2, Cyp3a, and combination knockout mice. Upon oral cabazitaxel administration, total plasma levels were greatly increased due to binding to plasma carboxylesterase Ces1c, which is highly upregulated in several knockout strains. Ces1c inhibition and in vivo hepatic Ces1c knockdown reversed these effects. Correcting for Ces1c effects, Abcc1a/1b, Abgc2, and Abcc2 did not restrict cabazitaxel oral availability, whereas Abcb1a/1b, but not Abgc2, dramatically reduced cabazitaxel brain accumulation (>10-fold).

Coadministration of the ABCB1 inhibitor elacridar completely reversed this brain accumulation effect. After correction for Ces1c effects, Cyp3a knockout mice demonstrated a strong (six-fold) increase in cabazitaxel oral availability, which was completely reversed by transgenic human CYP3A4 in intestine and liver. Cabazitaxel markedly inhibited mouse Ces1c, but human CES1 and CES2 only weakly. Ces1c upregulation can thus complicate preclinical cabazitaxel studies in some of the used knockout mouse models. As ABCB1 limits cabazitaxel brain accumulation it may also limit therapeutic efficacy against (micro)metastases or primary tumors positioned wholly or partly behind a functional blood-brain barrier. This can be reversed with elacridar coadministration, and similar effects may apply to ABCB1-expressing tumors. Since CYP3A4 profoundly reduces the oral availability of cabazitaxel, this might be improved by coadministering ritonavir or other CYP3A inhibitors, suggesting the option of patient-friendly oral cabazitaxel therapy.

**Genes and proteins involved in anticancer drug resistance and pharmacokinetics**

**Kort A, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Brain accumulation of the EML4-ALK inhibitor ceritinib is restricted by P-glycoprotein (P-GP/ABCB1) and breast cancer resistance protein (BCRP/ABCG2). Pharmacol Res. 2015;102:200-7**


**Ritonavir inhibits intratumoral docetaxel metabolism and enhances docetaxel antitumor activity in an immunocompetent mouse breast cancer model**

Docetaxel (Taxotere) is currently used intravenously as an anticancer agent and is primarily metabolized by Cytochrome P450 3A (CYP3A). The HIV protease inhibitor ritonavir, a strong CYP3A4 inhibitor, decreases first-pass metabolism of orally administered docetaxel. Anticancer effects of ritonavir itself have also been described. We aimed to test whether ritonavir co-administration could decrease intratumoral metabolism of intravenously administered docetaxel and thus increase the antitumor activity of docetaxel in an orthotopic, immunocompetent mouse model for breast cancer. Spontaneously arising K14cre; Brca1<sup>F/F</sup>; p53<sup>F/F</sup> mouse mammary tumors were orthotopically implanted in syngeneic mice lacking Cyp3a (Cyp3a<sup>−/−</sup> mice) to limit ritonavir effects on systemic docetaxel clearance. Over 3 weeks, docetaxel (20 mg/kg) was administered intravenously once weekly, with or without ritonavir (12.5 mg/kg) administered orally for 5 days per week. Untreated mice were used as control for tumor growth. Ritonavir treatment alone did not significantly affect the median time of survival (14 vs 10 days). Median time of survival in docetaxel-treated mice was 54 days. Ritonavir co-treatment significantly increased this to 66 days, and substantially reduced relative average tumor size, without altering tumor histology. Concentrations of the major docetaxel metabolite M2 in tumor tissue were reduced by ritonavir co-administration, whereas tumor RNA expression of Cyp3a was unaltered. In this breast cancer model, we observed no direct antitumor effect of ritonavir alone, but we found enhanced efficacy of docetaxel treatment when combined with ritonavir. Our data, therefore, suggest that decreased docetaxel metabolism inside the tumor as a result of Cyp3a inhibition contributes to increased antitumor activity.

**Hepatocellular shuttling and recirculation of sorafenib-glucuronide is dependent on Abcc2, Abcc3, and Oatp1a/1b**

Recently, we proposed an efficient liver detoxification process dubbed “hepatocyte hopping” on the basis of findings with the endogenous compound bilirubin glucuronide. According to this model, hepatocytic bilirubin glucuronide can follow a liver-to-blood shuttling loop via Abcc3 transporter-mediated efflux and subsequent Oatp1a/1b-mediated liver uptake. We hypothesized that glucuronide conjugates of xenobiotics, such as the anticancer drug sorafenib, can also undergo hepatocyte hopping. Using transporter-deficient mouse models, we showed that sorafenib-glucuronide can be extruded from hepatocytes into the bile by Abcc2 or back into the systemic circulation by Abcc3, and that it can be taken up efficiently again into neighboring hepatocytes by Oatp1a/1b. We further demonstrated that sorafenib-glucuronide excreted into the gut lumen can be cleaved by microbial enzymes to sorafenib, which is then reabsorbed, supporting its persistence in the systemic circulation. Overall, our results suggest broad relevance of the hepatocyte shuttling process known as “hepatocyte hopping” for detoxification of targeted cancer drugs that undergo hepatic glucuronidation, such as sorafenib. Most likely many other drugs glucuronidated in the liver will be subject to the same process.

**Other findings and outlook**

Our findings illustrate the wide impact of the multispecific detoxifying systems that we study on the in vivo behavior of many anticancer and other drugs, as well as dietary toxins and carcinogens. The insights and mouse models that we generate can be used to improve the development and application of new anticancer and other drugs, to optimize drug administration regimens, and to better predict risks of variable activities in the different detoxifying systems during chemotherapy, pharmacotherapy and exposure to environmental toxins and carcinogens.
Molecular and genetic breast cancer epidemiology

Therapy recommendations for breast cancer patients are based on the estimated average risk of tumor relapse and death, which is mostly based on tumor characteristics but not on patient genotype. Our work includes breast cancer risk factors in the broadest sense, but focuses on the effects of genetic factors, on the risk of breast cancer subtypes, and on prognosis and long-term outcome of breast cancer. To support this work, we set up several national cohort studies, and are involved in a number of international consortia, in total comprising over 150,000 breast cancer patients.

We use both candidate approaches for e.g. BRCA1, BRCA2, and CHEK2, and agnostic approaches for variants in other genes through GWAS. Studies include interactions between germline variants, tumor characteristics, breast cancer treatment and lifestyle factors on breast cancer prognosis, incidence of second tumors, and cause-specific mortality. Our work also entails the role of tumor immunohistochemical and genomic markers, morphology, and tumor differentiation on breast cancer outcome. Moreover, we study gene – environment interactions, e.g. diabetes and insulin exposure, on the incidence of specific breast cancer subtypes, and on breast cancer outcome. Our ultimate goal is to include these findings in guidelines or prediction tools for improved disease management, or in the pre-selection of women for breast cancer screening programs. End of 2014 we started a project, in collaboration with ErasmusMC, in which we are developing an online decision aid for the risk of contralateral breast cancer, including genetic, diagnostic, treatment and lifestyle factors. The main purpose is to facilitate shared decision-making by patient and physician so that adequate strategies can be chosen for treatment and follow-up, e.g., the prevention of unnecessary surgery among low risk women.

Related to this breast cancer research, a second research line focuses on biobanking and patient information, and the Ethical Legal and Social Issues, in scientific research using human materials. We are based in two divisions: Molecular Pathology, and Psychosocial Research and Epidemiology, and collaborate with clinical departments in this institute, the Core Facility Molecular Pathology and Biobanking (CFMPB, coordinated by Annegien Broeks), and with a large number of national and international research groups.

Selected results of progress in data collection

The GAME-ON effort including the Breast Cancer Association Consortium (BCAC; PI: Doug Easton) designed a novel 600k cancer SNP chip, named “OncoArray”, aiming to detect more cancer risk and outcome related variants, as well as fine-mapping for the detection of the actual functional variants. Our group curates the BCAC database containing the tumor characteristics and clinical information; numbers of breast...
cancer patients increase every year; this year from 85 studies with >113,000 patients to 76 studies with >135,000 patients; ~44,000 with OncoArray data. Data are ready now and analyses are ongoing.

In the EU CARING project we collected in collaboration with Danish colleagues immunohistochemical and RNA sequencing information on 300 patients with and without diabetes to address the question whether diabetic patients develop more aggressive tumor subtypes compared to non-diabetics.

**Selected research findings**

We have systematically reviewed all available literature concerning BRCA1/2 germline mutations and survival (PloS One 2015). Summarizing 66 relevant studies, there was a tendency towards a worse breast cancer-specific and overall survival for BRCA1/2 carriers, however, results were heterogeneous and the evidence was judged to be indecisive. Adjustment for tumor characteristics tended to shift the observed risk estimates towards a relatively more favorable survival. In pooled analyses of the BCAC studies with GWAS studies including 37,000 breast cancer patients, we found two imputed novel variants related to other genes to be associated with survival (J Natl Cancer Inst 2015). Also, we observed associations between a number of candidate genes and breast cancer survival, and specific treatment-related interactions (Breast Cancer Res, OncoTarget 2015).

In our breast cancer <50 years cohort, we detected 3.3% and 1.1% BRCA1/2 mutation carriers, respectively. An evaluation of the current Dutch criteria for referral of breast cancer patient to the Clinical Genetic Centers for BRCA1/2 using this cohort showed that these could be improved by including triple negative breast cancers under the age of 65 (Eur J Hum Genet 2015). We also showed that BRCA1/2 carriers have a 2-3 fold higher breast cancer risk, and that young age (<40 years) at diagnosis of the first breast cancer is a important predictor for BRCA1/2 mutation carriers, but not for non-carriers, to develop a second cancer in the contralateral breast (JCO 2015, in press).

Using BCAC data, combining 76 germline variants in a polygenic risk score, it was shown that women in the highest 1% of the polygenic risk score had a three-fold increased risk of developing breast cancer compared with women in the middle quintile (Nati Cancer Inst 2015). We observed that in a specific subgroup, i.e., the CHEK2 *1100delC carriers, the polygenic risk score similarly well predicted breast cancer risk (Muranen et al, under review).

Evaluating ANXA1 expression in breast tumors, we found, using data from nine breast cancer studies from BCAC and from one study of familial breast cancer patients with BRCA1/2 mutations, that ANXA1 was overexpressed in BRCA1/2-related breast tumors, and in tumors with poor prognosis features such as triple negativity and poor differentiation. ANXA1 might be a biomarker candidate for breast cancer survival prediction in high-risk groups such as HER2+ cases (BMC Medicine 2015).

Regarding non-genetic risk factors for breast cancer, we focused on reviewing the postulated association between insulin and insulin analogue treatment and breast cancer development, and plausible mechanisms. In total 16 in vitro, 5 animal, 2 in vivo human and 29 epidemiological papers were included. Insulin AspB10 showed mitogenic properties in vitro and in animal studies. Glargine was the only clinically available insulin analogue for which an increased proliferative potential was found in breast cancer cell lines. However, the pooled analysis of 13 epidemiological studies did not show evidence for an association between insulin glargine treatment and an increased breast cancer risk. We concluded that there is no compelling evidence that any clinically available insulin analogue, nor human insulin increases breast cancer risk. Overall, the data suggested that insulin treatment is not involved in breast tumor initiation, but might induce breast tumor progression by up regulating mitogenic signaling pathways.

![Figure 1: Van den Broek AJ, Schmidt MK et al PLoS One 2015](Image 312x501 to 417x572)

**Figure 1: Van den Broek AJ, Schmidt MK et al PLoS One 2015**


![Figure 2: Annexin A1 expression in breast tissue (Sobral-Leite M et al BMC Med. 2015)](Image 442x481 to 553x592)

Figure 2: Annexin A1 expression in breast tissue (Sobral-Leite M et al BMC Med. 2015)


The aim of our research is straightforward: 1) To design novel cell-based immunotherapies for a number of human cancers. We wish to dissect which antigens on tumor cells are key to cancer regression. Such knowledge may help decide which patient groups are most likely to respond to T cell-based immunotherapies. In addition, such knowledge may allow us to more selectively steer T cell reactivity towards these tumor-associated antigens.

We have previously developed a number of technologies that allow the high-throughput analysis of antigen-specific CD4+ and CD8+ T cell responses in clinical samples. Over the past years, we have exploited these platforms to assess whether recognition of patient-specific neo-antigens that arise as a consequence of mutations is common in human melanoma. The key biological finding from these studies is that in the vast majority of melanoma patients CD4+ and/or CD8+ T cell reactivity against neo-antigens is formed. In addition, T cell responses against these neo-antigens can increase upon both T-cell checkpoint blockade and TIL therapy. Collectively, these data provide strong evidence for a model in which recognition of tumor-specific neo-antigens is a major ingredient of the clinical activity of cancer immunotherapies. In further support of this hypothesis, in collaboration with N. Rizvi (MSKCC) we demonstrated that the average mutational burden of NSCLC patients who respond to PD-1 blockade is higher than that of patients who do not show clinical benefit.

With the relevance of tumor-specific neo-antigens now well established, we have also addressed two questions that will influence our ability to exploit neo-antigens for therapy. In a first project in collaboration with C. Kesmir, we have analyzed whether during natural tumor-immune interactions a selection occurs for tumor cells that lack many of the neo-antigens that could be seen by T cells, a process coined immune editing. Based on 3 bio-informatic analyses (figure 1) we conclude that recognition of tumor-specific neo-antigens is a major ingredient of the clinical activity of cancer immunotherapies. In further support of this hypothesis, in collaboration with N. Rizvi (MSKCC) we demonstrated that the average mutational burden of NSCLC patients who respond to PD-1 blockade is higher than that of patients who do not show clinical benefit.

In a separate project in collaboration with J. Olweus, we have analyzed whether during natural tumor-immune interactions a selection occurs for tumor cells that lack many of the neo-antigens that could be seen by T cells, a process coined immune editing. Based on 3 bio-informatic analyses (figure 1) we conclude that recognition of tumor-specific neo-antigens is a major ingredient of the clinical activity of cancer immunotherapies. In further support of this hypothesis, in collaboration with N. Rizvi (MSKCC) we demonstrated that the average mutational burden of NSCLC patients who respond to PD-1 blockade is higher than that of patients who do not show clinical benefit.

In the coming period we expect to further enhance our...
understanding of immune recognition of human neo-antigens, for instance by analyzing the functional properties of neo-antigen specific CD4+ T cells, and by testing whether loss of individual neo-antigens is likely to occur at the moment selective pressure is applied. In related efforts, we have analyzed whether tumor-infiltrating T cells in ovarian cancer and colorectal cancer – two tumor types for which T cell infiltrates form a positive prognostic marker – are commonly tumor reactive. Finally, with the broader use of cancer immunotherapeutics, it is becoming increasingly important to understand the molecular mechanisms that determine the sensitivity of tumor cells to T cell-based immunotherapies. With this in mind, we are conducting genetic screens that may yield factors that determine both T cell fitness and tumor sensitivity to T cell attack. Early data from this research line suggest a potentially important role of T-cell secreted IFNγ in immune-mediated inhibition of tumor growth.

Dissecting the basic concepts of immunity

In parallel to these studies of human tumor–specific immune responses, we aim to dissect immune processes within model systems, both to increase our fundamental understanding of these processes, and as a breeding ground for novel technologies. To highlight a few of the results obtained, we have used the lineage tracing technology we have previously developed to evaluate the single cell output of common myeloid progenitors (CMPs), a progenitor subset that was thought to give rise to both the erythroid and myeloid lineage. The central observation made in this work has been that individual CMP are highly biased in the cellular output that they yield (figure 2), arguing for a revised model of hematopoiesis. In parallel, we are continuing our development of systems that allow us to visualize the effect of T cell-secreted signals on surrounding tissue, a tool that may be helpful to assess possible long-range effects of T cell triggering, in particular in tissue-resident memory T cell subsets. Finally, in an effort to better understand adaptive immunity in prokaryotes, we have provided evidence for an important role of an additional component of the cas1-cas2 complex.

Epithelial cells and inflammation

In this research line led by senior post-doc Dr Ferenc Scheeren, we aim to understand inflammatory pathways in breast epithelial cells during homeostasis, regeneration and cancer. Previously, Ferenc Scheeren has shown that Toll Like Receptor-2 (TLR2) signaling play a role in regeneration of normal intestinal and breast epithelia, as well as in malignant transformation. He has also demonstrated a role for TLR2/CD14 signaling in bladder cancer and head and neck squamous cell carcinoma. Currently, we focus on the cancer cell-intrinsic role of the downstream NLRP3 inflammasome during normal breast development and breast cancer. We are collaborating on this project with the lab of Dr G. Nunez.

In addition, we are dissecting the effects of hormone therapy on the breast cancer microenvironment. We make use of an Estrogen Receptor (ER) Cre recombinase knock-in mouse that permits genetic tracing of ER expressing cells that are potentially regulated by estrogen signaling. Interestingly, the traceable cells include T cells, B cells and neutrophils in the blood. This genetic model provides evidence that hormone therapy directly affects the tumor microenvironment. Future work will elucidate these effects and is aimed at developing combined hormone- and immunotherapy approaches.

Translation of research findings into biotech

2015 was remarkable in that it witnessed the emergence of two biotech companies, Kite Pharma EU (Amsterdam) and Neon Therapeutics (Boston) of which the activities are in part based on the concepts and technological platforms developed within our group. Furthermore, the efforts to develop novel cancer immunotherapies are in both companies partly led by former group members. In parallel to the output that we generate in the form of our academic research findings, this type of biotechnological translation will hopefully further advance the development of cancer immunotherapy.
Development of cancer is generally due to errors that occur in cellular pathways. Understanding the mechanisms of underlying processes will help to determine where the errors occur and how they can be treated. We study proteins using a combination of biochemical and biophysical methods, including X-ray crystallography and cryo-EM (electron microscopy) to provide three-dimensional structures. This leads to insights in molecular mechanisms that we validate in cells. In addition, our structures provide targets for drug design studies. In this work, we focus primarily on proteins involved in ubiquitin conjugation, particularly in stress response and DNA repair pathways and DNA mismatch repair.

**DNA mismatch repair**

DNA mismatch repair plays a crucial role in maintaining genome stability. Defects in the mismatch repair proteins in humans predispose to Lynch syndrome (or hereditary non-polyposis colorectal cancer) and are associated with a variety of sporadic cancers. DNA mismatch repair is initiated by recognition of a mismatch or an unpaired base by MutS (in Escherichia coli) or its MSH homologs (in humans). Initial recognition of the mismatch is followed by an ATP-dependent conformational change of MutS into a sliding clamp state that can be recognized specifically by the next protein in the mismatch repair cascade, MutL (or its homologs). Intriguingly, both MutS and MutL are critical for correct repair and germline mutations in the human homologs of either of these proteins lead cancer predisposition.

We have studied the sliding clamp state and the contribution of MutL to the mismatch reaction. To trap this transient state, we have collaborated with the groups of Peter Friedhoff (Giessen) and Joyce Lebbink (Rotterdam). We make use of single cysteine variants produced in the Friedhoff laboratory that allow specific cross-linking of MutS to MutL only in the presence of a mismatch and a nucleotide. These cross-linked complexes stabilized the sliding clamp state sufficient for X-ray crystal formation. We have been able to determine the crystal structure of the complex between MutS and the LN40 N-terminal domain of MutL using a site-specifically crosslinked complex. This allowed the analysis of the large conformational changes upon activation and sliding clamp formation (figure 1). Interestingly, the structure also revealed how these changes lead to activation of MutL. The structure captures MutS in the sliding clamp conformation, and shows how tilting of the MutS subunits across each other pushes DNA into a new channel. This, together with reorientation of the connector domain creates an interface for MutL with both MutS subunits. We were able to validate this state using a larger number of different analyses, including single-cysteine FRET from different sites and mutagenesis. The new structure explains how the sliding clamp promotes loading of MutL onto DNA, to activate downstream effectors. Using stopped-flow analysis, we could show that this was a slow step, after the fast recognition of the DNA. Using in vitro nuclelease assays as
well as in vivo complementation assays we could show that this loading is essential for DNA mismatch repair. We thus elucidate a crucial mechanism that ensures that MMR is initiated only after detection of a DNA mismatch (Groothuizen et al, Elife 2015). In our crystal structure of the MutS sliding clamp state, we did not observe the DNA, in-line with fact that this complex does not bind at a specific site. Currently we are using cryo-EM to validate our structural data, in collaboration with Meindert Lamers at the LMB.

**Ubiquitin conjugation**

Ubiquitin conjugation in cells provides critical signals that change the fate of the target protein. It is important almost all cellular processes, including DNA repair, apoptosis, cell cycle, chromatin regulation and endocytosis. Since these processes are necessary for cellular integrity, deregulation of ubiquitin-dependent processes often leads to cancer. We focus on mechanisms of ubiquitin conjugation to aid the process of drug design critical pathways in ubiquitin conjugation. The process of conjugation by ubiquitin-(like) proteins involves covalent linking of one or more 76-amino-acid ubiquitins to a target protein by an E1/E2/E3 cascade of enzymes. Correct ubiquitination requires the complex spatial arrangement of ubiquitin, E2, E3 proteins and the target simultaneously in a precise but flexible manner. We are particularly interested in the factors that determine the rate, the specificity and the selectivity of the E2/E3 dependent reaction, especially in DNA-dependent processes.

Deubiquitinating enzymes (DUBs) control vital processes in eukaryotes by hydrolyzing ubiquitin adducts. Their activities are tightly regulated, but the mechanisms remain elusive. In particular, the DUB UCH-L5 can be either activated or inhibited by conserved regulatory proteins RPN13 and INO80G, respectively (figure 2). We have shown how the DEUBAD domain in RPN13 activates UCH-L5 by positioning its C-terminal ULD domain and crossover loop to promote substrate binding and catalysis. Intriguingly, the structurally related DEUBAD domain in INO80G does not activate, but rather inhibits UCH-L5. To do this it exploits similar structural elements in UCH-L5 to promote a radically different conformation, and employs molecular mimicry to block ubiquitin docking. This causes large conformational changes that create small but highly specific interfaces that mediate activity modulation of UCH-L5 by altering the affinity for substrates. Our results have established how related domains can exploit enzyme conformational plasticity to allosterically regulate DUB activity. These allosteric sites may present novel insights for pharmaceutical intervention in DUB activity.


Peulen H, Beelderbos J, Suckenberger M, Hooge A, Grills I, van Herk M, Sonke J-J. Target delineation variability and corresponding margins of peripheral early stage NSCLC treated with stereotactic body radiotherapy. Radiother Oncol. 2015


The correction comprised of an infinite response filter based on 3 exponents calibrated to a falling edge response of an un-attenuated beam. An additional extrapolation for saturated pixels was incorporated in our in-house developed clinical CBCT reconstruction software. In patient imaging, the artifact was minimal in scans reconstructed using image lag correction (figure 1). Quantitatively, there was a significant reduction of the apparent contrast of the artifact from 43 ± 16.7 HU to 9.6 ± 12.1 HU, depending on the date of the calibration. The image lag correction parameters were stable over a period of 3 months. The computational load was increased by approximately 10%, not endangering the fast in-line reconstruction.

Variability Characterization
An important source of geometrical uncertainty is target definition. To quantify this target delineation variability in peripheral early stage lung cancer 16 tumors were delineated by 11 radiation oncologists from 4 institutes. A relatively small target delineation uncertainty of 1.2mm-1.8mm (1SD) was observed for early stage NSCLC. A 3.4-5.9mm GTV-to-PTV margin was required to account for this uncertainty alone, ignoring other sources of geometric uncertainties. Deformable image registration (DIR) forms an important component of adaptive radiotherapy to automatically quantify anatomical changes over the course of treatment. We therefore validated an in-house developed B-Spline-based DIR approach for CBCT to CT registration in lung cancer patients. First, the consistency of the DIR was assessed using a full circle method for 10 patients with repeated CT (rCT) and CBCT scans. For the second validation method we used 12 patients in which gold fiducial markers had been implanted in mediastinal lymph nodes.
The residual displacement from both methods was $1.5 \pm 0.8 \text{mm}$ and $1.8 \pm 1.0 \text{mm}$ respectively. These inaccuracies of less than 2 mm are small relative to the underlying day to day geometric uncertainties.

**Treatment plan modification**

Rotations of the prostate gland induce considerable geometric uncertainties in prostate cancer radiation therapy. We therefore developed, implemented and validated a rotation correction strategy for volumetric modulated arc therapy (VMAT). To that end, we exploited the dynamic collimator rotation option to follow the beams eye’s view projection of the prostate rotation. On average, clinical target volume minimum dose (Dmin) decreased up to 10% without corrections while Dmin remained within 4% for corrected plans. Bladder S78 and rectum EUD of the corrected plans matched those of the original plans. The average pass rate for the corrected plans delivered to the phantom was 98.9% at 3% per 3 mm gamma criteria. Adaptive plan modifications have the potential to combine high plan robustness with small margins. This hypothesis was tested in head-and-neck cancer patients. Following automatic treatment plan optimization with uniform CTV to PTV margins of 5, 3 and 0 mm, CBCT-to-CT deformable registration was performed and the dose was recalculated, mapped and accumulated in the planning CT. Margin reduction from 5, 3 to 0 mm led to DAR sparing of $\sim 1 \text{Gy}$ average dose per mm. Despite online repositioning, substantial systematic deformations were present ($\geq 3 \text{ mm}$). Discrepancies in CTV coverage $> 2 \text{ Gy}$ were found in 1, 3 and 7 instances respectively, mainly elective CTVs. ROC analysis on intervention thresholds with 0mm plans showed that after 8 fractions all candidates for adaptive replanning were detectable without false positives. A single adaptive replanning restored the treatment intend in 70% of cases.

**Outcome Modeling**

A possible side effect of high dose per fraction radiotherapy of early stage lung cancer is rib fracture. To find the relationship between dose and fracture probability, we first developed an automatic rib segmentation algorithm. Subsequently, the dose effect of automatic and manual delineated ribs was compared in a small subgroup and found to be significantly equivalent (figure 2). The next step is to use the automatic segmentation algorithm in a large cohort of patients to find prognostic parameters for rib fractures. The late follow-up of a randomized radiation and concomitant high-dose intra-arterial or intravenous cisplatin trial for advanced head and neck cancer was evaluated. After a median follow-up of 7.5 years, locoregional control and overall survival were not different between the treatment arms. Late dysphagia was worse in the intravenous arm. Intra-arterial cisplatin did not improve tumor control compared to intravenous administered cisplatin, despite the higher dose in IA delivery of the drug.

**µIGRT**

The µIGRT system (X-RAD 225 CX, PXI, North Branford, USA) was decommissioned, disinfected, transported to the new animal facility and re-commissioned causing considerable delays in the research program. The high end pre-clinical imaging systems (MRI, PET, SPECT) available in the new animal facility enables to further enhance the pre-clinical image guidance program. Lung toxicity experiments with conformal avoidance of the heart demonstrated a clear dose effect relationship of CT density changes and provide a sensitive model for combined therapy assessments. In the µIGRT project we collaborate with Conchita Vens, Gerben Borst and Marcel Verheij.
Receptors for matrix adhesion

The main objective of our group is to study the mechanisms involved in cell adhesion. Specifically, we are interested in characterizing the interactions that take place between cells and the extracellular matrix (ECM) component laminin and determining their significance.

Mechanisms of integrin β1-induced cell scattering

Integrins are heterodimeric ab transmembrane receptors that link the ECM to the intracellular cytoskeleton. The integrin β1 subunit can dimerize with 12 different α subunits and is thus able to interact with a wide array of ECM ligands. Introduction of integrin β1 in β1-null GE11 epithelial cells induces cell scattering and leads to a dramatic reorganization of the actin cytoskeleton. These phenotypic changes resemble an epithelial-to-mesenchymal (EMT) process, observed in embryonic development and cancer progression. In this project, we aim to understand the role of β1 signaling in this process and to decipher the molecular mechanisms underlying integrin β1-induced cell scattering. For this purpose, we established a β1-inducible (Tet-ON system) GE11 epithelial cell line, which we will use to perform RNA-Seq and proteomic screens to identify β1 interactors involved in cell scattering.

The role of integrin α3β1 in skin tumorigenesis

The integrin α3β1 has been implicated as a promoter of tumorigenesis as well as an inducer of invasive and metastatic phenotypes in different types of tumors. Subjection of epidermis-specific Itga3 knockout mice (Itga3 eKO) to the two-stage model of chemically induced skin carcinogenesis (DMBA/TPA treatment) showed that efficient tumor development is critically dependent on the presence of α3β1. Because the Itga3 eKO mice showed strong reduction in the number of slow-cycling, label-retaining cells in the hair follicles (HFs) as well as an increased number of cells expressing the follicular stem cell marker keratin 15 in the infundibulum and interfollicular epidermis, it was hypothesized that DMBA-initiated cells lacking α3β1 exit their compartment and terminally differentiate before they can acquire additional mutations that would lead to the onset of cancer.

To further investigate the effect of α3β1 on the dynamics of different cell populations in skin, we have performed flow cytometry analysis of epidermal keratinocytes from Krt14 Itga3 eKO and wild-type skin. Compared to wild-type mice, Krt14 Itga3 eKO mice showed a consistent but small decrease in the size of the isthmus cell population, the upper region of HFs, positive in homeostatic conditions as well as during skin tumorigenesis, i.e. after short-term DMBA/TPA treatment. Furthermore, an increase in the size of the isthmus cell population, the upper region of HFs, was observed in the Itga3 eKO compared to WT mice. Together this data supports a role of α3β1 in retaining stem cells in their niches, thereby preventing their migration towards the isthmus and loss by terminal differentiation. However, it is arguable...
whether the differences in HF populations between Krt14\textit{Itga3}\textit{eKO} and control mice are sufficiently large to explain the near complete absence of papillomas in the Krt14\textit{Itga3}\textit{eKO} subjected to long-term DMBA/TPA treatment. In order to investigate the role of α3β1 in HF stem cells \textit{in vivo}, we have generated a Krt19-Cre\textit{ERT}, \textit{Itga3}\textit{floxed/\textit{Itga3}\textit{KD}} transgenic mouse strain that allows conditional deletion of \textit{Itga3} in HF stem cells. We have further introduced a mT/mG reporter gene in this strain, which will enable us to follow α3-deficient cells \textit{in vivo}.

The role of the integrin α3β1 in breast tumorigenesis
To assess the role of α3β1 in breast progression, we have conditionally deleted the \textit{Itga3} gene in the mammary epithelium of MMTV-Her2/Neu transgenic mice. The MMTV-Her2/Neu transgenic mouse tumor model closely mimics progression and gene expression profiles of human breast cancer. Our preliminary data suggest that in this model, α3β1 deletion does not alter tumor initiation or growth. However, the number of lung in the MMTV-Her2/Neu model is strongly increased upon \textit{Itga3} gene deletion. Furthermore, while most of the metastases in wild-type mice grew as tumor cell aggregates within blood vessels, in the \textit{Itga3} knockout mice the metastases were mostly present in the lung parenchyma and showed an invasive growth pattern. The size and number of the metastatic foci were also significantly increased in the \textit{Itga3} knockout mice. Thus, similar to what has been observed in the two-stage skin carcinogenesis model, in the MMTV-Her2/Neu model of breast cancer loss of α3β1 is associated with a more aggressive and invasive phenotype in late stage tumors. The fact that we could not detect an effect of α3β1 deficiency on tumor initiation and growth in this model when compared with the two-stage model of chemically induced skin carcinogenesis might be explained by the absence of a TPA-induced promotion phase of tumor formation in the breast cancer model.

Crosstalk between α3β1 and α6β4
In the skin hemidesmosomes (HDs) and focal adhesions (FAs) contribute to epidermal-dermal cohesion. A major component of HDs is the integrin α6β4, which binds to laminin-332 in the epidermal basement membrane and is connected to the intermediate filament system through plectin. On the other hand, FAs contain integrins, such as α3β1, that link the actin cytoskeleton to the ECM. The ability of cultured keratinocytes to form HDs is closely associated with their ability to form FAs. To study the role of FA proteins in HD formation, we used CRISPR/Cas9 technology to delete α3 and associated molecules in human keratinocytes and study the effects thereof on HD formation. Additionally, we performed proteomic screens to identify common and specific interactors of the integrin subunits α3, α6 and β4. Future research is focused on investigating the significance of newly identified and known interactors of the integrin α3 and β4 subunits in HD formation.

β4 phosphorylation at threonine 1736
Previously, we have demonstrated that in keratinocytes phosphorylation of T1736 in the C-terminal end of the β4 cytoplasmic domain disrupts the interaction of β4 with the plakin domain of plectin. Our recent studies have shown that T1736 phosphorylation of β4 in response to PMA treatment is mediated by PKD2 activation downstream of PKCδ. On the other hand, both the EGF-stimulated phosphorylation of T1736 and the EGF-induced dissolution of HDs are dependent on a functional MAPK signaling pathway, and treatment with the RSK inhibitor BI-D1870 prevented EGF-stimulated phosphorylation of β4-T1736. Moreover, phosphorylation of β4-T1736 is enhanced by an overexpression of wild-type RSK1, while it is reduced by the expression of kinase-inactive RSK1 or by siRNA-mediated depletion of RSK1. In summary, our data indicate that different stimuli can lead to the phosphorylation of β4-T1736 by either PKD2 or RSK1.
Genomic instability and carcinogenesis

Genomic instability, a hallmark of human cancer, has been attributed to defective DNA maintenance and cell cycle control mechanisms. Our research focuses on mutagenesis resulting from (1) defective DNA mismatch and crosslink repair pathways, and (2) perturbed DNA replication as a consequence of defective G1/S control. The principle tools include gene modification in murine embryonic stem cells (ESC) and analyses of the phenotypic consequences in ESCs, mice and cell lines derived thereof.

**DNA MISMATCH REPAIR**

Inherited defects in DNA mismatch repair (MMR) genes underlie the cancer predisposition Lynch syndrome (LS), which manifests as early onset colorectal and endometrial cancer. MMR corrects DNA replication errors, which are recognized by MSH2/MSH6 or MSH2/MSH3 dimers. Recruitment of another dimer, MLH1/PMS2, promotes exonucleolytic removal and resynthesis of the error-containing strand.

Mismatches also arise upon replication of damaged bases such as 6-O- or 5-O-methylguanine. Repetitive removal by MMR of the incorporated nucleotide rather than the lesion itself causes DNA breakage and cell death. Thus, DNA MMR acts anti-mutagenic and mediates the toxicity of methylating agents.

**Oligonucleotide-directed gene modification**

We recently developed a gene modification technique in mouse embryonic stem cells (ESCs) that allows single base pair substitution at any desired location in the genome without the need for prior generation of a DNA double-stranded break. The method uses short synthetic oligodeoxyribonucleotides (ssODN) that are complementary to an endogenous target sequence except for the centrally located nucleotide that comprises the desired modification (Aarts and Te Riele, NAR 2010;38:6956).

Gene modification involves annealing of the ssODN to a complementary sequence in the replication fork and its subsequent integration into the genome. However, the mismatch at the position of the mutating nucleotide elicits a MMR reaction that restricts gene modification to an efficiency of only 10⁻⁷ (Dekker et al., NAR 2003;31:e27). ESCs can be made permissive for ‘oligo targeting’ by transient suppression of MSH2 (Aarts et al., NAR 2006;34:e147) or MLH1 (Dekker et al., Mutation Res 2011;715:52), which increases the frequency to 10⁻⁵ but at the price of random, possibly confounding mutations. We found that this effect of MMR can be avoided when the mutating nucleotide contains a specific chemical modification.

Chemically-modified ssODNs (CMOs) of only 25 nucleotides allow highly accurate base-pair substitution at frequencies of 10⁻⁴ in wild-type cells, while MMR suppresses off-target effects (figure 1). We are currently extending our novel oligo targeting protocol to human cell lines and induced pluripotent stem cells (iPSC). In *vitro* experiments showed that chemical modification prevents mismatch binding by purified bacterial MutS. Consistently, also
in *Escherichia coli* the CMD design evades MMR during λ-mediated gene editing.

**Unclassified variants of MMR genes**

LS is caused by inherited mutations in MMR genes that fully disrupt gene function. Also single codon variants of MMR genes are found in (familial) cancer, however, as the open reading frame remains unperturbed, it is often unclear whether they are causative for disease. We have used oligo targeting to recreate suspected *MSH2* and *MSH6* gene variants in ESCs, permitting accurate assessment of their MMR capacity. Of twelve ‘variants of uncertain significance’ (VUS) five partially or fully abrogated MMR activity and could be classified as pathogenic (Wielders et al., Human Mutation 2011;32:389-96; J Med Genet 2014;51:245; Plos ONE 2013;8:e74766). Although reliable, the throughput of this approach is low. We have therefore developed a novel high-throughput procedure that allows rapid identification of pathogenic VUS of *MSH2*, *MLH1* and *MSH6*: among 135 VUS, we detected 46 overtly or weakly deleterious variants.

**A novel mouse model for Lynch syndrome**

We have made a novel mouse model in which, similar to LS patients, *MSH2*-defective crypts exist amidst an excess of MMR-proficient crypts (ratio 1:20) (Wojciechowicz et al. Gastroenterology 2014;147:1064). Half of these animals developed intestinal cancer from the *MSH2*-deficient compartment after ±1.5 year. Exposure of *Msh2*-Lynch mice to the methylation agent temozolomide caused 5-fold expansion of *MSH2*-deficient crypts, demonstrating a remarkable plasticity of the intestinal crypt structure: non-neutral competition can exist between stem cells from different crypts, allowing substitution of entire crypt structures. Furthermore, 11/13 temozolomide-exposed *Msh2*-Lynch mice developed multiple intestinal tumors at ±4 months, likely as a consequence of the increased number of *MSH2*-deficient cells and their susceptibility to temozolomide-induced mutagenesis. Exposure to methylation agents is clearly a risk factor for tumor development in LS patients. We are using this model to develop strategies to reduce cancer risk in LS patients.

**FANCONI ANEMIA**

Fanconi anemia (FA) is a recessive disorder characterized by skeletal malformations, progressive anemia and high incidence of AML and HNSCC. FA is caused by bi-allelic defects in either one of 17 FANC genes that are essential for DNA interstrand crosslink (ICL) repair. In collaboration with the VU University FA research group, we have generated Fancf- and Fancm-deficient mice (Bakker et al., J Pathology 2012;226:28-39; Bakker et al., Hum Mol Genet 2009;18:3484). Crossing the Fancf and Fancm knockout alleles into cancer prone *Apc*+/− and *Eμ-Pim* transgenic mice revealed tissue- and gene-specific acceleration of tumorigenesis by FA defects. The Fancf knockout allele comprises a 4 base pair insertion in the open reading frame. We have optimized oligonucleotide-template-directed repair of CRISPR/Cas9-induced DNA breaks in ESCs and used this to restore the Fancf open reading frame. We are currently transferring this approach to hematopoietic stem cells in an attempt to develop gene therapeutic strategies to counteract bone marrow failure in FA patients.

**LOSS OF G1/S CONTROL BY INACTIVATION OF RB PROTEINS**

Loss of G1/S control is frequently seen in tumors. G1/S control relies on the retinoblastoma proteins pRb, p107 and p130, which collectively regulate E2F transcription factors. Ablation of pocket proteins abrogates G1/S control but additional events are needed for oncogenic transformation. To identify these, we study the requirements for mitogen- and anchorage-independent proliferation of pocket-protein-deficient cells.

**Mitogen independence**

Mouse embryonic fibroblasts (MEFs) completely devoid of pocket proteins (TKO MEFs) still need mitogens for proliferation: mitogen deprivation of TKO MEFs caused G1 arrest effectuated by induction of p27KIP1 and p21CIP1, the latter pointing to activation of the DNA Damage Response (DDR) (Fojer et al., Cancer Cell 2005;B:455). Indeed, mitogen deprivation caused severe replication stress manifested by slow replication fork progression, reduced origin firing and accumulation of DNA double-strand breaks (DSBs) (Van Harn et al., Genes Dev 2010;24:1377). We are currently performing screens to determine genetic events that attenuate this response and allow mitogen-independent proliferation.

**Anchorage independence**

MEFs devoid of pRB and p107 or p130 (DKO) and TKO MEFs also remained dependent on a solid support (anchor), even if they expressed RASV12 (Vormer et al., MCB 2008;28:7263). By gain- and loss-of-function screening, we found that increased expression of *TBX2* or knockdown of p53 allowed anchorage-independent growth of RASV12-expressing DKO MEFs. To identify additional mediators of anchorless growth, we have developed a novel loss-of-function screening procedure that predominantly interrogates genes over-expressed in arrested cells. This approach uncovered two novel tumor suppressor pathways, one of which involving the anti-proliferative p38-mediated stress-response.

![Chemical modification of single-stranded oligonucleotides (CMOs) allows gene modification in mismatch-repair-proficient cells. Targeting efficiency of CMGs designed to correct a defective start codon in a neomycin reporter gene expressed as the ratio of G418-resistant colonies over the number of seeded Msh2+/− (light bars) or Msh2−/− (dark bars) cells. Chemical modifications (bold) were at (M) or immediately adjacent (X) to the mismatching base in the oligonucleotide.](Image)
We investigated the spatial distribution of tumor satellites in the prostate. In 61 patients with a median number of 3 satellite tumors, the median distance between the index lesion and the satellites was 1.0 cm, with a maximum of 4.4 cm. This indicates that a limited margin around the index lesion to account for subclinical disease does not effectively cover all satellites, suggesting that the entire gland should be considered clinical target volume (CTV) for radiotherapy treatment planning.

The focus of our research is the improvement of target definition in radiotherapy by application of MRI and the development and validation of quantitative imaging methods for tumor characterization for radiotherapy dose painting. To this end, strategies to integrate anatomical MRI in the radiotherapy workflow are designed and applied to a range of tumor sites. For radiotherapy dose painting, quantitative MRI techniques are investigated. Combining T2-weighted MRI, diffusion-weighted MRI and DCE-MRI, a prediction model was developed for prostate cancer, mapping the probability of tumor presence inside the gland. Validation of this model with whole-mount section histology is ongoing. Building on the experience in prostate cancer, currently the potential of quantitative multi-parametric MRI is investigated to guide radiotherapy of head and neck, rectal and gynecological cancers.

**Digital radiotherapy patient**

Within the EU-funded project `Digital radiotherapy patient (or THERAPAT)` the aim is to use quantitative multi-parametric MRI as input for models providing the probability of tumor presence and dose prescription for radiotherapy dose painting. Clinically relevant implementations for quantitative T2 mapping, diffusion-weighted MRI (DWI) and dynamic contrast-enhanced MRI (DCE-MRI) were validated in a multi-center setting and are currently used in the multi-parametric (mp) MRI protocols in a study of patients receiving two MRI exams prior to prostatectomy.

For radiotherapy dose painting, quantitative MRI techniques are used in the multi-parametric (mp) MRI protocols in a study of patients receiving two MRI exams prior to prostatectomy. The key deliverable of the Therapat project is an automated pipeline for generating radiotherapy dose painting treatment plans from mp-MRI. This pipeline has been made and evaluation of its stability is now investigated using the test-retest mp-MRI data acquired in the multi-center patient study.

**Multi-parametric MRI and histological properties of prostate cancer**

We investigated the spatial distribution of tumor satellites in the prostate. In 61 patients with a median number of 3 satellite tumors, the median distance between the index lesion and the satellites was 1.0 cm, with a maximum of 4.4 cm. This indicates that a limited margin around the index lesion to account for subclinical disease does not effectively cover all satellites, suggesting that the entire gland should be considered clinical target volume (CTV) for radiotherapy treatment planning.

We therefore investigated the potential for dose differentiation
between the visible tumor (GTV) and the CTV. Using the number of tumor cells derived from histopathology, we calculated the tumor control probability (TCP) for the visible tumor and for subclinical disease separately. According to the calculations, a dose reduction to 70 Gy to the CTV combined with a slight dose increase to 80 Gy to the GTV leads to the same level of tumor control as a homogeneous dose of 78 Gy to the entire gland.

**Analysis of dose painting in prostate cancer**

The FLAME trial, a multi-center phase III randomized trial of dose escalation in prostate cancer using external-beam radiotherapy, has finalized inclusion. In total 571 patients have been randomized.

For dose painting in prostate cancer delineation of the tumor on mp-MRI is required. We studied the agreement between tumor delineations by 6 teams of radiation oncologists and radiologists from 3 centers, and validated this with whole mount histopathology specimens. For 20 patients, 18 index lesions were consistently identified by all observers. The kappa indices for the agreement between the delineations of the teams were $0.61 \pm 0.19$ (mean ± SD) and the inter-observer contour standard deviation was 2.3 mm. In addition, 66 out of 69 satellites were missed by all observers. In the analysis of clinical studies of focal dose escalation, these uncertainties need to be considered.

For dose painting, usually external-beam radiotherapy is used. With low-dose rate brachytherapy with 125-Iodine seeds, we showed that it is technically feasible to achieve a large differentiation between the tumor (250 Gy) and the remainder of the gland (125 Gy). The dose reduction to most of the gland from the regular 145 Gy to 125 Gy results in a significant dose-reduction to urethra and bladder neck. However, for precise dose painting, matching the dose to the tumor probability on a voxel-by-voxel basis, external beam radiotherapy is preferable.

**MRI-guided radiotherapy for rectal cancer**

To improve the precision of dose delivery in the clinic, the department of Radiation Oncology introduces MRI guidance for radiotherapy. As part of the MR-linac consortium an integrated MRI-linear accelerator (MR-linac) will be installed at the NKI in the coming year. One of the applications where in-room MRI-guidance is expected to provide a benefit, is the treatment of rectal cancer with radiotherapy. To date, the variability in shape of the target volume, and mobility during the dose delivery is a challenge. To ensure adequate dose deposition in the target volume large margins are now required, leading to comorbidity and prohibiting dose escalation to improve the rate of pathologic complete response. With the MR-linac we expect to improve targeting accuracy, allowing us to reduce treatment margins and investigate the clinical benefit of dose escalation to the tumor.

However, the presence of a permanent magnetic field during irradiation influences the dose delivery, especially around air cavities. Here, electrons are able to return to the surface through which they entered the air cavity (Electron Return Effect, ERE) locally resulting in dose hot- and cold-spots. To investigate if this could pose a problem for rectal cancer, where air pockets appear and disappear frequently, we evaluated the dose in a group of 10 patients showing large changes in air content on repeat CT scans. We found that the presence of a 1.5 T magnetic field has an impact on the dose distribution when the air content changes, but this effect is limited to of a few percent and therefore we conclude that despite these daily variations the dose delivery in an MR-linac is safe for patients with rectal cancer.
Individualized therapy in bladder cancer: molecular targets and biomarkers

Bladder cancer is the fifth most common cancer worldwide, with a prevalence of 2.7 million patients. Although bladder cancer is often superficial at diagnosis, 30-40% of patients present with more advanced disease or progress to more aggressive disease. For patients with locally advanced or metastatic bladder cancer, platinum-based chemotherapy is the mainstay of treatment. Unfortunately, virtually all patients with metastatic cancer and a substantial proportion of patients with locally advanced bladder cancer will eventually present with platinum-refractory disease. At this stage, therapeutic options are (at best) limited. Molecular pathways activated in bladder cancer could provide targets for new treatments. We aim to advance the development of targeted therapies in platinum-refractory bladder cancer by exploring novel molecular targets, mechanisms of resistance and biomarkers that can guide therapy. Through the large number of bladder cancer patients and broad availability of early phase clinical trials with molecularly targeted therapies at the NKI/AVL, discoveries can rapidly be translated into clinical trials.

Genetic determinants of response to neo-adjuvant platinum-based therapy

Pathologic complete response to neo-adjuvant platinum-containing chemotherapy (NAC) is a strong prognostic determinant for patients with muscle-invasive bladder cancer (MIBC). Despite comprehensive molecular characterization of bladder cancer, associations of molecular alterations with clinical outcome and treatment response are still largely unknown. Recently, we found ERBB2 mutations to be associated with complete response to neoadjuvant chemotherapy. ERCC2 missense mutations, previously found associated with response to NAC, were enriched in responders, however this association did not reach statistical significance in our cohort. In addition, we analysed a group of complete responders and non-responders to NAC for DNA copy number alterations. Copy number gain of the E2F3 locus was enriched in responders, whereas copy number gain of the MYC locus and copy number loss of the CDKN2A locus were both associated with non-response. In order to provide a definitive answer on the association of genetic aberrations with response to NAC in bladder cancer, we assembled a validation cohort composed of pre-chemotherapy bladder cancer samples from multiple centres. These samples will be genetically analysed for copy number alterations and mutations in ERCC2, ERBB2 and several other genes that have been reported to be associated with NAC response.

Histone modifications in bladder cancer

Of all cancer types investigated by the TCGA consortium, urothelial cancer shows the highest incidence of mutations in chromatin modifying enzymes, with genetic alterations found in over 95%
of all tumours. Chromatin modifiers aberrant in bladder cancer include the histone methyl transferases KMT2D and KMT2C and the histone demethylases KDM6A and KDM6B. Inactivating mutations of these genes are expected to increase the levels of histone 3 lysine 27 trimethylation (H3K27me3), a marker for repressed genes, and decrease the levels of the marker for actively transcribed genes; histone 3 lysine 4 trimethylation (H3K4me3). These epigenetic abnormalities are believed to result in repression of multiple regions in the chromatin thereby affecting transcriptional output and phenotype. The extent and localization of these repressions in primary bladder tumours has not been resolved yet and their functional role in tumorigenesis awaits further research. We are dissecting the epigenetic status of bladder tumour tissue from patients to identify epigenetic biomarkers for prognosis and understand the biological consequences of these changes. We have selected a cohort of bladder cancers having good or poor clinical outcome. On these tumours we have done ChIP-sequencing for various histone marks, in collaboration with the laboratory of Wilbert Zwart. In addition, we have established CRISPR knockouts for genes that are commonly mutated in bladder cancer to test the functional consequences of these alterations.

Circulating cell-free tumour DNA
Molecular pathways activated in bladder cancer could provide targets for new treatments. Establishing the most relevant target in metastatic cancer can be a challenge for various reasons: 1) Metastases can be hard to biopsy due to proximity to vital structures. 2) A biopsy will provide information for only one location, which may not represent the bulk of the metastatic burden. 3) Therapy can induce genetic changes or select for certain genetic aberrations that were of low abundance in the pre-treatment tumour. Therefore, the ideal molecular test to guide treatment would represent the bulk of the metastatic burden at the moment of treatment initiation and would be relatively easy to obtain. Cell-free tumour DNA circulates in the plasma of cancer patients and can comprise as much as 1-10% or more of total circulating DNA in patients with advanced stage malignancies. Circulating tumour DNA (ctDNA) contains information on tumour mutations and possibly reflects metastatic burden. The relative abundance of mutations has been shown to change as a result of therapy and could possibly serve to guide treatment. Perhaps more importantly, specific mutations in ctDNA could reflect changes in abundance of specific clones, making it possible to adapt molecularly targeted therapies to dominant clones. Urinary cell-free DNA is another source of easily accessible DNA to provide a mutation spectrum of the primary tumour and potentially also of metastatic burden. In our ctDNA project, we longitudinally collect cell-free DNA in the urine and blood of bladder cancer patients treated with systemic therapy to establish the dynamics of genetic alterations during treatment. Samples were analysed by our collaborators in Cambridge (Rosenfeld lab) using the Tam-seq method. Tumor-specific mutations were found in urine as well as in blood. Currently, a larger cohort of bladder cancer patients is investigated to establish dynamics of cancer mutations in cfDNA during therapy.

Enhancement of sensitivity to FGFR-inhibitors
The FGFR3 gene is activated in 10-15% of advanced bladder cancers, and FGFR inhibitors are currently tested in clinical trials. Using shRNA based genetic screens, we found modulators of response to FGFR inhibitors in bladder cancer cell lines. Through several upstream feedback activations, resistance mechanisms appear to converge on the PI3K pathway. We are currently investigating inhibition of this pathway in vitro as well as in vivo.
Early stage technology assessment, operations research and cancer rehabilitation

In this group three research topics are covered: Early Stage Technology Assessment, Improving Oncology Services and Cancer Rehabilitation.

**Early Stage Technology Assessment**

As healthcare costs are continuously increasing and demographics and technologic developments in oncology cause especially high service and financial burdens on health systems, sustainability of future oncology services will inevitably become an issue. Gradually we can expect Health Technology Assessment (HTA) not only to be involved in policy and coverage decisions, but also in an earlier stage in the translational research process.

From 2003 through 2006, a technology assessment study was conducted on the introduction of a 70-gene micro array test as a prognostic tool in the treatment of node negative breast cancer (the RASTER-study) and as a side study of the European MINDACT-study. We ended this series with a Cost Effectiveness Analysis and will propose new health economic evaluations on the results of MINDACT from 2016 onwards. In 2015 an early stage technology assessment of TIL-transfer technology in advanced melanoma started in a Coverage with Evidence Development project until 2018. In 2015 Anna Miquel Cases finalized the early stage technology assessment in the application of diagnostic/prognostic markers in neo-adjuvant breast cancer treatment. This is part of the CTMM program BREASTCARE. We foresee to be increasingly involved in translational research projects and in 2015 Valesca Retèl started as post-doc 50% employed by NKI and 50% by the Health Technology Assessment, Improving Oncology Services and Cancer Rehabilitation.

**Improving Oncology Services**

Together with the University of Twente and the Integraal Kanker Centrum Noord-Dost, PhD student Melvin Kilsdonk is finalizing a project to evaluate the added value of accrediting oncology departments in General Hospitals; he will defend his thesis in 2016. As part of the Eurocan Platform project, work package 12 is directed towards the development of scientometrics and a designation system for excellent Comprehensive Cancer Centers. PhD student Abinaya Rajan finalized an Excellence Designation System for Comprehensive Cancer Centers that was...
presented at the closing meeting and accepted by the European Agency of Cancer Sciences. Benchmarking is a possibly powerful tool to inform management on improvement options and patients on the quality of services. In 2013 the EU-subsidized project BENCH-CAN started in order to develop and pilot a European benchmarking system on Comprehensive Cancer Care.

In 2015 all pilots and site visits were performed, as well as a project to develop a European Cancer Consumer Quality Index. In 2015 we organised a symposium on organising fast track diagnostics on a larger scale; this was sponsored by the National Cancer Charity (KWF). Melanie Lindenberg drafted a value analysis on breast cancer from patient perspective as a first step towards an AVL perspective on Value Based Payments. Christel Spooler performed a logistic analysis on the diagnostic trajectory in immunotherapy for lung cancer.

**Rehabilitation, Physical Activity and Cancer**

Survivorship care and rehabilitation are important elements of a cancer centre’s program. In a stepwise approach various rehab and survivorship services have been developed and by mid 2016 a cancer survivorship and rehab centre will open its doors. A major Alpe d’Huzes/KWF project was started early 2011, focusing on patient empowerment, return to work, tele-monitoring and implementation of relevant findings and programs related to physical exercise and supported by innovative IT. This program, totalling up to 2,8 million euros, will formally end in 2016, though the evaluation runs until mid 2017. The adapted portal site for lung and breast cancer patients was launched by mid 2015, after a (two-phase) feasibility study started early 2014. As further development in this field Laura Kooij performs research into e-health interventions and survivorship care, such as IT-supported stepped care and video consultation. Furthermore Janne Mewes, a PhD student, is working on the cost effectiveness and budget impact of multidisciplinary/multi faceted rehabilitation interventions; she is located at the University of Twente. Also a PhD student co-supervised by Wim van Harten works at IQ-Healthcare in Nijmegen on the structured implementation of ACARE projects’ findings in ten Dutch hospitals.
Cancer epidemiology

The cancer epidemiology group is currently concentrating on two principal research lines: (1) the long-term health consequences of cancer treatment, particularly in terms of the risk of developing second malignancies or cardiovascular disease (CVD); (2) the etiology of hormone-related cancers, with a focus on gene-environment interactions.

Late effects of cancer treatment

Now that curative treatment is available for a substantial group of cancer patients, it is increasingly important to evaluate how the occurrence of late complications of treatment affects their long-term survival. We aim to evaluate the risk of second cancers and CVD after radiotherapy (RT) and chemotherapy (CT) for Hodgkin’s lymphoma (HL) (n=8,500), testicular cancer (n=7,100) and invasive breast cancer (n=100,000) over a period of up to 40 yrs after primary treatment.

As part of a program grant awarded in 2011 a comprehensive assessment is made of the late effects of treatment for HL in close collaboration with Berthe Aleman, Radiation Oncology. In 2015, we completed a large study on the risk of subsequent malignant neoplasms (SMNs) after HL. Since the early 1980s it is known that HL survivors experience a substantially increased risk of treatment-related SMNs. However, since the late 1980s HL treatment has changed towards smaller radiation target volumes, lower radiation doses and more effective, generally less toxic CT schemes. We assessed the impact of these changes on long-term SMN risk in a cohort of 3,905 5-year survivors treated between 1965-2000 at ages 15-51 years. With a median follow-up of 20 years, 1,055 SMNs were diagnosed in 908 patients, resulting in a 4.6-fold increased risk compared to the general population. Risk was still 3.9-fold increased at 35 years after treatment and the cumulative incidence reached 48.5%. The cumulative incidence of solid SMNs did not differ between patients treated in 1965-1976, 1977-1988 and 1989-2000. While less extensive supradiaphragmatic radiation fields, compared to mantle field, were associated with significantly lower breast cancer risk (hazard ratio (HR) 0.38), risk did not decrease in more recently treated patients, due to lower prevalence of CT-induced premature menopause and more breast cancer screening. A cumulative procabazine dose >4.2 gram/meter² strongly decreased breast cancer risk (HR 0.57, compared to no chemotherapy), but was associated with a 2.7-fold increased HR for gastrointestinal SMNs. We concluded that risk of solid SMNs does not appear to decrease in more recently treated patients, potentially due to changes in CT regimens and more breast cancer screening.

Awareness of increased SMN risk remains crucial for HL survivors and their treating physicians.

Since we observed decreased risk of RT-induced breast cancer after CT-induced premature menopause, we are now investigating the effects of endogenous and exogenous...
hormones after HL treatment on breast cancer risk. In a matched case-control study of 174 cases and 466 controls we determined the duration of intact ovarian function, as well as the duration and timing of exogenous hormone use, after HL treatment. In ongoing analyses we assess the modifying effects of these hormonal factors on the RT dose-response relationship. Furthermore, we are investigating the modifying effects of SNPs on the risk of radiation-induced breast cancer, based on an international breast cancer case-control study of 418 cases and 520 controls. Through collaboration with the Breast Cancer Association Consortium we have also obtained SNP data (ICOGS panel) of an age-and country-matched control group of 5000 first primary breast cancers without a history of RT (case-only design for validation).

To study the influence of HL treatment on CVD risk in depth, two case-control studies were performed. The first study included 325 patients with coronary heart disease and 1,204 matched controls. A new method for retrospective radiation dosimetry was developed to estimate the mean heart dose (MHD) for all patients included in the study. A linear radiation dose-response relationship with MHD was established, with an increase of coronary heart disease rate of 7.4% per Gy. This results in a 2.5-fold increased risk of for patients receiving a MHD of 20 Gy from mediastinal RT (compared to no mediastinal RT). Excess relative risks per Gy appeared to decrease with age at treatment. Having one or more classical CVD risk factors (diabetes mellitus, hypertension or hypercholesterolemia) independently increased the risk of coronary heart diseases. A high level of physical activity was associated with a 50% decreased risk.

To study the risk of heart failure in this population, a similar case-control study was performed, including 91 patients with heart failure and 278 matched controls. A nonlinear radiation dose-response relationship with upward curvature was derived for MHD, resulting in rate ratios of 1.2 and 2.5 at MHDs of 20 and 30 Gy, respectively. Anthracycline-containing CT increased heart failure rate 2.8-fold, without evidence for interaction with radiation dose. In patients treated without anthracyclines, modelled 20-year cumulative risk of heart failure following MHDs of 0-25 Gy, 26-30 and >31 Gy were 0.9%, 1.9% and 3.6%, respectively, and in patients treated with anthracyclines these risk were 3.4%, 6.8% and 12.9%, respectively. These findings can be used to predict CVD risk for HL patients before treatment, during RT planning and during follow-up. With a large grant from KWF-Alpe d’HuZes, the Dutch BETEr consortium (Better care after Hodgkin Lymphoma: Evaluation of long-term Treatment Effects and screening Recommendations) is developing a nationwide survivorship care program for HL survivors. The project is coordinated by the NKI and the Radboud UMC. So far, survivorship clinics have started in ten hospitals, including the NKI. The remaining 13 participating centres are preparing to start in 2016. The identification of survivors is nearly complete, resulting in approximately 10,500 HL survivors and 2,400 NHL survivors of whom approximately 70% are eligible for the BETEr program. Screening guidelines have been formally approved by nearly all involved organizations for medical specialists, and last revisions are currently being made. After laborsome negotiations with the Dutch Care Authority a financial care product (Diagnosis Treatment Combination=DBC-DOT) has been developed that can be used from 2016 onwards.
For our study of ischemic CVD and SMN risk among testicular cancer survivors, in collaboration with Prof. Dr. Gietema at UMC, we performed a linkage of the expanded cohort (diagnosed 1976-1995) of 7,105 patients with the Netherlands Heart Intervention Registry, through which we identified 203 new ischemic heart disease or chronic heart failure events, in addition to 336 reported by the general practitioner (in total 484 cases).

Linkage with the Netherlands Heart Intervention Registry identified 148 patients with a first ischemic heart event who were younger than 50 years at testicular cancer diagnosis and are therefore candidates for the TACKLE study. This multicentre case-cohort study aims to shed light upon the association between testicular cancer treatment and development of the metabolic syndrome and hypogonadism, and ultimately the risk of developing CVD. Testicular cancer survivors receive a questionnaire and are asked to participate in a cardiovascular risk assessment during a site visit, a home visit or a visit to their general practitioner. In 2015 the TACKLE study started in the AVL and Erasmus MC (UMCG already started including patients in 2014). So far, 323 patients have participated, with response rates of 80% for UMC, 52% for AVL and 40% for Erasmus MC. Currently, preliminary analyses are conducted on solid malignancy risk following treatment with platinating agents.

To evaluate long-term morbidity from CVD in breast cancer survivors treated with contemporary regimens we conducted two large cohort studies. The first is a population-based cohort (obtained through the Netherlands Cancer Registry) of patients with invasive breast cancer (n=70,230). We acquired CVD data through linkage with the cause of death registry and two population-based registries for CVD morbidity, the nationwide hospital discharge registry and the Netherlands Heart Intervention Registry. The second cohort is hospital-based and consists of 27,000 patients treated 1970-2009 in the NKI or the Erasmus MC. For this cohort more detailed treatment information and CVD risk factors was collected. In 2015 we reported on CVD risk in the population-based cohort. RT regimens used in invasive breast cancer treatment between 1989-2005 slightly but significantly increased the risk of CVD. We reported increased CVD risks (including ischemic heart disease, valvular heart disease, and heart failure) after left- versus right-sided radiation therapy following mastectomy (hazard ratio (HR) =1.19). Risks were more strongly increased in patients aged <50 years at breast cancer diagnosis (HR=1.48). Left- versus right-sided RT after wide local excision only increased the risk of ischemic heart disease (HR=1.14). A subgroup analysis with information on RT fields showed increased CVD risks after left-sided chest wall RT alone, left-sided breast RT alone, and internal mammary chain field RT (all compared to right-sided breast RT alone). In addition, we reported a significantly increased heart failure risk after anthracycline-based CT (HR=1.35).

**Etiology of hormone-related cancers**

In our nationwide cohort study among families tested for a **BRCA1**/2 mutation (HEBON study; 40,461 relatives, including 6,019 **BRCA1**/2 mutation carriers), we are studying whether 1) hormonal/life-style factors modify cancer risk in **BRCA1**/2 families, and 2) common genetic alterations are associated with the risk of breast cancer among **BRCA1**/2 carriers. While we have been coordinating the study so far, this year we actively involved Erasmus Medical Center (M. Hooning) and Leiden University Medical Center (P. Devilee) for coordinating the treatment and DNA parts of HEBON, respectively. Since 2011, 37 Hebon research plans, submitted by one of the Dutch Academic Medical Centers have been approved by the Steering Committee of HEBON.

We evaluated whether the breast cancer risk reduction of approximately 50% after risk-reducing salpingo-oophorectomy (RRSO) as reported by retrospective studies among **BRCA1**/2 mutation carriers, may have been due to bias. First, we replicated the analytical methods as previously applied in four major studies on risk of breast cancer after RRSO. Secondly, we analyzed the data in a revised design in order to further minimize bias. The most important differences between our approach and those of previous studies were the requirement of (i) no history of breast cancer at date of DNA diagnosis and the inclusion of person-time preceding RRSO. Applying the four previously described analytical methods and the data of 551 to 934 **BRCA1**/2 mutation carriers with a median follow-up of 2.7 to 4.6 years, the hazard ratios were significantly decreased, i.e., 0.36 to 0.62, being similar to earlier findings. For the revised analysis, we obtained a non-significant hazard ratio of 1.09. Therefore, we concluded that in previous studies, breast cancer risk reduction after RRSO in **BRCA1**/2 mutation carriers may have been overestimated. Using a design that maximally eliminated bias, we found no evidence for a protective effect.

Annual MRI and mammography is recommended for **BRCA1**/2 mutation carriers to reduce breast cancer mortality. Less intensive screening is advised above age 60 years. In HEBON we compared tumor stage at breast cancer diagnosis between different screening strategies in **BRCA1**/2 mutation carriers above age 60. We included 148 women with breast cancer detected above age 60. With biennial mammography 53% (30/57) of carcinomas were detected in unfavorable stage (≥T2, positive lymph nodes or distant metastases), compared to 21% (12/56) with annual mammography. With biennial screening 40% of breast cancers were interval cancers, compared to 20% with annual screening. We concluded that if life expectancy is good, continuation of annual breast cancer screening of **BRCA1**/2 mutation carriers above age 60 is worthwhile.

Clinical genetic testing is commercially available for rs61764370, an inherited variant residing in a KRAS3’ UTR microRNA binding site, based on suggested associations with increased ovarian and breast cancer risk as well as with survival time. However, previous studies were small. Therefore, we evaluated cancer risks and outcome associated with rs61764370, an inherited variant residing in a KRAS 3' UTR microRNA binding site, based on suggested associations with increased ovarian and breast cancer risk as well as with survival time. However, previous studies were small. Therefore, we evaluated cancer risks and outcome associated with rs61764370, an inherited variant residing in a KRAS 3' UTR microRNA binding site, based on suggested associations with increased ovarian and breast cancer risk as well as with survival time. However, previous studies were small. 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new study design led to the discovery of six new ovarian cancer susceptibility loci. Variants at 1p36 (nearest gene, WNT4), 4q26 (SYNPO2), 9q34.2 (LABO) and 17q11.2 (ATA5D) were associated with risk of epithelial ovarian cancer, and at 1p34.3 (RSPD1) and 6p22.1 (GPKX) variants were specifically associated with risk of serous ovarian cancer. Incorporating these variants into risk assessment tools will improve clinical risk predictions for BRCA1 and BRCA2 mutation carriers.

Two cohorts of women with specific exposures have been initiated in the past and are followed prospectively, i.e. the Nightingale Study among 59,947 nurses on shift work and breast cancer and the DESNET study among 12,091 DES daughters on DES exposure and cancer. New analyses on incident cancer cases will be conducted in 2016.

The aim of the nationwide OMEGA study is to assess the long-term risk of hormone-related cancers after fertility treatment, such as ovarian stimulation for in-vitro fertilization (IVF). The cohort comprises 19,158 women treated with IVF and 5,950 women treated with subfertility treatments other than IVF between 1983 and 1995 (OMEGA I), and 12,173 women treated with IVF and 4,427 women treated with subfertility treatments other than IVF between 1996 and 2000 (OMEGA II).

In 2015, we performed analyses on the risk of several hormone-related cancers with updated cancer incidence data until 2014 from the Netherlands Cancer Registry. After a median follow-up of 21 years, 893 breast cancers and 109 colorectal cancers were observed in the full OMEGA I cohort, yielding similar risks in the IVF group and the general population. Breast cancer risk did not increase with longer time since treatment (relative risk of 0.92 after >20 years, compared to the general population). Risk was significantly decreased after >7 IVF cycles (HR=0.55, adjusted by 1-2 cycles), which might be explained by longer periods of down-regulation preceding IVF, with low estradiol and progesterone levels, and higher doses of human chorionic gonadotropin. Breast cancer risk was also decreased after poor ovarian response at first IVF cycle (HR=0.77 for ≤4 versus >4 collected oocytes), probably due to earlier menopause in women with a poor response. We conclude that, reassuringly, ovarian stimulation for IVF does not appear to increase long-term risk of breast cancer. Women in the IVF group had a significantly increased colorectal cancer risk compared with women in the non-IVF group (HR=1.80). However, colorectal cancer risk was not increased after more IVF cycles, more ampules of gonadotropins administered or with longer follow-up periods. Further research is warranted to examine whether ovarian stimulation for IVF contributes to development of colorectal cancer.

In 2015 we used the OMEGA cohort to examine the role of several aspects of physical activity (timing, duration, frequency, and intensity) on the risk of premenopausal breast cancer. After a median follow-up of 10.6 years after questionnaire completion, 232 incident cases of premenopausal breast cancer were identified in 13,892 OMEGA I participants. Whereas the frequency of relatively recent sports activity appears to decrease the risk of premenopausal breast cancer, duration, timing and intensity did not. Each additional hour/week of exercise at baseline reduced the risk by 10% (HR=0.90, P trend 0.03). Women exercising >3 hours/week at baseline had a 43% reduced risk of breast cancer compared to women exercising one hour/week.


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Chromatin dynamics

Each time a cell divides the genetic information encoded in the genome is duplicated and segregated between the two daughter cells. In addition, cells can inherit non-genetic or “epigenetic” information. Epigenetic refers to variations in function that are heritable without an underlying change in DNA sequence. For example, during the development of a human body, a single genome gives rise to a diverse range of cells with very different functions and shapes. These cell types can subsequently be maintained during cell divisions later in life when the initial signal that instructed the formation of the cell type may no longer be present. In addition to normal development, epigenetic inheritance underlies processes ranging from oncology to aging to evolution, and is therefore highly relevant for many areas of biology. The research in the Van Leeuwen lab is centered around two key topics in the rapidly evolving field of epigenetics: 1. How do epigenetic regulators control gene expression in normal development and cancer? 2. What is epigenetic information, how stable is it, and how is it transmitted and copied?

Epigenetic regulation of gene expression

Switching genes on or off and keeping them in that state involves ‘packaging’ of the DNA in the nucleus by wrapping it around proteins called histones. This packaged form of DNA is called chromatin. Histones carry different chemical modifications that affect the packaging state of chromatin, which in turn affects the ease with which the cellular machinery can access the DNA code and regulate gene expression. The Van Leeuwen lab is investigating the stability and dynamics of chromatin states and how the accessibility instructions are involved in epigenetic memory. By taking advantage of budding yeast as a model system the Van Leeuwen lab is developing new tools to investigate the basic principles of chromatin-based gene regulation.

Histone dynamics and inheritance

It is widely believed that the long-lived histone proteins that package eukaryotic genomes can carry non-genetic or epigenetic information and thus transmit information about genome activity from one cell generation to the next. However, inheritance of genomic packaging status is hard to study and still poorly understood. Studies on the behavior of long-lived proteins in dividing cells have been hampered by the lack of biochemical and genetic technologies to analyze them. To solve this problem, we take advantage of budding yeast genetics and develop tools to simultaneously monitor old and new proteins in living cells. One of the tools we developed is Recombination-Induced Tag Exchange (RITE). Using RITE in combination with genomics and proteomics methods, we unexpectedly found that histone protein inheritance can vary along the genome, that histone proteins may walk along active regions, and that histone proteins carrying epigenetic information can be replaced by new naive histone proteins, potentially erasing information. Since the
inheritance and dynamics of histones are expected to influence the epigenetic landscape and epigenetic memory, we are searching for the mechanisms responsible for these processes. We recently developed a barcode-sequencing method tool (Epi-ID) that will allow us to systematically identify novel histone dynamics factors in genetic screens. In collaboration with the Neefjes lab (Division of Cell Biology) we have applied fluorescent versions of RITE to study the spatiotemporal inheritance of organelles in dividing cells. Our current investigations are aimed at determining the rules, mechanisms, and functions of histone protein turnover and inheritance. These studies are facilitated by our yeast robotics infrastructure that enables high-throughput manipulation and analysis of many genetic mutants in parallel.

Role and regulation of histone methylation
Errors in the chemical modifications of histones, such as acetylation and methylation, can lead to changes in gene expression and cause cancer. We are particularly interested in histone methylation, which plays a critical role in maintaining cell identity and in tumor development. Here we use budding yeast as a discovery platform, and translate our knowledge to mouse models and human cells. We previously discovered a histone methyltransferase Dot1, which can add one, two, or three methyl groups to lysine 79 of histone H3 (H3K79). Dot1 influences chromatin structure and the DNA damage response, and has been implicated in oncogenic transformation in mammals. A major goal of our research is to understand the regulation of H3K79 methylation and its function in gene regulation and DNA damage response. Our recent studies have shown that Dot1 is a non-processive enzyme in vivo. This uncommon mechanism affects the function of the methylated lysine and determines how methylation can be regulated, for example by trans-histone crosstalk to ubiquitination of histone H2B. In yeast the degree of methylation of H3K79 increases progressively on histones and depends on the growth rate. Together with results obtained by mathematical modeling, these findings suggest that H3K79 methylation constitutes a timer mechanism. Using the RITE tool, we found that genomic locations that show high turnover of histones show lower degrees of H3K79 methylation, indicating that histone turnover and inheritance fine tune the levels of H3K79 methylation. We are currently developing genome-wide screens to identify additional mechanisms of regulation of Dot1. Candidate regulators are being studied in yeast as well as human cells and mouse models.

Together, our studies will provide a deep molecular understanding of the inheritance and dynamics of protein-based information in dividing cells and the impact of chromatin-based information on gene regulation. These fundamental processes are not only relevant for epigenetics but also for evolution, aging, and disease.
Role of polycomb-group genes in transcriptional repression, stem cell fate and tumorigenesis

Our lab has a long-standing interest in epigenetic gene regulation dictated by chromatin modifications. We study the mechanism of transcriptional repression by Polycomb-group (Pc-G) protein complexes, and the effects of deregulation of Pc-G genes on development, Cell cycle control, cancer formation and stem cell maintenance. In addition, we are performing large-scale genetic screens in primary cells and in cancer-predisposed mice to identify cancer-relevant networks of oncogenes and tumor-suppressor genes.

Functional characterization of Pc-G protein complexes

Repressive Pc-G proteins and counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in maintenance of proper gene expression patterns during development at the level of chromatin structure. Pc-G protein complexes control large sets of genes including Hox gene clusters and the INK4a/ARF tumor suppressor locus. At least two biochemical distinct evolutionary highly conserved Pc-G protein complexes can be distinguished. The first (PRC2) contains Ezh1/Ezh2 (SET domain proteins acting as Histone H3 methylases), Su(z)12, Eed and histone deacetylases. The second complex (PRC1) is represented by several subcomplexes all carrying the central Bmi1/Ring1B or Mel18/Ring1B ubiquitin E3 ligase that can monoubiquitinate H2A or H2Ax at K119. We focused on key PRC1 and PRC2 enzymes in gain- and loss-of-function studies in mice. Conditional Ring1b and Bmi1 loss-of-function experiments indicate an essential role for maintenance of Pc-G repression in development and stem cell maintenance. An outstanding question is how the activity of PcG enzymes is regulated; we showed that phosphorylation of Bmi1 is required for E3 ligase activity of PRC1 and by mutating the essential phosphorylation sites demonstrated that these are important for Bmi1’s oncogenic capacity and also for its role in double strand break repair. In collaborative experiments with Dr S. Ganesan, New Jersey Cancer Institute, we demonstrated rapid recruitment of a specific subclass of PRC1 to sites of induced DNA damage and using functional assays showed that this contributes to optimal DNA DS break repair. In addition we showed that the deubiquitinating enzyme Usp3 can remove the K119ub mark and conditional loss of USP3 in mice leads to increased DNA damage and chromosomal instability and leads to increased cancer incidence and hematopoietic stem cell defects. A major question is where and how Pc-G complexes bind to chromatin. After establishing genome wide binding profiles for PRC1 and PRC2 in Drosophila we recently showed that the ± 400 Polycomb domains encompass conserved developmental regulatory genes controlling differentiation. With In vivo 4C (chromatin conformation capture on Chip) we demonstrated that these domains interact in vivo in 3D nuclear space in Drosophila neural tissues. An emerging theme is the unique functions of dynamically changing Pc-G protein complexes.
during differentiation. In this regard, we recently demonstrated a specific role for the chromodomain helicase Chd4 associating with PRC2 to define a specific narrow developmental time window for inhibiting astroglial differentiation in the mouse brain.

**Connections between Pc-G gene repression, control of stem cell fate and cancer**

We originally identified Bmi1 as a cMyc-cooperating lymphoma-inducing oncoprotein in mice. We have recently exploited new conditional knockout and transgenic mouse models to extend the observed widespread Pc-G deregulation to models of solid cancer. This indicated important roles for overexpression of both Bmi1 and Ezh2 in breast- prostate- and non-small cell lung cancer as well as in glioblastoma. In contrast, using tissue specific loss of function for Bmi1, Ezh2 or the essential PRC2 component Eed in mice we recently showed profound effects on stem cell/progenitor compartments in the breast, small intestine and brain, often associated with premature induction of proliferation arrest and aberrant differentiation (in several cases at least in part due to induction of the prominent Pc-G target and tumor suppressor Ink4a/Arf), highlighting a dual role for Pc-G in controlling both proliferation and differentiation. To select the critical cancer-inducing Bmi1 target genes among the many Pc-G targets in the genome we performed high-throughput ChIP to define a conserved set of Bmi1 targets in mouse and human glioblastoma cells. We subsequently used these to generate a custom shRNA library that was used in in vivo glioma-inducing shRNAi screens to select shRNAs that are specifically gained or lost. This robust screening method yielded novel tumor suppressors and candidate oncogenes several of which could be independently validated and which form the basis for investigating possible new intervention strategies exploiting glioma-subtype specific synthetic lethal interactions (figure 1). In light of the widespread deregulation of Pc-G in diverse cancers we are currently investigating using both small molecule inhibitors and in parallel inducible shRNAi for Bmi1 and Ezh2 their possible use in preclinical intervention studies in mouse models for basal breast cancer, colon cancer, Glioblastoma and lung cancer.

**Genome-wide Chromatin profiling using a novel transposon-reporter system**

In collaboration with the Wessels and Van Steensel labs we have developed high-throughput chromatin profiling by using Thousands of PiggyBac transposon-based Reporters In Parallel (TRIP) in embryonic stem cells. The power of this approach lies in combining different (inducible) transcriptional reporters in transposons with random barcoding and high throughput sequencing to study genome-wide position effects and influences of local chromatin and epigenetic states on reporter expression. The modular and flexible set up of TRIP allows for the efficient probing of different features of chromatin context on a wide variety of molecular processes including transcription, chromatin dynamics, DNA methylation effects and RNA splicing and stability (figure 2).
Chromatin genomics

Chromatin is probably the most complex molecular ensemble in the cell. It consists of hundreds of proteins that package the DNA and interact with regulatory elements such as enhancers and promoters. All of these components work in concert, and cannot be fully understood unless they are studied in their native context. In addition, the spatial organization of interphase chromosomes is thought to be of key importance for genome expression and maintenance. Yet, this three-dimensional chromosome organization and its impact on gene regulation and other functions are still poorly understood.

In order to gain insight into these fundamental processes, we develop and apply new genomics techniques to reveal the interplay of chromatin and regulatory elements, and to visualize the architecture of chromosomes inside the nucleus. We use Drosophila and mammalian cultured cells as model systems.

New genomics assays to study chromatin function

Most existing genome-wide tools to study gene regulation are descriptive and correlative by nature, and do not permit the inference of causal relationships. Therefore, we have begun to develop new methods to assay mechanism and function, while maintaining a genome-wide view.

We previously reported a powerful multiplexing approach for the parallel monitoring of transcriptional activity of thousands of randomly integrated reporters (TRIP). Complementary to this, we have now developed a genome-wide method to study how regulatory elements are functioning when taken out of their natural chromatin context. In this approach millions of random genomic DNA fragments are tested for their ability to act as an enhancer or promoter (depending on the precise design of the assay) when moved to a new context. This method provides fundamental insights into the biology of regulatory elements and their interplay with chromatin.

We also developed a cheap and simple assay to monitor the efficacy of genome editing by CRISPR/Cas9. The assay, named TIDE, only requires a pair of PCR reactions and two standard capillary sequencing runs. A specially developed decomposition algorithm then identifies the major induced mutations and accurately determines their frequency in a cell population (figure 1). An interactive web tool for automated decomposition of the sequence traces is available at http://tide.nki.nl. This web server is now widely used by the research community, with ~100 visits per day. TIDE greatly facilitates the testing and rational design of genome editing strategies.

Together, these new tools enable us to systematically dissect the mechanisms and functions of chromatin and chromosome organization.

Roles of chromatin in gene regulation and DNA repair

In several ongoing projects we are investigating the regulatory functions of chromatin. We are using a combination of RNA metabolic labeling, cell fractionation and mathematical modeling
to construct a coherent set of genome-wide maps of mRNA transcription, nuclear export and stability. We are overlaying these maps with our chromatin maps to infer how chromatin context may guide these processes. In addition, we are using a modified TRIP approach to study how the activity of regulatory proteins is affected by their local chromatin environment, by tethering specific proteins to a reporter gene that is integrated in thousands of random genomic locations.

We have set up an inducible Cas9 system in human cells to trigger a double-strand break (DSB) at a single genomic locus of choice, and then employ TIDE, direct detection of broken DNA ends and mathematical modeling to determine the kinetics and fidelity of DSB repair at this locus. This approach can be combined with manipulation of chromatin components to study how DSB is precisely modulated by the local chromatin environment. In addition, we are implementing a variant of TRIP to extend this approach to many genomic locations in parallel. The results are expected to provide new insights in the impact of chromatin context on the process of DSB repair.

**Mechanisms and single-cell dynamics of genome-nuclear lamina interactions**

The nuclear lamina (NL) is a protein layer at the nucleoplasmic surface of the nuclear envelope. By DamID mapping we previously found that the genome of mammalian cells is associated with the NL through ~1,300 Lamina-Associated Domains (LADs). These LADs tend to be large (median size 0.5Mb) and harbor thousands of genes that are transcriptionally repressed. We hypothesize that the NL provides an anchoring scaffold that helps to organize the genome in nuclear space. We previously reported an approach to visualize and track LADs by microscopy. This revealed that LAD-NL interactions are dynamic and to some degree variable from cell to cell. In order to study the apparently stochastic LAD-NL interactions in more detail, we have recently adapted the DamID method for use in single cells, thus breaking the “single cell epigenomics barrier”. We have obtained genome-wide maps of NL interactions in ~400 single cells (figure 2). Analysis of these data revealed a core architecture consisting of gene-poor LADs that contact the NL with high cell-to-cell consistency, interspersed by LADs with more variable NL interactions. The variable contacts tend to be cell-type specific and are more sensitive to changes in genome ploidy than the consistent contacts. Single-cell maps indicate that NL contacts involve multivalent interactions over hundreds of kilobases. Moreover, we observe extensive intra-chromosomal coordination of NL contacts, even over tens of megabases. The consistency of NL contacts is inversely linked to gene activity and correlates positively with the heterochromatic histone modification H3K9me3. The single-cell DamID mapping method opens up exciting new venues to study cell-to-cell variation and consistency in genome organization.

We have also generated a large collection of genome-wide NL interaction maps from a broad diversity of human and mouse cell types. We are analyzing this “LAD atlas” to identify principles that are conserved or variable between species and between cell types. Furthermore, we are developing a “scrambling” strategy to induce many random rearrangements and deletions in the genome of mouse cells, in order to identify cis-determinants of NL interactions. Finally, we systematically evaluated whether lamins – the main structural proteins of the NL – are necessary for LAD organization in murine embryonic stem cells. Surprisingly, removal of essentially all lamins does not have any detectable effect on the genome-wide interaction pattern of chromatin with emerin, another marker of the NL. This suggests that other components of the NL mediate these interactions. We aim to identify these factors in the future. Collectively, these studies highlight principles of the dynamic spatial architecture of chromosomes in relation to gene regulation.
Targeted radiosensitization

Increased understanding of the molecular mechanisms underlying tumor and normal cell radiosensitivity have led to the identification of a variety of potential targets for rational intervention. These are based on the “hallmarks of cancer”, eight biological capabilities acquired during the multistep development of human tumors. Among these, blockade of growth factor signaling, interference with new blood vessel formation, enhancement of apoptosis, inhibition of DNA damage repair, and immunomodulation represent attractive strategies in combination with radiation to increase tumor response while sparing the normal tissue. We aim to translate such novel combination strategies from bench to bedside with a focus on cell death and DNA repair/response modulation.

Manipulation of cell death

Many tumor types, including head and neck cancer, (HNSCC) frequently overexpress anti-apoptotic Bcl-2 family members, which has been associated with radio- and chemoresistance and poor clinical outcome. We tested the pan-Bcl-2 inhibitor AT-101 for its capacity to enhance radiation-induced tumor cell kill in HNSCC cell lines in vitro. Additionally, we aimed to compare the effective in vitro concentrations with human serum levels of AT-101 obtained from a phase I/II trial, to substantiate therapeutic opportunities. Our in vitro results showed that AT-101 synergistically enhances radiation-induced apoptosis in a sequence-dependent manner. Clonogenic survival assays demonstrated a radiosensitizing effect at sub-apoptotic concentrations of AT-101. Pharmacokinetic analysis of patient blood samples showed a dose-dependent increase in plasma AT-101 concentration. Thus, AT-101 is a competent enhancer of radiation-induced apoptosis in HNSCC in vitro. In addition, in vitro radiosensitization was observed at clinically achievable serum levels. These finding support further evaluation of the combination of AT-O1 with radiation in Bcl-2- overexpressing tumors.

DNA damage response modulators

DNA damage repair and response inhibition are attractive strategies to potentiate radio- or chemotherapy. Among such approaches, PARP inhibitors are particularly attractive as radio-enhancers due to the cellular replication-dependent radiosensitizing and vasodilatory properties. Three collaborative phase I-II studies evaluating the safety and tolerability of the PARP inhibitor olaparib, in combination with radiotherapy in locally advanced breast cancer (with Gabe Sonke), non-small cell lung cancer (NSCLC; with M van den Heuvel) and HNSCC (with Michiel van den Brekel) are run in our clinic to test this promising combination. Biomarkers that assess the activity of drugs or the combination are important to guide such trials. In collaboration with Jan Schellens, we developed and elaborated a PARP inhibitor pharmacodynamics assay that allows sensitive assessment of PARP inhibitor activity.
Identification and exploitation of DNA repair defects

In view of the combination trials, we extensively analyzed PARP inhibitor mediated radiosensitization with respect to radiation and drug dose in vitro. We found that PARP inhibition provided robust and significant radiosensitization in multiple HNSCC cell lines. Radiosensitization was achieved at PARP inhibitor concentrations that were consistently much lower than those that caused toxicity. PARP inhibitor drug dose dependence of this radiosensitization was dependent on DNA repair integrity as shown by using genetically modified BRCA2-deficient and complemented cells. Importantly, with relation to the clinical trials these data indicate that cellular radiosensitization can be achieved at much lower PARP inhibitor drug dose levels than previously assumed from the single agent activity profiles seen in monotherapy trials.

Exploitation of tumor specific DNA repair defects for tumor targeted radiosensitization

The base excision repair/single strand break repair (BER/SSBR) pathway targets DNA lesions such as induced by radiation or from endogenous sources. DNA polymerase beta (polβ) has a crucial role in this repair pathway. Previously, in collaboration with RW Sobol (Pittsburgh), we found that the cellular levels of polβ are tightly regulated. Consistent with our previous reports, this regulation appears to support a selective use of BER sub-pathways during different cell cycle phases. We found that polβ overexpression is common in human tumors. Cell lines that have been genetically modified to overexpress polβ have been used to analyze the cellular impact of such an overexpression. Interestingly, we identified a DNA repair defect that is manifested in increased radiosensitization. We also found that these repair defects can be further exploited by using DNA repair inhibitors in combination with radiation.

Evaluation of tumor-specific radiosensitization strategies and their therapeutic gain

Novel strategies that combine radiotherapy with targeted drugs harbor the risk of increased normal tissue toxicity that may counteract the desired dose intensification. With our image-guided mouse irradiator (μGRT) we are able to provide accurate dose deposition to the organs and target normal tissues of interest. We demonstrate the development of fibrosis after unilateral lung radiation and radiation dose dependent changes in CT densities. In longitudinal studies functional end points have been monitored and associated with CT images and radiation dose over time. We analyzed multiple breathing parameters that provided new insights into radiation induced lung toxicities.

Response prediction

This research line is aimed to improve prediction of radiosensitivity in both cell lines and pre-treatment biopsies from human tumors. In a HNSCC panel, consisting of 32 cell lines, we found that epithelial to mesenchymal transition (EMT) status, determined by combined microRNA and messenger RNA analyses, was the best predictor of intrinsic radioresistance. These findings were validated in two independent HNSCC lines, in which induction of EMT reduced radiosensitivity. MicroRNA sequencing data on formalin fixed paraffin embedded material from patients with T2-3 larynx carcinomas, treated with single modality radiotherapy in the NKI over the last 10 years were used to show that low expression of the most important miR (miR-203) correlated with local disease recurrence after. More recently we have been working on combining 3 different HNSCC series with RNA expression data to analyze the value of published signatures. In addition, we developed a new high-throughput screening method for determining radiation sensitivity (collaborator: VW van Beusechem, VUmc, Amsterdam). Fast and uniform irradiation of batches up to 30 microplates was achieved using a Perspex container and a clinically employed linear accelerator. Assay performance was compared to that of the CFA and the CellTiter-Blue homogeneous uniform-well cell viability assay, and validated in a whole-genome siRNA library screening setting using PC-3 prostate cancer cells. On 4 different cancer cell lines, the automated cell counting assay produced radiation dose response curves that followed a linear-quadratic equation and that exhibited a better correlation to the results of the CFA than did the cell viability assay.

Improved drug delivery

The cellular plasma membrane is an effective barrier for exogenous compounds to accumulate in tumor cells. In collaboration with G Koning (Erasmus MC, Rotterdam) we previously established that well-defined short chain sphingolipids (SCS), including N-octanoyl-glucosylceramide (GC), effectively counteract this membrane barrier function. GC facilitates diffusion over the tumor cell membrane of the widely used anti-cancer drug, doxorubicin. In order to identify more anti-cancer drugs that, in addition to doxorubicin benefit from lipid analogue-mediated cellular accumulation, we have used a high-throughput approach, screening libraries of both existing and newly developed (targeted) cytostatic agents in different cell lines. Evident hits included mitoxantrone (MTO), which served as our next candidate drug. MTO was encapsulated in liposomes containing C8-GluCer or C8-GalCer in their bilayer. These new SCS-liposomes containing MTO (SCS-MTOL) were tested in vivo for tolerability, pharmacokinetics, biodistribution, tumor drug delivery by intravital microscopy and efficacy, and compared to standard MTO liposomes (MTOL) and free MTO. Liposomal encapsulation decreased MTO toxicity and allowed administration of higher drug doses. Intratumoral liposomal drug delivery was heterogeneous and rather limited in hypoxic tumor areas, yet SCS-MTOL improved intracellular drug uptake in comparison with MTOL. The increased MTO availability correlated well with the improved antitumor activity of SCS-MTOL in a MDA-MB-231 breast carcinoma model.
Host responses to systemic anti-cancer treatment and Personalized Medicine

Emile Voest is director of the Netherlands Cancer Institute, medical oncologist and translational scientist. In addition to his clinical and managerial responsibilities he is leading his own research group. His laboratory work is devoted to bringing personalized medicine to patients and is focused on the impact of the host response on treatment outcome and the development of biomarkers that predict treatment efficacy. The results from these studies are subsequently translated in clinical studies. These translational approaches are performed across tumor types with emphasis on epithelial tumors.

**Host responses, genomics and (tumor)organoids**

Fatty acids and fish oil in chemoresistance

In 2015 my group has been able to further unravel an intriguing fatty acid signaling network that renders tumor cells refractory to genotoxic chemotherapy. We have identified additional receptors that mediate signaling to confer chemoresistance in multiple mouse models. While we previously identified BLT2 the receptor for 12-S-HHT we now succeeded to identify GPR120 on splenic macrophages as the receptor for 16:4.

Interestingly, we have also looked at the content of 16:4 in supplements such as fish oil. Since a significant group of patients use fish oil during chemotherapy we performed both mouse studies and translated these to the clinical situation. We showed that a minimal amount of fish oil could confer chemoresistance in mice. A subsequent phase 1 study in healthy volunteers showed that intake of fish oil or raw fish resulted in detectable concentrations of 16:4 in the circulation. Fatty fish (herring and mackerel) induced higher levels than tuna and salmon. This has raised concerns about the safety of fish oil without any studies showing a potential benefit. Our findings have led to an advice to refrain from fish oil the day before and after chemotherapy.

**Genomic guided personalized medicine**

In 2015 we have made great progress in further facilitating DNA guided personalized medicine. In the Netherlands, a collaborative consortium was founded in 2010 by the three largest cancer centers and in 2015 all academic cancer centers and several large teaching hospitals have joined. I have had the honor to chair the consortium named The Center for Personalized Cancer Treatment (www.cpt.nl), which primarily focuses on next generation sequencing approaches to personalize cancer treatment. Since 2015 I am member of the executive board. We have now established a state of the art national sequencing center with all possible sequencing technologies including an Xten setup. To be able to professionally organize this we have created the Hartwig Medical Foundation. This is a not-for-profit foundation that will create a database of genomic and clinical data and treatment outcome that may be used by the scientific community. It has funding to sequence more than 7000 patients with metastatic cancer both whole genome DNA
and RNA sequencing. This will yield an unprecedented database for research. The Hartwig Medical Foundation will not perform research itself but merely supports research.

Genomics guided personalized medicine has also important ethical consequences and little is known about this. We therefore started a project, in collaboration with Annelien Breedenoord, to identify the needs and wishes of patients who are offered DNA sequencing including germ line data. This will help to prepare and inform patients better for important decision and also improve our informed consent procedures.

**Organoids as a tool to personalize medicine**

Finally, in the context of our personalized medicine efforts and in collaboration with the HUB Foundation, Hubrecht Institute and UMC Utrecht we have started to investigate whether organoids derived from tumors from individual patients have the capacity to predict treatment responses. We have initiated several clinical trials to investigate this. These trials include validation studies in patients with chemotherapy and targeted therapy in lung and colorectal cancer (TUMOROID), organoid-guided experimental treatment studies (SENSOR) and more. We also use the organoid platform to screen for the functionality of fusion genes that, in collaboration with Wigard Kloosterman, were identified by RNA sequencing in a large set of colorectal cancer patients.

In summary, my group is committed to better understand host responses to treatment and to define a genetic tumor signature that reflects the responsiveness of cancer cells to treatment which will lead to improving treatment outcome in cancer patients.
Molecular pathology of breast cancer

Breast cancer is a heterogeneous disease ranging from being non-hazardous to life threatening. Accurate pathological and molecular analyses are key to make accurate predictions regarding prognosis and response to treatment. Therefore, we aim to find, validate, and implement biomarkers to make more precise and personalized predictions regarding prognosis and response to treatment.

Finding the balance between over- and undertreatment of breast Ductal Carcinoma In Situ (DCIS)

Mammographic screening carries a risk of overdiagnosis and hence overtreatment of ductal carcinoma in situ (DCIS), a non-obligate precursor lesion of invasive breast cancer. We analyzed the risk of ipsilateral and contralateral invasive breast cancer in a large population-based cohort of 10,090 women treated for DCIS with a median follow-up of 10.7 years. Unique features of our study are the availability of complete treatment data for the entire population-based cohort and the long-term and complete follow-up for subsequent invasive breast cancer.

First, we compared women treated with breast conserving surgery and radiotherapy, breast conserving surgery alone, and mastectomy. Our results highlight that the beneficial effect of radiotherapy after breast conserving surgery diminishes with longer follow-up. Further, less benefit from adjuvant radiotherapy was seen in younger women. Finally, our data show that the risk of subsequent contralateral invasive breast cancer is low and does not justify contralateral prophylactic mastectomies.

Second, we studied whether the risk of subsequent ipsilateral and contralateral invasive breast cancer and all-cause mortality differed among women with screen-detected, interval, or nonscreening-related DCIS. We found that screen detection was non-hazardous to life threatening. Accurate pathological and molecular analyses are key to make accurate predictions irrespective of DCIS treatment. However, the very low absolute risk difference at 15 years does not support different treatment strategies for women with screen-detected, interval, or non-screening-related DCIS. Further, having a screen-detected DCIS was associated with better prognosis for overall survival. The latter is most likely due to a healthy user effect and not caused by a difference in number of breast cancer-related deaths.

Third, we will evaluate the risk of cause-specific mortality, and assess factors associated with breast cancer-specific mortality in patients treated for DCIS in the coming year.

Within our nationwide cohort we are conducting a nested case-control study to identify subsets of DCIS patients with a very low risk on developing an invasive ipsilateral breast cancer. We will compare DCIS samples from 200 patients that developed an ipsilateral invasive breast cancer after DCIS (the “cases”), and 400 matched DCIS samples of women that remained recurrence free during a ten year follow up period. Central pathology revision, staining for an 8-marker immunohistochemistry panel,
as well as molecular analysis is currently performed to identify biomarkers predicting an invasive recurrence. Furthermore, we designed a randomized controlled, phase 3, open-label, non-inferiority trial to evaluate the safety of active surveillance in 1240 women with Low-Risk DCIS (LORD). The LORD trial is coordinated by the BOOG and EORTC. The interest and feasibility of this trial was evaluated with positive results. Up to now three grants have been awarded. The study is expected to open in 2016.

Mechanisms of response and resistance in patient-derived xenograft models of triple-negative breast cancer

In close collaboration with the group of Jos Jonkers, we have generated patient-derived xenograft (PDX) models for BRCA1-deficient triple negative breast cancer (TNBC) and used these to test response to alkylating agents and PARP inhibitors. Initially, these models respond well to such treatments, but eventually resistance develops frequently. For a BRCA1-mutated model, resistant tumors harbor additional small deletions restoring the BRCA1 reading frame, similar to the mechanism found in patients. For the hypermethylated BRCA1-deficient models, we found treatment-induced demethylation of the BRCA1 promoter or complex genetic rearrangements resulting in fusion transcripts with the BRCA1 mRNA downstream of exons of an unrelated gene. Both mechanisms restore full length BRCA1 expression. Strikingly, response was not directly correlated to BRCA1 expression in a series of 24 TNBC PDX models treated with cisplatin (collaboration with the Curie Institute, Paris, France). In a small subset of these models, response did correlate with RAD51 focus formation. Additional analyses of candidate predictive factors are ongoing.

Development of clinically useful molecular tests to predict chemotherapy response of primary breast cancers

Within the neoadjuvant chemotherapy program, we aim to develop tests predicting response to preoperative chemotherapy. Since 2004 we collect pre-treatment biopsy (PDX) material from all patients scheduled to receive neoadjuvant chemotherapy in the NKI-AVL. In addition, we collect all clinical and pathological data of the patients, resulting in a database with over 1,400 patients registered, and from most of them biopsies for translational research are available, as well as resection specimens in case of remaining disease at surgery. In the past years we made significant progress in our search for biomarkers. Highlights are the association between a BRCA-like genomic profile and a remarkably better response to high dose chemotherapy. To assess this BRCA-like genomic profile in routine diagnostics we developed an easy to perform MLPA assay (collaboration with Petra Nederlof). This assay is now used in three different clinical trials for high dose chemotherapy. Another highlight is the identification of five chemotherapy resistance genes using the “outlier approach”. This bioinformatics algorithm was developed in close collaboration with the computational biology group (Lodewyk Wessels). Another project performed in this collaboration is the deep sequencing of matched samples taken before and after neoadjuvant chemotherapy of 21 patients. By comparing somatic mutations, copy number alterations and gene expression levels between ‘before’ and ‘after’ samples from the same patient, we aim to study the effect of chemotherapy on breast tumors and to identify potential resistance mechanisms (figure). Since these samples can only be collected from therapy resistant tumors (sensitive tumors would not yield enough tumor material after therapy, if any), the relevance and implications of results coming from these analyses will have to be assessed in the pre-treatment samples of our full neoadjuvant cohort. Another close collaboration exists with the medical oncology department (Sjoerd Rodenhuis, Gabe Sonke). In one of the projects we aim to identify biomarkers for trastuzumab response (primary endpoint is pathologic complete response in the breast and lymph nodes). For this purpose we will analyze expression data of the primary tumor (pretreatment) in a cohort of -100 patients with stage II or III HER2-positive breast cancer homogenously treated with neoadjuvant PTC (paclitaxel, trastuzumab, carboplatin).

In addition to the expression data we will analyze the PIK3CA mutation status of the tumors and the mutation status of some other common breast cancer genes as well.

Defining radiotherapy sensitivity of breast cancer

We collaborate with Harry Bartelink, Marc van de Vijver, and Paula Elkhuzien to find biomarkers predictive of radiotherapy response (see radiotherapy section for further detail). First, the Preoperative Accelerated Partial Breast Irradiation (PAPBI) trial was initiated in which patients are irradiated preoperatively. Response to radiotherapy is measured 6 weeks after treatment by pathological and radiological response (MRI, PET, CT). Of 70 patients, RNA sequencing data of fresh frozen material from pre-treatment biopsies and post-treatment surgery specimen are available. This data is being analyzed in order to find a signature for radiotherapy response. In addition, radiation induced genetic alterations will be studied and existing profiles validated.

Second, samples will be analyzed from the completed Young Boost Trial regarding 2400 patients under 50 years randomized between normal boost dose vs. a higher boost dose after breast-conserving therapy. The profiles related with radiotherapy response from the PAPBI trial will be validated on this material to assess whether a response profile is associated with local control.

Third, we analyzed trends in treatment and outcome in a large cohort of 8507 patients treated with breast conserving therapy between 1980 and 2008. A very low local recurrence (LR) rate over the past decades was observed. Although young age is an important risk factor for LR, also in young patients (<40 yrs) LR rates were low. We set up a nested case-control study to find risk factors for LR in young patients and will collect tissue blocks for revision, immunohistochemical and molecular analysis.
Computational cancer biology

The Computational Cancer Biology group develops novel computational approaches and performs state-of-the-art analyses of a wide array of data types to further our basic understanding of cancer and to translate these findings to the clinic. We follow a two-pronged approach. On the one hand, we focus on employing computational approaches to chart the cancer landscape by integrating heterogeneous molecular data sets. On the other, we employ drug response data from model systems, such as cell lines and organoids, to map the cancer landscape to treatment strategies that are most likely to effectively combat cancer. A number of exemplary presents below in more detail.

Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer

Invasive lobular carcinoma (ILC) is the second most frequently occurring histological breast cancer subtype after invasive ductal carcinoma (IDC), accounting for around 10% of all breast cancers. The molecular processes that drive the development of ILC are still largely unknown. We have performed a comprehensive genomic, transcriptomic and proteomic analysis of a large ILC patient cohort and generated an integrated molecular portrait of ILC. Mutations in CDH1 and in the PI3K pathway are the most frequent molecular alterations in ILC. We identified two main subtypes of ILCs: 1) an immune related subtype with mRNA up-regulation of PD-L1, PD-1 and CTLA-4 and greater sensitivity to DNA-damaging agents in representative cell line models; 2) a hormone related subtype, associated with Epithelial to Mesenchymal Transition (EMT), and gain of Chromosomes 1q and 8q and loss of Chromosome 11q. Using the somatic mutation rate and eIF4B protein level, we identified three groups with different clinical outcomes, including a group with extremely good prognosis (figure 1). We generated a comprehensive overview of the molecular alterations driving ILC and have explored links with therapy response. This molecular characterization may help to tailor treatment of ILC through the application of specific targeted, chemo- and/or immune-therapies.

RUBIC: An algorithm to detect recurrent DNA copy number breaks to identify driver genes

The frequent recurrence of copy number aberrations in genomic loci across tumor samples is a reliable hallmark of a subset of cancer driver genes. However, state-of-the-art algorithms for detecting recurrent aberrations fail to detect several of these drivers. We developed RUBIC, a novel approach that effectively combat cancer. A number of exemplary projects are presented below in more detail.


RUBIC: An algorithm to detect recurrent DNA copy number breaks to identify driver genes

The frequent recurrence of copy number aberrations in genomic loci across tumor samples is a reliable hallmark of a subset of cancer driver genes. However, state-of-the-art algorithms for detecting recurrent aberrations fail to detect several of the known drivers. We developed RUBIC, a novel approach that detects recurrent copy number breaks, rather than recurrently amplified or deleted regions. This change of perspective allows for a vastly simplified approach as recursive peak splitting procedures and repeated re-estimation of the background model are avoided. Furthermore, the false discovery rate is controlled
on the level of called regions, rather than at the probe level, as in competing algorithms. We benchmarked RUBIC against GISTIC2 (a state-of-the-art approach) and RAIG (a recently published approach) on simulated copy number data and on three SNP6 and next generation sequencing copy number data sets from TCGA. We show that RUBIC calls more focal recurrent regions and identifies a much larger fraction of known cancer genes as benchmarked against the Cancer Gene Census and a set of bona fide validated breast cancer genes (figure 2).

**Multilevel models improve precision and speed of IC50 estimates**

The drug sensitivity of pre-clinical models such as cell lines and organoids is often determined based on experimentally determined dose-response curves. Experimental variation in these curves result in inaccuracies in the estimate of a key drug sensitivity characteristic: the concentration at which 50% cell kill occurs (IC50). We proposed a multilevel mixed effects model that takes advantage of all available dose-response data, rather than using a single drug–cell line response. This approach significantly improves the precision of the IC50 estimates by simultaneously employing all dose-responses across all cell lines and drugs. The model exploits the fact that certain characteristics, such as the slope of the dose-response curve are, in general, cell line specific characteristics and can hence borrow information for the data fit across all drugs to which a cell line was exposed. The new estimates are highly concordant with the currently used Bayesian model when the data are well behaved, i.e. when adhering to the expected sigmoidal shape for the dose-response curve. Otherwise, the multilevel model is clearly superior. The multi-level model yields a significant reduction in outliers, a significant increase in precision, and runs orders of magnitude faster. A very useful characteristic is that it allows the automatic identification failed experiments by identifying experiments associated with a large residual error, i.e. poor model fit.

**LOBICO: Logic models to predict continuous outputs based on binary inputs with an application to personalized cancer therapy**

Mining large datasets to find ‘actionable knowledge’ represented by easily interpretable associations between a small number of input variables (e.g. mutations) and an output variable of choice (drug response) is becoming more commonplace and more challenging as data bases are rapidly growing. We present ‘Logic Optimization for Binary Input to Continuous Output’ (LOBICO), a computational approach that infers small and easily interpretable logic models of binary input features that explain combinations of multiple mutations are more predictive of drug sensitivity and specificity. As such, it represents an important step towards practical application of interpretable logic models.

![Figure 1](image1.png) **Figure 1:** A) The decision tree, classifying the samples based on their somatic mutation rate and eIF4B protein level. B) Kaplan-Meier curves of the groups of samples defined by the decision tree. Samples with high mutation rate have a poor survival, while samples with low eIF4B level have a good survival.

![Figure 2](image2.png) **Figure 2:** Genomic representation of recurrent amplifications on Chromosome 6q detected by RUBIC, GISTIC2 and RAIG in SNP6 copy number profiles of 1880 Breast Invasive Carcinoma from the TCGA. The main panel represents the RUBIC segmented aggregate profiles across 6q. The rows at the top with labels ‘RUBIC’, ‘GISTIC2’ and ‘RAIG’ show the genomic locations of called recurrent regions of each respective method. The row with label ‘Census’ shows the locations of Cancer Gene Census genes. The annotated genes (CGSor203, BEND3, MYB, ESR1) represent validated breast cancer genes. RUBIC calls all these validated genes, while GISTIC2 and RAIG both fail to call MYB and ESR1.


Seventy percent of all head and neck squamous cell carcinoma (HNSCC) patients present with advanced staged disease with an overall 5-year survival of approximately 40-50% in case of radical and often mutilating surgery and/or (chemo-) radiation therapy. The addition of high-dose cisplatin to radiotherapy (CCRT) increases the percentage of ≥ grade 3 acute toxicity up to 89%, in stark contrast to the 6.5% of patients that actually experience a 5-year survival benefit after CCRT. Apart from cisplatin and some other classic chemotherapeutic agents, only cetuximab has been shown to increase the response to radiotherapy in HNSCC, while Olaparib, a PARP-inhibitor, is currently tested for its clinical efficacy in HNSCC patients. It remains a major challenge in head and neck cancer to find novel compound based treatment schemes that help to achieve higher cure rates while preserving function of nearby tissues and organs at risk.

Anti-cancer drugs and HNSCC

Automated drug screens were used to identify novel radiosensitizing compounds to improve drug based RT outcome in patients with head and neck cancer in the Neefjes cell biology laboratory. Different chemical libraries were tested for their biological effects on different HNSCC cell lines. These included two kinase inhibitor libraries donated by Pharma (from the Ovaa lab). Compounds were tested at multiple concentrations, either with or without additional radiation, for their potential radiosensitizing effects based on a fluorescence metabolic read-out. We identified a promising lead compound that showed selective radiosensitizing activity against a panel of HNSCC cell lines, while having no effect on cell viability in the absence of radiotherapy (see figure). This effect was confirmed by a colony-forming assay with a DEF (dose-enhancement factor) of 1.8. Recently, the target of our lead compound was found to be ATM, part of the DNA repair pathway. In addition, our compound did outperform Olaparib in several (but not all) HNSCC cell lines. We are currently writing a protocol to test our novel ATM inhibitor in mice. These studies should define better options for radiotherapy of HNSCC patients.

Fresh primary tumor cultures of HNSCC

Fresh tumor biopsies of previously untreated HNSCC patients are taken at the Head and Neck Surgery Department of the Antoni van Leeuwenhoek Hospital and cultured at the Neefjes’ cell biology laboratory following a Histoculture Drug Response Assay (HDRA), consequently maintaining architecture, heterogeneity and cell-cell interactions. Tumor fragments are each placed on a Gelfoam sponge and cultured for a maximum of 7 days at 37 °C in 5% CO₂ atmosphere. Biopsies of 121 patients have been taken into culture for analysis, with a success rate of 89.5% (technical error 2.2%, contamination of the culture 2.2%, benign tissue fragments 7.1%). This study aims to establish a culture system for testing novel drug ex vivo.
Radiosensitizing effect of the selected compound
Head and neck cancer cell line UT24 incubated for 30 min. with the appropriate concentration of the designated radiosensitizer and subsequently irradiated for 30 min.

After 7 days cell viability is determined by a cell titer blue assay. Normalized to radiation without the designated radiosensitizer. Upper line shows cell survival without radiation. Lower line shows cell survival after exposure to 4 Gy of radiation.

Microenvironment of HNSCC
HNSCCs can be categorized by two distinct aetiologies: tobacco and/or alcohol use in combination with genetic predisposition, or infection and activity of viral oncogenes. HPV positive oropharyngeal HNSCC tumors were described to have increased numbers of tumor infiltrating CD8+ and FOXP3+ lymphocytes compared to HPV-negative tumors, correlating to favorable clinical outcome in HPV positive HNSCC. In Schumacher’s laboratory, we aim to assess hypoxia as a determinant for T cell capacity in human tumors. The net effect of hypoxia or HIF1α accumulation on T-cell differentiation or effector function in human malignant solid tumors in vivo is unknown, and may very well differ per T-cell subset. Tumor hypoxia may interfere with the effect of aPD1 on adaptive immunity, due to 1. hypoxia-mediated altered local T cell fate (gene transcription and/or RNA translation), and 2. the local balance between different T cell subsets and myeloid cells. When we will be able to reveal key consequences of hypoxia on T cell capacity both at steady-state and after immunotherapy in clinical setting, the results of our study may have large implications for the development of combination treatments that aim to further enhance tumor-specific T cell activity and consequently treatment response in HNSCC during check-point blockade therapy.

Cisplatin and radiotherapy-induced ototoxicity in patients with HNSCC
Ototoxicity is a commonly seen adverse event after cisplatin treatment and radiotherapy to the head and neck area, with incidences reported up to 88% after CCRT and 54% after RT. Although ototoxicity is not a life threatening disease, it may have a major impact on a patient’s quality of life. Therefore, counseling patients about this adverse event is important and attention is particularly needed in patients who rely heavily on auditory input (e.g. teachers, musicians, the visually impaired). The main objective of our research (Theunissen, Balm) is to improve our knowledge of (chemo-) radiation induced ototoxicity in patients with head and neck cancer.
Translational endocrinology and pathological genomics in breast and prostate cancer

Our lab is specialized in the field of translational endocrinology, studying steroid hormone receptor behavior in relation to cancer. As major fields of interest, we focus on response to therapeutic interventions in breast and prostate cancer, in search of biomarkers for response prediction and treatment outcome. For both tumor types, different treatment options are available and selecting the most optimal treatment for the individual patient has great clinical potential. Steroid hormone receptor structures, transcription complex composition and downstream signaling cascades are evolutionary highly conserved, creating strong and ample opportunity for synergism between projects on different hormonal tumor types.

We study alterations in chromatin-binding features of hormone receptors and epigenetic signatures in tumors and cell lines, in order to identify distinct genomic profiles that bear clinically relevant data on patient outcome. Additionally, since transcription factors and epigenetic programs dictate expression of genes that ultimately drive cell behavior and tumor progression, we identify driver genes for outcome, yielding potential biomarkers as well as novel therapeutic targets for small molecule inhibitor design.

Androgen Receptor/DNA signatures in prostate cancer predict outcome

Prostate cancer is the second most prevalent malignancy in men. Biomarkers for outcome prediction are urgently needed, so that high-risk patients could be monitored more closely postoperatively. To identify prognostic markers and to determine causal players in prostate cancer progression, we assessed changes in chromatin state during tumor development and progression, in close collaboration with the labs of Andre Bergman and Lodewyk Wessels (Stelloo et al., 2015). Based on these analyses, we studied the genome wide androgen receptor/chromatin binding profiles that lead to the identification of a distinct androgen receptor/chromatin binding signature, that was distinct between primary prostate cancers and tumors with an acquired resistance to therapy (figure 1A, B). These differential androgen receptor/chromatin interactions dictated expression of a distinct gene signature with strong prognostic potential. Further refinement of the signature provided us with a concise list of nine genes that hallmark prostate cancer outcome since transcription factors and epigenetic programs dictate expression of genes that ultimately drive cell behavior and tumor progression.

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transcriptomics after neoadjuvant androgen ablation; Henk van der Poel, Andre Bergman) and the PRESTO study (Predicting Response to Enzalutamide as a Second Line Treatment for Metastasized Castration Resistant Prostate Cancer Patients; Andre Bergman). Furthermore, in collaboration with Carmen Jeronimo and Rui Henrique (Portuguese Oncology Institute, Porto), we are currently executing a follow-up project, in which extensive epigenetic analyses are performed on 100 prostate cancers, in search for novel prognostic biomarkers and to further increase our understanding of transcriptional regulation in prostate cancer.

**Estrogen Receptor coregulators in resistance and sensitivity to therapy**

Each year, 1.4 million women are diagnosed with breast cancer. About 75% of all breast tumors are Estrogen Receptor (ER\(\alpha\))-positive and are thought to depend on hormonal stimuli for tumor cell proliferation.

The steroid receptor coactivator SRC3 is essential for the transcriptional activity of ER\(\alpha\). SRC3 is posttranslationally modified by phosphorylation, but these events have to date not been investigated with regard to functionality or disease association. We found phosphorylated SRC3 to possess selectivity for binding the chromatin on a genome-wide scale in cell lines and tumors; while total SRC3 is selectively found at enhancer regions, SRC3-pS543 is recruited to promoters of ER\(\alpha\) responsive genes (Zwart et al., 2015). SRC3-pS543 was associated with improved disease-free survival in tamoxifen untreated high-risk patients, while such a correlation was not seen in tamoxifen-treated cases, with a significant test for interaction \(p = 0.001\), figure 2). Multivariate analysis showed SRC3-pS543 to be an independent prognostic factor. Follow-up studies are focused on other phosphorylation events on SRC3 in relation to its genomic action, its direct downstream target genes in relation to outcome and to further understand the functional crosstalk between SRC3 and its family members in breast cancer.

In a separate project, the molecular mechanisms underlying resistance to tamoxifen were studied. We have shown in the past that tamoxifen resistance is induced by activation of the Protein Kinase A (PKA) pathway (de Leeuw et al., 2013). PKA induces the phosphorylation of ER\(\alpha\) on Serine 305 and subsequent receptor activation after tamoxifen binding. Together with the labs of Sabine Linn and Jacques Neefjes, we showed that the PKA-anchoring protein AKAP13 is essential for ER\(\alpha\)/PKA interaction (Bentin Toaldo et al., 2015). Stratifying breast tumors on ER\(\alpha\) Serine 305 phosphorylation status resulted in the identification of a gene network centered upon AKAP13. AKAP13 mRNA expression levels correlate with poor outcome in patients who received tamoxifen treatment in the metastatic setting. In a luminal breast cancer cell line, AKAP13 interacts with ER\(\alpha\) as well as with a regulatory subunit of PKA. Knocking down of AKAP13 prevented PKA-mediated Serine 305 phosphorylation of ER\(\alpha\) and abrogated PKA-driven tamoxifen resistance, illustrating that AKAP13 is an essential protein in this process.
Division of Diagnostic Oncology

The division of Diagnostic Oncology is at the hub of interactions between clinical departments and research departments. The division comprises the departments of Radiology, Pathology, Clinical Chemistry, Clinical Physics, Family Cancer Clinic and Nuclear Medicine. Diverse as the departments are, they have a common focus: to improve diagnostics, and thereby treatment, as well as follow-up of different types of cancer using the latest technologies. In this respect, investments in 2015 in Next Generation Sequencing, digital MRI and digital PET/CT systems stimulates new scientific opportunities in diagnostic oncology. Extra impuses in 2015 for the departments of pathology and radiology have resulted in appointments for research groups headed by Prof. dr. Gerrit Meijer and Prof. dr. Regina Beets-Tan, respectively. All efforts in the division have led to many publications, presentations and invited lectures worldwide. The introduction of new staff and new techniques will definitely increase the scientific output of the Division of Diagnostic Oncology in the upcoming years. Finally, Stijn Heijmink, Lisa Klopmanhouwer and Kenneth Pengel from our division successfully defended their thesis this year.

DEPARTMENT OF CLINICAL CHEMISTRY

The department of clinical chemistry is responsible for the routine collection of patient materials, such as blood and urine, and biochemical and haematological analysis for patient care. Furthermore, the department of clinical chemistry has the objective to bridge basal biomarker research and routine clinical application of meaningful biomarkers. For this purpose we develop and validate new analytical assays, investigate new clinical applications of biomarkers and implement new meaningful assays in routine patient care.

Biomarker method development

In collaboration with Olaf van Tellingen and Margot Tesselaar, Division of Medical Oncology

One focus of the department of clinical chemistry is the development of new analytical methods for biomarker analysis. Recently a liquid chromatography tandem mass spectrometry (LC-MS/MS) based assay was developed for the analysis of serotonin, a marker for neuroendocrine tumors. This method was used to investigate the relevance of the blood matrixes whole blood, platelet-rich plasma and serum that are all used in daily practice. It was shown for the first time that serotonin determined in these matrixes was interchangeable. The assay is now implemented for routine serotonin analysis in daily patient care.

Another project aims to develop a finger prick PSA (prostate specific antigen) method without losing any analytical performance. This should allow patients with prostate cancer...
to collect a blood sample at home and relieve them from venous blood drawing and a trip to a blood collection point.

Future method development will focus on the development of LC-MS/MS based assays for the analysis of androgens and estrogens. Androgens are important drivers of prostate cancer and primary therapy is focused on reducing the endogenous androgen concentration. In hormone receptor positive breast cancer estrogen exposure and signaling is the basis of many therapeutic strategies. Currently, the limited analytical sensitivity and specificity of routinely used immunoassays for these compounds significantly limits monitoring of androgen levels in prostate cancer patients and estrogen levels in postmenopausal women with breast cancer.

Circulating tumor DNA
In collaboration with Division of Medical Oncology (Michel van den Heuvel, Michiel van der Heijden) and Department of Pathology (Kim Monkhorst, Martjae Vogel)

The department of clinical chemistry is focused on translating new technologies and biomarkers from research to clinical care and explores the application of new biomarkers. Circulating tumor DNA (ctDNA) could improve personalized treatment of tumors by allowing frequent measurement of tumor derived DNA fragments in blood. In 2015 we have explored the possibility of measuring ctDNA in plasma. This work was primarily aimed at three questions: validation of ddPCR for measurement of ctDNA, identification of emerging resistant clones and at the identification of targetable mutations in the absence of a result from a tissue biopsy.

Technical validation showed a high sensitivity for ctDNA in a variable background of wild-type circulating DNA. Our initial validation included the most common EGFR mutations, BRAF and NRAS mutations. In more than 20 patient cases (NSCLC, Melanoma) we retrospectively measured ctDNA in time. These results showed the possibilities of ctDNA measurements for early detection of emerging resistant clones, therapy efficiency, residual disease, recurrence, etc. The ctDNA results correlated with the clinical disease stage. Based on the technical validation and our experience ctDNA measurements were made available for patientcare at the end of 2015.

Measurements on liquid biopsies were not limited to measurements in plasma. Driver mutations were studied and identified in cell free liquor, circulating tumor cells (CTC’s), pleural effusion, etc. For the identification of Leptomeningeal metastasis we have shown the possibility to identify driver mutations in cell free liquor and on isolated CTC’s. A limited amount of work has been done on copy number variations of EGFR and HER2 on CTC’s or FFPE.

Further technical developments aimed at the screening of multiple mutations in a single run. We have designed a method to identify multiple individual mutations and to identify groups of similar mutations. To further increase the amount of data obtainable from plasma we started to explore the possibilities to sequence circulating tumor DNA from plasma using capture based assays.

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PUBLICATIONS


Baars JE, Ausems MGE, Riel E van, Kars MC, Bleeker EM. Communication between breast cancer patients who received inconclusive genetic test results and their daughters and sisters years after testing. J of Genetic Counseling 2015 (in press)


The pathophysiological changes that occur after Risk reducing salpingo-oophorectomy (RRSO) are largely unknown. Therefore, we prospectively collected blood samples and questionnaires of women who underwent RRSO. The objective of our study is to improve our knowledge about vasomotor, mood and sexual complaints and its relationship with pathophysiological changes. Prospectively, we included 147 pre- and postmenopausal women who underwent a RRSO. Questionnaires about quality of life, sexuality and its relationship with pathophysiological changes. We started to measure the Anti-Mullerian hormone (AMH) in women who underwent a RRSO. The objective of our study is to improve our knowledge about vasomotor, mood and sexual complaints and its relationship with pathophysiological changes. We started to measure the Anti-Mullerian hormone (AMH) in women who underwent a RRSO. Therefore, we prospectively collected blood samples and questionnaires of women who underwent RRSO. We collected blood samples one day before, one day after, six weeks after and six months after RRSO. We are evaluating the concentrations of HE4 in different body fluids, such as serum, ascites, urine and saliva in patients with gynaecologic cancer. CA125 is the most commonly used tumor marker in the diagnosis of ovarian cancer, but has limitations in both sensitivity and specificity. Human epididymal secretory protein (HE4) is a promising biomarker and is included with CA-125 in the Risk of Ovarian Malignancy Algorithm (ROMA) score, which is suggested to further increase the diagnostic accuracy than either marker alone. HE4 demonstrated the highest discrimination between benign and malignant pelvic masses compared to ROMA, Risk of Malignancy Index and CA125 alone. HE4 can be used in combination with CEA to make the distinction between EOC and ovarian metastases.

Risk reducing salpingo-oophorectomy: physical and psychological consequences

In collaboration with Gyneecologic Oncology (Marc van Beurden, Ravi Vermeulen) and Division of Psychological Research and Epidemiology (Neil Aaronson)
SPECT device for SN imaging will be delivered December 2015 and installed probably next year (Lightpoint Medical). A new freehand Cerenkov radiation and the first prototype of a new device will be end of that year. New collaborations were explored in the field of a new device for FDG-guided biopsy of breast lesions. This system Mammocare (Grant 606017) an international consortium ofIn the context of the European Union supported project Mammocare (Grant 606017) an international consortium of

In 2015, the department of nuclear medicine has focused its research program on two main topics: image guided treatment and personalized medicine. This has resulted in a variety of presentations and publications worldwide. A summary of all activities is presented in this overview.

**Technological innovation and international network cooperation**

Activities in the context of the European educational and research network on radioguided surgery and interventional molecular (Radioguide) were also in 2015 continued within the organization of international training courses during the congresses of the World Federation on Nuclear Medicine and Biology (WFNMB) in Cancún and the European Association of Nuclear Medicine (EANM) in Hamburg. In addition, current research program has also led to a strong collaboration with the IAEA and the EANM in Vienna and subsequent invited lectures in several European countries.

In the context of the European Union supported project Mammocare (Grant 606017) an international consortium of partners from different European countries (Spain, France, United Kingdom, Netherlands) was formed for the development of a new device for FDG-guided biopsy of breast lesions. This system was installed in 2015 and the first patients were studied at the end of that year. New collaborations were explored in the field of Cerenkov radiation and the first prototype of a new device will be installed probably next year (Lightpoint Medical). A new freehand SPECT device for SN imaging will be delivered December 2015 and further developed in 2016 in close collaboration with XStrahl.

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**Biobank**

In collaboration with the Division of Experimental Therapy (Annegien Broeks)

In 2011, we started with the biobank for storage of serum and whole blood for future investigations in a broad research area. Every new patient in the NKI-AVL is asked by an informed consent to donate 2 tubes of blood. In 2015, we expect to collect 2300 serum and 2500 whole blood samples from new patients. Since 2014 we also store EDTA-plasma and platelets during follow-up of different tumours. These components will be used for developing circulating tumour DNA assays and RTA-transcripts, respectively. In 2015, we expect to collect 2000 EDTA-plasma’s and 1400 platelet samples. In October 2015, the isolation of the peripheral blood mononuclear cells moved from the department of Experimental Immunotherapy to the department of Clinical Chemistry.

**DEPARTMENT OF NUCLEAR MEDICINE**

In collaboration with: Kenneth Gilhujs, Simon Horenblas, Claudette Loo, Hester Oldenburg, Marie-Jeanne Vrancken Peters, Kenneth Pengel, Henk van der Poel, Sjoerd Rodenhuis, Emiel Rutgers, Jelle Teertstra, Jelle Wesseling, Uulke van der Heijde, Theo Ruers, Jeffrey Steinberg, Warner Prevos, Martin Klop, Margot Tesselaar, Jose Belderbos, Gabe Sonke, Jan-Jakob Sonke, Jeroen van de Kamer, John Haanen, Alexander van Ackooi, Robert Poel, Angela Collarino, Pablo Borrelli, Lisanne Bonsen, Daphne Huizing

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**For references, please refer to the original document or a citation database.**


Preclinical imaging

In 2014 a joint effort of various preclinical and clinical disciplines has resulted into the emergence of an imaging facility at the new mouse cancer clinic. Several modalities have been purchased, including a SPECT/CT scanner (USPECT+, MLabs), a PET/CT scanner (Albira, Brucker), a low-field MRI system (ICON, Brucker) and the star of the show a 7 Tesla MRI (BioSpec, Bruker). In 2012 a study on cisplatin imaging in mice was initiated. Cisplatin is often used in combination with radiotherapy for the treatment of several types of cancers (e.g. head-and-neck cancer, lung cancer). Radiolabeled 195mPt-cisplatin was developed in collaboration with NRG in Petten for SPECT imaging of cisplatin. After proof-of-principle work with phantom studies, experiments were performed to test this new imaging method in mouse models. The biodistribution of 195m-platinum could be visualized at multiple time-points after administration, with signal quantification that correlated well with the standards of ex vivo gamma counting and atomic absorption spectroscopy. Also 195mPt-cisplatin imaging of tumor-bearing animals was performed. As a first application the influence of hydration state on the biodistribution of cisplatin was tested. This demonstrated that, in animals, hydration prior to chemotherapy reduced nephrotoxicity but also lowered the dose delivered to the tumor. The same trend is seen retrospectively in patients. The ultimate goal of this project is to predict survival and toxicity of patients who received cisplatin-based therapies, and to optimize factors that influence the biodistribution of cisplatin in concurrent chemoradiation.

Radioguided iodine seed localisation and surgery

In the framework of the EU-Eurostars project “Et1703 Real-time fhSPECT” the use of freehand-SPECT (fh-SPECT) for optical tracked real time 3D radioactivity mapping was further evaluated. In a series of studies this method was introduced for radioactive seed localisation with multiple iodine seeds. The aim was to introduce freehand-SPECT for visualisation and navigation in the operating theatre to improve the current radioguided intraoperative methods for resection of non-palpable breast cancer. For 125I-seed localisation and navigation in the breast a short freehand-SPECT acquisition with an optical tracked gamma probe can be used. A 3D reconstruction of the radioactivity distribution visualises the location and guides the direction to pinpoint the target. Our research was focussed on 125I-seed guided breast conserving procedures only. This application of freehand-SPECT was a novel way to utilize this device and was not demonstrated before. It started with a proposed training protocol for freehand-SPECT acquisitions to improve scanning accuracy and intraobserver variation. When an accurate scan protocol with appropriate training encompassing an average of 30 minutes per novice user was applied, accurate clinical use of the system was insured. The next study uses this technique to estimate the distance to the 125I-seed in resected breast cancer specimens and thereby predict the status of the resection margins. This proved useful with accuracy acceptable for in vivo use. Accordingly, freehand-SPECT was used to guide 125I-targeted 99mTc-albumin nanocolloid administrations used for SN procedures. This led to the conclusion that simultaneous use of (125)I-seeds and (99m)Tc-nanocolloid is possible under well-standardised conditions: Non-palpable breast lesions can be safely excised using the (125)I-seed in the same session with a SN procedure.
On the basis of a meta-analysis the RSL method was reviewed including 16 articles and 3188 patients. The overall irradiality rate varied from 3% to 30.3% with a weighted average of 10.3%. The clinical adaptation shows growing confidence in RSL and further growth is expected.

Finally, we performed a study where the additional value of freehand-SPECT is evaluated in an intraoperative setting for RSL procedures. RSL procedures using multiple markers can be of specific difficulty while using a gamma probe only in terms of separating the markers transcutaneously because of relative positioning. This study demonstrated that for RSL procedures with multiple 125I-seeds radioguidance with freehand-SPECT proved useful. We are now examining the possibilities to implement this technique in standard practice for these procedures, and to use the RSL method in other cancer types such as thyroid carcinoma and head-neck cancer.

In addition to the above-mentioned study and in the context of the application of portable gamma cameras (PGC) in sentinel node (SN) procedures for breast cancer surgery, several aspects of PGC’s were explored. First, three PGCs were compared based on their technical performance. The test protocol was designed based on NEMA tests for gamma cameras with a clinical application in mind. Second, a PGC was clinically evaluated in patients scheduled for breast conserving surgery and a SN procedure who had also an I-125 seed in situ. The goal of this study was to find whether the performance of this PGC is equally or better compared to imaging using the conventional gamma camera in terms of finding SNs. Also the additional value of dual-isotope imaging (I-125 of the seed and Tc-99m of the SN tracer) during surgery was evaluated. Patient inclusion will continue in 2016. At last, an activity-based costing analysis on both imaging system was accomplished that will be published shortly.

**PET guided breast cancer biopsy**

A novel device for high-precision 18F-FDG-guided breast cancer biopsy was developed by a consortium of investigators, participating in the European Project MAMMOCARE, and placed at the Netherlands Cancer Institute. Breast cancer (BC) biopsy is used for assessment of risk estimation, molecular subtypes, and gene expression to tailor chemotherapy.

The first clinical project assessed the accuracy of a new Positron Emission Tomography (PET)-guided breast biopsy system, which enables tumour sampling from areas with high 18F-FDG uptake. The prototype system consists of two stacked rings each containing 12 plane detectors, forming a dodecagon with an aperture of 186mm, to acquire a truly 3D PET breast image reconstructed with a 1mm³ voxel size. A vacuum-assisted biopsy needle is used on a robot-controlled arm. To test the accuracy of needle placement, the needle tip was labelled with 18F-FDG and positioned at 78 target coordinates within the PET detector field of view. At each position a PET acquisition was obtained from which the needle positioning accuracy was calculated. Five BC patients were scanned to evaluate intratumoural 18F-FDG uptake and 3D automated tumour localization. The needle positioning tests revealed that the system had an average accuracy of 0.5mm (range 0-1mm), 0.6mm (range 0-2mm), and 0.4mm (range 0-2mm) for the x-, y-, and z-axis, respectively. Dedicated PET images of all patients showed accurate 3D computer generated localization and


Horlings HM, Flanagan AM, Huntsman DG. Categorization of cancer through genomic complexity could guide research and management strategies. J Pathol. 2015;236:397-402


Sentence node detection in clinical practice

Nynke van den Berg, Hervé Simon, Gijs KleinJan, Thijs Engelen, Anton Bunschoten, Mick Welling, Bernard Tijnik, Simon Horenblas, Jaques Chambron, Fij van Leeuwen

The opto-nuclear probe for combined radio- and fluorescence SN detection was further evaluated in 41 (ex vivo) and nine (in vivo) patients scheduled for SN biopsy following administration of ICG-99mTc-nanocollod. During the operation, all nodes were evaluated with the opto-nuclear probe using different sensitivity settings and count rates were noted for both radioactivity and fluorescence. Ex vivo, the gamma tracing function of the opto-nuclear probe correctly identified the SN in all 150 evaluated (non)SN samples. Ex vivo fluorescence tracing in the low-sensitivity mode correctly identified 71.7 % of the samples. This in-creased to 98.9 % when fluorescence tracing was performed in the high-sensitivity mode. In vivo fluorescence tracing (high-sensitivity mode) accurately identified the SNs in all nine patients (20 SNs evaluated; 100 %).

Cenk Acar, Gijs KleinJan, Nynke van den Berg, Esther Wit, Fij van Leeuwen, Henk van der Poel

The most important feature of sentinel node (SN) biopsy for prostate cancer procedure is that staging can be improved. SNs might be found outside the extended pelvic lymph node dissection (ePLND) template that renders SNs IS additive of ePLND. At the same time, staging within the template can be further refined. We reviewed the literature regarding the SN biopsy procedure for prostate cancer. PubMed and Embase were searched for all English-language publications from January 1999 to September 2014 by using the keywords as “prostate cancer” and “sentinel lymph node” plus “biopsy” or “dissection” and/or “procedure.” This review discusses step-by-step SN biopsy for prostate cancer. Topics of discussion are 1) Preoperative SN mapping (tracers and imaging); 2) Intraoperative SN identification (surgical procedure and outcome); and 3) Novelities to improve SN identification (pre- and intraoperative approaches). Conventional SN mapping is performed following the injection of a 99mTc-based tracer and subsequent preoperative imaging, e.g. lymphoscintigraphy and SPECT/CT. This approach allowed the detection of SNs outside the eLND template in 3.6-36% of men with intermediate and high-risk prostate cancer. Hereby an overall false-negative rate of SN was reported between heterogeneous intratumoural 18F-FDG uptake, suitable for PET-guided biopsy. Accuracy testing demonstrated a high-precision of this PET-guided breast biopsy system, which enables its clinical feasibility evaluation in BC patients scheduled for chemotherapy.

The next objective will be to assess the feasibility and safety of the PET guided biopsy device to get representative histologic biopsies from the breast tumour in breast cancer patients (stage II/III) who are referred to our institute and scheduled for neo-adjuvant chemotherapy treatment. Further objectives are: 1) the comparison in gene-expression and molecular subtending between the breast biopsies taken from the tumour region with the highest 18F-FDG uptake and the tumour border, 2) obtaining information about the duration of the biopsy procedure, and 3) measuring the radiation exposure for the physicians during the biopsy procedure.
PET/CT in BRAFV600 mutated melanoma: response monitoring and resistance prediction of combined BRAF/MEK-inhibitors (REPOSIT-study)

In patients with unresectable stage III or metastatic melanoma harbouring a BRAF-mutation, combined treatment with BRAF and MEK inhibition has shown an improved progression free survival and overall survival compared to monotherapy with BRAF inhibition alone. Recent results of the coBRIM-study in which patients with unresectable stage IIIC/IV are treated with BRAF-inhibitor vemurafenib combined with MEK-inhibitor cobimetinib have shown a progression free survival of 12.9 months compared to 7.2 months when treated with vemurafenib alone. However, despite these results resistance to therapy arises in most patients. At this point a predictive marker for response assessment or resistance prediction is lacking. By performing PET shortly after treatment initiation, metabolic alterations in metastases can be visualized and quantified. With this technique, responders might be distinguished from non-responders at an early phase and these metabolic alterations might predict the development of resistance to vemurafenib/ cobimetinib treatment.

The department of nuclear medicine initiated in close collaboration with the melanoma research group the REPOSIT-study. In this phase II, open-label, multicentre study ninety patients with unresectable stage IIIC or stage IV melanoma patients harbouring a BRAFV600E or BRAFV600K mutation will be included and will be treated with vemurafenib plus cobimetinib treatment.

D-24.4%. To further refine the intraoperative sampling procedure, novel imaging methods such as fluorescence imaging have been introduced. Prospective randomized comparison studies are needed to confirm the added benefit of sentinel template directed nodal dissection. A proper and obtainable end point of such a study could be the number of removed positive nodes performing nodal dissection with or without sentinel template directed dissection. Similarly the clinical impact of novel imaging technologies needs further investigation.

Pablo Borelli, Maarten Donswijk, Renato Valdés Olmos, Marcel Stokkel

Compared to primary breast cancer, the lymphatic drainage in patients suffering from breast cancer recurrence is often altered either because of prior surgery and/or radiotherapy. This can strongly influence sentinel node (SN) detection and surgical approach. The aim of the study was to identify if SPECT/CT could improve detection rates and provide a better surgical approach than planar lymphoscintigraphy in this subgroup of breast cancer patients. From December 2006 to date 131 patients with breast cancer relapse were included. All patients were candidates for sentinel node surgery and potential curative treatment. Preliminary results show that SPECT/CT improved SN detection rates in 9%. More importantly, in patients with SN visualization on planar scintigraphy, a SPECT/CT versus planar lymphoscintigraphy mismatch was found in 63% of the cases. This led to a surgical adjustment of the SN procedure in 31% patients. SPECT/CT appears to be mandatory in patients with breast cancer recurrence scheduled for sentinel node biopsy. Based on these results, new studies are currently developed related to injection techniques and scan protocols.


Pouw B, Van der Ploeg IM, Muller SH, Valdés Olmos RA, Janssen-Pinkelse LK, Oldenburg HS, Vrancken Peeters MT. Simultaneous use of an (125)I-seed to guide tumour excision and (99m) Tc-nanocollloid for sentinel node biopsy in non-palpable breast-conserving surgery. Eur J Surg Oncol. 2015;41:71-8


For primary tumour visualisation and response monitoring magnetic resonance imaging (MRI) is the golden standard, but PET/CT has also been described to be useful, although it is still not proven to what extent. We performed a study in 188 female patients with primary breast cancer in which both techniques were tested. It was found that in ER-positive tumours PET/CT images in combination with MRI images resulted in the most optimal response monitoring (AUC: 0.818; sensitivity 55.8%). For HER2-positive MRI had the highest sensitivity (AUC: 0.735; sensitivity 36.2%), outperforming the PET/CT (AUC: 0.543; p=0.04). In triple negative primary tumours MRI (AUC: 0.855; sensitivity 45.4%) and PET/CT (AUC: 0.844) provided comparable results. (Monitoring Tumour Response to Neoadjuvant Chemotherapy using MRI and 18F-FDG PET/CT in Breast Cancer Subtypes. AMTh Schmitz, SC Teixeira, CE Loo et al.)
**Immuno-PET**

An ongoing study that utilizes Immuno-PET is the M13CEA, in which the pharmacokinetics and therapeutic activity of RO6895882, a variant of interleukin-2 targeting CEA, was evaluated. In a subpopulation, the radioactive 89zr-IL2CEA was co-administered with the investigational drug RO6895882. In close collaboration with the department of pharmacology and VUMC this study aims to assess the in vivo biodistribution and organ pharmacokinetics at several time points post-infusion. With this imaging approach, potential differences in accumulation between CEA-positive and CEA-negative tumors can be visualized. Studies like the M13CEA enable the department of pharmacology to study this distribution in vivo at different dose regimes in greater detail. Additionally, the relationship between the tumor accumulation and an observed pharmacodynamic effect can be established. Hence, immuno-imaging will play an important role in the future of drug development. Also, this treatment strategy in which a therapeutic option is directly linked to a diagnostic test (also known as theragnostics) is of great value for proper patient selection. Currently, 18 patients were already included of which interim analyses are expected in 2016.

**Radioembolization**

Selective administration of 90Y-loaded micro-spheres into the hepatic artery (a technique known as radioembolization) enables treatment of liver tumors while limiting the dose to the normal liver tissue. This novel therapeutic option in hepatocellular carcinoma and liver metastases has been performed at the NKI-AVL for over two years. The treatment requires a meticulous work-up and close collaboration between the departments of Nuclear Medicine and Radiology. In 2015, the RELAPSE study (M13REL) has been initiated by these departments, in collaboration with the UMC. The objective of this study is to assess the efficacy of radiofrequency ablation (RFA) in combination with Yttrium-90 (90Y) radioembolization in patients with irresectable colorectal liver metastases. Conventionally, stereotactic radiotherapy, RFA or surgery were the treatment options in this setting, however, local liver recurrence is considered a frequent phenomenon. It is hypothesized that combining RFA with radioembolization will reduce the chance of liver recurrence, and increase survival. At present 8 patients have already been included at the NKI-AVL and the final results will be expected in 2016.

**Ga68-Dotatate PET/CT**

In 2015, approximately 18 patients were included in the “Study on timing Lanreotide in patients with metastatic or unresectable neuroendocrine tumours undergoing Ga68-DOTATATE PET/CT-scanning” (N14SRO). This study aims to influence the analysis of Lanreotide on the uptake of Ga68-DOTATATE on PET/CT in which patients with a neuroendocrine tumor undergo a diagnostic study prior to and shortly after the administration of Lanreotide. The end of the study is expected June 2016.

A second study with this tracer was developed in 2015 in collaboration with the Academic Medical Center in Amsterdam. The objective of this randomized placebo-controlled study (N15NFM) is to investigate the efficacy of Lanreotide autosolution during 18 months, as compared to placebo, to reduce and/or stabilize adenoma size in patients with NFMA and positive pituitary somatostatin receptor imaging using Provenzano E, Bossuyt V, Viale G, Cameron D, Badve S, Denkert C, MacGroggan G, Pearlstone-Llorca F, Boughey J, Curigliano G, Dixon JM, Esserman L, Fastner G, Kuehn T, Paintering F, von Minckwitz G, White J, Yang W, Symmans WF. Residual Disease Characterization Working Group of the Breast International Group-North American Breast Cancer Group Collaboration. Standardization of pathologic evaluation and reporting of postoperative specimens in clinical trials of breast cancer: recommendations from an international working group. Med Pathol. 2015;28:1185-201

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The DNA-diagnostic laboratory

The DNA-diagnostic laboratory has celebrated its 20th anniversary this year and screened over 5000 families for germline mutations in the BRCA1/2 genes since the start in 1995. These families have received genetic counseling at our Family Cancer Clinic. As from September 2014, the lab is routinely testing for large deletions and duplications in the BRCA2 gene although these mutations are very rare in contrast to BRCA1 and for testing of the CHEK2 1100delC mutation. We do not detect any BRCA2 rearrangement yet, but the 1100delC mutation in CHEK2 seems to be as frequent as BRCA 1 and 2 mutations together. The CHEK2 mutation is considered to be a moderate risk variant and is relatively frequent in the Netherlands.

The implementation of NGS s for BRCA1/2 in routine diagnostics is scheduled for February 2016. The delay is due to a ≥17 % increase in requests for routine testing. We also started to introduce a PCR based BRCA1/2 NGS test for FFPE tumor samples, especially for ovarian cancers. When ovarian cancers harbor either a germline or somatic mutation in these two genes patients can be treated by PARP inhibitors. Several clinical trials require rapid testing of tumor DNA for a BRCA1 specific or a BRCA2 specific profile. In this respect we can now offer a complete test panel for BRCA1: germline and somatic BRCA1, 2 testing, BRCA1 promoter methylation and CNV seq (also introduced in February 2016).
diagnostics this year) to assess the genomic tumor profile for BRCAAness features. The BRCAAness testing is performed in close collaboration with Petra Nederlof, head Molecular Diagnostics. For families with Lynch Syndrome, we perform Sanger sequencing for analysis of mismatch repair genes MLH1, MSH2 and MSH6. The microsatellite instability test contains a multiplex of 5 mononucleotide markers. Immunohistochemistry for the mismatch repair genes is carried out in collaboration with the pathologist Snaebjornsson. About 50% of the microsatellite instable(MSI)-high tumors with absent staining of MLH1 have a methylated MLH1 promoter. We now also test MSI high tumors detected by the Molecular Diagnostic team. In case no methylation is detected, so no indication for a somatic cause of the MSI-high pattern, the patient is referred to the clinical genetics department as this may indicate a possible hereditary predisposition. This result has direct consequences for the patient and it family management.

Research projects

The Family Cancer Clinic contributes data to several multi-center national and international research projects, e.g. HEBON Resource (Hereditary Breast and Ovarian Cancer Research Group Netherlands see Division Psychosocial Research and Epidemiology), DNA-profiling by CNV seq or BRCAAness of breast and ovarian cancer patients (in close collaboration with Lips, Division of Molecular Pathology), and the BCAC and CIMBA consortiums which focus on the contribution of SNPs to cancer risk, (HEBON resource, Rookus, dept of Psychosocial Research and Epidemiology (PSOE)). In this respect we have started a project using We also contribute to two EU HORIZON 2020 studies, BRIDGES and BCAST, both focus on the further molecular analysis of non BRCA1/2 breast cancer prone families and tumors resp. Furthermore, we participate in studies which assess the biological significance of so called unclassified variants (DNA changes of which it is uncertain whether they be pathogenic mutations or polymorphisms) in collaboration with the dept Biological Stress Response (te Riele on MMR UVs), in national and international collaborations with other DNA-diagnostic and research labs, e.g. ENIGMA for BRA1/2 UVs (Hogervorst), psychosocial studies, in collaboration with the PSOE (Bleiker) and clinical and genetic research in families with gastrointestinal cancer, including stomach cancer and pancreatic cancer (Cats, Division of Medical Oncology). Furthermore, Rosenberg is involved in the molecular characterization of colon tumors that were found in Hodgkin patients (collaboration with Snaebjornsson (Pathology) and Leerdam (Division of Medical Oncology)

Further collaborations are in a study on premature aging in BRCA1/2 carriers (BRAVA study, in collaboration with prof. dr. Asuens, UMC Utrecht) and on the clinical significance of variants within the BRCA1 and BRCA2 genes (collaboration with prof. dr. Van Asperen, LUMC).

New initiatives are a project - funded by KWF in 2014 - on informing family members in hereditary tumour syndromes. In close cooperation between the PSOE (Bleiker), Family Cancer Clinic (Menko) and the national organizations Erfocentrum and Levenmetkanker new methods for informing family members will be developed aimed at improved communication of cancer risk and better use of preventive measures. New web site information will be available early in 2016. For colorectal cancer families with Lynch-like syndrome a project has been


DEPARTMENT OF PATHOLOGY

Pathology is all about diagnosing the nature of disease processes, to guide clinical decision-making and optimize personalized and precision treatment of cancer patients. The department of pathology of NKI has the mission to provide along these lines cutting edge diagnostics for current patients visiting our institute, while at the same time focusing research efforts aimed at developing diagnostics of the future, which we foresee to be very much biomarker based. Here our challenge is to generate as much information as possible from tissue, cell and DNA samples, which is relevant to patients. Important questions to be answered relate to finding, validating, and implementing prognostic and predictive biomarkers, combined with tumor classification issues.

Under its new head Gerrit Meijer, the department will sustain and extend its high level of diagnostic service and is boosting the role of pathology as a key player in the field of translational medicine and research. Important key assets to this are the Core Facility Molecular Pathology that is key to tissue biobanking as well as support of clinical and translational studies. Most staff members are actively involved in multidisciplinary research activities. The medium and long-term team efforts are briefly described below. The translational research by the Translational Gastrointestinal Oncology group (led by Gerrit Meijer as principal investigator) is carried out in the Division of Diagnostic Oncology and the research by Jelle Wesseling as principal investigator is carried out in the Division of Molecular Pathology. The progress of these research lines can be found in the first part of this report.

set up for the detection of causative variants in DNA mismatch repair genes (e.g. Rieke, van Leerdam, Menko) and this subject is explored in close cooperation with the ErasmusMC group in Rotterdam (Dirjens). Hereditary renal cell cancer is the common denominator for a dozen different syndromes. For one of these syndromes, hereditary leiomyomatosis and renal cell cancer (HLRCC), a nation-wide second evaluation is set up. At present, more than 40 families have been identified at the DNA level (Mensenkamp, UMCN).

The possible hereditary background of a large group of patients with multiple primary tumours is evaluated in cooperation with Lok (NKI) and Maher (Cambridge, UK).

TP53-mutation carriers from Li-Fraumeni syndrome families are screened by total body MRI in the NKI. Data will be collected on the MRI-results and on the psychosocial impact of this screening tool (Ruijs, Bleiker, Sonke (Division of Medical Oncology) and Loo (Division of Radiology)). In 2015 a project is started by Van der Kolk, Bleiker and Lok (Division of Gynaecology, funded by Astra Zeneca) investigating the effect of online information prior to the genetic counseling (Genova Project). The goal of this study is to increase the efficiency of the counseling (measuring the duration of the face to face contact) while maintaining the degree of satisfaction and knowledge and without increasing perceived stress.

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**BREAST PATHOLOGY**

**Discovery, evaluation, and validation of prognostic and predictive factors to tailor diagnosis and treatment of breast cancer patients**

Sabine Linn, Claudette Loo, Emiel Rutgers, Wilbert Zwart, Mila Donker, Bas Koolen, Joyce Sanders, Petra Nederlof, Jelle Wesseling

Breast cancer is a heterogeneous disease requiring an individualized approach for optimal diagnosis and treatment. Therefore, we collaborate with a variety of research groups, mainly within the institute, but also worldwide, to ensure that our concerted action ultimately will benefit breast cancer patient survival. As such, we team up on improvement of existing biomarker assessment (e.g. HER2 testing), as well as development of new biomarker tests to determine for example the tamoxifen sensitivity in breast cancer positive for the estrogen receptor.

**Subtyping of second tumors after breast cancer**

Jelle Wesseling, Loes van Velthuysen, Sabine Linn, Neeltje Steeghs, Petra Nederlof, Marjanka Schmidt

With the increased incidence of breast cancer and the improved survival, studies on breast cancer outcome have become exceedingly important. Survivors of breast cancer are at an increased risk for developing second cancers, but the risk of these cancers may vary based on the type of the first breast cancer, age at first cancer diagnosis, and therapy for the first breast cancer. It is still largely unknown which are the predominant morphological and molecular subtypes of these second primary cancers that occur after breast cancer, and whether the development of these subtypes is affected by patient characteristics and treatment of the primary breast cancer. This is an important question because of the worse prognosis for certain subtypes. The aim of this pilot study is to characterize second primary breast and gastro-intestinal tumors that developed after a primary breast cancer diagnosis by immunohistochemical markers and Comparative Genomic Hybridization. Protein expression and genomic profiles of the primary breast tumors will be compared with those of the second primary breast and gastrointestinal tumors. Information from the clinical genetic center and molecular pathology about known germline or tumor mutations will be taken into account. Patient characteristics, clinic-pathological factors and treatment will be investigated to explore the driving factors for the similarities or differences of these first and second tumors.

**Characteristics of hereditary non-BRCA-mutated breast cancer**

Esther Lips, Rashmie Debipersad, Frans Hogervorst, Petra Nederlof

In our research line we try to characterize hereditary breast cancers. Since 2005 we are performing genomic profiling of those tumors, resulting now in a database with over 1000 of tumors profiled. Using these data we are currently working on the following projects:

1.) From over 200 patients with a BRCA variant of unknown significance we performed genomic profiling of the tumor material. We determined if the genomic profile of these tumors resembled the genomic profile of BRCA1 or BRCA2 mutated tumors by applying our BRCA-like genomic classifiers. We are...
now assessing relationships between specific BRCA-unclassified variants, bioinformatics prediction algorithms and genomic profiles.

2.) We characterized a group of estrogen receptor (ER) positive BRCA1 mutation carriers, and found that on a genomic level these tumors are highly similar to ER-positive BRCA2 mutated tumors and should not be considered sporadic cases. This suggests that the new classes of BRCA1 deficiency-targeting drugs can also be applied in ER-positive BRCA1 mutated cancers.

3.) Germline mutation testing for CHEK2 has entered the clinic last year. We are currently analyzing genomic profiles of CHEK2 mutated tumors. This analysis could identify specific genomic aberrations associated with a higher breast cancer risk.

In addition to our research in hereditary breast cancer, we are continuously updating and refining our BRCA-like classification tools, which are both used for treatment prediction benefit as well as better characterizing tumors for clinical and research purposes.

**UROPATHOLOGY**

**Tumor characteristics in radical prostatectomy specimens to optimize focal radiotherapy**

Ghazale Ghobadi, Jeroen de Jong, Uulke van der Heijde

Dose escalation and focal radiotherapy of primary prostate cancer can reduce toxicity. To optimize recognition of tumors on MR imaging – which is needed for focal therapy –, digital delineation of primary tumors and assigning Gleason scores to separate tumor foci on histopathological slides will be used as a reference.

**Correlation between the location of tracer injection in the prostate and the observed lymphatic drainage pattern**

Nynke van den Berg, Simon Horenblas, Jeroen de Jong, Henk van de Poel

For prostate SN procedure a hybrid tracer (ICG-99mTc-nanocolloid) that is both radioactive and fluorescent, will be injected in the peripheral zone where most tumors occur. Location of the tracer injection will be correlated to tumor location examined by pathological evaluation. The goal of this study is to determine where the tracer was injected in relation to the location of the tumor and how this affected lymphatic drainage; were more often “true” tumor draining (metastasis containing) SNs identified when the tracer deposits were placed near/in the tumor compared to the intra-prostatic injection.

**Integrative Androgen Receptor genomics as a read-out for recurrence risk and treatment**

Suzan Stelloo, Henk van de Poel, Jeroen de Jong, Wilbert Zwart

Tumor material from prostate cancer patients will be tested for Androgen Receptor/chromatin binding profiles using ChIP-seq. In addition, other transcription factors and epigenetic histone modifications will be mapped, next to global gene expression profiles. These data will be integrated and correlated with response to treatment, to identify ADT resistance biomarkers and predictive markers for tumor recurrence.

We aim to generate the most comprehensive overview of
primary prostate cancer genomics. This will include Androgen Receptor/chromatin binding patterns, histone modifications and gene expression data derived from prostate tumor samples and prostate cancer cell lines. This may enable us to identify distinct predictive markers for prostate cancer recurrence and treatment resistance.

**Predicting Response to Enzalutamide as a Second Line Treatment for Metastasized Castration Resistant Prostate Cancer Patients: a biomarker design study (PRESTO-study)**

Suzan Stelloo, Henk van der Poel, Jeroen de Jong, Walter Prevo, Michael Hauptmann, Ekatarina Nevedomskaya, Andre Bergman, Wilbert Zwart

Enzalutamide is a new anti-hormonal drug, which showed excellent activity as a second line treatment for patients progressing after docetaxel treatment. However, 46% of patients did not reach a >50% response on Enzalutamide and eventually all patients progressed. Biomarkers that can identify patients who will have excellent, and long lasting responses are highly needed. In this study, we will explore the exact actions of Enzalutamide on Androgen Receptor (AR) regulation, design predictive AR/DNA binding signatures and examine the role of AR mutations and alternative expression growth in prostate cancer growth. Therefore, biopsies from bone metastases, lymph nodes or visceral metastases will be taken prior to start of Enzalutamide treatment. Possible biomarkers will be further validated in Formalin Fixed Paraffin Embedded (FFPE) primary tumor material.

**Dynamics of Androgen Receptor genomics and transcriptomics after neoadjuvant androgen ablation (DARANA)**

Wilbert Zwart, Suzan Stelloo, Jeroen de Jong, Andre Bergman, Henk van der Poel

To identify AR chromatin binding patterns and downstream responsive genes that hallmark distinct sensitivity to enzalutamide treatment in prostate cancer, as well as the dynamics thereof before and after treatment. The role of third generation antiandrogens in the neoadjuvant setting has not been studied. To analyze the effects of the novel antiandrogen enzalutamide on AR-responsive gene expression, tumor samples will be assessed before (biopsy) and after (resection) 6 weeks of neoadjuvant androgen ablation, and PSA and Ki-67 will be determined by immunohistochemistry. Patients will be stratified on alterations in PSA and/or Ki-67 to identify alterations in AR genomic profile and downstream regulated genes that correlate with response to androgen ablation.

**THORACIC PATHOLOGY**

**Lungscape-project designed by European Thoracic Oncology Platform (ETOP)**

Kim Monkhorst, Petra Nederlof, Paul Baas

This project aims for molecular characterization of a large European cohort (2000-2400) of stage I-IIIB resected NSCLC. NKI-AVL archival FFPE material of resection specimens of non-small cell lung carcinomas of a group of approximately 70 patients is analyzed for a panel of molecular characteristics. To start with, EML4-ALK translocation was analyzed by IHC and positive cases were analyzed by FISH. Currently, c-MET, PTEN and PDL1 analyses were implemented as a next step in this project.

**Deletion of p16 as a biomarker in cytological specimen to identify mesothelioma**

Petra Nederlof, Josine Quispel

We will investigate if 9p21deletion detection by FISH is a valuable biomarker for mesothelioma. Approximately 70-85% of the malignant mesothelioma (epithelial type) show deletion of CDKN2A. Using this marker the diagnosis mesothelioma in cytological specimen could possibly be improved. This study is ongoing.

**Identifying novel targets in solid malignancies: FGFR1-3 and PD-L1**

Willemijn Theelen, Jeroen de Jong, Stefan Willems, Hugo Horlings, Ed Schuuring, Hans Blauweeers, Annegien Broeks, Michel van de Heuvel

There is a strong incentive for improvement of systemic treatment of many solid malignancies. Personalized treatment, using drugs that target activating mutations have shown to improve therapy. Also novel immunotherapeutics targeting immune checkpoints have shown promising results. Often extensive molecular profiling of the tumor is required to identify the right target that predicts.

FGFR3 was recently identified in our first NSCLC next generation sequencing screens to be a potential novel target. In the initial cohort two samples showed a FGFR3-TACC3 translocation. In one of these samples the FGFR3-TACC3 fusion protein was already confirmed by PCR. Both samples showed to have a high FGFR3 expression as tested by immunohistochemistry.

TMA’s of head and neck squamous cell carcinoma, urothelial carcinoma and NSCLC samples will be used to select positive cases which will be further investigated.

PD-L1 was recently identified as a target for immunotherapy. Currently clinical trials are being performed in advanced stage NSCLC to assess the efficacy of at least six clinically active anti-PD1 and PD-L1 monoclonal antibodies.

For PD-L1 a series of early stage NSCLC will be screened (>300patients), but also TMA’s of other thoracic malignancies, H&N, and bladder carcinoma will be screened. Data will be used to design a neo-adjuvant trial in NSCLC. If data on other solid malignancies are promising a proof of principal trial in other solid malignancies can be designed.

**GASTROINTESTINAL PATHOLOGY**

**Radiotherapy- and chemotherapy-associated colorectal cancer**

Lisanne Rigter, Petur Snaebjornsson, Winald Dinjens, Efraim Rosenberg, Annegien Broeks, Berthe Aleman, Jelle ten Hoeve, Inez Goossens-Beumer, Mireille Snel, Gerrit Meijer, Hein te Riele, Floor van Leeuwen, Monique van Leerdam

Hodgkin lymphoma (HL) survivors who have been treated with infra-diaphragmatic radiotherapy and/or high dose procarbazine have an approximately 5-fold relative risk to develop colorectal cancer (CRC) compared with the general population. The mechanism behind the progression of normal
Frequent sequence, possibly in an established collaboration with the cohort will be used for matching and whether we will perform the treatment-induced gastric carcinomas will determine which available for matching and comparison. The characteristics of stable. [TCGA, Nature 2014].

Characterized and annotated as MSI, CIN, EBV or genetically CIN (shallow sequencing/MLPA). Gastric carcinomas will be assessed for MMR protein expression. EBER in situ hybridization will be used to detect Epstein-Barr Virus (EBV). Molecular analyses will address microsatellite stability (MSI) testing, and evaluation of immunohistochemical stains (CD3, CD8, PD1, PD-L1, CD45). The outcomes of this study will determine whether immunotherapeutically targeting these disease entities is justified.

Studies on the outcomes after oesophageal cancer treatment

Rosa van der Kaaij, Francine Voncken, Petur Snaebjornsson, Berthe Aleman, Johanna van Sandick

Results of gastric cancer resection differ per histological subtype (e.g. Lauren classification: intestinal versus diffuse type adenocarcinoma). In oesophageal adenocarcinomas the same histological subtypes exist, but there are no data on the relation between these subtypes and their outcome after surgery. The aim of this study is to study the long-term outcomes per histological subtype of patients operated for oesophageal adenocarcinoma in our institute between May 1998 and December 2014 (n=115). All oesophageal adenocarcinomas will be retrospectively classified per histological subtype. Oesophageal cancer patients show a wide diversity in (I) response to neoadjuvant chemoradiotherapy and (II) long-term outcome. The aim of this study is to develop a solid nomogram that can help in response prediction and potentially in patient tailored therapy. Known response predictors are pathological criteria as Mandard score, differentiation grade, lymphvascular invasion, histological subtype. These pathological data are retrospectively studied and revised on resection specimens and biopsies of oesophageal cancer patients treated in our institute between May 1998 and December 2014 (n=254).

Gynaecological Pathology

Role of the peritoneal elastic lamina in tumor invasion of epithelial ovarian cancer

Juliette van Baal, Koen Van de Vijver, Mijke Bol, Willemien van Driel, Marrije Buist, Gemma Kenter, Christianne Lok

The aim of this study is to determine anatomical components of the peritoneum that possibly contribute to the inhibition of tumor cell invasion in epithelial ovarian cancer. In this study we attempt to demonstrate the integrity of the peritoneal elastic lamina (PEL) in relation to tumor invasion and growth pattern. In 11 patients we performed immunohistochemical staining with HE and EVG staining. All samples demonstrated a well distinguishable PEL. In some patients the tumor depositions did not grow through the continuous fibre network of the PEL, but demonstrated a more pushing growth pattern. In other patients, tumor depositions grew through a destructed or fragmented PEL, demonstrating an infiltrative growth pattern. These results suggest a role of the intact PEL in the function of

Radiotherapy- and chemotherapy-associated gastroesophageal cancer

Lisanne Rigter, Petur Snaebjornsson, Nicole van Grieken, Annemieke Cats, Efraim Rosenberg, Annegien Broeks, Berthe Aleman, Gerrit Meijer, Hein te Riele, Floor van Leeuwen, Monique van Leerdam

Hodgkin lymphoma survivors and testicular cancer survivors have an increased risk of developing gastric cancer. This increased risk shows a dose-response relationship with irradiation and alkylating agents. The etiology of treatment-associated gastric cancer remains unknown. Hypermethylation or defects in DNA alkylation repair mechanisms (MGMT, mismatch repair (MMR) system) might be present in alkylating-agent-associated gastric cancer, whereas irradiation might induce chromosomally stable (CIN) gastric cancer. Therefore, we will evaluate histopathological and molecular characteristics of gastroesophageal cancer diagnosed in Hodgkin lymphoma survivors and gastric cancers in testicular cancer survivors. FFPE material of gastric carcinoma biopsies and resections will be collected through the Nationwide Pathology Database (PALGA). We expect to collect 80% of requested material. Histopathological revision will be performed, and signs of radiation-induced changes will be evaluated (mucosal atrophy, fibrosis, vascular changes, inflammatory infiltrate). Helicobacter pylori will be assessed. A tissue microarray will be constructed and immunohistochemistry will be performed for MMR protein expression. EBER in situ hybridization will be used to detect Epstein-Barr Virus (EBV). Molecular analyses will address microsatellite stability (Pentaplex PCR), CpG island methylator phenotype (MLPA) and CIN (shallow sequencing/MLPA). Gastric carcinomas will be characterized and annotated as MSI, CIN, EBV or genetically stable. (TCGA, Nature 2014).

Three Dutch cohorts of sporadic gastric carcinomas are available for matching and comparison. The characteristics of the treatment-induced gastric carcinomas will determine which cohort will be used for matching and whether we will perform DNA sequencing, possibly in an established collaboration with the US National Cancer Institute.
the peritoneum as barrier against tumor depositions. We will perform a retrospective study with a larger study population to investigate the prognostic significance of the integrity of PEL and its correlation with tumor behavior and growth pattern.

**Genome sequencing of low-grade serous ovarian cancer and serous borderline tumor with and without micro-papillary structure**

Juliette van Baal, Koen Van de Vijver, Seth Coffelt, Willemien van Driel, Rene Bernards, Lorena Mittempergher, Gemma Kenter, Christianne Lok

Little is known about the mutational profile of the low-grade serous ovarian cancer, and about the mutational differences between serous borderline tumor with and without a micro-papillary structure. The aim of this study is to achieve more understanding of the stepwise mutational pathways of the development of a benign tumor into an invasive (low-grade) serous carcinoma. We will perform Sequenom analysis for determination of hotspot mutations, followed by deep sequencing of the kinome.

**Incidence of lymph node metastasis in mucinous ovarian cancer**

Juliette van Baal, Christianne Lok, Willemien van Driel, Gemma Kenter, Koen van de Vijver

There is an ongoing discussion concerning the complete staging procedure in mucinous ovarian carcinoma. Lymphadenectomy may be omitted from this procedure, especially in low grade (grade 1) mucinous ovarian carcinoma. Via the national pathology record system (PALGA), a search was performed to identify all patients with mucinous ovarian cancer, diagnosed from 2002-2014. Pathology records are intensively studied and lymph node metastases are recorded.

**Serum Human Epididymis Protein 4 and myometrial invasion in endometrial cancer patients**

Anna Stiekema, Tiny Korse, Koen van de Vijver, Annemarie Bruining, Willemien van Driel, Gemma Kenter, Christianne Lok

The primary treatment of endometrial cancer is surgery with adjuvant radiotherapy if risk factors for local recurrence or lymph node metastases are present. High grade endometrial cancer is more likely to invade deeply into the myometrium, increasing the possibility of lymph node metastases. If deep myometrial invasion can be predicted preoperatively, lymphadenectomy can be considered to avoid post-operative radiotherapy in the absence of metastases. MRI-scanning can be used to predict myometrial invasion. Also, serum HE4 is suggested to increase in the presence of deep myometrial invasion. In this endometrial cancer study, the correlation of serum HE4 and depth of myometrial invasion is evaluated and compared to MRI.

**Core Facility Molecular Pathology and Biobank**

Anniegien Broeks, Donne Majoro, Joyce Sanders, Ingrid Hofland, Dennis Peters, Sten Cornelissen, Linde Braaf, Chantal Curiali, Renate de Groot, Esther Holman, Jose Overwater

In recent years, the need for controlling the ‘secondary use’ of human biospecimens for research purposes at the NKI-AVL became more apparent. To ensure human material is used properly and efficiently, especially in the case of scarce, valuable samples, a facility for issue and use of NKI-AVL biobank materials was desired. Therefore, in 2010, the Core Facility Molecular Pathology & Biobanking (CFMPB) was founded. The CFMPB registers, coordinates, assists and facilitates research involving archived human/patient material (biospecimens), using the online Application & Request tool (ART). This concerns all research using (secondary use) biobank material both from the department of Pathology (the paraffin-block archive, frozen tissue bank, fresh tissue) and the department of Clinical Chemistry (the serum and blood biobank). The facility provides professional expertise, appropriate samples and tissue based experimentation in the context of optimally controlled medical-ethical issues.

The CFMPB has a fully equipped and dedicated histology/immunohistochemistry (IHC) lab. Nearly all IHC is performed using the BenchMark Ultra (Ventana, Roche) automated stainer, in close collaboration with the diagnostic pathology department. Additionally a Discovery Ultra (Ventana, Roche) automated stainer, adaptable to a broad array of tissue testing capabilities, is available. From developing novel assays on low-expressing biomarkers to enabling the highest level of experimental complexity. All routine IHC diagnostic protocols can also implemented at the CFMPB and can be requested for research studies. Ample experience is available for the development of new antibody staining protocols. Elaboration and optimizing of staining protocols is performed in close collaboration with the requesting scientist and the involved pathologist.

In 2015 70 new studies were registered in ART, of ~ 4500 T-numbers archived HEs and tissue blocks have been requested and using the Ventana stainers 13000 IHCs slides have been stained. 180 different antibody protocols are up and running in the facility, for details see the ART website: www.nki.nl/topmenu/molecular-pathology-biobanking-core-facility/

**Department of Radiology**

**Colorectal cancer Imaging**


After her start in fall 2015 as chair of the department of radiology, the Netherlands Cancer Institute and Professor of Radiology, University of Maastricht, the imaging research line of Regina Beets-Tan, was transferred from Maastricht to Amsterdam and will be continued in the Netherlands Cancer Institute.

The main areas of research interest are clinical and translational research in MRI and the use of functional MRI for innovative treatment in rectal cancer (organ preservation), the latter of which is in synergistic collaboration with the research group of colorectal surgeon Geerard Beets, MD, PhD, Professor of Surgical oncology who also moved from Maastricht to Amsterdam.
The work of this colorectal team consists of the following research lines:

**Functional MRI for Organ Preservation in Rectal cancer**

The clinical colorectal cancer research line is focused on MR imaging of rectal cancer. This multidisciplinary research aims to develop and validate new functional MR techniques to assess and predict response to treatment and select complete responders after preoperative chemoradiotherapy of rectal cancer. Accurate selection of complete responding patients may allow for organ preserving treatment or even deferral from surgery (‘watch and wait’ policy) with less morbidity and mortality than standard resection. Both research lines in functional MRI and Organ Saving Treatment of Rectal Cancer have a world-leading role. A national multicenter imaging trial validating diffusion MR imaging, funded by the Dutch Cancer Society, are in the final inclusion of 450 patients and results will be analyzed in the coming year. A multicenter observational ‘watch and wait’ trial based on selection with endoscopy and MRI is ongoing since 2014 and has been continued in NKI. The group recently obtained funding from the Dutch Cancer Society to commence a large cohort imaging and therapy study to implement organ preservation nationwide. Centers of expertise (coordinated by the NKI as the principal investigating centre) will include patients in a cooperative effort with the STAR-TREC trial. Apart from this national trial, an international prospective registry initiative has been started (www.iwwd.org) to register imaging and clinical data of ‘watch and wait’ patients worldwide. This project has recently been awarded with the Bas Mulder Award for Denise Hilling. The clinical imaging research in colorectal cancer is in close collaboration with surgical and radiotherapeutical imaging research teams in NKI-AVL and the imaging work will be expanded to oesophageal cancer within NKI-AVL. Worldwide collaboration exists with multidisciplinary colorectal research teams including that of the National Cancer Center in Tokyo, MSKCC in NYC, Fudan University Hospital in Shanghai, Karolinska University Hospital in Stockholm and Catholic University Hospitals in Rome and Leuven. In 2015 this research line has resulted in 25 peer-reviewed papers, has been awarded 5 times the 2015 BJU Society Prize from the European Society of Coloproctology. The project has also been awarded with the Dutch Cancer Society a clinical fellowship for Miranda Kusters. This multidisciplinary work has resulted in the past year with the completion of two PhD theses of Luc Heijnen and Milou Martens entitled: “Rectal cancer imaging: how about the nodes” and “Organ preservation in rectal cancer: can new MRI techniques improve patient selection?”. A third, fourth and fifth PhD theses will be completed in 2016/2017 and a sixth PhD work on this subject has just started in NKI.

**Image segmentation and multiparametric imaging**

Ongoing projects are image segmentation of imaging biomarkers from functional MRI. Histogram analysis of response assessment and prediction are the subject of research in this line. Changes to treatment that cannot be eyeballed are well perceived by extracting quantitative data of diffusion, perfusion and signal intensities from MRI. Another related imaging research is in close collaboration with Philips R & D Best and Aachen. The project consists of analyses of multiparametric MR (and CT) imaging data of the rectum and liver, including diffusion-weighted imaging, dynamic contrast-enhanced MRI, magnetization transfer imaging and texture analysis. This project is in colorectal, breast, prostate and brain tumors. The results of this retrospective project will be prospectively validated in a new patient cohort at the NKI.

**Radiomics**

Radiomics entails the extraction and usage of quantitative data from routine scans to better predict the heterogenic characteristics and prognosis of certain tumors. The aim of our project is to determine whether it is possible to predict the response of rectal tumours to neoadjuvant chemoradiotherapy (CRT), using the radiomics features extracted from the primary MRI imaging of the rectum. To achieve this, the tumors of a large number of patients are delineated and divided into two datasets. The first set will be used to test a large number of radiomics features. A resulting selection of the most promising features will be used to create a biomarker model, which will be validated using the second dataset. Using such a biomarker model in rectal tumors may allow a better selection of rectal patients with early stage tumors who are likely to respond to CRT with complete response. Using a similar approach, we will investigate whether it is possible to predict the likelihood of metachronous metastasis in the liver, by using a radiomics analysis of the liver in patients without livermetastasis at primary staging.

The imaging research in multiparametric imaging and radiomics is in close collaboration with NKI-AVL imaging research teams of the dept of radiation oncology.

**Whole body Imaging and image fusion**

This project is an institutional multidisciplinary collaborative project between the departments of Radiology and Nuclear Medicine and representatives of the departments of Radiation Oncology, Medical Oncology, Surgery, Gynaecology, Internal Medicine, Urology and Thoracic Surgery. It focusses on the combined, multiparametric imaging assessment of different tumour types (e.g. colorectal, melanoma, bladder, prostate, esophagus, breast, lung) in order to boost diagnostic performance for tumour staging, response evaluation/prediction and prognostication. Data from MRI, CT and PET will be incorporated and retrospectively combined (and fused) to be analyzed from a whole-body, multi-modality and multiparametric perspective.

**BREAST IMAGING**

Claudette Loo, Gonneke Winter-Warnars, Sjoerd Rodenhuis, Kenneth Gilhuijs, Thiemo Nijnatten, Marjolein Smidt, Lisa Klompenhouwer, Kenneth Pengel, Emile Rutgers

October 2015 Kenneth Pengel completed his work in two research projects with a thesis “ multimodality imaging of breast cancer for selection and monitoring of treatment”. This thesis investigated if, and how, the application of magnetic resonance imaging (MRI) and positron emission tomography in combination with computed tomography (PET/CT) contributes to improved personalized treatment strategies. The study group
in the first project was composed of patients who were eligible for breast conserving therapy. The results showed that MRI could accurately depict the disease extent and that MRI detected more disease than conventional imaging in 11% of the patients. Surgical precision was improved in patients with infiltrating ductal carcinoma. MRI was useful to identify breast cancer of limited extent. Furthermore Pengel et al presented guidelines to select women in whom a preoperative breast MRI can be avoided. A preoperative MRI scan appears to have little value if all of the following conditions are met: the patient is older than 60 years, there is no substantial discrepancy between the tumor diameter on mammography and ultrasound, there is no evidence of affected lymph node, the ACR-code (a measure for the percentage of glandular tissue on mammography) is 1 or 2 and the tumor is no invasive lobular carcinoma. In the second project (CTMM breast care) the use of PET/CT and MRI was investigated for treatment response evaluation in women who were treated with primary chemotherapy. PET/CT and MRI showed comparable value for tumor response monitoring but PET/CT was less accurate in HER2-positive tumors. Combined use of PET/CT and MRI showed complementary potential but this strongly depends on breast cancer subtypes as well.

Other on-going breast cancer research of the breast imaging group: The department participates in the CTMM breast care program (the Choice project). Furthermore collaboration with the department of nuclear medicine in studies for validation of a prototype biopsy system for PET positive breast tumors. Other collaborative studies are the FAMrisc study, The PAPBI study, The percuspect study (“smart needle”), The DENSE study together with the UMCU; the supine breast MRI (feasibility study) and the under-estimation study was started with close collaboration with the group of Wesseling and Gurdeep S. Mannu from the Research group of Cancer Surgery University of Oxford. In 2015 cooperation with the group of J. Wesseling and E. Rutgers in the LORD study, resulted in an international multi-center non-inferiority study for low grade DCIS, which will start in the first trimester of 2016. Collaboration is also with research teams from the University Medical Center Utrecht and Maastricht University Medical Center in breast imaging studies on axillary nodal staging which will result in 2 theses completed in 2016 (Claudette Loo) and 2017 (Thiemo Nijnatten).

**Prostate Imaging**

**Stijn Heijmink, Annemarie Fioole-Bruining, Petra de Koekkoek-Doll**

In 2015 Stijn Heijmink obtained his PhD for his work in prostate imaging. The prostate imaging group investigated the ability of MR imaging to determine the location of prostate cancer within the prostate gland. A prospective case-control study was designed in collaboration with the radiotherapy department. This project focusses on salvage brachytherapy for patients with MR guided biopsy proven recurrence after external radiotherapy. With the department of Urology the MR imaging features of the prostate were correlated with functional outcome after robot-assisted laparoscopic radical prostatectomy. A prospective project in this subject has been submitted for funding correlating MR imaging features with functional outcome.

**Figure 4:** Extracting radiomics data from images. (a) Tumours are different. Example computed tomography (CT) images of lung cancer patients. CT images with tumour contours left, three-dimensional visualizations right. Please note strong phenotypic differences that can be captured with routine CT imaging, such as intratumour heterogeneity and tumour shape. (b) Strategy for extracting radiomics data from images. (I) Experienced physicians contour the tumour areas on all CT slices. (II) Features are extracted from within the defined tumour contours on the CT images, quantifying tumour intensity, shape, texture and wavelet texture. (III) For the analysis the radiomics features are compared with clinical data and gene-expression data. (Aerts HJ et al. Nat Commun. 2014;5:4006)
The Division of Medical Oncology comprises a growing number of research activities with focus on translational research, early drug development and clinical research. In this report only research from the largest groups is presented. Much of the work is multidisciplinary and involves groups from different divisions or different institutions both in The Netherlands and abroad. Two of the major common themes between the different research groups within the Division are personalized medicine and immunotherapy. We expect that cancer treatment will be more and more individualized, so that patients can be offered the optimal treatment for their specific type of cancer, be it chemotherapy, targeted therapy or immunotherapy.

**Clinical Pharmacology of Anticancer Drugs**

Jan Schellens, Bastiaan Nuijen, Hilde Rosing, Henk Boot, Annemieke Cats, Serena Marchetti, Frans Opdam, Dieta Brandsma, Neeltje Steeghs, Sabine Linn, Baukellen van Triest, Alwin Huitema, Jos Beijnen, Joost van den Berg (AmBTU)

Research activities of the department of Clinical Pharmacology, the department of Pharmacy & Pharmacology and the division of Molecular Pathology (group Schellens) are closely integrated. There is close collaboration with the departments of gastro-enterology, chest oncology, neuro-oncology and radiotherapy. We continued clinical research fully compliant with ICH-GCP guidelines, previously certified by the Dutch Ministry of Health. The department of Pharmacy & Pharmacology successfully continued its official governmental GLP (in the field of analytical chemistry), GMP (formulation and manufacturing of investigational cytotoxic drugs) and GDP (ISO9002 for worldwide distribution of clinical supplies) licenses.

### Pharmaceutical formulation

Development of oral dosage forms of docetaxel and paclitaxel and/or clinical manufacturing of cyclohextrin, liposomal formulations and slow-release formulations of various (anticancer) compounds is ongoing. The Amsterdam Biotherapeutics Unit (AmBTU), the biotech facility located in the Pharmacy, is producing Tumor Infiltrating Lymphocytes (TIL) infusions for melanoma patients enrolled in the first multi-center phase III trial with TIL therapy in the world. AmBTU, in close collaboration with clinical immunotherapy, has manufactured two MART-1 T cell receptor modified T cell infusions in 2015. We have observed high on-target reactivity of these cells in melanoma patients, indicating that the developed production protocol results in highly reactive T cells. AmBTU is currently also producing DNA vaccines for HPV induced malignancies, in the context of the FP7 RAIDs program.
II Bioanalytical method development + implementation in PK studies

Our therapeutic drug monitoring (TDM) services, for the tyrosine kinase inhibitors to optimize the treatment of these drugs, has been implemented for imatinib, sunitinib (and its metabolite N-desethyl sunitinib), pazopanib, vemurafenib, erlotinib, gefitinib, dasatinib, nilotinib, lapatinib, and sorafenib. We provide results to the clinic with a turn-around time of 1 week. Additionally we support the TDM for optimizing the tamoxifen treatment of estrogen receptor positive breast cancer patients. In our laboratory dried blood spots DBS methods were developed and validated for the determination of tamoxifen and endoxifen, pazopanib and vemurafenib. The clinical validations showed that DBS and plasma concentrations were well correlated. The DBS concentrations were equal to the plasma concentrations after correction for the haematocrit and blood cell-to-plasma partitioning. We concluded that DBS sampling is feasible at home for treatment individualization.

The mass balance study with 14C-labelled omacetaxine started this year and 6 patients were included. The radioactivity measurements in whole blood, plasma, urine and faeces using Liquid Scintillation Counting showed that 85% of the administered radioactive dose was recovered and equally excreted in urine and faeces. In plasma, omacetaxine and its metabolite 4'-desmethylomoharringtonine were found. Omacetaxine, 4'-desmethylomoharringtonine and cephalotaxine were measured in urine. Furthermore a mass balance studies (also with 14C-labelled compounds) is ongoing with vosaroxin (a topoisomerase II inhibitor).

Three intracellular formed metabolites are held responsible for the anti-cancer effect of 5-FU: 5-fluorouridine 5'-triphosphate (FUTP), 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP), and 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP). We developed an assay to determine these metabolites in peripheral blood mononuclear cells (PBMCs). The assay was successfully applied to quantify 5-FU nucleotides in PBMC samples from patients treated with capecitabine and patients receiving 5-FU intravenously. FUTP amounts up to 3054 fmol/10^6 PBMCs, and FdUMP levels up to 169 fmol/10^6 PBMCs were measured. FdUTP concentrations were below the lower limit of quantification.

III-1 Novel approaches to improve oral bioavailability

The ‘boosting’ concept of oral docetaxel plus ritonavir, an efficacious inhibitor of gut wall and hepatic CYP3A4, has been further developed this year. In the two ongoing phase I studies with oral docetaxel (ModraDoc006) boosted by ritonavir (r), i.e. ModraDoc006/r the advised dose-level is 60/100 QD and 30 mg ModraDoc006 in the morning and 20 mg in the afternoon plus 100 mg ritonavir. In the ongoing phase II study with the novel oral paclitaxel tablet formulation ModraPac005 boosted by ritonavir (ModraPac005/r) daily low-dose or metronomic dosing the MTD is 30 mg in the morning and 20 mg in the afternoon each plus 100 mg ritonavir.

III-2 Pharmacology (PK/PD, ADME, mass balance) of novel anticancer drugs

Currently we perform fifty-six phase I/II, pharmacological and proof of concept studies, which number has slightly increased compared with last year. We recruited more than 300 new patients for these studies this year. Thirty-nine of these studies

### Publications


Blank CU, Erikk T. Therapeutic use of anti-CTLA-4 antibodies. Int Immunol. 2015;27:3-10


De Goeje PL, K. Bezemer K, Heurers ME, Dingemans AMC, Groen HJM, Smit EF, Hoogsteden HC, Hendriks RW, Aerts JGV, Hegmans JJ. Immunoglobulin-like transcript 3 is currently investigated. The phase I study with the bispecific antibody (MoAb) CEA-IL2v, CEA-TCB is currently investigated. The phase I study with the bispecific MoAb targeting CEA and the CD3 T cell receptor is ongoing. A new phase I study with the CEA-IL2v and anti-PDL1 MPDL3280A (atezolizumab) was initiated. 7

c. Phase Ib study with the PD1-antagonist pembrolizumab (MK3475)

High antitumor activity and long responders were observed in selected patients with head and neck cancer, pleural mesothelioma and ovarian cancer. Toxicity was as expected with anti-PD1 mAb therapy.

d. Treatment of BRAF mutant colorectal cancer by BRAF inhibitor plus anti-EGFR based combination therapies

The clinical trial developed by us to determine the safety, tolerability and efficacy of LGX181 (BRAF inhibitor) in combination with cetuximab (EGFR inhibitor), or in combination with both cetuximab and BYL719 (PI3K inhibitor, alpelisib) in patients with BRAF mutant CRC is ongoing. High activity and good safety were confirmed in a randomized phase II study. In the multicenter phase I and II studies with a total of 150 patients more than one third worldwide was recruited at the Netherlands Cancer Institute. We started a new phase I study in BRAFm CRC with accompanying mutations in the Wnt pathway with cetuximab, encorafenib and WNT74.

e. Treatment of KRAS mutant colorectal cancer (CRC), pancreatic and non-small cell lung cancer (NSCLC) by MEK inhibitor combined with pan-HER inhibitor

The study employing dacomitinib (panHERi) with MEKi PD-0325901 is ongoing. Diarrhea and skin toxicity were the most prominent side effects. A second study employs the combination of lapatinib and trametinib and a third study the combination of afatinib and selumetinib. Preliminary pharmacodynamic studies revealed almost complete pERK reduction at the highest dose of PD-0325901.

f. Other phase I studies

Execution of the phase II study of AZ1775 plus carboplatin in p53 mutant advanced platinum refractory or resistant ovarian
cancer progressed. The drug-safety study with olaparib in hepatic and renal insufficiency is ongoing. We started a new immunotherapy study with the OX40 agonist PF-04518600 and another phase I study with the anti-PD1 PDR00. We also started a new phase I study with the DNA-PKi MS2490484A. Another phase I study was started with the HER2-HER3 bispecific MCLA-128.

### III - Pharmacokinetic and pharmacodynamic (PK/PD) modelling and simulation

A modelling framework relating PK, toxicity, tumor size response, survival and cost-effectiveness for metastatic prostate cancer was developed informed by phase II data of eribulin. PSA dynamics in response to eribulin was related to survival measures enabling optimizing phase III design for novel agents in this disease and early estimation of cost-effectiveness. We developed semi-mechanistic PK models for selected oral anticancer agents with poor absorption PK (docetaxel and paclitaxel in combination with ritonavir and the tyrosine kinase inhibitor pazopanib). Within the oral taxane program, modelling and simulation was used to estimate the impact of different oral formulations on the PK of these agents in combination with ritonavir. We showed that the novel solid dispersion tablets of both paclitaxel and docetaxel had superior absorption characteristics compared to the capsule formulation and had an almost similar profile as the drinking solutions.

### III-4 Pharmacogenomics to identify patients at risk for toxicity or undertreatment

Pharmacogenetic screening of patients treated with 5-FU/Capecitabine

Two key publications from our department in J Clin Oncol and Lancet Oncology this year delivered level I evidence that: upfront genotyping of DPYD and genotype directed dosing is life-saving and cost effective, results in exposure to 5FU equal to standard dosing in other patients with SNPs in DPYD and that four SNPs should be tested for adaptive dosing. The nationwide prospective study employing this strategy plus upfront uracil-based phenotyping recruited this year over 200 patients (figure 1).

### III-5 Combined modality studies

Three studies are ongoing employing PARPi olaparib in combination with radiation in NSCLC, breast and head and neck cancer. Our IP protected PARPi pharmacodynamic assay in white blood cells supports low daily dosing of 2x25 mg of olaparib in this strategy. A novel concept was started employing ModraDoc006/r plus radiation in locally advanced prostate cancer. A new phase I study employing the DNA-PKi MS2490484A as radiosensitizer was started in patients with head and neck cancer.

### IV Collaboration with the Dutch Medicines Evaluation Board

We performed (co-)rapporteur reports for the EMA/MEB: Regorafenib (STIVARGA) for metastatic and/or unresectable GIST after prior treatment with 2 tyrosine kinase inhibitors; Sonidegib (ODOMZO) for adult patients with locally advanced basal cell carcinoma (BCC) who are not amenable to curative surgery or radiation therapy and metastatic BCC; S1 (TEYSUNO) for advanced gastric cancer in combination with platinum-combinations other than cisplatin (i.e., oxaliplatin, carboplatin);
Dekker N, Hermens RP, de Wit JH, van Zelst-Stams WA, Hoogerbrugge N, group Rs. Improving recognition and referral of patients with an increased familial risk of colorectal cancer: results from a randomized controlled trial. Colorectal Dis. 2015;17:499-510


Ramucirumab (CYRAMZA) monotherapy or in combination with paclitaxel in locally advanced or metastasized GEJ carcinoma. We gave Scientific Advice about: Palbociclib in breast cancer; VX970 in SCLC; Peretinoin in HCC; Everolimus (new formulation); Mepolizumab (SB-240563) for the treatment of Hypereosinophilic Syndrome (HES).

We reviewed as Concerned Member State (CMS) procedures: Bevacizumab in combination with platinum in locally advanced or metastatic cervical cancer; Ibrutinib in patients with recurrent or refractory mantle cell lymphoma (MCL) or CLL /SSL; Nintedanib for treatment of idiopathic pulmonary fibrosis (IPF); Leuprolreotide acetate for high risk advanced hormone-refractory prostate cancer.

**CLINICAL IMMUNOTHERAPY AND TARGETED THERAPY**

**Background and objectives**

The clinical immuno- and targeted therapy group is primarily involved in the treatment of melanoma and renal cell carcinoma patients. Translational immunotherapy research focuses on adoptive cellular therapies, such as T-cell receptor gene therapy and treatment with tumor-infiltrating lymphocytes (TIL) for melanoma, DNA and peptide vaccination studies for HPV-related squamous cell cancers, and dissection of immunological changes upon immune checkpoint inhibition. For renal cell cancer our group is leading in the development of investigator-initiated phase II/III trials to improve the treatment with small molecule receptor tyrosine kinase inhibitors (RTKI), mTOR kinase inhibitors, combinations of cytokines and anti-angiogenesis drugs, and novel immunological approaches using combination therapy with immune checkpoint inhibition.

**Melanoma**

**Clinical adoptive T cell transfer program in melanoma**

As of September 2008 the Division of Immunology, Medical Oncology and Pharmacy have worked closely together to prepare a TIL trial at the NKI-AVL. (see also Clinical Pharmacology Section). After receiving approval from the Central Research Committee involving Human Subjects (CCMO) for a pilot study (n=10), we have enrolled 10 patients in this study. Toxicity was as expected and objective clinical responses were observed in 5/10 patients (including 2 ongoing complete remission). This study was initiated based on results from single arm, first institution phase II trials performed at the Surgery Branch of the NIH, Bethesda, USA, and Sheba Medical Institute, Tel Aviv, Israel, which showed a 40% or more objective response rate in sometimes heavily pre-treated stage IV melanoma patients. Because of the consistency of these results, the oftentimes long durations of these responses, and the good tolerability of this treatment, we have initiated a European randomized controlled phase III trial, comparing TIL therapy to ipilimumab as first or second line treatment for patients with stage IV melanoma. This trial will be collaborative effort with centers in Copenhagen,
Denmark and Manchester, UK, and for TIL production with Sanquin blood transfusion services in Amsterdam. The primary objective is improvement in PFS at 6 months.

Between 2011 and 2015 we have enrolled 5 patients in a phase I/II trial with T cell receptor (TCR) gene therapy. HLA-A*0201 positive patients with MART-1 expressing metastatic melanoma and no further treatment options are infused with genetically modified autologous peripheral blood T lymphocytes. These modified cells express a TCR specific for the melanocyte differentiation antigen MART-1, expressed in 80% of melanomas.

**Immune checkpoint inhibition in melanoma**

As of end of 2011 anti-CTLA4 (ipilimumab) has become available in the Netherlands for metastatic melanoma, originally as second line therapy, but in 2013 also as first line therapy. As of June 2014 the anti-PD1 drug pembrolizumab became available in a named patient program for patients that have failed prior anti-CTLA4 treatment. In 2015 both nivolumab and pembrolizumab became approved for metastatic melanoma. We developed a phase I biomarker study for patients with stage IIIB/C melanoma with the combination of ipilimumab and nivolumab as neoadjuvant versus adjuvant treatment. The study has included 6 patients in 2015. Furthermore, in collaboration with other groups we are developing biomarkers of response to immune checkpoint inhibitors, such as lymphocyte counts at baseline, LDH levels at baseline, mutational load, type of inflammation in the tumor micro-environment. We are participating in a biomarker study with ipilimumab and nivolumab. In addition blood samples from HLA-A*0201 positive patients are being analysed for changes in cellular immune responses upon pembrolizumab treatment (see Division of Immunology). In metastatic uveal melanoma ipilimumab combined with radiofrequency ablation of a single liver metastasis has finished accrual of the dose escalating phase I part and approval has been obtained to continue the phase II part of the study. Partial responses have been observed and translational research is being done in collaboration with the Division of Immunology.

**BRAF V600 and NRAS mutated melanoma**

As of September 2012 vemurafenib and as of end 2013 dabrafenib has been approved for the treatment of BRAF V600 mutated melanoma. From many of these patients, blood and tumor samples have been collected for translational research (see also Division Immunology and Division Molecular Oncology). Recently published results from two phase 3 RCT in which we participated with this combination showed a higher response rate, PFS and OS compared to single agent dabrafenib or vemurafenib indicating that resistance to BRAFi may be rate, PFS and OS compared to single agent dabrafenib or vemurafenib indicating that resistance to BRAFi may be

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Haenen JB, Thijen Hv, Blank CU. Toxicity patterns with immunomodulating antibodies and their combinations. Semin Oncol. 2015;42:423-8


Haas R, Baas P. Hemithoracic radiation therapy after extrapleural pneumonectomy for malignant plural mesothelioma: Applicable to all and by all. J Med Imaging Radiat Oncol. 2015;59:353-4


Ho VK, Gittenbeek JM, Brandsma D, Beerekoot LV, Sonke GS, van der Heiden-van der Lee M. Survival of breast cancer patients with synchronous or metachronous central nervous system metastases. Eur J Cancer. 2015;51:2508-16


Renal cell carcinoma

In the renal cell carcinoma program we closely collaborate with Dr Axel Bex from the urology- oncology group.

In 2005 we started participation in a treatment-use program of the small molecule sunitinib, a multiple receptor tyrosine kinase inhibitor with high affinity for VEGF-R, PDGF-R, c-KIT and FLT3. Since VEGF and PDGF play prominent roles in the pathogenesis of sporadic clear cell renal cell cancer, inhibition of kinase activity of their receptors appeared to be a rational therapy in this patient population. In 2012, the Working Party for immunotherapy in oncology (WIN-D) initiated the ROPETAR trial. This investigator initiated phase II trial compares 2 monthly rotations of pazopanib followed by everolimus to pazopanib until progression followed by everolimus until progression. This design is based on translational research showing that resistance to a TKI can be the result of epigenetic changes in the tumors. By interrupting TKI treatment this resistance mechanism may be hampered. As PFS is the primary endpoint, we hope to find an improvement in PFS in the rotation arm of the study. In 2014 accrual was stopped after having reached the target number of patients. We are awaiting the PFS and safety analysis. In 2015 we also participated in one second line RCC phase II/II study, combining everolimus with cyclophosphamide (to reduce regulatory T cells), one RCT as first line treatment comparing combination ipilimumab plus nivolumab with sunitinib. In addition, we also treated metastatic RCC patients in a phase I study with IFN-a plus atezolizumab.

The idea of defining the optimal timing for nephrectomy in primary metastatic RCC was adopted by the EORTC GU group and has been approved as a randomized controlled multicenter phase III trial (NCT1099423) that has started enrolment in 2010. Patients are being randomized to receive either 3 cycles of sunitinib treatment prior to tumor nephrectomy or immediate nephrectomy. Post surgery both groups will (re)start sunitinib treatment until disease progression. Study endpoint: Progression-free and overall survival. The NKI-AVL has delivered both principle investigators (Axel Bex and John Haanen) for this study. 95 patients have been enrolled. Blood and tumor material is being stored for translational research in collaboration with the PREDICT consortium.

BREAST CANCER THERAPY AND RESPONSE PREDICTION

Sabine Linn, Sjoerd Rodenhuis, Carolien Smorenburg, Gabe Sonke, Jacqueline Stouthard, Jos Beijnen, Joke Foekema, Marjo Holtkamp, Alwin Huitema, Merel Jebbink, Inge Kemper, Marleen Kok, Rutger Kornstra, Esther Lips, Ingrid Mandjes, Lennart Mulder, Jette Muris, Petra Nederlof, Mette van Ramshorst, Annelot van Rossum, Margaret Schot, Philip Schouten, Tessa Steenbruggen, Jelle Wesseling, Lodewyk Wessels

Background and objectives

The objective of this program is to develop and improve potentially curative therapy for patients with locoregional or oligometastatic breast cancer and to improve treatment options in metastatic breast cancer. Close collaborations are maintained
with many other clinical departments and research divisions of the Netherlands Cancer Institute, the Dutch Breast Cancer Research Group (BOOG), the EORTC Breast Cancer Group and the Breast International Group (BIG). In 2015, some 125 patients were entered in sixteen clinical studies in breast cancer, either focusing on the treatment of early breast cancer or on advanced disease.

**Preoperative chemotherapy**

The preoperative chemotherapy program continued to accrue over 120 patients in 2014. The program is the core of a multidisciplinary research effort to optimize response prediction and to improve response monitoring. It consists of separate studies for ER+/HER2- breast cancer (single institution), triple negative tumors (Neo-TN) and HER2+ tumors (TRAIN-2). A DNA-based BRCA1-like test developed by our group to detect a ‘BRCA1-like’ genomic scar is undergoing prospective testing in the Neo-TN study (NCT01057069). This trial has now achieved its targeted inclusion and will be closed for patient accrual by the end of 2015. The TRAIN-1 study established weekly carboplatin, paclitaxel, and trastuzumab as a highly effective neo-adjuvant regimen with manageable toxicity in HER2+ disease. Median follow-up is four years and long-term results are now being compared with pCR rates and biomarkers for resistance will be analyzed. The TRAIN-2 study (NCT01996267) is a randomized multicenter trial, which investigates the efficacy of neoadjuvant chemotherapy with or without doxorubicin in the presence of dual HER2 targeting with trastuzumab and pertuzumab. The trial opened for inclusion in December 2013 and had included 395 patients by December 1 2015. We developed the NRI (neoadjuvant respons index) in 2009 as a measure of downstaging and it turned to be even more useful as a predictor of recurrence-free survival in triple-negative breast cancer than the rate of pCR (pathological complete remission). We have shown that the NRI is also predictive in patients who do not achieve a pCR. The translational preoperative AFTER trial to study responsiveness of hormone-receptor positive breast cancers to tamoxifen, anastrozole or fulvestrant in postmenopausal patients has been extended to other centers (NCT00738777), and 85 patients have been accrued. Changes in Ki67 mRNA expression are used as a read-out for hormonal therapy responsiveness. In collaboration with the Dutch Cancer Registry IKNL and the NABON Breast Cancer Audit (NBCA) we are investigating the considerable variation between hospitals in the Netherlands in the use of preoperative chemotherapy in patients with breast cancer. While 70% of patients with locally advanced (stage III) breast cancer is treated with neoadjuvant chemotherapy, considerable variation is observed between 98 Dutch hospitals participating in breast cancer care, even after case-mix correction.

**Adjuvant systemic therapy, prognosis and prediction**

The primary objective of the Matador study is to identify predictive gene expression profiles for a disease-free survival benefit of either a docetaxel-containing regimen (docetaxel-doxorubicin-cyclophosphamide (TAC)) or an accelerated doxorubicin-cyclophosphamide (ACdd) regimen (ISRCTN1893718). Accrual of 664 patients has been completed. Median follow-up of 5 years will be reached by Q2 2016 and...
first analyses will then be initiated. In collaboration with Leiden University Medical Center and the TEAM study group, the TEAM Iib trial has been completed and has included 1118 patients. The study addresses the question whether the addition of three years adjuvant oral ibandronate to standard adjuvant systemic therapy in postmenopausal patients with hormone-receptor positive early breast cancer improves outcome (ISRCTN17633610). The number of events needed for the primary endpoint is expected in Q2 2018 and analyses will follow soon afterwards.

Oligometastatic breast cancer

The OLG0 study (NCT01646034) randomizes patients with limited metastatic disease (up to three distant metastases amenable for radical local treatment) between conventional chemotherapy and high dose alkylating treatment. Only patients whose tumor harbors homologous recombination deficiency as determined by the BRCA-like aCGH profile or by germline BRCA1/2 mutation are eligible for randomization. The aim of the study is to provide randomized evidence to support an aggressive oligometastatic approach and to validate the BRCA-like aCGH profile in this population. Thirty-two patients have been included in the study.

Metastatic breast cancer

In collaboration with Cambridge University and Hospitals (UK) and Vall d’Hebron Institute of Oncology (Barcelona, Spain), under the auspices of two European projects (the RATHER project and the EUROCAN Platform), and with support from Genentech, the NKI has initiated a phase lb/prospective randomized phase II trial of the safety and efficacy of tamoxifen in combination with the isomir selective PI3K inhibitor GDC-0032 (tasesibi) compared with tamoxifen plus placebo in hormone receptor positive, HER2-negative, metastatic breast cancer patients with prior exposure to endocrine treatment (POSEIDON trial). The phase lb part has almost been completed. The TONIC trial is a single center phase II trial in metastatic triple negative breast cancer, which we recently initiated to determine the activity of anti-PD1 (nivolumab) treatment and its impact on different immune response induction treatments in TNBC patients with metastatic disease. We hypothesize that short-term induction treatment with low dose doxorubicin, metronomic cyclophosphamide, cisplatin, or radiation induces an anticancer immune response resulting in increased activity of nivolumab as compared to unprimed, single agent nivolumab. Repeated biopsies are included in the study design to monitor immune response to induction treatment and nivolumab.
centered care, which is based on the newest preclinical and clinical developments. The main focus is on clinical trials with a major translation component, both in non-small cell lung cancer, mesothelioma and neuro-endocrine tumors. A new field of interest is smoking prevention, and emerging fields involve potential new developments in thymus tumors and small cell lung cancer.

**Immune Checkpoint Inhibition**

Recently, many immunology studies have been initiated and finalized in non-small cell lung cancer (NSCLC) and mesothelioma. For instance, we addressed the possible impact of high-dose radiotherapy SBRT on anti-PD1 treatment in stage IV recurrent NSCLC in our investigator-initiated trial (PembroRT). Translational research on tumor biopsies focusing on predictive factors and immunological mechanisms is ongoing with the collaboration of the group of Ton Schumacher. The collaboration with Free University Medical Center (Amsterdam) and Erasmus University Medical Center (Rotterdam) has already resulted in an unexpectedly fast patient accrual in this study. Furthermore, first-line studies have been initiated with immune-checkpoint inhibitors. One of our single institution studies involves the combination of ipilimumab with MPDL3280A (anti-PDL1).

With the registration of one of the anti-PD1 monoclonal antibodies in Europe, our institution has taken the lead in treating patients in a compassionate-use program. In the last semester of 2015, over 120 patients have joined this program. All patients have been asked to participate in studies with organoids and fluid phase biopsies again focusing on predictive markers through research on circulating tumor DNA and other serum markers.

**Malignant Pleural Mesothelioma (MPM)**

We participated in two maintenance trials for MPM, contributed with the majority of our patients in an international trial, and are still running our national investigator initiated trial on switch-maintenance with gemcitabine. Another investigator-initiated trial investigates anti-PD1 treatment in MPM, and has included over 20 patients in four months’ time. This study is expected to be finalized in the first quarter of 2016. Analysis of pre- and post-immunotherapy treatment biopsies will be performed. A grant has been obtained to investigate the appearance of neoantigens in this patient cohort together with the group of Ton Schumacher.

In collaboration with the Division of Cell Biology the primary short-term cultures for tumor cells in pleural fluid have been continued. Tumor cells, cultured from pleural fluid are exposed to a library of single and combination chemotherapy compounds to identify the best combination and to predict if a patient will respond to this treatment. In parallel, RNA-Seq analysis is being used to identify sensitive or resistant tumors to treatment. The thoracic oncology group has been recognized as one of the expertise centers for rare intra thoracic diseases.

**Neuroendocrine Tumors**

This year the center of excellence status for neuro-endocrine tumors for the NKI was confirmed. The first international randomized trial concerning pulmonary NET malignancies has been finalized with a major contribution from the NKI and results are expected at the end of 2016. Investigator initiated trials are expected to start in 2016.
Non-Small Cell Lung Cancer (NSCLC)

In addition to the current standard of screening patients for activating mutations, we have expanded the panel with gene sequencing. We have attracted many niche studies currently covering the whole spectrum of targetable mutations in non-small cell lung cancer, consolidating our role as national center for this disease.

Within the European Thoracic Oncology Platform (EOTP) we have initiated several studies. The prevalence of ALK translocations has been analyzed retrospectively in a large European cohort of 2,300 patients who underwent surgical treatment. This year another study addresses the effect of adjuvant immune checkpoint inhibition in stage III patients treated with concurrent chemoradiation.

Smoking Cessation

Active involvement of the department of Thoracic Oncology has led to implementation of smoking-cessation programs in the Netherlands Cancer Institute. Members of our group are leading in national and international (IASLC) committees, in order to influence the political level to compel politicians refrain from interaction with tobacco lobbyists.

Small-Cell Lung Cancer (SCLC)

Patients with extended-stage SCLC were treated with or without additional chest radiotherapy in a large international (CREST) study. Although the primary endpoint was not met, subgroup analysis indicated that some patients may benefit from this approach. In 2016, the CONVERT study, comparing two schedules of chemoradiotherapy in patients with limited-stage SCLC will be presented in major meetings. In our institute, we will focus on the new molecular targets in subgroups of patients with recurrent SCLC.

Thymic tumors

Last year we have updated our institutional database, joined an international database of the EOTP (ThymoScan) and have made the first plans for two investigator initiated studies.

GASTROENTEROLOGY

Henk Boot, Annemieke Cats, Jolanda van Dieren, Cecile Grootscholten, Monique van Leerdam, Margot Tesselaar, Wieke Verbeek, Linda Henricks, Didier Meulendiks, Elvira Nuijten, Lisanne Rigter, Jan Schellens, Esther Toes, Marcel Verheij, Francine Voncken

Background and objectives

The department of Gastroenterology is involved in different phases of research, with emphasis on early detection and prevention of and innovative multimodality treatments for GI cancers including neuro-endocrine tumors (NET) and hereditary GI-cancer syndromes.
**Upper Gl cancer**

For esophageal cancer several imaging studies are being performed including the evaluation of fiducials, MRI and 4D-PET. We are reference center for hereditary diffuse gastric cancer (HDGC) families. Endoscopic characteristics and results of prophylactic gastrectomies of 25 CDH1 mutation carriers (2008-2015) are described. The results of 340 surveillance gastroscopies (2005-2015) in first degree relatives at risk for HDGC are described in a second cohort study.

In 2015, all intended 788 patients with primary resectable gastric cancer were enrolled in the international, randomized, phase III CRITICS study. First results are expected in spring 2016.

In gastric cancer patients with a R1-resection postoperative chemoradiotherapy was beneficial in a retrospective study of 110 patients. Long-term follow-up showed decreased spleen-volume and elevated risk of infection.

For patients with advanced cancer of the stomach or the GE-junction we determined the safety and preliminary activity of docetaxel, oxaliplatin and captecitabine in a phase Ia/Ib study. The overall response rate was comparable to other reports. Toxicity was relatively mild. Subsequently, we initiated a multicenter phase II study with this regimen combined with bevacizumab and trastuzumab in Her2-positive tumors. In 25 advanced gastric cancer patients one year PFS and OS were 52% and 79%.

In gastric cancer patients with limited peritoneal carcinomatosis a dose-finding study of cytoreductive surgery with HIPEC, using oxaliplatin and increasing doses of docetaxel is started.

**Lower Gl cancer**

In collaboration with the Erasmus MC, Rotterdam, we are responsible for the monitoring and evaluation of the Dutch population-based CRC screening program (www.rivm.nl). Furthermore, we are a Dutch NFU expert center for hereditary Gl cancer syndromes. Several research projects are going on in high-risk groups including patients with hereditary CRC syndromes, serrated polyposis syndrome and Hodgkin Lymphoma survivors (MLDS grant).

Several studies are focusing on DPD activity. A total of 2038 patients were prospectively screened for DPYD*2A. DPYD*2A was strongly associated with fluoropyrimidine-induced severe and life-threatening toxicity. Genotype-guided dosing resulted in adequate systemic drug exposure and improved safety.

For advanced squamous cell carcinoma of the anal canal the human papilloma virus (HPV) DNA was determined in tumor tissue of 107 patients treated with (chemo)radiotherapy. HPV- /p16- status was a strong predictor for reduced locoregional control and OS.

**NET**

In close collaboration with the UMCU Utrecht, we are a ENETs center of excellence and a Dutch NFU GEP-NET expertise center. As of December 2015, with the start of PRRT we now have all techniques to diagnose and treat patients with a GEP-NET.

Several research projects are ongoing including participation in a randomized phase 3 trial placebo versus everolimus (RADIANT 4).


Schouten PC, Linn SC. Challenges in the use of DNA Repair Deficiency As a Biomarker in Breast Cancer. J Clinical Oncol 2015;33:1867-1869


UROLOGIC ONCOLOGY

André Bergman, Martijn Kerst, Michiel van der Heijden, Elsbeth van der Laan

Background and objectives

The urologic oncology group is dedicated to the treatment of prostate, bladder, testicular and penile cancer. This subdivision of the division of Medical Oncology aims to contribute to international trials and to play a leading role in initiation of national trials and translational research.

Prostate cancer

Predicting Response to Enzalutamide: a biomarker design study (PRESTO-study)

In this national study, biopsies of metastatic sites of castration resistant prostate cancer are taken prior to enzalutamide treatment. In the biopsies, binding profiles of the androgen receptor to the chromatin are assessed, which may hold biomarker properties as a predictor of response to enzalutamide.

Registry of Treatment Outcomes of Patients Treated with Radium-223 (ROTOR-registry)

Radium-223 is the newest life-prolonging therapy for metastasized prostate cancer patients. This national study aims to assess the course of pain of patients treated with radium-223, the positioning of this treatment among other treatment options in a non-study population and identification of bone metabolism specific biomarkers for response.

Cabazitaxel in combination with Budesonide (CABARESC)

Cilutis causing diarrhea is a common toxicity of Cabazitaxel treatment. Budesonide might prevent this toxicity. In this national randomized open-label Phase III study patients are randomized between Cabazitaxel treatment with or without Budesonide. At closure of this trial, our site included 14 patients.

Phase IIb Study of the Efficacy and Safety of Continuing Enzalutamide during docetaxel treatment

DNA damage as a result of taxanes is under control of the androgen receptor. In this international trial, patients with chemotherapy naïve castration resistant prostate cancer are treated with enzalutamide. At the time of disease progression patients are all treated with docetaxel and randomized between simultaneous treatment with enzalutamide or placebo.
who are platinum-ineligible. Thirteen patients were included at the NKI. First results were presented at ESMO 2015, reporting a response rate of 27% in PD-L1 IHC-positive patients and 15% in unslected patients. A follow-up phase 3 trial was opened in 2015 (IMvigil 211). Patients are randomized between MPDL3280A and standard chemotherapy (vinflunine or taxane). NKI enrolled 27 patients in this trial so far.

Other trials in urothelial cancer patients that opened in 2015 were:
- The RANGE study: docetaxel +/- ramucirumab in second line advanced urothelial cancer
- The IMvigil 010 study: adjuvant MPDL3280A vs observation in high-risk PD-L1 IHC-positive muscle-invasive bladder cancer
- The DANUBE study: chemotherapy vs durvalumab vs tremelimumab + durvalumab as first line treatment in advanced bladder cancer.

**Molecular characterization of urothelial cancer**

Using next-generation sequencing, a large cohort of bladder cancers treated with pre-operative platinum-based chemotherapy was analyzed. An unexpected association between ERBB2 mutations and response to chemotherapy was found. A large multi-institution validation cohort was assembled to test the association between ERBB2 and several other molecular alteration and chemotherapy response.

**Testicular cancer**

Our department continued its role as a reference center for testicular cancer, covering the treatment of all subtypes and all clinical stages, including relapse treatment using high dose chemotherapy and autologous stem cell transplantation.

**TIGER study**

This is an international trans-Atlantic prospective randomized trial for patients relapsing after BEP chemotherapy, in which Conventional Dose Therapy (TIP) is compared with sequential High Dose Therapy (TICE).

**M14RAC study**

This as a multi-centre retrospective study to analyse the outcome (OS and DFS) of patients with initial clinical stage I seminoma, who relapse after adjuvant treatment with carboplatin (characteristics of treatment and outcome).

The TACKLE study, a national study to identify risk factors for development of cardiovascular disease after treatment is currently recruiting patients.

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Van Driel WJ, Lok CA, Verwaal V, Sonke GS. The role of hyperthermic intraperitoneal intraoperative chemotherapy in ovarian cancer. Curr Treat Options Oncol 2015;16:1


Van der Heiden-van der Loo M, de munck L, Sonke GS, van Dalen T, van Dielet PJ, van den Bongard HJ, Peeters PH, Rutgers EJ. Population based study on sentinel node biopsy before or after neoadjuvant chemotherapy in clinically node negative breast cancer patients: identification rate and influence on axillary treatment. Eur J Cancer 2015; 51:915-21


In 2015, the department continued to expand its research activities along two main themes: image-guided adaptive radiotherapy (lIGART) and targeted radiosensitization. By combining geometric, biological and (more and more functional) imaging information before, during and/or after irradiation, we are able to adjust the treatment plan if necessary. This adaptive radiotherapy, in combination with 
vivo dosimetric verification, contributes to the ultimate ambition of state-of-the-art radiotherapy: individualized, highly precise and safe treatment delivery.

As member of the international MRI-Linac Consortium, we conduct tumor site-specific studies on MRI-guided treatment planning and delivery, and prepare the introduction of this new imaging/treatment machine in our clinic.

In addition to chemoradiotherapy, the combined use of targeted agents and radiation (“bioradiotherapy”) is an attractive and rational strategy to improve outcome and limit toxicity. Our lab research aims to translate these targeted radiosensitizers from bench to bedside and back. Several of these agents are already part of our clinical program, including DNA repair inhibitors (lung, head and neck, breast), EGFR-blockers (bladder), angiogenesis inhibitors (soft tissue sarcoma) and immune-modulators (lung, prostate).

In November the department held its 2-yearly clinical retreat, offering an inspiring program on lessons learned and future developments within the fields of radiation oncology, biology and physics.

The Radiation Oncology department continues to promote the introduction of particle therapy in The Netherlands. The Amsterdam Proton Therapy Center (APTC), a joined initiative of NKI-AVL, VU Medical Center and the Academic Medical Center, aims at realizing a beyond-state-of-the-art proton facility for treatment and research in Amsterdam (location NKI-AVL) in collaboration with the Prinses Maxima Center for Pediatric Oncology (PMC) and the University Medical Center Utrecht. Uulke van der Heide was appointed Professor in Imaging Technology in Radiation Oncology at the University of Leiden per August 1st.

Harry Bartelink received the prestigious ECCO Lifetime Achievement Award at the ECCO congress in Vienna. Jasper Nijkamp won the yearly NWO’s Bessensap Science- meets-Press pitch, Ingar Seemann successfully defended her thesis on “Radiation and anthracycline induced cardiovascular damage”.

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Dose painting uses two properties of radiotherapy that set it apart from other treatment modalities: first, radiotherapy offers a high degree of spatial control over the deposition of radiation dose. With in-room imaging devices such as the cone-beam CT and the MR-linear accelerator, we can ensure that these fields are targeted correctly. Secondly, radiotherapy has a differential capacity, in which different parts of the target can be irradiated with different dose levels within a single treatment. The implication of these properties is that in radiotherapy the decision about what to treat is not binary. To guide decisions about what to treat, and to which level, functional imaging techniques can be used to characterize the tumor and its heterogeneity.

Our research focuses on the development and validation of quantitative imaging methods that allow tumor characterization for radiotherapy dose painting. Strategies to integrate anatomical and quantitative MRI in the radiotherapy workflow are designed and applied to a range of tumor sites.

Probabilistic planning for liver SBRT. Treatment planning for Stereotactic Body Radiation Therapy (SBRT) of liver tumours is often challenging due to large respiratory motion and nearby OARs such as great vessels or duodenum. In such cases a section of the PTV is edited to save nearby OARs. We evaluated a PTV-less probabilistic planning technique which directly incorporates respiratory motion and other geometric uncertainties of the GTV in the dose optimization process. This technique might result in improved balance between OAR exposure and the confidence of proper target dosage, and requires less human interaction.

Four liver SBRT plans (3x20 Gy prescription), in which OARs were nearby the PTV, were re-planned using an in-house developed research plug-in for Pinnacle (version 9.100). The plug-in combines the tumour trajectory extracted from the 4D planning CT (-1 cm amplitude for these cases) with the Gaussian distribution of random errors (0.25 cm) into a dose blurring kernel, and incorporates a Gaussian distribution of systematic errors as GTV offsets (0.34 cm). Our probabilistic objective aims for a 90% confidence of GTV minimum dose. Clinical and probabilistic plans were compared using in-house software that accurately simulates the effects of motion and uncertainties on an optimized dose distribution by explicitly sampling three daily errors for each systematic error, and 100 positions along the breathing trajectory for each daily error. OARs were evaluated via a traditional DVH.

**Publications**


Aleman BM, van Leeuwen FE. Hypofractionated adjuvant radiotherapy for breast cancer: no signs of increased risk of cardiotoxicity. Ned Tijdschr Geneeskd. 2015;159:A8856

In three out of four cases the probabilistic plan showed a clear benefit compared to the conventional plan. In two cases the GTV coverage in the probabilistic plan was significantly improved, while maintaining a low enough dose on the OAR (see figure 1). In the third case the dose to an OAR (great vessels) was significantly reduced while still reaching 90% target dose confidence. In the fourth case all clinical constraints were met both in the clinical and probabilistic plan.

From these results we conclude that probabilistic planning can make a valuable contribution to treatment planning in those cases in which it is difficult to meet all clinical criteria. VMAT for intracranial SRS: coplanar or non-coplanar arcs – high dose conformity versus accurate delivery. In stereotactic radiosurgery of intracranial lesions, high plan quality and accurate dose delivery are imperative. Noncoplanar techniques typically result in plans with improved conformity, but couch rotations may affect the accuracy of the dose delivery. The aim of this study is to compare noncoplanar with coplanar VMAT plans with respect to plan quality while taking into account the possible effect of couch rotation inaccuracy.

Five intracranial SRS cases with a single lesion (PTV 0.64–3.96cc) were planned with 1) noncoplanar VMAT; two single arcs – couch 0° and couch ±90°; 2) coplanar VMAT; dual arc – couch 0°. Prescribed dose (PD) was 24 Gy in a single fraction. Plan acceptance criteria were such that VPTV,24Gy>95% and Dmax, PTV < 150% PD. Organs at risk (OARs) included the brainstem, optic nerves, chiasm, and eyes. Parameters used for plan evaluation included: VPTV,24Gy, Paddick conformity index (CIP), Paddick gradient index (GIP), volume receiving 10Gy (V10Gy), and Dmax to OARs. To simulate couch rotation inaccuracy for noncoplanar plans, the isocenter of the noncoplanar arc was shifted by using a vector translation in the coronal plane of 1mm (based on requirements at linear accelerator acceleration). All original coplanar and noncoplanar plans satisfied the PTV and OAR plan acceptance criteria. In all cases the OARs were adequately spared (highest Dmax was 2.2Gy). For small PTVs (PTV <1.45 cc), noncoplanar plans showed little improvement regarding the dose fall off outside the target (improved GIP and reduced V10Gy, table 1).
The robustness of single isocenter VMAT treatment plans for multiple brain lesions

In an increasing number of patients, multiple brain lesions are treated simultaneously using stereotactic techniques and VMAT. For lesions that are close together, treatment plans can be made that use only a single isocenter, without compromising plan quality in terms of conformity and DVH parameters, compared to multi-isocenter plans. Since every isocenter requires its own setup procedure at the linac, a single isocenter plan increases efficiency of treatment delivery and reduces patient discomfort. However, single isocenter plans do not allow for separate correction for ‘independent’ motion of the lesions, due to rotations of the head. Moreover, the associated placement of the single isocenter between targets introduces sensitivity to residual patient rotations after setup. Both effects decrease the robustness of the plan. We investigate and quantify this loss of robustness.

For 7 patients the robustness of single-fraction treatment plans with 2 mm PTV margins were evaluated assuming different distances (in the range 0-10 cm) between CTV-centroid and isocenter positions. These plans satisfy V100% Dpres = 95% for the PTV, where Dpres is the prescribed dose (18 or 24 Gy) and a max dose in the PTV that does not exceed 150% Dpres. The positions of the CTVs were shifted with respect to the planned dose distribution distribution to simulate setup uncertainties due to couch positioning and rotations. The simulated shifts were sampled from non-setup systematic uncertainties, $\Sigma = 0.8$ mm SD, in combination with positioning uncertainties of the setup. Our clinical practice is to take two CBCTs prior to the treatment, the first to obtain the position of the patient on the table, the second after the couch shift which corrects for translations but not for rotations with respect to the plan CT. For the simulations we used the SDs of the residual translations and rotations from the second CBCT registration.

The residual setup errors were 0.5 mm SD for translations and 1.1 degree SD for rotations around the isocenter. The maximal separation dmax between the CTV-centroid and isocenter that lead to a 90% probability that the patient receives a dose D99% = 95% Dpres to the CTV was determined. We obtained dmax = 5.0 ± 0.9 cm. The results for this small group of patients suggest that with the current clinical margins lesions with centroid positions up a maximum of 10 cm apart would result in treatment plans that are robust for translational and rotational setup uncertainties.

**Table 1. Plan evaluation parameters of the coplanar and noncoplanar VMAT plans. Patients are ranked from large to small tumour volume.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Parameters</th>
<th>Volume (cm³)</th>
<th>VPTV,24Gy [%]</th>
<th>Plan conformity</th>
<th>Paddick CI</th>
<th>Paddick GI</th>
<th>V10Gy (cm³)</th>
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**EPI D D O S I M E T R Y**

Roel Rozendaal, Hanno Spreeuw, Igor Olaciregui-Ruiz, Patrick González, René Tielenburg, Marcel van Herk, Ben Mijnheer, Anton Mans

**Overview of clinical experience**

In our institution, almost all external beam radiotherapy treatments are verified using in vivo portal dosimetry, for reasons of efficiency and patient safety. The total 3D dose distribution is reconstructed using a back-projection algorithm and compared with the planned dose distribution using 3D gamma evaluation. If a deviation exceeds our alert criteria, manual inspection is required. We performed an analysis of...
Haas RL, Baas P. Hemithoracic radiation therapy after extrapleural pneumonectomy for malignant plural mesothelioma: applicable to all and by all? J Med Imaging Radiat Oncol. 2015;59:353-5


Portal dosimetry for the MR-linac

The complexity of online treatment adaptation makes independent dosimetric verification in the Elekta MR-linac combination indispensable. One of the challenges for MR-linac portal dosimetry is the presence of the MRI housing between the patient and the EPID. A study was therefore started to adapt our back-projection algorithm, used for in vivo dosimetry at the other linacs, for the presence of the MRI scanner. These adaptations concerned a subtraction of scatter from the MRI housing to the EPID, a correction for the attenuation in the MRI housing, and a compensation for changes in the photon beam spectrum. Furthermore, an additional set of commissioning data of the parameters for the back-projection model was implemented. Experiments using a 12 cm aluminum plate (mimicking the MR-linac geometry) showed excellent agreement between planned and EPID-based reconstructed dose distributions. This result is an essential step towards an accurate, independent, and potentially fast field-by-field IMRT dose verification tool for the MR-linac.

IMAGE GUIDED ADAPTIVE RADIATION THERAPY

Mehrsima Abdoli, Olga Hamming-Vrieze, Lisa Hartgring, Uulke van der Heide, Marcel van Herk, Nienke Hoffmans-Holtzer, Nelly Kager, Simon van Kranen, Tessa van de Lindt, Floris Pos, Angelina Protik, Peter Remeijer, Anne Lisa Wolf, Jan-Jakob Sonke

An MRI based mid-ventilation approach for radiotherapy of liver cancer on an MR-linac

With an integrated MRI and linear accelerator (MR-linac) under development, it will become possible to visualize even low contrast tumors at the time of irradiation and new MRI-based image guidance strategies for moving targets are required. The purpose of this work was to develop an efficient strategy to acquire a high quality MRI image in mid-ventilation. In midV, however, the velocity of the liver is high and images are thus sensitive to motion artifacts. Therefore, a fast but low resolution (LR) image was acquired in midV (single-shot TSE, TE=100ms, TR=222ms, shot length=166ms), while interleaved, a high resolution (HR) image was acquired in the more stable exhale phase (multi-shot TSE, TE=100ms, TR=2180ms, shot length=234ms, res=1x1x4mm, NSA=3). Both acquisitions were triggered using a navigator channel placed around the diaphragm. The HR image was subsequently deformably registered (DR), using B-splines with mutual information, to the midV anatomy. In this way a high quality image in midV was obtained. All measurements were performed on a 1.5T whole body MR system (Philips Healthcare, Best) on healthy volunteers. After deformable registration of the HR exhale scan to the midV anatomy, a good quality image in midV (DR midV) was obtained. It shows minimal motion artifacts and vascular structures are clearly visible (figure 2a). In a sagittal view it is visible that the DR midV fits the midV anatomy much better than the exhale scan (figure 2b). Before clinical implementation, the robustness and accuracy needs to be validated in a cohort of patients.
Validation of a Deformable Image Registration for Adaptive Radiotherapy of Lung Cancer

Radiation therapy for lung cancer patients is subject to several geometrical uncertainties due to baseline shifts, differential motion and anatomical changes over the course of treatment. Adaptive radiotherapy (ART) aims to mitigate these uncertainties by adjusting the treatment plan. Deformable image registration (DIR) forms an important component in this strategy. The goal of this study was to validate an in-house developed B-Spline-based DIR approach for CBCT to CT registration in lung cancer patients. To that end, two validation methods have been applied to verify the performance of the DIR technique. First, the consistency of the DIR was assessed using a full circle method. 10 patients with repeated CT (rCT) and CBCT scans were used and combinations of pCT, rCT and CBCTs were selected to form circles consisting of 3 scans each (24 circles in total). Each scan was deformably registered to the next, which over the full circle ideally results in a zero deformation vector field (DVF). The voxel-wise residual displacements (RDs) were summarized by mean and SD calculated in LR, CC and AP directions, as well as for the vector length. For the second validation method we used 12 patients in which gold fiducial markers had been implanted in mediastinal lymph nodes. The distance between the markers on the pCT and a CBCT was measured before and after DIR. The residual displacement from both methods was 1.5±0.8mm and 1.8±1.0mm respectively. In conclusion, acceptable accuracy of less than 2 mm was found which is small relative to the start to finish day to day geometrical uncertainties. Despite these relatively small RDs, caution must be taken in the presence of large low-contrast variations of anatomy in the course of treatment.

Rapid creation of intermediate bladder shapes for a library of plan strategy

Bladder patients are typically treated with an empty bladder protocol to reduce the treatment volume and limit toxicity. Despite clear instructions before treatment, patients still show large inter-fraction motion due to bladder filling variation. This inter-fraction bladder filling variation can be anticipated by a library of plan strategy. In this study, a robust and fast method was developed to generate a library of CTs. After rigid registration of a full and empty bladder planning CT, the CTVs were delineated on both CTs. Subsequently, (1) the center of gravity (CoG) of the empty bladder was calculated, (2) for 50 equiangular values of phi and theta the distance from the CoG to both the empty and the full bladder were sampled and stored in rectangular “distance maps”, (3) starting from the empty bladder distance map, a maximum of 3 intermediate distance maps were interpolated such that the largest difference between subsequent maps was 10mm; (4) from the intermediate distance maps dots were distributed in rectangular “distance maps”. The created contours were successfully validated visually by a radiation oncologist. Additionally the spacing of intermediates was checked and found to be 1 cm along the largest straight line segment that connects the full and empty bladder, approximately perpendicular to the surface. The computation time of the algorithm is approximately 2 seconds. In conclusion, the developed interpolation method on full and empty bladder volumes was shown to be robust and fast. The method has been successfully implemented in the clinical workflow.
Quantifying the impact of respiratory parameters in the delivery of spot scanning proton therapy in free breathing lung cancer patients

Respiratory motion cause significant dose errors in Intensity Modulated Proton Therapy (IMPT) for lung cancer patients due to induced variations in range and the interplay effect. This aim of this study was to investigate the relation between the respiratory amplitude and the dosimetric consequences of the interplay effect. To that end, IMPT plans were optimized for 4 NSCLC lung cancer patients on mid-position CT with iGTV density override, spot size s=6 mm and spot spacing 1s. The proton treatment delivery was simulated by an in-house developed time dependent simulation platform. The resulting dose distribution was compared to a simple shift-invariant blurring of the dose. Dose errors (1SD within the PTV) introduced by the interplay effect showed a linear relationship with the amplitude while the blurring effect caused dose error with quadratic dependence on the amplitude (figure 4). In conclusion, the dosimetric consequences from interplay and range effects in proton therapy are related to the amplitude. Large tumors with higher amplitude of motion seem to be more sensitive to dose errors.

Robustness of H&N treatment plans against deformations and anatomy changes: opportunities for margin reduction

Safety margins increase the robustness of treatment plans against geometrical errors while margin reduction improves OAR sparing. With adaptive interventions robustness in plans with small margins may be restored. The aim of this study was to investigate 1) how much coverage during treatment is lost as a result of deformations and anatomy changes in H&N cancer patients, 2) if early detection is possible. To that end, VMAT treatments plans (SIB, 35x2Gy) were automatically optimized with uniform CTV to PTV margins of 5, 3 and 0 mm for 19 oropharyngeal patients. Subsequently, BSpline CBCT-to-CT deformable registration was performed, the dose was recalculated, mapped and accumulated in the planning CT. Margin reduction from 5à3à0 mm led to OAR sparing of ~1Gy Dmean per mm. Despite online repositioning, substantial systematic deformations were present (≥3 mm). Nevertheless, increase in OAR dose generally was small (Dmean≤1Gy ), independent of applied margins. In 5 patients PG Dmean increased ≥2Gy. Discrepancies in CTV coverage ≥2 Gy were found in 1, 3 and 7 instances respectively, mainly elective CTVs. ROC analysis on intervention thresholds with 0mm plans showed that the Area Under the Curve ranged from 0.73 to 1.00. After 8 fractions all candidates for adaptive replanning were detectable without false positives. In conclusion, Large deformations and anatomy changes not necessarily led to unacceptable loss of CTV coverage and/or increase of dose to OARs, especially in plans with 5 and 3 mm margins. Eminent underdosage was early and efficiently predictable, enabling patient selection for adaptive interventions in plans with small margins.

Figure 3: Schematic overview of bladder model generation. From the empty bladder 50 equiangular distances from the CoG to both the empty and the full bladder were sampled and stored in rectangular “distance maps”. The distance map was used to interpolate intermediate structures.

Figure 4: Dose errors caused by blurring (left) and interplay effects (right) as a function of the respiratory peak-to-peak amplitude.


Nestel U, Belderbos J. Rebuttal from Dr Nestle and Dr Belderbos. Transl Lung Cancer Res. 2015;4:629

Nestel U, Belderbos J. Cons: should a medically inoperable patient with a T2N0M0 non-small cell lung cancer central in the lung hilus be treated using stereotactic body radiotherapy? Transl Lung Cancer Res. 2015;4:629-6


Paemsans M, Garcia C, Wong CD, Patz EF, Jr., Komaki R, Eschmann S, Govindan R, Vansteenkiste J, Meert PAPBI. Response of the tumor will be evaluated by MRI scan before radiotherapy and at time of surgery. The mRNA gene expression profiles, the miRNA expression profiles and the biological studies performed are gene expression profiling from RNA and DNA isolated from biopsies taken of the tumor. To qualify for the trial, patients must be 60 years or older, and have a unifocal cT1-2 (<3 cm) pN0 MD breast cancer; sentinel node procedure before irradiation. 78 patients were treated by PAPBI consisting of delivering 10x4 Gy over 12 days. The radiation schedule has changed to 5 x 6 Gy in a 1 week schedule, to reduce the overall treatment time, this shorter schedule is commonly used in post-operative radiation partial breast irradiation. Six weeks after PAPBI, a wide local excision is performed. As the tumor remains ‘in situ’ during irradiation, accurate tumor delineation and control of accurate radiation dose delivery to the tumor becomes possible by treating these patients with a cone BT linear accelerator. To identify a subgroup of breast cancer radiosensitivity, biological studies performed are gene expression profiling from RNA and DNA isolated from biopsies taken of the tumor before radiotherapy and at time of surgery. The mRNA gene expression profiles, the miRNA expression profiles and the DNA copy number changes will be correlated with response to radiotherapy, defined as pathologic response at the time of the lumpectomy (i.e. 6 weeks after the completion of the PAPBI). Response of the tumor will be evaluated by MRI scan and PET (before radiotherapy and before surgery) and classical pathology. In total 120 patients are needed. At this time, 106 patients are included in the study (NKi-AVL n=60; IGR n=25; Karolinska n=10; UMCU n=11). The first 70 patients (all treated with 10x4 Gy) were analyzed. Post-operative complications were noted in 11 of 70 patients (16%) The overall postoperative infection rate was 11%. The majority of patients had no or mild fibrosis and fibrosis improved over time. At 1, 2 and 3 years of follow-up respectively 90%, 98% and 100% of patients had no or mild fibrosis. Fibrosis was only found in a small volume of the breast. The global cosmetic outcome, scored by the physician, was good to excellent in 77% at 6 months to 100% at 3 years. The majority of the Dutch patients

BREAST CANCER

Berthe Aleman, Harry Bartelink, Naomi Boekel, Sophie Bosma, Milla Donker, Kenneth Gilhuys1, Floor van Leeuwen, Femke van der Leij, Claudette Loo, Hester Oldenburg, Marion Scharpfenecker, Sanne Schagen, Ingar Seemann, Fiona Stewart, Marc van de Vijver2, Corine van Vliet-Vroegindeweij, Wouter Vogel, Sandra Vreeswijk, Erik van Werkhoven, Jelle Wesseling, Astrid Scholten, Nicola Russell, Paula Elkhuizen & BOOG and EORTC collaborators

Defining radiotherapy sensitivity

(A) In 2010 the Image guided Preoperative Accelerated Partial Breast Irradiation (PAPBI) started. This trial is directed at implementing pre-operatively given image guided accelerated partial breast irradiation without compromising local control in early breast cancer patients. By assessing tumor response to radiotherapy, the goal of the study is to develop a gene expression profile that predicts the breast cancer radiosensitivity. This gene signature of breast radiosensitivity would further design optimal treatment strategies for individual breast cancer patients treated with BCT.

To qualify for the trial, patients must be 60 years or older, and have a unifocal cT1-2 (<3 cm) pN0 MD breast cancer; sentinel node procedure before irradiation. 78 patients were treated by PAPBI consisting of delivering 10x4 Gy over 12 days. The radiation schedule has changed to 5 x 6 Gy in a 1 week schedule, to reduce the overall treatment time, this shorter schedule is commonly used in post-operative radiation partial breast irradiation. Six weeks after PAPBI, a wide local excision is performed. As the tumor remains ‘in situ’ during irradiation, accurate tumor delineation and control of accurate radiation dose delivery to the tumor becomes possible by treating these patients with a cone BT linear accelerator.

To identify a subgroup of breast cancer radiosensitivity, biological studies performed are gene expression profiling from RNA and DNA isolated from biopsies taken of the tumor before radiotherapy and at time of surgery. The mRNA gene expression profiles, the miRNA expression profiles and the DNA copy number changes will be correlated with response to radiotherapy, defined as pathologic response at the time of the lumpectomy (i.e. 6 weeks after the completion of the PAPBI). Response of the tumor will be evaluated by MRI scan and PET (before radiotherapy and before surgery) and classical pathology. In total 120 patients are needed. At this time, 106 patients are included in the study (NKi-AVL n=60; IGR n=25; Karolinska n=10; UMCU n=11). The first 70 patients (all treated with 10x4 Gy) were analyzed. Post-operative complications were noted in 11 of 70 patients (16%) The overall postoperative infection rate was 11%. The majority of patients had no or mild fibrosis and fibrosis improved over time. At 1, 2 and 3 years of follow-up respectively 90%, 98% and 100% of patients had no or mild fibrosis. Fibrosis was only found in a small volume of the breast. The global cosmetic outcome, scored by the physician, was good to excellent in 77% at 6 months to 100% at 3 years. The majority of the Dutch patients

1 UMCU, Image Sciences Institute, Utrecht
2 AMC, Department of Pathology, Amsterdam
was satisfied to very satisfied with the cosmetic result; 81%, 86%, 80% and 79% after respectively 0.5, 1, 2 and 3 years. Three patients developed a local recurrence. Other important aspects of this study are collections of fresh frozen tissue (i) to assess the radio-induced genetic alterations on the surgical post-radiation specimen compared to the tumor response 6 weeks after the end of radiotherapy; (ii) to study the early changes in gene-profiling and (iii) to evaluate the early functional imaging modifications.

Target volume delineation of patients treated in the PAPBI trial will be evaluated by comparing preoperative delineation with postoperative delineation. Previously we showed less inter-observer variation in the preoperative situation versus the postoperative situation and comparable target volume sizes in a dataset of 24 breast cancer patients. A new phase III study is in preparation comparing pre- versus postoperative Partial Breast Irradiation (schedules 5x5.7 Gy). This will be an international multicenter study that will start after closure of the PAPBI study (study protocol has been accepted by the Ethical committee of the NKI-AVL, and a KWF grant has been applied for).

(B) Radiosensitivity breast cancer cell lines: In addition, with the clinical PAPBI study, 30 human breast cancer cell lines will be investigated for their radiosensitivity profile. Until now we performed colony assay experiments for 7 breast cancer cell lines (T47D, MCF7, SKBR3, BT 474, BT 549, SUM 159, BT 20). This will also be implemented in the study described in C.

(C) In the completed Young Boost Trial 2400 patients under 50 years have been randomized between normal boost dose vs. a higher boost dose after breast-conserving therapy. From these patients fresh frozen material as well as paraffin embedded material is collected. Follow up is available for the first years. Histological revision of tumor material is performed and tissue micro arrays of these tumors are being made. RNA and DNA isolation is done of paraffin- and frozen material. Gene expression profiling is planned. The profiles related with radiotherapy response found in the studies A-B will be evaluated on this material to assess whether a response profile is associated with local control. So far 21 patients had an isolated local recurrence, of which 18 patients from the Netherlands. A case-control study with Dutch patients was set up with 3 controls per case, matched on age, patients from the Netherlands. A case-control study with Dutch patients was set up with 3 controls per case, matched on age. All patients were radiated at the NKI-AVL.

(D) In a large cohort of 8507 patients treated with breast conserving therapy between 1979 and 2008 we studied trends in treatment and outcome. All patients were radiated at the NKI-AVL, surgery was performed at the NKI-AVL or referring hospitals. Low locoregional recurrence rates of 2 and 5% after respectively 5- and 10-years were found in the whole study population, already in the beginning period of breast conserving therapy in earlier years. Even in young patients (<40 years), known as an independent risk factors as seen in many series, a low locoregional recurrence rate was found of 4% and 9% after respectively 5 and 10 years. Also in the present study young age was a significant risk factor for developing local relapse. The risk of a locoregional recurrence was significantly


Reduced by the use of radiotherapy boost (HR 0.52) and the use of adjuvant systemic therapy (HR 0.50). While the use of a radiotherapy boost declined over time, the locoregional recurrence rate remained stable. This can be explained by the observed increased use of adjuvant systemic therapy (from 19% between 1980-1987 to 41% between 1989-1998 to 51% between 1999-2008). Additional analyses on young patients (<40 years) will be performed.

(E) The 20-year follow-up of the EORTC Boost-no Boost trial has been reported. This trial included more than 5000 patients who had undergone breast cancer surgery with complete excision for stage I and II primary breast cancer. Demonstrated a benefit in local control on addition of a local boost of 16 Gy to the tumor bed (HR at 20 years: 0.63; p<0.0001), with the largest absolute effect in younger patients. The boost increased the risk of breast fibrosis. There was no significant impact on overall survival at 20 years however (HR 1.05; 99% CI: 0.92-1.19, p=0.323). The clinical implication of these findings is that the boost dose can be safely omitted in most patients over 60 years of age, but is of benefit for local control in the younger age groups.

(E) PET scan use in breast cancer. It was found that even small tumors < 1 cm are adequate visible on PET scan screening. In higher stage breast cancer it was found that PET scan adds to conventional staging. In breast cancer patients scheduled for neo-adjuvant chemotherapy (NAC), PET/CT renders pre-chemotherapy sentinel lymph node biopsies unnecessary in case of an FDG-avid axillary node, enables axillary response monitoring during or after NAC, and leads to changes in radiotherapy for a substantial number of patients (8-10%) because of detection of occult N3-disease. Based on these results, we recommend a PET/CT as a standard staging procedure in breast cancer patients scheduled for NAC. The presence and number of FDG-avid nodes were evaluated and the proportion of patients that would be upstaged by PET/CT, based on detection of >4 FDG-avid axillary nodes defined as cN2(4+) or occult N3-disease, was calculated. In total, 87 of 278 patients were considered high-risk based on conventional staging. PET/CT detected occult N3-disease in 5 (11 %) of 47 low-risk patients. In 144 intermediate-risk patients, PET/CT detected >4 FDG-avid nodes in 24 (17 %) patients and occult N3-disease in 22 (15 %) patients, thereby finally upstaging 38 (26 %) of intermediate-risk patients. Of 43 (23 %) upstaged patients, 18 were ypNO, 12 were ypN1, and 13 were ypN2-3. Pre-chemotherapy PET/CT is valuable for selection of breast cancer patients at high risk for LRR. In our population, 23 % of patients treated with NAC were upstaged to the high-risk group based on PET/CT information, potentially benefiting from regional radiotherapy.

Defining radiation toxicity

Quantifying patient reported outcome measures (PROM) for radiation dermatitis in breast cancer treatment. In a prospective cohort study, 333 consecutive breast cancer patients filled in a Likert-type questionnaire to assess the experience of radiation dermatitis as a result of breast cancer radiotherapy. The study was designed to determine the inter-patient variation in response and to define the number of patients needed to identify a significant difference in PROM for this end point in a comparative trial of management strategies for radiation dermatitis. For all treatment regimes, the peak reaction was
recorded at 5-6 weeks after start of treatment. The variance at 4 weeks was 399.2 with a standard deviation of 20, from which we could derive the number of patients required for a comparative trial as being 200 to detect a significant difference with a power of 80%.

(A) Cardiac toxicity. In a cohort study the cardiac toxicity (morbidity and mortality) of 10,468 Dutch patients treated for ductal carcinoma in situ between 1983 and 2004 was evaluated and compared to age-matched population rates. The database for the treatment of DCIS was coupled to the heart intervention registry of the Netherlands and the hospital discharge registry. Reassuringly, no increase in cardiac events was observed in the 5-year survivors of DCIS at 10 years follow-up.

(B) Cardiac toxicity. In a population-based cohort of 72,045 5-year breast cancer survivors, a linkage study was performed with population-based registries of cardiovascular disease. Left-sided radiotherapy after mastectomy increased the risk of any cardiovascular event compared to both surgery only (hazard ratio (HR)=1·23 95%CI=1·11-1·36) and right-sided radiotherapy (HR=1·19 95%CI=1·04-1·36). Radiation associated risks were found not only ischemic heart disease (IHD), but also for valvular dysfunction and congestive heart failure (CHF). Risks were more pronounced in patients aged <50 years at BC diagnosis (HR=1·48 95%CI=1·07-2·04 for left- versus right-sided radiotherapy after mastectomy). Radiotherapy after wide local excision did not increase risk of any cardiovascular event, yet an increased IHD-risk was found (HR=1·14 95%CI=1·01-1·28). Detailed radiotherapy information showed an increased CVD risk for left-sided chest wall irradiation only, left-sided breast irradiation only, and internal mammary chain field irradiation, all compared to right-sided breast irradiation only. Compared to patients not treated with chemotherapy, chemotherapy used >1997 increased the risk of CHF (HR=1·35 95%CI=1·00-1·83).

(C) Cardiac toxicity. In a series of experiments using mouse models with endoglin heterozygote (modified microvascular response and ApoE-/− mice (accelerated atherosclerosis) the effects of radiation alone and in combination with systemic therapies given for breast cancer (an anthracycline and Her-2 inhibition) on cardiac tissues and function were evaluated. Her-2 inhibition with lapatinib did not appear to worsen functional or histological damage caused by either radiation or Adriamycin in the model used.

(D) Microvascular (skin) toxicity. Building on mouse models of radiation-induced telangiectasia, we have performed observational studies on the skin of irradiated breast cancer patients, including a clinical intervention study of the effects of bisphosphonate on microvascular damage. This was to test the hypothesis that telangiectasia result from an influx of a macrophage lineage derived from bone marrow derived monoclonal cells, and that this can be inhibited by administration of a bisphosphonate. From the results we could conclude that radiation injury to the microvasculature is mediated though TGF-β, whereas the repair is modulated by the co-receptor endoglin and promoted by macrophages. (E) Radiation breast cancer carcinogenesis. BRIGHT study - breast cancer induction from Hodgkin Therapy - is a national nested case control study and forms part of an international collaboration in the European Gene-Rad-Risk Programme including 65S cases and controls.
In total 206 cases of breast cancer following Hodgkin lymphoma treatment have been included. Utilizing a novel method, detailed dosimetry of the radiation treatment and the dose delivered to the site of subsequent breast cancer has been estimated. This work is almost complete. Each patient has 3-4 controls matched for calendar year and age. Treatment and lifestyle factors are collected from the medical files and patient questionnaires. In addition patients donate blood for DNA and proteomics analysis of radiation sensitivity genes or other predisposing factors for radiation carcinogenesis. Using the same method we will also analyze the dosimetry for 220 UK patients.

Breast Cancer clinical trials consortia and collaborations

Within the BOOG network and the EORTC, the department takes part in a number of clinical trials and studies of breast cancer. These include the EORTC DCIS boost- no boost trial, the IRMA trial of post-operative external beam 3D conformal accelerated partial breast irradiation, and the BOOG male breast cancer study (prospective registration study). Trials that have finished accrual and are in active follow-up include the EORTC / BIG SUPREMO trial of post mastectomy chest wall irradiation in intermediate risk breast cancer, to which we have contributed 47 patients of the 1680 total inclusion (Co-chief Pt. Russell). Our department has made significant contributions to two EORTC breast cancer trials for which the final results have recently been published: the internal mammary – medial supraclavicular radiotherapy trial E22982 which demonstrated a benefit for the radiation arm with a HR for recurrence of 0.87 and an absolute survival benefit of 3%. The E1098 trial AMAROS after axillary mapping radiation or surgery was recently reported. The axillary survival benefit of 3%. The E1098 trial AMAROS after axillary radiation arm with a HR for recurrence of 0.87 and an absolute survival benefit of 3%. The E1098 trial AMAROS after axillary radiation arm with a HR for recurrence of 0.87 and an absolute survival benefit of 3%

COMBINATION OF RADIOTHERAPY AND CHEMOTHERAPY/BIOLOGICALS


Several clinical trials during the last decades clearly show that (concurrent) delivery of both chemotherapy and radiotherapy (chemoradiotherapy; CRT) significantly improves local control in a variety of advanced solid tumors. Major further improvement can be expected from the combination of radiotherapy and/ or chemotherapy with biological agents that specifically target deregulated pathways in tumor cells (bio-chemo/radiotherapy).

Head and neck

In September 2012, the ARTFORCE trial started. The original design of this randomized international phase II study was a 2x2 randomization between CRT with cisplatin or cetuximab and between adaptive dose redistribution or standard radiotherapy (268 patients with locally advanced oropharynx, oral cavity or hypopharynx squamous cell cancer). In May 2014, after inclusion of 24 patients, an amendment for this trial was accepted excluding the cetuximab treatment arms. Randomization between adaptive dose redistribution or standard radiotherapy is identical allowing analysis of all patients for the primary endpoints loco-regional control and toxicity. Furthermore, a new imaging modality, the HX4-labeled-PET scan, will be performed for all patients included in the study in the Netherlands to evaluate its predictive value for treatment outcome. To date, 61 patients have been included of which 23 were treated in the NKI. In October 2014, we started an olaparib dose escalation study in combination with radiation in patients with HPV-negative oropharyngeal or laryngeal squamous cell carcinoma. Olaparib inhibits PARP, an enzyme that is involved in the repair of DNA single strand breaks. Preclinical work shows that the inhibition of PARP by olaparib leads to radiosensitization. The main objective of this trial is to establish the maximum tolerated dose (MTD) of olaparib in combination with high dose accelerated radiotherapy (DAHANCA schedule). The accrual is ongoing. To date, 4 patients have been included. We are preparing an amendment to extend the inclusion criteria to patients with HPV-positive heavy smokers, N2b oropharyngeal or laryngeal cancer and all patients with locally-advance disease where cisplatin-based chemoradiotherapy is not possible. In addition a new phase I study enrolling the DNA-PKi MS2490484A as radiosensitizer was started. In this trial patients with an indication for palliative treatment in the head and neck area are treated with the combination of RT and the DNA-PKi. Patient accrual is ongoing and so far no extra toxicity was encountered. Both olaparib and DNA-PKi are examples of strategies to improve the effect of RT in combination with biological targeted drugs related to the DNA repair pathway.

Breast

In 2013 we started with an olaparib dose escalation study and radiation treatment in patients with inoperable, metastatic or inflammatory breast cancer. The primary endpoint of this study is to establish the MTD of the treatment of the breast and regional lymph nodes. Secondary endpoints are safety and pharmacodynamic endpoints in blood- and tumor samples. Patients are stratified for with or without skin sparing treatment. Three patients without skin sparing treatment and one patient with skin sparing treatment have been treated with respectively 50 mg BID and 25 mg BID. So far, no toxicity due to the addition of olaparib has been encountered in these patients.

Gastroenterology - Esophageal cancer

CRT followed by surgery is nowadays considered the standard of care for most patients in a good general condition with
Both postoperative CRT and perioperative chemotherapy are evidence-based strategies to improve outcome in operable esophageal cancer patients. The primary aim of this study is to quantify motion-based variation of the target volume over the course of CRT in esophageal cancer patient, and to use this information to calculate appropriate PTV (planning target volume) margins. As secondary objective of this study we aim to optimize imaging of the esophageal tumor by assessing the functionality of 4D-PET-CT and MRI for the esophageal tumor and to evaluate these modalities for staging, for early response assessment during CRT and for response monitoring after completion of treatment. These secondary endpoints will be analyzed in a multi-institutional setting, in collaboration with the University Medical Center Utrecht (UMCU) and MD Anderson Cancer Center. Furthermore, we started a pilot study in the NKI-AVL to explore four-dimensional imaging of PET-CT in esophageal cancer patients. FDG PET-CT image acquisition in the abdominal and thoracic region is influenced by organ motion. Respiratory movement blurs the metabolic signal of the esophageal tumor and lymph nodes. We hypothesize that the metabolic signal obtained with motion compensation results in higher SUV-max values and clearer demarcation of the esophageal tumor and lymph nodes.

In addition, we compared long-term outcomes of CRT between young and elderly (>70 years) esophageal cancer patients treated with curative intent. All esophageal cancer patients (n=253) treated between 1998 and 2013 in our institute with neoadjuvant or definitive CRT were retrospectively analysed. We found that elderly esophageal cancer patients (>70 years) treated with neoadjuvant CRT followed by surgery or definitive CRT had long-term outcomes, which did not differ from the outcomes of their younger counterparts. We concluded that for esophageal cancer patients, advanced age alone should not be a contraindication for CRT as a part of treatment with curative intent.

Currently, we are analysing the results of a prospective multicenter delineation study. The aims of this study are: (1) to study the possible additional value of PET to CT for GTV delineation; (2) to study institutional differences in CTV delineation and to improve uniformity of CTV delineation; (3) to study the possible additional value of MRI to PET-CT for GTV delineation. All Dutch institutes have participated in the parts of the study relating to aims 1 and 2. The MRI delineation will be performed in the MR dedicated institutes only, i.e. NKI-AVL and UMCU. Most delineations have been performed and analyses of the results are currently on going.

Finally, we are accruing patients into the national multicenter randomized trial of dose escalation in definitive CRT for patients with esophageal cancer (ART DECO) and a multicenter phase I trial evaluating the safety and efficacy of the addition of Trastuzumab to neoadjuvant CRT (CROSS regimen) for Her2+ esophageal cancer patients.

Gastroenterology – Gastric cancer

Both postoperative CRT and perioperative chemotherapy are evidence-based strategies to improve outcome in operable gastric cancer. The international phase III CRITICS study randomized patients with resectable gastric cancer to receive preoperative chemotherapy, surgery and postoperative chemoradiation, or preoperative chemotherapy, surgery and postoperative CRT. In April all 788 patients have been accrued and the first results are expected early 2016. Based on the observations that preoperative chemoradiotherapy is associated with better patient compliance as compared to postoperative regimens and preoperative treatment results in a high chance of obtaining disease downsizing/downstaging and microscopically radical resections, we designed the CRITICS-II trial to identify the optimal preoperative regimen in operable gastric cancer.

Together with our co-workers from the department of Surgery we have also demonstrated that postoperative CRT has a beneficial effect on both local control and survival after a microscopically incomplete (R1) resection. Studies on normal tissue toxicity after postoperative CRT for gastric cancer have demonstrated that IMRT limits renal damage in comparison to AP-PA and 3D-conformal radiation techniques. Liver damage was reversible. Furthermore we have demonstrated a radiation dose-dependent and progressive effect on the spleen. Vaccination policies could be based on this finding.

Gastroenterology – Rectal cancer

MRI-based functional imaging and response monitoring are now major subjects of interest for research in rectal cancer. More information on (differential) tumor and nodal movement is needed for practice changing treatment on the MR linac. For patients with rectal cancer this new treatment option is very promising, hopefully leading to more organ sparing treatment and less toxicity. The studies N13MRI (weekly multiparametric MRI), M14FMRI (marker near the tumor) and our project on library of plans for rectal cancer patients are all predicated studies for the MR linac. More information is needed on anatomical variation (position, shape, size) of the GTV. Furthermore, the value of functional MRI parameters to evaluate treatment response is subject of ongoing research. Based on these data, clinical studies for the MR linac are being developed with the aim to better select poor responders for boost treatment and margin reduction based on daily imaging. The library of plan strategy is feasible for rectal cancer patients and is being introduced in the clinic.

Lung - NSCLC

In the N11ORL phase I study that started accrual in 2012 our standard concurrent CRT (CCRT) regimen for locally advanced NSCLC is combined with dose escalation of the PARP inhibitor olaparib. The aim of the N11ORL study is to define the recommended dose of olaparib when combined with CCRT for locally advanced NSCLC. Since the opening of the trial in May 2012, ten patients entered the trial. Four patients entered the first dose level, olaparib 25 mg BID. New dose constraints for the Dmax of the esophagus were applied because of severe late esophagus toxicity. We switched to a TITE-CRM design. TITE-CRM has several advantages above the normally used 3x3 dose escalation design, as late toxicity can be included as a dose limiting toxicity and TITE-CRM allows continuous patient accrual. The third adaptation we made in the N11ORL was dose-de-escalation of olaparib of the CCRT treatment arm.

So far, six patients have entered the dose level of olaparib 25
mg QD in combination with CCRT. In this dose level, one patient experienced a thrombocytopenia grade 4 that was considered to be treatment-related and evaluated as a DLT. Another patient developed an esophageal ulcer grade 3 more than 3 months after end of treatment. This toxicity was also considered treatment-related and evaluated as a DLT. All together, the lowest dose level of olaparib 25 mg QD in combination with CCRT was considered to be above the maximum tolerated dose. The CCRT arm of this trial was therefore closed.

As we know that concurrent chemotherapy (cisplatin) substantially increases the risk of esophageal toxicity and olaparib sensitizes both cisplatin and radiation, we opened a second treatment arm in the N110RL study for NSCLC patients treated with sequential CRT. In this arm, patients are first treated with chemotherapy and if they have a favorable response they are treated in the study with radiation and olaparib (without concurrent chemotherapy). We included five patients at the dose level of olaparib 25mg QD without having seen any severe toxicity. Therefore, the dose is re-escalated to olaparib 25mg BID. In this dose level, so far two patients have been included. In both no additional acute toxicity is seen.

In the multicenter “PET-Boost trial” (M09PB0) dose-escalation is implemented by boosting the radiation dose within the primary tumor based upon biological activity on pre-treatment FDG-PET. Patients are randomized to receive the standard 66 Gy in 24 fractions with a dose-escalation of minimum 3 Gy to the primary tumor as a whole or to the volume with >50% SUVmax within the primary tumor. In both treatment arms, patients are irradiated to the same MTD to the lung and may receive CCRT. The study opened this year in Manchester (The Christie). Other contributing European sites are Leuven, Copenhagen, Maastricht, Amsterdam (AMC) and Eindhoven. Karolinska, Stockholm finished the dummy run procedure. A total of 10 randomizations were accomplished this year (total 90 randomizations). The trial was temporarily put on hold for data monitoring.

In 2015, a total of 85 locally advanced NSCLC patients were treated with CCRT consisting of 66 Gy in 24 fractions and daily low dose cisplatin. These patients are treated with standard daily 1 liter saline pre-hydration since 2013 and showed a tendency to a lower progression free survival compared to patients receiving pre-hydration on indication. The effect of pre-hydration on cisplatin kinetics was further investigated in mouse models. Pre-hydration reduces tumor platinum levels in mice, comparable to giving only half a dose of cisplatin. Further research is ongoing to elucidate this phenomenon.

Since June 2015 we started using a Simultaneous Integrated Boost technique for all patients with mediastinal lymph node involvement. While 66 Gy in 24 fractions is delivered to the primary tumor, 58 Gy in 24 fractions of 2.42 Gy is delivered to the involved lymph nodes (Biological Equivalent Dose 60 Gy). The reason the start with the SIB technique was twofold: the results from the RTOG 0617 and our “Big-Donut” analysis. This study investigated differences between local failure (LF) and regional failure (RF). In total, 226 patients treated with CCRT were analyzed. LF and RF as first event was seen in 37 primary tumors (16%) and 14 lymph nodes (6%). Volume, location and SUVmax were significantly associated with failure in univariate analysis. In multivariate analysis, volume remained the only significant factor. Because the (mostly) smaller volumes of the involved lymph nodes compared to the volume of the primary tumor we decided to de-escalate the dose to the mediastinal lymph nodes.

We also investigated long-term OS of 102 patients in the RADITUX trial (randomized between February 2009 and May 2011), a multi-center randomized phase II (NTR2230) with the initial aim to assess the effect of additional cetuximab to CCRT in LA-NSCLC patients (stage II/IIIA/B). Arm A received high dose radiotherapy (24 x 2.75 Gy) and concurrent daily low-dose cisplatin (6 mg/m2). Arm B received an identical treatment regimen with additional weekly cetuximab (400 mg/m2 loading dose one week prior to radiotherapy followed by weekly 250 mg/m2). OS from February 2009 was calculated as time from randomization until death from any cause or January 2015. Median OS was 31.5 months, not significantly different between arms A and B; 33.0 and 30.0 months. 1-, 2- and 5-year OS rates were 74.5%, 59.4% and 37.3%, respectively. Although the addition of cetuximab was not associated with increased OS, the median OS for the entire group was remarkably high; 31.5 months. This excellent 5 year OS has not been reported before in an unslected group of LA-NSCLC patients treated with CCRT. The results were presented at the World Conference on Lung Cancer in Denver.

In the N12LPR protocol weekly FDG PET/CT scanning in NSCLC patients during CCRT is investigated to correlate early FDG-PET/CT responses during CCRT with outcome. Currently, 25 patients had a weekly FDG-PET/CT during treatment.

**Lung - SCLC**

Small cell lung cancer (SCLC) accounts for approximately 15% of lung carcinomas. At the time of diagnosis, approximately 30% of patients with SCLC will have limited-stage disease (LS), i.e. tumors confined to the hemithorax of origin, the mediastinum, or the supraclavicular lymph nodes. Patients with tumors that have spread beyond these areas are staged as extensive-stage disease (ES).

For LS-SCLC, the combination of chemotherapy and thoracic radiotherapy is the standard treatment. Two meta-analyses have shown that thoracic radiotherapy given concurrently with chemotherapy improves both local control and survival. Nevertheless, several important questions including the optimal total radiation dose and radiation fractionation remain unanswered. In 2009 the EORTC started an international, multicenter randomized phase III trial comparing twice daily radiotherapy with high dose radiation delivered once daily, both given concurrently with standard cisplatin and etoposide chemotherapy (CONVERT trial). In July 2013 the target of 532 patients (4 patients from the NKI-AVL) was reached and recruitment was closed. Results of this trial are expected in 2016.

For ES-SCLC, chemotherapy is the cornerstone of treatment. However, over 75% of patients have persisting intra-thoracic disease after initial chemotherapy, and about 90% manifest intra-thoracic disease progression at 1 year after completing initial chemotherapy. In the absence of promising systemic agents that can improve local response, the role of thoracic irradiation in patients with ES-SCLC was evaluated in a multicenter phase III randomized trial initiated by the VUmc (CREST trial). The objective of this study was to investigate whether thoracic radiotherapy can improve 1-year survival, following a response to chemotherapy. A total of 498 patients...
have been treated (50 patients from the NKI-AVL) and the trial was closed in December 2012. In 2014, the results of this trial were published in the Lancet. Although the difference was relatively small, a significant overall and progression-free survival benefit for thoracic radiotherapy was demonstrated. An additional analysis, published in the Lancet in 2015, showed that in patients with residual intrathoracic disease, the overall survival was significantly longer in the thoracic radiotherapy group. This suggests that the presence of residual intrathoracic disease after chemotherapy is a factor that should be considered in patient selection. Currently, further analysis of the CREST study data is being done and plans for a subsequent trial are under preparation.

Prophylactic cranial irradiation (PCI) is the standard treatment in patients with small cell lung cancer (SCLC) without progression after chemo-radiotherapy in stage I-III disease and after having a remission after chemotherapy in stage IV. PCI results in a significant decrease in the incidence of brain metastases, and also has a minor but significant effect on overall survival. Adverse neurocognitive effects (fatigue, nausea, neurocognitive decline, and ataxia) however may be mild and transient, but can also be progressive and persistent. In collaboration with the division of Psychosocial Research and Epidemiology (Sanne Schagen) we initiated an international phase III trial (NCT01780675) (NKI 2013-6096). Investigating Hippocampal Avoidance-Prophylactic cranial irradiation (HA-PCI) in SCLC patients (M12PHA). Patients with SCLC are randomized to receive PCI with or without Hippocampal Avoidance (HA). The primary objective is assessment of hippocampus dependent memory functioning at 4 months after PCI with or without hippocampal sparing by the Hopkins Verbal Learning Test-Revised. Secondary objectives are assessment of other neurotoxicity and quality of life, radiological brain abnormalities, evaluation of the incidence and location of brain metastases and overall survival. The trial opened also in Eindhoven, Groningen, Leuven, Antwerp and Ghent and this year Amsterdam (AMC), Rotterdam and Tilburg opened as well. In 2015, 38 patients have been randomized (total 61) (figure 5).

Accurate delineation of the hippocampus is crucial for this trial. We evaluate the hippocampus delineation variability on 5 trial patients among 7 radiation oncologists according to the RTOG guideline and investigated the influence of delineation variability on dose planning. Although a high variability among observers was detected especially in dorsoventral direction (figure 6) the 5 mm margin around the hippocampus seems to cover the delineation variation (figure 7).

**SBRT**

High precision, high dose per fraction SBRT is increasingly applied in our department for early stage NSCLC or metastatic sites from other cancers. In 2015 a total of 280 patients were treated. We analyzed the overall survival of 634 patients treated with SBRT from 2006 until 2013. A total of 407 T1 tumors, 110 T2 tumors and 117 metastases were irradiated. The follow-up was until Oct 2014. The collaboration with the ELEKTA research group (together with Toronto, Wuerzburg, Beaumont and Philadelphia) resulted in several multi-center analyses of patients treated with SBRT. Within this group the VOLUMES trial (M11VOL) for peripheral tumors larger than 5 cm or 2
tumors, accrued 46 patients (of which 43 from NKI-AVL): 25 in group A (>5 cm) and 21 in group B (>2 tumors). Starting from this year Toronto opened for accrual. As a result, all centers are now actively involving. The phase I “Hybrid study” (N12HYB) investigating the safety of combined stereotactic radiotherapy and conventional fractionated CRT in stage II and III NSCLC with peripheral tumors (<5 cm) accrued in total 11 patients. Results will be presented at ESTRO 2016.

Urology – Bladder
The standard treatment for muscle invasive bladder cancer is cystectomy. For those not eligible for this type of treatment, or for those opting to preserve their bladder, the alternative treatment is radiotherapy preferably in combination with chemotherapy. Although CRT provides excellent bladder preservation rates and survival comparable with those following cystectomy, CRT is rarely applied which prompted us to search for less toxic and more effective bladder-preserving strategies, with a special focus on new radiosensitizers. Like in several other tumor types, in bladder cancer EGFR status is associated with poor outcome and radiosensitivity. Given the very high affinity for EGFR in combination with the favorable toxicity profile, the combination of radiotherapy with concurrent panitumumab is very attractive to explore in bladder cancer. In close cooperation between the departments of Urology, Medical Oncology and Radiotherapy, we initiated a phase I trial evaluating the combination of fractionated radiotherapy with panitumumab. Primary endpoint of the study is the acute toxicity rate. Secondary endpoints are the complete response rate at 3 months, local control rate at 6, 12, and 18 months, and at 2 years, bladder preservation rate and any grade 3 or 4 adverse event during and within one month after completion of therapy. Exploratory endpoints are % EGFR expression, RAS mutational status, response rate in correlation with EGFR and RAS status and response rate in relation to treatment path. This trial has been completed and the and the final results will be out soon. The “panitumumab” trial has led to renewed interest for bladder preservation by the urologist community and we recently initiated a multicenter prospective phase II trial evaluating the outcome of induction chemotherapy followed by extended lymph node dissection and CRT for high risk invasive bladder cancer, the CHEMORAD trial. This trial has recently been approved by our medical ethical committee and will soon be open for accrual.

Gynaecology
In locally advanced cervical cancer, the treatment of choice is a combination of external beam CRT for 5 weeks followed by (chemo) brachytherapy. Since 2012 patients in our institute are treated with Image-Guided Adaptive Radiotherapy with library of plan (LoP) VMAT irradiation and MRI based adaptive brachytherapy for the treatment of cervical cancer. We evaluated the clinical use and early outcome of this adaptive treatment and concluded that imaged guided adaptive LoP protocol for cervical cancer based on daily CBCT is feasible with similar early clinical outcome as the non-LoP treated patients. Online CBCT prior to treatment are of sufficient quality to select one out of four plans at the accelerator.

In 2015 our goal was to reach a D90 of the HR-CTV >90 Gy EQD2 for most patients, a prerequisite for participation in the international EMBRACE 2 study. We succeeded in that and in 2016 we participate in the EMBRACE 2 study. A sub study in the EMBRACE 2 will be plan of the day radiation. Together with Aarhus and Leuven we participate in an repetitive MRI-based functional imaging and response monitoring study in locally advanced cervical cancer. This M13IMA study evaluates the sensitivity and specificity of DWI-MRI to identify patients who will develop local failure after cisplatin-based CRT of cervix cancer. Before treatment, in week 3 of treatment and every brachy insertion an extra functional MRI is performed to establish functional MRI parameters of the tumor during radiation treatment and in follow up 3 months and 1 year after treatment for response evaluation. So far, 11 patients have been entered in the study. This data will give us data for predicting local tumor control after radio-chemotherapy. Currently we are in the designing phase for MR linac studies. Patients with endometrial and cervical vaginal recurrences are often treated with a combination of (chemo) radiation and brachytherapy using the multichannel vaginal cylinder (MVC). We evaluated our first experience with the MVC. Since the clinical introduction of image guided adaptive MRI based brachytherapy using a MVC, 25 patients with vaginal recurrence were treated with curative intent in our department. The clinical treatment protocol accounts for the uncertainty in rotation of the cylinder with respect to the target area. By means of an imaging study, we have found the magnitude of the interfraction rotation to be 6 degrees (1 SD). We performed a simulation study to assess the impact of rotations on the main plan parameters (target D90 and OAR D2cc). Results show that the impact of the rotations is limited.

Soft tissue sarcoma
In 2012 we started a multicenter phase I study in sarcomas of the extremities and head and neck, evaluating the safety and feasibility of standard preoperative radiotherapy combined with dose-escalated pazopanib, a small molecule inhibitor of VEGFR. The rationale behind this combined modality treatment is multifactorial: VEGF targeted therapy results in normalization of tumor vasculature which might improve the oxygenation of the tumor and greater efficacy of radiation; both radiation and VEGF-blocking agents target tumor-associated endothelial cells to induce apoptosis; radiation induced upregulation of VEGF is counteracted by VEGF-targeted therapy. By including the last patient in the highest dose level, the study was successfully completed in 2014.

In 2015 a 35 patient confirmatory prospective phase II study will expand the highest dose level of once-daily 800 mg pazopanib concurrent with 25x2 Gy radiotherapy. Primary endpoint will be the induction of a pathological response. As side studies repeat DWI-MRI and repeat biopsies will be performed during treatment. The study will also be opened in the Royal Marsden Hospital, London, UK. Myxoid liposarcomas are known for their radiation sensitivity on preoperative radiotherapy to 50 Gy in 5 weeks. A marked volume reduction can already be appreciated from the third week and this shrinkage continues until definitive surgery. Based on these clinical observations a multicenter prospective phase II clinical trial is opened to investigate the feasibility of lowering the neoadjuvant radiotherapy dose to 36 Gy. Due to the rarity of the disease the study is accruing slowly but steadily. As of autumn 2014, large international sarcoma hospitals are activated as participating centers (London and Boston); other European medical centres in the USA are following this study. 

Together with Aarhus and Leuven we participate in an repetitive MRI-based functional imaging and response monitoring study in locally advanced cervical cancer. This M13IMA study evaluates the sensitivity and specificity of DWI-MRI to identify patients who will develop local failure after cisplatin-based CRT of cervix cancer. Before treatment, in week 3 of treatment and every brachy insertion an extra functional MRI is performed to establish functional MRI parameters of the tumor during radiation treatment and in follow up 3 months and 1 year after treatment for response evaluation. So far, 11 patients have been entered in the study. This data will give us data for predicting local tumor control after radio-chemotherapy. Currently we are in the designing phase for MR linac studies. Patients with endometrial and cervical vaginal recurrences are often treated with a combination of (chemo) radiation and brachytherapy using the multichannel vaginal cylinder (MVC). We evaluated our first experience with the MVC. Since the clinical introduction of image guided adaptive MRI based brachytherapy using a MVC, 25 patients with vaginal recurrence were treated with curative intent in our department. The clinical treatment protocol accounts for the uncertainty in rotation of the cylinder with respect to the target area. By means of an imaging study, we have found the magnitude of the interfraction rotation to be 6 degrees (1 SD). We performed a simulation study to assess the impact of rotations on the main plan parameters (target D90 and OAR D2cc). Results show that the impact of the rotations is limited.

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and US centers are considering participation as well (including PMH Toronto, Canada, the entire Scandinavian Sarcoma Study Group and the Universities of Mannheim, Germany and Leuven, Belgium).

Within the European Organization for Research and Treatment of Cancer a randomized phase III clinical trial called “STRASS”, has been opened to investigate the role of neoadjuvant radiotherapy in retroperitoneal sarcomas. The study was opened in the beginning of 2013 and is accruing well (as per November 2015 180 of 256 patients randomized).

**OUTCOME MODELING**

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Heart dose is associated with shorter overall survival for patients treated with chemoradiation for locally advanced NSCLC

Traditionally, sparing the heart in chemoradiotherapy of locally advanced lung cancer has a low priority compared to the lungs and esophagus. Recently, however, the randomized phase III trial RTOG 0617 showed that the volume of the heart receiving a dose of at least 5Gy (V₅) was associated with a lower overall survival (OS). The aim of the current study is to validate this in an independent database. To that end, 375 patients treated with IMRT (24x2.75Gy and daily low-dose cisplatin) were retrospectively selected. For the heart both Vₓ (5≤x≤50) was calculated. Median follow up was 16 months and median OS was 26 months. Using univariate proportional hazard modeling Vₓ for x≤40Gy was significantly associated (p≤0.05) with OS. For V₅, which was most significant in the analyzed set, the hazard ratio (HR) was 1.008. When pts are split at the median V₅ = 37.0%, the median OS was 29 ± 2.5 months versus 19 ± 2.4 months for pts below and above the median respectively (p=0.03, Log Rank). Similarly, the illustrates significant separation in Kaplan-Meier plots of OS with the pts divided in V₅ quartiles. Both GTV (p≤0.001, HR=1.001) and V₅ (p=0.003, HR=1.007) were significant in multivariate analysis. In the multivariate analysis the correlation between GTV (median volume 109 cc) and Vₓ was less than 0.15, indicating that a higher heart dose is not the effect of larger tumor volumes and hence a worse survival due to more advanced decease. In conclusion, the dose received by the heart is strongly associated with overall survival in locally advanced lung cancer patients treated with chemoradiation (figure B).

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TCP modeling in a large cohort of NSCLC patients inclusive of geometric uncertainties

TCP model parameters are typically derived using the CTV planned dose which do not reflect the presence of geometric uncertainties. In this study TCP model parameters were derived including geometric uncertainties. To that end, a multi-institutional database containing 693 NSCLC patients of which 530 SBRT (stage-I), and 163 treated with concurrent chemoradiation with volumetric image guidance in all fractions was used. The median follow-up was 1.13 and 1.5 years respectively in the chemoradiation and SBRT regiments. To account for day-to-day geometric uncertainties, the dose of the PTV and the volume of the GTV (to calculate the total clonogens in the GTV) were used to optimize the ‘Marsden TCP’ model. The α/β-ratio and clonogen density was fixed to 10 Gy and 1e7 cells/cc respectively. The best-fit parameters were estimated at $\alpha=0.38(0.32-0.45)$Gy$^{-1}$, $\sigma_{\alpha}=0.18(0.14-0.26)$Gy$^{-1}$ and equivalent uniform dose (EUD) $a=-0.3(-1.1-\infty)$. The AIC of a PTV based method was 32.8 units better than a CTV based method. The separate analysis of SBRT and chemoradiation did not yield a superior TCP model ($p=0.28$). In conclusions, the tumor control probability of chemoradiation and SBRT regiments were successfully described by a single model. An EUD approaching the PTV mean dose best described the TCP indicating that geometric uncertainties play a role despite accurate image guidance.

Early detection of esophagitis using FDG PET/CT during lung cancer chemoradiotherapy

Biologically adaptive radiotherapy depends on early detection of response of tumor and/or normal tissue to allow timely adaptation of the remaining fractions. To that end, we are performing an ongoing study with weekly FDG PET/CT in non-small cell lung cancer patients during therapy. The aim of this study was to investigate the feasibility to detect early response of the esophagus. All nine patients received 24x2.75 Gy concurrent with daily low dose cisplatin. A region of interest (ROI) was defined as the part of the esophagus planned to receive more than 40 Gy, excluding the GTV and lymph nodes with a 5 mm margin. For this ROI cumulative FDG uptake histograms were made for each weekly scan. In 7 out of 9 patients, a significantly increased SUV in the esophagus (SUV≥4) could be seen before fractions 16 and/or 21. The increased uptake occurred in regions of the esophagus planned to receive more than 63 Gy. However, the cumulative minimum dose at which esophagitis was first detected on PET ranged from 34 to 54 Gy over the 7 patients. The patients without PET esophagitis had a planned esophagus dose of <50 Gy. In all follow-up scans the FDG uptake in the esophagus had subsided. In conclusion, this study shows that FDG uptake in the esophagus increases during chemoradiation at varying delivered doses and can be used to gauge early radiation damage. There was, however, no evidence of early PET or CT changes in the healthy lung receiving >40 Gy.

Effect of prehydration on progression free survival in NSCLC patients treated with low dose chemoradiation

Renal toxicity is a frequently occurring side effect in patients with locally advanced non-small cell lung cancer (LA-NSCLC) treated concurrently with daily low dose cisplatin and radiation. Previously, only patients with a decline in renal function were pre-hydrated with saline prior to treatment in order to reduce this toxicity. Since 2011 daily prehydration (PH+) has become standard practice for all low-dose cisplatin chemoradiation patients treated for LA-NSCLC. The aim of this study was therefore to evaluate the effect of prehydration on progression free survival (PFS). To that end, a total of 418 LA-NSCLC patients treated with chemo-radiotherapy in were included in this retrospective study. The patients were divided into two time periods; 2007-2010 without standard prehydration (PH-) and 2011-2013 with PH+. The median OS for PH- vs PH+ was 25 and 22 months, respectively ($p=0.53$) with a median follow up of 56 and 20 months. The median PFS for PH- vs PH+ was 14 and 11 months respectively ($p=0.11$, see figure 8). There was, however, also a significant difference in the median GTV of 100 cc in the PH- and 81 cc in the PH+ groups ($p<0.05$). Therefore multivariate Cox regression analysis was performed, in which PH+ was associated with a hazard ratio (HR) of 1.24 (CI: 0.98-1.58; $p=0.08$) and GTV with a HR=1.008 per 1 cc (CI: 1.016; $p=0.06$). In conclusion, prehydration in LA-NSCLC patients treated with daily low dose concurrent chemoradiation appears to reduce PFS. Further research into the effect of prehydration on cisplatin kinetics within the patient is required.

Significant reduction of acute toxicity after IG-IMRT compared to 3D-CRT in prostate cancer patients

Image-guided IMRT (IG-IMRT) is associated with significant dose reductions to organs at risk (OAR) compared to 3D-conformal radiotherapy (3D-CRT) in prostate cancer patients. However, clinical data identifying the benefits of IG-IMRT in patients treated in daily practice are scarce. The purpose of this study was to compare dose distributions and acute gastrointestinal (GI) and genitourinary (GU) toxicity levels of prostate cancer patients treated to 78 Gy (39x 2 Gy) with either IG-IMRT or 3D-CRT. To that end, 215 patients treated with 3D-CRT and 260 patients treated with IG-IMRT both to 78 Gy were included in this analysis. Applied margins were 10mm (3D-CRT) and 5-8mm (IG-IMRT). Dose surface histograms and toxicity questionnaires were compared. IG-IMRT resulted in significant lower median volumes receiving 5-75 Gy (all $p$ values <0.001) for anorectum (figure 9a), anal canal and bladder. The mean dose to the anorectum was 34.4 Gy vs. 47.3 Gy, 23.6 Gy vs. 44.6 Gy for the anal canal and 33.1 Gy vs. 43.2 Gy for the bladder (all $p<0.001$). Acute toxicity reached a maximum at fraction 30 for most endpoints, as shown for proctitis grade ≥2/≥3 in figure 9b. After adjusting for risk factors at MV analysis, IG-IMRT resulted in significantly lower overall GI grade ≥2 RT0G toxicity (29% vs. 49%, $p=0.002$, odds ratio (OR) 0.49) and overall GU grade ≥2 toxicity (38% vs. 48%, $p=0.009$, OR 0.59). In conclusion, clinically relevant reductions were observed in acute GI and GU toxicity for patients treated with IG-IMRT compared to 3D-CRT. This is the result of significantly lower doses to OARs, achieved by improved techniques and tighter margins.
This research line aims to optimize surgical procedures by better surgical guidance during operative procedures. To this end new imaging technologies are developed and tested to improve tumor mapping and staging pre and intra-operative. These imaging and surgical guidance procedures should lead to more radical resections while sparing normal tissue and organ function. The research line is a strong collaboration between the NKI-AVL, Technical University Twente and industrial partners. An innovative concept was developed and it concentrated around the sequential steps within the surgical work flow: tumor localization, tumor treatment and assessment of tumour treatment. For the first step an electromagnetic navigation system was designed which guides the surgeon with high accuracy to the place of tumour tissue. The second step, surgical resection, is monitored by optical spectroscopy, able to differentiate real time tumour tissue from normal tissue at the tip of the surgical instrument. Ex vivo tissue specimens from more than hundred patients were analyzed showing an accuracy of the technique of over 90%. These results were confirmed by extensive in vivo testing in a controlled setting in breast cancer, liver metastases and lung tumors. Currently, we concentrate to incorporate the developed technology into surgical tools which should enable radical resection of all tumour tissue. In the third step, success of treatment is evaluated by multispectral camera images of the specimen and resection area to assure that all tumour tissue has been removed. We succeeded to bring these techniques from bench to bedside and currently we are introducing this technology within the surgical work flow. Technology development was allied to the design and realization of a new operating theatre complex with specific facilities for image guided surgery. After safely bringing the technology for image guided surgery into the surgical work flow, the technology is now being evaluated within a broad range of surgical procedures and tested for efficacy and cost effectiveness. 2015 was highlighted by the first intra-operative abdominal navigation procedure after years of experimental work. The board of directors decided to support image guided surgery with extra funding for the next 4 years. This funding will be used to further implement image guided surgery within the wide variety of disciplines within the section of surgical oncology.
**SURGICAL ONCOLOGY**

**Breast cancer**
Hester Oldenburg, Marie-Jeanne Vrancken Peters, Frederieke van Duijnhoven, Emiel Rutgers, Jos van der Hage, Matthijs Nijenhuis, Lotte Elshof, Suzana Teixeira, Bas Pouw, Iris van der Ploeg, Natasja Janssen, Marieke van der Noordaa

**Ductal Carcinoma in Situ (DCIS)**
We are setting up a large worldwide randomized controlled, phase 3, open-label, non-inferiority trial to evaluate the safety of active surveillance in 1240 women with Low-Risk DCIS (LORD). The LORD trial is coordinated by the BOOG and EORTC. The study is expected to open in Q1 2016.

**Sentinel node studies**
Final results of the AMAROS were published. In this trial it was shown that axillary radiotherapy instead of axillary lymph node dissection in patients with primary breast cancer and a positive sentinel node provide comparable regional control with fewer side effects. Ongoing research is been done on treatment or not of axillary and extra-axillary lymph nodes in breast cancer.

**Neoadjuvant chemotherapy (NAC) and local treatment**
Following NAC, a pathologic complete response (pCR) of the breast tumor is seen in 10-70% of patients, depending on tumor subtype. In pCR patients, surgical resection of (part of) the original tumor bed will likely not contribute to local control. However, current imaging techniques are not sufficiently reliable to identify pCR patients. We therefore initiated a diagnostic study in which the value of post-NAC biopsies in assessing pCR is investigated. First results are expected in 2016.

**Radioactive Seed localisation**
We have shown that the usage of the 125I seeds is a valuable tool to fine-tuning breast-conserving therapy (BCT) in the primary surgery setting and after neoadjuvant chemotherapy. We evaluated its use in combination with the sentinel node procedure and published a meta-analysis of its international use at present. Furthermore we evaluated the use of multiple I125 seeds in extensive DCIS to facilitate BCT. To facilitate resection of non-palpable lesions we investigated the use of intra-operative 3D navigation.

**Psychosocial effects of breast cancer treatment**
In cooperation with the PSOE division a RCT on internet-based cognitive behavioral treatment of sexual dysfunction after breast cancer was initiated, funded by the KWF. An interactive portal and empowerment study for breast cancer patients is been done, funded by KWF/Alpe d’HuZes. Forth going studies are been done on cognitive behavioral treatment for menopausal complaints due to breast cancer treatment. (KWF and Pink Ribbon funded).

**Nabon Breast cancer audit**
As board members of the NBCA we have contributed to the annual report of DICA. Furthermore, we have received two KWF grants for investigating the variation of the use of direct reconstructions after ablative surgery and the use of...
Aletta Houwink MD Academic staff
Lenie Hulshoff MD Academic staff
Sefik Karagozoglu MD Academic staff
Anne Lukas MD PhD Academic staff
Anita Rothenatter-Oplop MD Academic staff
Peter Schutte MD Academic staff
Michael Šrámek MD PhD Academic staff
Liang Tjoa MD Academic staff
Ingeborg Vergouwe MD Academic staff
Marchien van der Weide MD Academic staff
Esther Wolthuis MD PhD Academic staff

Publications

Amant F, Verheescke M, Wlodarska I, Dehaspe L, Brady P, Brison N, van Den Bogaert K, Dierickx D, Vandenabeele V, Tousseyn T, Moerman P, Vanderheecke A, Vergote I, Neven P, Bertelsot P, Putseys K, Dannes S, Van Denberghe P, Legius E, Vromens J, VandenHerpe M, Pierpont M. Prognostic biomarkers for prognosis and more importantly predictive biomarkers for response to treatment. A research grant has been received from GSK/Novartis for this project. For this purpose, there is also a strong collaboration with the NKI for Translational Research together with Daniel Peepers. In parallel, the group is reporting on important clinical topics. Finally, the group is developing new techniques, such as the Robot surgery for the groin (JOS van der Hage leads this Robot program).

Specific Studies:
- EORTC 1325 (Adjuvant Pembrozulmab versus placebo for stage III/IV melanoma)
- MSLT-2 study (CLND vs. OB after Positive SN) will report first results in 2016-2017
- EORTC 1208 Minitub registry for minimal SN Tumor Burden, Alexander van Akkooi is the world-wide PI.
- EORTC 1325 (Adjuvant Pembrozulmab versus placebo after CLND for stage IIIA (>1 mm), IIIB/C melanoma)
- OPACIN study (neo-adjuvant combination IPI+NIVO versus adjuvant IPI+NIVO for palpable groin/axilla metastases)
- REDUCTOR study (8 weeks of Dabrafenib & Trametinib for irresectable stage IIIB/C).

In-transit Metastases
As 1 of the 3 National ILP (Isolated Limb Perfusion) Centers, we get frequent referrals and we are updating the database. For the non-extremity, we will participate in a study with T-VEC (Oncolytic Virus) + Pembrozulmab vs. Pembrozulmab alone.

DMTR
Nowadays, all stage IIIIC/IV patients in the Netherlands are registered in the Dutch Melanoma Treatment Registry (DMTR). The objective is to assure quality of patient selection and treatment with these drugs and to provide real world data for their (cost)effectiveness. Michel Wouters is co-founder and board member of the DMTR.

Colorectal liver metastases
Theo Ruers, Koert Kuhlmann, Niels Kok, Frits van Coevorden
Research is focused on imaging and local tumor destruction. Local tumor destruction: This year the long term results became available from the CLOCC study, the first randomized multicenter study evaluating the efficacy of RFA in colorectal liver metastases. Median OS was significantly prolonged in the RFA arm versus the control arm, respectively 45.6 and 40.5 months. We also analyzed the data on local recurrence after liver resection or radiofrequency ablation (RFA) as observed in two international practice changing randomized studies (EORTC-
EPDC and EORTC-CLOCC). It was observed that for lesions smaller than 4 cm local recurrence was similar between RFA and resection. In addition, we developed a new method for effective monitoring of RFA ablations by a new optical spectroscopy device. Another clinical trial (Silent) on the effect of primary tumor resection on the outgrowth of colorectal liver metastases is still running.

Imaging: In collaboration with the University Twente and industry we have developed 3D segmentation models for visualizing colorectal liver metastases during surgery. We introduced imaging software on the iPad that can be used for intraoperative orientation during liver surgery. We are now focusing on superimposing detailed pre-operative anatomical images and tumor positions on real time video screens during liver surgery creating an augmented reality setting during surgery. In collaboration with industrial partners the use spectroscopy for tumor diagnosis and guidance of minimal invasive procedures is investigated.

The research line is funded by CTMM, KWF, University Twente, EORTC and industrial partners.

Colorectal surgery and HIPEC
Arend Aalbers, Geerard Beets, Koert Kuhlmann, Theo Ruers, Karlijn Woensdregt, Niels Kok, Alexander Veenhof, Hanneke van Eden

In 2015 Geerard Beets joined our team and Koert Kuhlmann came back after his KWF fellowship. By welcoming Geerard Beets we broadened our expertise in “Wait-and-See” policy after (near-)complete response to chemoradiotherapy for distal rectal carcinoma. Our research projects are divided in three categories: Clinical outcome studies, imaging studies and translational studies. Considering rectal cancer the main research focus has been on organ sparing therapy after chemoradiotherapy for patients with distal rectal carcinoma. Main focus in HIPEC has been on establishing a nationwide standardized protocol and setting up a randomized prophylactic HIPEC study for patients with T4 colon carcinoma. Imaging studies with perioperative navigation techniques have been carried out and optical techniques for the imaging of tumor tissue have been studied. Translational research is done by our PhD student Hanneke van Eden. She developed 3D organoid cultures from tumor cells derived from resection specimens. Chemoresistance- and sensitivity studies are being done with the organoid cultures.

Oesophageal and gastric cancer
Johanna van Sandick, Frits van Coevorden, Koen Hartemink, Xander Veenhof, Jurriën Stiekema, Rosa van der Kaaij

An important topic in oesophagogastric cancer research is improvement of pre-treatment patient selection. Various ways towards individualised therapy are being explored. In an earlier series, we have demonstrated that intestinal and diffuse type gastric cancer differ in biological behaviour and treatment outcome. In oesophageal adenocarcinoma the same subtypes are found. Efforts are being made to investigate epidemiological differences between these subtypes by means of the National Cancer Registry and the nationwide pathological registry. Also, to study the impact of histological subtype on treatment outcome, a database was developed involving all patients treated in our institute since 1998 for oesophageal cancer with curative intent (n=253).


Bex A. Classification of renal cell carcinoma subtypes: there is more than meets the eye. Eur Urol. 2015;67:98-9


Bleeker MCS, de Rooij J, Trum JW. Giant Condyloma of the Uterus: Migrating Disease? Gynecol obstet res obst. open J 2015:2:45-48


De Brée R, van den Brekel MW. Elective neck dissection versus observation in the clinically node negative neck in early oral cancer: Do we have the answer yet? Oral Oncol. 2015;51:963-5


In a potentially curative setting, oesophageal cancer is treated with a combination of neoadjuvant therapy and surgery or definitive chemoradiotherapy. These treatments are often withheld from elderly patients. In our series (n=253), similar long-term outcomes were obtained in younger and elderly (≥ 70 years) oesophageal cancer patients. These data show that advanced age alone should not be a contraindication for treatment with curative intent in oesophageal cancer patients. In a surgical case series (n=13), the use of a 3D scope for a thoracolaparoscopic oesophageal cancer resection proved to be safe and feasible. The surgical technique is currently in practice.

Standard treatment of gastric cancer patients with peritoneal carcinomatosis is palliative systemic chemotherapy. In a feasibility study, Hyperthermic Intraperitoneal Chemotherapy (HIPEC) combined with cytoreductive surgery and gastrectomy is being investigated as treatment option. After identifying the optimal dose level of intraperitoneal chemotherapeutic agents (oxaliplatin and docetaxel) in this patient group, a randomised multicenter study will be conducted.

The clue in individualised cancer therapy is expected to be found in genetic profiling. Fresh frozen endoscopic biopsy samples of oesophageal cancer patients have been collected in our institute since 2008. Tissue sampling protocols were optimised and tumor biopsies of gastric cancer patients are now also collected.

**Thoracic surgery**

Johanna van Sandick, Houke Klomp, Michel Wouters, Koen Hartemink, Xander Veenhof, Hes Broxk, Rachel Numan, Chris Dickhoff, Niels Vos, Pleunie Hooijman, Matthijs van Gool, Wilson Li, Jelmer Oor, Carolien Bulte, Idris Bahce

Clinical innovations in surgical treatment for NSCLC include minimally invasive surgical techniques such as video assisted thoracoscopic surgery (VATS), 3D-VATS and robotic surgery. Scientific research includes activities related to changes in clinical and shared decision making (for example surgery vs. stereotactic radiation for stage I NSCLC). Several studies were done focusing on diaphragmatic function, cardiac function and anesthetic aspects during lung cancer surgery.

**Soft Tissue Sarcomas**

Frits van Coevorden, Jos van der Hage, Houke Klomp, Winan van Houdt

National and international collaboration has been the focus of our group in the last years.

At the national level, we have as AVL actively participated and initiated the setup of a national GIST registry and have been actively involved in the design and analysis of the data from national and international GIST studies focusing on TKI treatment. At the national level, we have as AVL actively participated and initiated the setup of a national GIST registry and have been actively involved in the design and analysis of the data from national and international GIST studies focusing on TKI treatment. At the national level, we have as AVL actively participated and initiated the setup of a national GIST registry and have been actively involved in the design and analysis of the data from national and international GIST studies focusing on TKI treatment.

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Quality assurance
Michel Wouters, Johanna van Sandick, Arend Aalbers, Koen Hartemink, Marie-Jeanne Vrancken-Peeters, Emiel Rutgers
Several medical specialists from the Netherlands Cancer Institute are involved with the nation-wide clinical audits of the Dutch Institute for Clinical Auditing (DICA), which was founded in 2011. DICA facilitates 20 nation-wide clinical audits, in which colorectal, breast, esophageal, gastric, hepatic, pancreatic, gynaecologic cancers as well as melanoma treatments in the Netherlands are evaluated. The audits are based on a web-based data-collection program that generates continuous and benchmarked feedback to the medical teams in Dutch hospitals. The multidisciplinary experts in the scientific committees evaluate the results of the audit periodically, develop sets of quality indicators, initiate and supervise analyses of the data with the aim to monitor and improve quality of cancer care on a national, regional and individual hospital level. Substantial improvements in guideline adherence and patient outcome have been demonstrated after initiation of the audits. The results are published in peer-reviewed scientific journals on a regular basis. Especially, the detailed population-based clinical data and methodological aspects of quality measurement, make an important contribution to medical literature. In the last year a multidisciplinary audit has been developed for lung cancer, the Dutch Lung Cancer Audit, that unites the existing radiotherapy and surgical audits, with a new audit comprising diagnostics and systemic treatments of lung cancer patients. Also, important initiatives have been taken to add information on Patient Reported Outcomes to the existing clinical audits, in order to provide a more complete insight in the quality of care provided by Dutch hospitals.

Ferres DM, Kema IP, Buist MR, Nijman HW, Kenter GG, Jordanan ES. Indoleamine-2,3-dioxygenase (IDO) metabolic activity is detrimental for cervical cancer patient survival. Oncology. 2015;84:114-57
Fisher SG, Kuhlmann KF, Poston GJ. Defining resectability of colorectal liver metastases. Colorectal Cancer. 2015;4:55-57


**HEAD AND NECK SURGERY AND ONCOLOGY**

Alfons Balm, Michiel van den Brekel, Frans Hilgers, Baris Karakulukcu, Martin Klop, Peter Lohuis, Ludi Smeele, Rob van Son, Bing Tan, Charlotte Zuur

The department is a national referral center and one of the larger clinical departments in this field treating around 500 new patients annually. The department is active in clinical and translational research. Currently, B staff members are working in the department, all with part-time appointments at the Department of Oral and Maxillofacial Surgery of the Academic Medical Center (University of Amsterdam). In 2015 the ties with the department of Head and Neck Surgery at the UMC in Utrecht have been strengthened further. Being a multidisciplinary field, there are many clinical and research connections within the institute and internationally.

In 2015 4 PhD theses and over 60 publications and chapters in textbooks were published.

**Translational research**

In 2015 the head and neck department was involved in several translational research projects. Together with the group of Conchita Vens next generation sequencing was further explored to study DNA repair defects. A clinical phase 2 trial using PARP inhibitors in combination with radiotherapy has been started. In a collaborative project with the department of radiotherapy, the search for predicting outcome, using deep sequencing miRNA and mRNA profiles is continued. In an international study lead by Olga Hamming (Artforce) adaptive radiotherapy techniques in combination with cisplatin and several translational side-studies are conducted. Together with Jacques Neefjes and Huib Ovaa, Charlotte Zuur studies the value of short term cultures in predicting response and also has characterised the radiosensitising characteristics of many available drugs. Tumor micro-environment and immune response studies have been initiated for both HPV positive and negative tumors. Macrophage, gammadelta T-cells, and the role in tumor response are evaluated. Immunotherapy studies are initiated in collaboration with John Haanen and Ton Schumacher.

**Rehabilitation**

Rehabilitation research focuses on several topics. One concerns prevention and treatment of swallowing and communication problems in patients treated with chemoradiation and surgery for advanced head and neck cancer. Research on postlaryngectomy vocal and pulmonary rehabilitation, in cooperation with Atos Medical in Sweden, has resulted in several medical devices. A project on cost effectiveness of this rehabilitation has been initiated. Together with the Amsterdam Centre for Language and Communication (ACLC) and the Ghent University and University of Antwerp, automatic speech analysis tools are clinically tested to assess the intelligibility of pathologic voices. A project on prediction of voice after laryngectomy has been initiated. Also with the ACLC a project on physician-patient communication is being pursued.

**Clinical research**

Clinical research is diverse. Fields of interest are sentinel node detection in melanoma and oral cancer, using modern techniques
such as image guided surgery and fluorescence. Epidemiology and outcome as well as patient counselling, are studied in several national and international studies. Together with the MAASTRO clinic a decision aid tool is developed. Waiting times for treatment and organization of optimal patient care as well as audit studies on the quality of care are being conducted. Prediction of inoperability and development of virtual tools to predict the functional outcome after surgery as well as radiotherapy are conducted in cooperation with the Technical University Twente. In cooperation with UI-Jakarta and UGM-Yogyakarta several studies on detection and antiviral treatment in EBV related nasopharyngeal cancer as well as clinical studies on NPC education of doctors are running. Otoxicity of chemoradiation and prevention by injection of Thiosulphate in the middle ear are studied. The department is active in research on photodynamic therapy. Currently the focus of this research is on dosimetry and interstitial PDT, especially in paranasal sinus carcinoma.

UROLOGIC ONCOLOGY

Simon Horenblas, Axel Bex, Henk van de Poel, Bas van Rhijn, Esther Wit, Kees Hendriksen, Elies Fransen van der Putte, Sarah Ottenhof

Research in urologic oncology has been centred on the following themes:

Improved staging of urologic tumors, organ and function sparing, fundamental research in prostate, kidney, bladder and penis cancer.

Improved staging of urologic tumors

In all urological tumors, apart from kidney cancer, sentinel node biopsy has proven to be extremely useful. In collaboration with the department of nuclear medicine at the NKI-AVL and the dept. of clinical imaging in the LUMC, fruitful clinical trials have been done assessing the use of SPECT/CT scans, intra-operative imaging with a mobile gamma camera (Sentinella®) and especially the use of a hybrid tracer. The sentinel node strategy together with the Sentinelia was effective in localizing sentinel nodes in patients with prostate cancer in a variety of anatomical locations, otherwise not removed during standard lymph node dissection. The rationale of patent blue, ICG and ICG with technetium is assessed and compared in various clinical trials (N09IGF, N12IGP, M13PSN). With the “firefly” system fluorescence imaging is integrated into the robot camera and acoustic-articulary articylary changes after chemo-IMRT treatment for advanced head and neck cancer. Eur Arch Otorhinolaryngol 2015 (in press)


bladder preservation trial has been opened (M15CRB) evaluating chemotherapy, lymph node dissection and chemo-radiation. A new treatment strategy for bladder preservation in non-muscle invading bladder cancer was added recently to our armamentarium: instillations with hyperthermic Mitomycin-C. Also for penis cancer chemo-radiation is being used as a tissue sparing modality in advanced cases. In non-metastatic kidney cancer an increasing number of small renal masses is treated by nephron sparing strategies such as robot assisted partial nephrectomy, thermal ablation and active surveillance. In addition we use a neoadjuvant strategy with tyrosine kinase inhibitors to downsize tumors to allow resection in surgically complex lesions or nephron sparing approaches to preserve renal function in imperative cases.

**Translational research in prostate, kidney and penile cancer**

**Prostate cancer**

The role of neoadjuvant Enzalutamide is analysed, especially the effect on positive surgical margins after prostatectomy. Moreover basic research is done in order to elucidate the effects of Enzalutamide on the interaction of androgen receptor DNA.

**Penis cancer**

A research project was started in collaboration with the dept. of head and neck surgery, gynaecologic surgery and dept. of immunotherapy to assess the role of the microenvironment.

**Kidney cancer**

In collaboration with Christian Blank we demonstrated that pretreatment of clear cell renal carcinoma with Sunitinib reduces myeloid derived suppressor cells which may be further exploited by a combination with immune checkpoint blockade. In addition, we cooperate with the international PREDICT consortium funded by an FP7 framework program of the European Union which analyses among other material tissue from the E30073 study for molecular signatures of response and progression.

**Bladder cancer**

Fundamental research in characteristics of urachal cancer is being done funded by a grant from the EAU. Immunotherapy with check-point inhibitors is extensively assessed in collaboration with colleagues from the medical oncology department.

Improved quality control of treatment results using prospective data collection, almost all uro-surgical treatments at the NKI-AVL can be analysed now. Quality of life analyses were done by the NKI-AVL and patients with penile cancer.

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The department focuses on innovative treatment for ovarian cancer, on immunotherapy for HPV related pre/malignant neoplasms and on interventions to improve quality of life for patients with gynaecological malignancies. Research takes place in close cooperation with the other centers from the Center for Gynaecologic Oncology Amsterdam (CGOA) i.e. AMC and VUMc. The department participates in several nationwide studies within the Dutch GOG and in the EORTC. Close International cooperation exists with Institute Curie and University of Leuven as well as all partners in the RAID-EU project.

The role of the micro-environment in primary tumors and draining lymph nodes in cancer of the cervix as well as cancer of the vulva is being studied by gathering lymph node scrapings during surgery (KWF 2013-6015) (Heeren, Samuels, Zijlmans, de Hartemink). Patient inclusion is currently almost completed and data will be analysed during 2016 and 2017. KWF CKTO2006-16.

The beneficial effect of a laparoscopy in order to predict the operability in high stage ovarian carcinoma is being studied in a multicenter randomized trial coordinated by the CGOA-AMC. Patients inclusion is completed and data are currently being analyzed (ZON MW doelmatigheid 80-B2310-97-11056),(Buist, Rutten, van Meurs, de Vrie, Mol, Kenter)

Several biomarkers in ovarian cancer are being studied to analyse their capacity as discriminative or prognostic tool. This involves not only He4, but also microvesicles that are being collected from body fluids in patients with gynaecological malignancies (Lok, Stiekema, Korse, Kenter).

The underlying immunological, molecular, genetic and epigenetic mechanisms that influence growth and invasion of peritoneal metastases of high grade serous ovarian carcinoma are virtually unknown. We investigate the ultrastructure of the different layers of the peritoneum such as the peritoneal elastic lamina (PEL) by means of several tecanics. (Lok, van Driel, Amant, van den Broek).

The effect of hormonal replacement therapy on menopausal complaints related to biochemical changes in surgically and naturally postmenopausal women is investigated in a prospective observational comparative study. (M06HRT Novaria) (Vermeulen, van Beurden, Korse).


PLASTIC AND RECONSTRUCTIVE SURGERY

Marieke Van Den Berg, Brigitte Drost, J. Joris Hage, Marije Hoornweg, Martine Van Huizum, Sanne Moolenburgh, Leonie Woerdeman

Our research is focused on innovative reconstructive techniques after ablative surgery by other specialists. Additionally, (micro-) vascular and muscular functional studies are executed in collaboration with the Department of Radiation Oncology (dr. Nicola Russell) at our institute and with the Faculty of Human Movement Sciences at the Vrije Universiteit, Amsterdam (prof. dr. Dirk-Jan Veeger).
Breast conserving therapy is defined as a breast conserving, wide local excision (WLE) of a mammary tumor combined with postoperative radiotherapy. Immediately oncoplastic restoration of the mammary form by use of breast reduction techniques (volume displacement) or immediate restoration of mammary volume by use of tissue replacement techniques (volume replacement) are gaining popularity to prevent this malformation. As an innovative technique, we are applying an internal mammary artery perforator (IMAP) flap for immediate tissue replacement after WLE of the medial part of the breast. Our experience shows that the IMAP flap is a reliable flap with good cosmetic outcome after oncoplastic reconstruction. Donor site revision often proved necessary but likely may easily be prevented by use of the propeller variant of this flap. Microvascular damage is an important component of late radiation-induced morbidity. In pre-clinical models, it was demonstrated that repair of vessel injury is dependent on proper endoglin-mediated transforming growth factor-beta (TGF-β) signalling and that it can be affected by infiltrating macrophages. In collaboration with dr. Russell, the Division of Cellular Stress Response and the Department of Biometrics, these findings were extended in irradiated patients, using skin as a model system, and assess whether bisphosphonates could modulate the response. Therefore, paired skin biopsies from irradiated and non-irradiated sites were obtained from 48 breast cancer patients. In 8 patients, biopsies were repeated after 4 months of bisphosphonate treatment. Immunohistochemistry was used to assess vascular alterations and leucocyte infiltration. Western Blot and qPCR were used to assess expression of growth factors and their receptors. It was observed that decreased blood vessel numbers at early time points were followed by increased endoglin expression and restoration of vessel number. Loss of small lymphatic vessels was associated with increased TGF-β levels, whereas dilation of lymphatic vessels correlated with increased macrophage infiltration. Bisphosphonate treatment reduced leucocyte infiltration, but also prevented restoration of blood vessel infiltration. It was concluded that radiation injury of the microvasculature is mediated through TGF-β, whereas repair is modulated by the co-receptor endoglin and promoted by macrophages.

**ANESTHESIOLOGY, INTENSIVE CARE MEDICINE AND PAIN MEDICINE**

Sandra Huissoon, Karin Ariese-Beldman, Marloes Bolman, Dirk Buitelaar, Sanneke Buma, Katina Efthymiou, Tjome de Graas, Christoph Hahn, Aletta Houwink, Lenie Hulshoff, Anja Karagozoglu, Anna Lukas, Anina Rothengatter-Ophof, Michael Šrámek, Julia Ten Cate, Liang Tjoa, Marchien van der Weide, Ingeborg Vergouwe, Esther Wolthuis

The principal aim of our department is to deliver the highest standard of anesthesiological and intensive care, pain therapy and supportive care. In the anesthesiology department research focuses on the role of peripheral blockades as an adjuvant to general anesthesia.
The purpose of these blockades is threefold: a reduction in perioperative opioid consumption, the prevention of chronic pain and shortening of length of hospital stay.

A study comparing the effects of a transverse abdominal plane block versus continuous epidural analgesia on the inflammatory response and pain after laparoscopic hysterectomy has been started.

There is increasing interest in the role the choice of anesthetic plays in cancer metastasis and recurrence. Future research will focus on the neutrophil- to lymphocyte ratio as a prognostic marker in this field.

Intensive Care research consists of participating in multi-centre clinical trials focusing on invasive monitoring.

In the pain therapy department current research focuses on post mastectomy pain. Since 2011 a RCT is ongoing about the efficacy of pulsed radiofrequency treatment on the intensity of postmastectomy pain. Postmastectomy pain (PMPS) is a frequently occurring long term complication of the surgical treatment of breast cancer with an incidence of 30-50%. As a result of the study additional trials have been started including an analysis of sensory disturbances in patients with PMPS and a clinical review about treatment options for PMPS.

The department participates in a multicenter trial investigating the validation of an assessment tool for breakthrough cancer pain.

In the supportive care department a retrospective study has been started examining emergency admissions at the end of life.


TUMOR REGISTRIES

The tumor registry is responsible for completing and maintaining three important registries for the institute. Since 1977, the department maintains a Tumor Register database containing information on patients visiting the hospital with benign tumors, pre-malignant, and malignant tumors. Depending on the clinical involvement at the hospital with respect to the diagnosis and therapy of the tumor, the number of items collected ranges from minimal to very extended. Every year about 9000 patients are added to the full register. This database is a valuable source for research and contains more than 225000 registrations. A selection of cases, i.e. patients who have been diagnosed primarily in the Netherlands Cancer Institute is sent to the National Cancer Registry at regular intervals. A second series of registries belong to the category of quality registers. Most of these registries are developed by the Dutch Institute for Clinical Auditing (DICA). DICA aims at creating valid monitoring systems for quality in healthcare by collecting a fixed set of items per area of interest over time. The system would make it possible to continuously auditing quality of care through online benchmarking taking patient- and disease characteristics into account. Currently, the tumor registration group participates in audits for breast cancer (NBCA), colorectal cancer (DSCA), upper gastro-intestinal cancer (DUCA), lung cancer (DLSA), lung radiotherapy (DLRA), melanoma treatment (DMLR), gynecologic cancer (DGOA), liver cancer (DHBH) and head and neck cancer (DHCN). The Dutch Breast Implant Registry (DBIR) has been launched recently and the prostate cancer registry (ProjZib) may be starting soon. In 2015 more than 2100 patients were registered for this purpose.

The third type of registry that has started in the hospital in July 2015 is the Landelijke Basisregistratie Ziektenhuiszorg (LBZ). This is a registry of medical, administrative and financial data of patients at the outpatient clinic, the day care department or who have been hospitalized. Key aspects are the use of ICD-10, an international coding system for diagnosis, and a standardized list of medical activities. The LBZ is a compilation of the former National Medical Register (LMR) and the National Outpatient Care

Biometrics Department

The Biometrics Department serves as the data center of the institute and provides the infrastructure for clinical and fundamental research on bio-statistical support, centralized patient data collection and documentation, data processing and coordinated administration and monitoring of clinical trials. The statisticians and data managers collaborate in clinical and research projects both within the institute and for national and international multicenter studies, guaranteeing Good Clinical Practice and reporting of safety data following National and International laws and guidelines.

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Jesse Roos Technical staff
Einnie van Schaffelaar Technical staff
Arda Scholten Technical staff
Helga Schrijver Technical staff
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Publications

Blank C, Poppen MG, Prevoo W, Meier M, Van Thielen H, Kivistorg P, et al., editors. Combined radiofrequency ablation (rfa) and iodipilum (ipi) in uveal melanoma: Phase 1b results from the secira-um trial. Eur J Cancer 2015
Register (LAZR). The idea of the LBZ is that it may serve as a general source for quality indicators among hospitals nationwide. In full progress, the registry will include 20,000 entries on a yearly basis.

**CLINICAL STUDIES AND SERVICES**

The Biometrics Department provides support for clinical trials performed in and by the institute. Clinical Project Managers facilitate the development of protocols and presentation to Medical Research-Ethics Committee (MREC). Local data managers facilitate the initiation of studies and perform the registration of pre-screening, screening and entry of patients into clinical trials. Central data management takes care of the quality of the central data base and monitors ensure that the clinical trials are conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s). Every year more studies are activated and patients are registered as shown in figure 1.

In 2015, almost 100 single and multicenter studies, falling under the Medical Research Involving Human Subjects Act (WMO), were activated (enrolling at least one patient). Currently more than 235 clinical trials are open for patient accrual. Just over half of the studies were presented primarily to the MREC of the Antoni van Leeuwenhoek.

By November 2015 a total of 2263 patients were registered centrally by the trial office in projects and trials, which comes down to more than one patient per hour. Besides using the service of the trial office, patients were also registered or randomized directly online into the different studies. The department has developed and maintains an online service (Trials on line: TRION) to facilitate the accrual of patients to clinical trials where all documents related to clinical trials approved and to be carried out at the institute, including the patient information and informed consent, are maintained. Currently other products are being evaluated, including self-developed applications for services to make studies available for mobile devices and easy-access.

**Collaborative groups**

The department not only provides services for the studies initiated by investigators from within the institute, but also collaborates with many national and international groups and consortia. Table 1 shows the portfolio of groups and trials with whom the department collaborates with their services. The involvement may range from providing statistical and methodological services, preparing submissions to MRECs or funding boards, building (e)CRFs, Central Data management, Safety Desk and Monitoring. Among the groups are BOOG, DCCG, DUCG, WIN-O, NVALT, CPCT and others. In addition, but not shown, the department provides services to large multicenter trials that are not directly associated with an established study group.

![Figure 1. Number of studies activated at the Antoni van Leeuwenhoek and the number of patients registered in the context of the 'Medical Research Involving Human Subjects Act' (WMO).](image-url)


Lam SW, Nofa MM, de Groot SM, Jager A, Bos MM, Linn SC, et al., editors. Plasma biomarker analysis in patients with HER2 negative locally recurrent or metastatic breast cancer (HRMBC) treated with first-line bevacizumab (a) and paclitaxel (t) without or with capcitabine (c). Cancer Res 2015


NVALT cancer studies

The most extensive collaboration in terms of services is with the Dutch Association of Chest Physicians (NVALT). Since 1999 the department is involved in the planning, designing, running, monitoring, handling administration of adverse events, and analyzing the clinical trials of this group in lung cancer and mesothelioma. In 2015 the support has been extended with a clinical trial coordinator and a legal expert taking care of all the contracts and legal issues. More than 75 hospitals in the Netherlands participate in these clinical trials. Funding has been obtained from the KWF and pharmaceutical company grants.

The NVALT 8 is a randomized phase II study of adjuvant chemotherapy with or without low-molecular weight heparin in completely resected non-small-cell lung cancer patients. Accrual has finished in the summer of 2013 and data are collected continuously. However, a final analysis is planned when sufficient events have been observed which is estimated to be in early 2016.

The NVALT11 study was closed in April 2014 with 174 patients randomized by 15 participating hospitals. In this study, run by the NVALT in cooperation with the Dutch Lung Cancer Research Group (DLCRG) and the National Platform for Radiotherapy in Lung Tumors (LPRL) the value of prophylactic cranial irradiation (PCI) versus observation is studied in radically treated patients with stage III non-small cell lung cancer. The necessary number of events is expected later in 2015. When the data are sufficiently mature, the trial will be included in a meta-analysis of PCI versus no PCI.

The NVALT12, a randomized study of docetaxel, cisplatin, bevacizumab with or without nitroglycerine patches in patients with stage IV non-squamous non-small cell lung cancer, has been completed and the manuscript was published in 2015.

The NVALT15 is a phase II study in squamous and large cell lung cancer with harboring fibroblast growth factor receptor-1 gene (FGFR1) amplification. Patients are to be treated with oral fibroblast growth factor-1 inhibitor BIBF1120 200 mg bid as second line chemotherapy. The study has a one-stage design to elucidate any activity of BIBF1120. To detect a PFS difference between historical control and the BIBF1120 group requires 76 patients. If this stage is successful then a large trial is considered. If unsuccessful the study will be terminated. It is planned to collect tumor material from all participants and to carry out exploratory analyses to assess the relation between FGFR1 copy numbers in primary tumor and outcome in terms of tumor response and progression-free survival. Accrual has started in 2014. Six Dutch centers and twelve Spanish centers are currently screening and entering patients into the study.

The NVALT17 was developed in 2013 opened for accrual in 2014. Thirteen sites have received official approval to enroll patients. This is a multicenter, randomized, open-label, phase III study in patients with EGFR mutated NSCLC receiving first line treatment. Each year at least 500 patients with non-squamous cell lung cancer will be screened to come to 50 patients per year with activating EGFR mutations during 3 years. Patients with these mutations will be randomized to receive erlotinib monotherapy daily or intercalated erlotinib (day 2-16 of each
cycle) with chemotherapy (pemetrexed 500 mg/m² administered intravenously on day 1 and cisplatin 75 mg/m² administered after prehydration on day 1 every 3 weeks) and after a maximum of 4 cycles followed by maintenance pemetrexed and erlotinib.

The NVALT19 in mesothelioma patients has started accruing patients in 2014. The aim of this randomized study is to characterize the potential clinical benefit, toxicity, and biomarkers of outcome for maintenance therapy with gemcitabine in patients with malignant pleural mesothelioma who have completed first line chemotherapy without progression. The choice of gemcitabine is based on previous work in mesothelioma and non-small cell lung cancer, which proposes a non-cross resistant switch of maintenance agent. The protocol also includes translational research. By now, 33 patients have been enrolled from 8 different centers.

In 2015 two new protocols have been developed and are awaiting approval of the METC. The NVALT18 is randomized phase II study of docetaxel versus intercalated erlotinib docetaxel combination therapy in patients with relapsed EGFR wild type, ALK negative non squamous cell carcinoma. A total of 230 patients need to be enrolled and the two treatments will compared with respect to progression-free survival. The NVALT 22 study is a phase III comparing cisplatin-pemetrexed with carboplatin-paclitaxel-bevacizumab as first line chemotherapy in KRAS mutated non-small cell lung cancer patients.


Legend to the format of the numbers:
1:   closed and completed
2:   currently open for patient enrolment
3:   closed and in the process of reporting
4:   in preparation
5:   in preparation

# Clinical Trial Supportive Services
TC:  Presenting to MREC, funding
CDM:  Central Data Management
eCRF: CRF on eCRF development
SEM:  Statistics and Methodology
SD:  Safety Desk
M:  Monitoring
R:  Registration/ Randomisation
C:  Contract negotiation/ Legal

**TABLE 1. PORTFOLIO BIOMETRICS DEPARTMENT (2000-2015)**

<table>
<thead>
<tr>
<th>STUDY GROUPS</th>
<th>STUDIES</th>
<th>NUMBER OF INSTITUTES</th>
<th>TARGET NUMBER OF PATIENTS</th>
<th>SERVICES OF THE DEPARTMENT</th>
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<tbody>
<tr>
<td>BODG</td>
<td>ATX</td>
<td>31</td>
<td>312</td>
<td>TC, SEM, R, CDM, CRF</td>
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<tr>
<td></td>
<td>Young Boost</td>
<td>32 / 3 countries</td>
<td>2400</td>
<td>TC, SEM, R, CDM, CRF, SD</td>
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<td></td>
<td>TRAN-2</td>
<td>36</td>
<td>437</td>
<td>TC, SEM, R, CDM, eCRF, SD</td>
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<tr>
<td></td>
<td>TRIPLE-B</td>
<td>&gt;20</td>
<td>304</td>
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</tr>
<tr>
<td></td>
<td>StepGo</td>
<td></td>
<td></td>
<td>SEM</td>
</tr>
<tr>
<td>DCCG</td>
<td>CAR01-3,5</td>
<td>64</td>
<td>&gt;1000</td>
<td>TC, SEM, eCRF, R</td>
</tr>
<tr>
<td></td>
<td>SALT0</td>
<td>9</td>
<td>90</td>
<td>TC, CDM, eCRF, SEM, SD, R</td>
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<tr>
<td></td>
<td>B-DOCT</td>
<td>55 / 2 landen</td>
<td>788</td>
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</tr>
<tr>
<td></td>
<td>CRITICS-1</td>
<td>&gt;20</td>
<td>&gt;1000</td>
<td>TC, CDM, eCRF, SEM, SD, M, R,C</td>
</tr>
<tr>
<td>NVALT</td>
<td>NVALT 1,2,3,4,6,7,8,9,10,12,14,15,16,17,18,22,24</td>
<td>&gt;20</td>
<td>&gt;1000</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
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<tr>
<td>WIN-O</td>
<td>REPOSIT</td>
<td>10</td>
<td>90</td>
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<tr>
<td></td>
<td>RDPEPETAR</td>
<td>16</td>
<td>100</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
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<td>DUCG</td>
<td>PERISCOPE</td>
<td>8</td>
<td>20-30</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
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<tr>
<td>DUGS</td>
<td>CHEMORAD</td>
<td>4</td>
<td>50</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
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<tr>
<td>SIOP-RTSG</td>
<td>P9301</td>
<td>126 / 22 countries</td>
<td>3000</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
</tr>
<tr>
<td></td>
<td>P2001</td>
<td>251 / 26 countries</td>
<td>4000</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
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<tr>
<td>ITCC</td>
<td>Diverse</td>
<td>&gt;40 / Europa</td>
<td>&gt;100</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
</tr>
<tr>
<td>CPCT</td>
<td>CPCT-01,-02,-05</td>
<td>&gt;10</td>
<td>&gt;1000</td>
<td>TC, CDM, eCRF, SEM, SD, M, R</td>
</tr>
</tbody>
</table>

**TABLE 2. OVERVIEW OF NVALT-STUDIES**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Stage</th>
<th>Studies</th>
<th>Number of Institutes</th>
<th>Target Number of Patients</th>
<th>Services of the Department</th>
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</thead>
<tbody>
<tr>
<td>NSCLC</td>
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<td>NVALT 2, 8, 24</td>
<td>312</td>
<td>TC, SEM, R, CDM, CRF, SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>NVALT 6, 11, 25</td>
<td>437</td>
<td>TC, SEM, R, CDM, eCRF, SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NVALT 1, 4, 12, 17, 21, 22</td>
<td>&gt;20</td>
<td>TC, SEM, R, CDM, eCRF, SD</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>IV first line</td>
<td>NVALT 10, 12, 15, 16, 18, 24</td>
<td>&gt;1000</td>
<td>TC, SEM, R, CDM, eCRF</td>
<td></td>
</tr>
<tr>
<td>Palliative Care</td>
<td>IV beyond first line</td>
<td>NVALT 19</td>
<td>&gt;1000</td>
<td>TC, SEM, R, CDM, eCRF</td>
<td></td>
</tr>
</tbody>
</table>


Reesink DJ, Fransen van de Putte EE, Vegt E, de Jong J, van Werkhoven E, Martens LS, et al., Clinical Relevance of Incidental Prostatic Lesions on FGD- PET/CT Scan. Should patients receive further evaluation? J Ultrasound 2015


Slotman BJ, van Tinteren H, Which patients with extensive stage small-cell lung cancer should and should not receive thoracic radiotherapy? Transl Lung Cancer Res 2015;4:292


By invitation of the National Cancer Institute of Canada the NVALT has started a collaboration on a world-wide, multi-centre, randomized, placebo-controlled trial of the human monoclonal antibody directed against programmed cell death ligand 1 (PD-L1) MEDI4736 in completely resected primary stage IB (> 4cm), II and IIA non-small cell lung cancer patients. The primary endpoint is disease-free survival (DFS) for patients with NSCLC that is PD-L1 positive. The study will run in 12 centers.

December 2015 the first national NVALT study meeting will be organised. This will bring principal investigators, research nurses, data managers, monitors and other relevant people involved to discuss current studies and new studies.

Other collaborations

The department collaborates with the Dutch Colorectal Cancer Group (DCCG) in several large phase III randomized studies in patients with advanced colorectal cancer, especially by providing statistical expertise and analysis capacity. This year, the CAIRO3 study, investigating the value of maintenance treatment with ceftarbine and bevacizumab versus observation after induction treatment in patients with advanced colorectal cancer was published in the Lancet. In June 2014, the CAIRO3 study was approved by the MREC and the trial is currently accruing patients. This study investigates neoadjuvant treatment strategies in colorectal cancer patients with initially irresectable liver-only metastases and is planned to enroll 640 patients. Like with the other CAIRO studies, the statistical methods are developed at the Biometrics Department and the analyses will be performed by the department. The study is also using the ALEA™ eCRF system for central data management. Of particular interest is the use of a national review panel evaluating the eligibility of patients before being included. ALEA™ serves as a central tool for screening new patients. For each potentially eligible patient a radiologist uploads the relevant scans onto the system and the system automatically invites members of the surgical panel to give their opinion on the electronic forms.

With the BOOG collaboration the department runs several large studies in breast cancer. The Young-boost study, a randomized phase III study in young women, investigating the value of additional radiation to the tumor bed, was closed in 2012 with over 2400 patients. The central data management of the department is currently cleaning and querying the data and a full analysis is expected in Q2 2016. The Train-2 study investigates neo-adjuvant systemic treatment in HER2+ breast cancer and aims at including 437 patients. Most of the study activities are performed by the department, including statistics and central data management and monitoring. This is also true for the Triple-B study, a biomarker discovery randomized phase II-b study in advanced triple negative breast cancer.

Immunotherapy studies

Although the first investigator initiated study with one of the now popular immunotherapies was already activated in 2011, especially in 2015 there was a tremendous boost in the number of studies presented to the MREC. Currently 10 studies are awaiting the first patient to be enrolled and another 29 have started actually registering patients in 2015. For most of the studies, a pharmaceutical company still acts as the sponsor, but
in a large number of studies the institute is the sponsor and for those studies the Biometrics department is heavily involved.

**The SIOP-renal tumor study group**

Almost all children with nephroblastoma or Wilms’ tumor are treated within prospective, randomized multicenter trials conducted either by the International Society of Pediatric Oncology (SIOP-Europe) or by the Children’s Oncology Group (COG-North America). The SIOP Renal Tumour Study Group (RTSG) is an established international collaborative group (21 European countries, the Brazilian national study group and centres in Argentina, Australia, New Zealand, United Arab Emirates, South Africa, Soviet Union and Ukraine). Since 1971, prospective randomised clinical trials have been conducted. In 1993, the sixth randomized controlled trial was launched and the statistical secretariat moved to the Biometrics department of the NKI-AVL in Amsterdam. The SIOP 9301 trial was the first to study a reduction of therapy in Wilms’ tumors. The most recent trial, SIOP 2001, continued the SIOP philosophy of applying pre-nephrectomy chemotherapy to allow a response-adapted risk stratification of post-operative treatment intensity. The primary question of this study was whether doxorubicine could be safely omitted from chemotherapy for stage II-III intermediate risk histology Wilms’ tumor. The trial enrolled 583 children from 261 centers in 26 countries in 8 years. In 2015 the manuscript was published in the Lancet.

In 2016 a new protocol and therapy guide will be initiated. The protocol contains the most up-to-date guidelines for diagnosing and treating Wilms’ tumors and recommendations for non-Wilms’ renal tumors. Over time research proposals will be developed and integrated into the protocol. One of the areas of interest will be new therapies in stage IV patients. Because of the small number of both patients and events, novel designs, e.g. Bayesian approaches and stopping rules will be applied to address the therapeutic questions.

To register and perform data collection of all patients enrolled into the Umbrella-protocol, it has been decided to use the ALEA eCRF-system that will be centrally organized and maintained at the Biometrics Department. The study also includes prospective collection of biological material in all patients. Prospective collection of high quality material will be used to confirm results of studies on the current data and to allow further improvements in risk stratification treatment protocols. Finally, in collaboration the Children’s Oncology Group pooled data analyses are carried out studying the incidence and outcome of patients who experienced a late recurrence more than 5 years after diagnosis across several clinical trials, and to develop evidence-based recommendations for follow-up surveillance.

The statisticians have collaborated with many departments of the institute and other, academic and non-academic institutes in the region in a variety of studies. All these collaborations resulted in co-authorships of more than 55 peer-reviewed publications in 2015 (see reference list).

**ICT DEVELOPMENTS**

Between 1988 and 2011, the WA department developed ALEA™, a comprehensive software solution supporting data collection for clinical research. The system and further programmatic developments were then taken over by FormsVision. Since 2011, FormsVision has brought ALEA into an industry ‘software-as-a-service’ (SaaS) solution providing integrated Interactive response technology (IRT), Electronic data capturing (EDC) and Electronic Patient-Reported Outcome (ePRO). IRT includes registration and randomization of patients in clinical trials and the Drug Supply Management functions required in double blind studies with Investigational Medicinal Products. EDC provides Electronic Data Capturing, including electronic Case Record Forms, local monitoring and central data management and has integrated imaging functions for radiology review of VT, MRI and PET scans. The system includes an audit trail in compliance with the requirements of the International Committee for Harmonization and produces standard based export facilities (CDISC, ODM and SDTM) as required by statistical best practice. Finally, ePRO provides electronic Patient's Reported Outcome facilities. With ePRO, patients or participants of epidemiological research or clinical trials may login to ALEA using a computer or tablet and complete one or more questionnaires. All systems are in use in the Antoni van Leeuwenhoek, but are also available to other researchers and groups in and outside the Netherlands.

In 2015 we have started the development of a number of high-level requirements together with FormsVision, the department of research ICT of the institute and PSOE (for the privacy solution). These functionalities are:

- Implementation of a local privacy gateway in compliance with privacy legislations and research requirements
- Implementation of a radiology gateway based on CTP to support the incorporation of DICOM imaging objects in clinical research projects
- Implementation of a LIMS gateway to enable the direct import of laboratory measurements in clinical trials
- Implementation of a SDM gateway, which provides a standardized description of AVL clinical trials suitable to transmit to a common clinical trial repository and which serves as a content source for a new version of TRION.

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**References**


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| Research facilities (per ultimo 2015) |

| ANIMAL FACILITY | MARCO BREUER, HEAD  
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ANNE MARIE RHEBERGEN  
ROEL SNEEPERS  
AND OUR ANIMAL CARE-TAKERS |
| ANIMAL PATHOLOGY AND HISTOLOGY FACILITY | SJOERD KLAARENBEEK, HEAD  
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MARJA NIEUWLAND  
ARNO VELDS |
| GLASSWARE | ANITA DE BOIS-BAKKER  
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NIELS DE WIT |
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MARIEKE VAN DE VEN, HEAD
UTE BOON
LEVI BUIL
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RENSKE DE KORTE-GRIMMERINK
NATALIE PROOST
STEPHAN FRERIKS
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LIBRARY
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PATRICK BAIRROSING
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PEPTIDE SYNTHESIS FACILITY
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JOHN DE WIDT
MAGDA STADNIK

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THEO LAMERS

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WILLIAM PETERS*
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JESSICA DEL BRAVO
PAUL KRIMPENFORT
LONA KROESE
BISHENG LIU
COLIN PRITCHARD
FINA VAN DE AHÉ
TANYA VERMEEREN-BRAUMLULLER

Detailed information about the services provided by each of the facilities can be found on the NKI website (www.nki.nl).
The Netherlands Cancer Institute offers a variety of opportunities for practical and theoretical training to (trainee) technicians, University Master students, PhD students and post-doctoral fellows. Research and clinical staff and their group members are involved in theoretical and practical training. Many staff members have joint appointments as professors at Dutch universities and even more contribute to the regular curriculum at various universities. The research divisions attract students from universities throughout the country. The NKI has a formal affiliation with the Science faculty of the University of Amsterdam (UvA) and is committed to make a contribution to Master student teaching. The institute participates in the Oncology Graduate School Amsterdam, together with the medical faculties of the UvA and the Free University (VU), referred to as Academic Medical Center (AMC) and VU medical center (VUmc), respectively. All educational activities are supervised by the Teaching Committee, which consists of Jannie Borst (chair and dean Master students), Hein te Riele (general affairs and dean PhD students), Fred van Leeuwen (dean post-docs), Roderick Beijersbergen (Master course), John Hilkens (HLO students and publicity), and Fons Balm (clinical teaching).

**MASTER STUDENTS**

The program in Experimental Oncology attracts Master students of all national universities (see www.nki.nl/topmenu/master-students/). Students generally have a background in (Medical) Biology, Health Sciences, Chemistry, Pharmacology, Medicine, or Psychology. The program offers combined practical and theoretical training in various aspects of experimental oncology. Practical training includes participation in ongoing research projects for a minimum of 4 months.

In 2015, 43 Dutch university Master students and 5 students from abroad completed a placement of 5-9 months at the biomedical research divisions. The students came primarily from the University of Amsterdam (UvA) (8) and the Free University Amsterdam (VU) (18) and, but also from the universities of Utrecht (5), Leiden (6), Rotterdam (2), Groningen (2), Nijmegen (1) and Wageningen (1). The institute also provides practical training opportunities for Bachelor students of the HLO (Universities of Applied Science), who stay for similar periods of time as the university students and like these, often make significant contributions to research progress of the PhD students and post-docs who supervise them. There is an increasing demand from universities for placing Bachelor students for the three-month internship that concludes their program, but we have difficulty to find supervisors for these students.

The core element of theoretical training is the course in Experimental Oncology, given twice yearly (Table 1). This course is compulsory for Master students who do an NKI internship of more than 4 months in a biomedical discipline, but in addition attracts many students from throughout the country. We routinely host about 50 students per course. It includes lectures and tutorials given by our highest level clinical and research professionals and is rated very highly in university evaluations.
PHD STUDENTS

PhD students at the NKI-AVL participate in the Oncology Graduate School Amsterdam (OOA), an alliance of the oncology research divisions of the NKI-AVL and the two Amsterdam universities. The number of PhD students has been rising rapidly in the past years. In 2015, the NKI-AVL had 224 PhD students registered at the OOA. 24 students defended a PhD thesis at a Dutch university.

Besides joining interdepartmental work discussions, the students follow the OOA training program that offers courses, meet-the-expert sessions and an annual retreat (Table 2). The OOA course program includes in-depth courses on different topics in cancer research, but also technical courses in English writing, biostatistics and -informatics, microscopy and animal handling. Students with an insufficient background in cancer research can attend the Experimental Oncology course for Master students. PhD students also have the opportunity to meet with experts in the field of oncology: the Friday morning seminar speakers are invited to take their lunch with a delegation of PhD students. Each PhD student can apply for such a lunch meeting several times a year.

The annual PhD student retreat is entirely focused on the research of the PhD students themselves. First year students present their work in the form of a poster, more advanced students give an oral presentation. Importantly, students are in charge of chairing sessions, monitoring discussions and selecting prize winners for the best poster and best presentation. In this manner, the retreat not only provides an overview of the research in the OOA at an early stage of the student’s career, but also training in presentation and interaction skills. In this way, we hope to stimulate translational interactions and bottom-up research, in which PhD students actively establish collaborations with other research groups.

### TABLE 1

<table>
<thead>
<tr>
<th>COURSES IN EXPERIMENTAL ONCOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPILOGUE</td>
</tr>
<tr>
<td>SURGERY</td>
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<tr>
<td>MEDICAL IMAGING</td>
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<tr>
<td>PATHOLOGY</td>
</tr>
<tr>
<td>GENETIC ANALYSIS OF CANCER</td>
</tr>
<tr>
<td>MOLECULAR DIAGNOSTICS</td>
</tr>
<tr>
<td>CONVENTIONAL PHARMACOTHERAPY</td>
</tr>
<tr>
<td>RATIONAL DRUG DEVELOPMENT</td>
</tr>
<tr>
<td>RADIONUHERAPY*</td>
</tr>
<tr>
<td>DNA DAMAGE RESPONSE</td>
</tr>
<tr>
<td>CELL DEATH</td>
</tr>
<tr>
<td>TELOMERASE AND CANCER</td>
</tr>
<tr>
<td>GENOMIC INSTABILITY</td>
</tr>
<tr>
<td>GENOMICS IN CANCER</td>
</tr>
<tr>
<td>EPIGENETICS IN CANCER</td>
</tr>
<tr>
<td>CELL CYCLE</td>
</tr>
<tr>
<td>IDENTITY AND TARGETING OF ONCOGENIC PATHWAYS</td>
</tr>
<tr>
<td>EMT AND METASTASIS</td>
</tr>
<tr>
<td>TUMOR MICROENVIRONMENT</td>
</tr>
<tr>
<td>MOUSE MODELS OF CANCER</td>
</tr>
<tr>
<td>IMMUNOLOGY AND IMMUNOTHERAPY</td>
</tr>
<tr>
<td>ADAPTIVE T-CELL THERAPY</td>
</tr>
<tr>
<td>ANALYSIS OF PROTEIN STRUCTURE</td>
</tr>
<tr>
<td>DRUG DELIVERY</td>
</tr>
<tr>
<td>RECENT ADVANCES TO STUDY GENOME FUNCTION</td>
</tr>
</tbody>
</table>

* INCLUDING TOUR
Senior graduate students can participate in a joint retreat with other cancer institutes in Europe. In 2015, this event was held in Manchester, UK, organized by the students form the CRUK, Manchester Institute, with participants from:
- The CRUK Institutes (Cambridge, Glasgow, London, Manchester and Oxford)
- The Institute of Cancer Research (ICR)
- German Cancer Research Center (DKFZ)
- The Max Delbruck Center for Molecular Medicine (MDC)
- The Netherlands Cancer Institute (NKI)
- The European School of Molecular Medicine (SEMM; IFOM-IEO)
who attended and contributed to a program of scientific lectures and posters as well as an enthusiastic social session. This retreat gives students the opportunity to become acquainted with oncology centers of excellence throughout Europe.

Once a year, the PhD student meets with a supervisory committee to evaluate the progress of research. Each committee has independent members (PIs) from within and outside the division. The committee discusses progress with the supervisor and the student jointly and separately. Two years after the appointment of the PhD student, a midterm review takes place. At this more elaborate meeting the likelihood of achieving a PhD within a reasonable time frame is discussed. This meeting can be used to redefine goals if necessary.

Each research division of the NKI-AVL has a delegate in the PhD student council that meets with the Dean of PhD students on a regular basis, as well as upon special request. They also mediate communication between the PhD students and the research manager or the board of directors.

**TABLE 2**
**OQA PHD STUDENT COURSES AND EVENTS 2015**

<table>
<thead>
<tr>
<th>DATE</th>
<th>COURSE</th>
<th>SPEAKERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEBRUARY 16, 23</td>
<td>HOW TO WRITE HIGH IMPACT PAPERS AND WHAT TO DO WHEN IT IS REJECTED</td>
<td>M van der Linden (VU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 participants</td>
</tr>
<tr>
<td>MARCH 5</td>
<td>MEET THE EXPERT K HODIVALA-DILKE AT VU</td>
<td>A Griffioen (VUMC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 participants</td>
</tr>
<tr>
<td>MARCH 23-APRIL 2</td>
<td>MOUSE MORPHOLOGY, GENETICS AND FUNCTION</td>
<td>M Edvardsen (AMC)</td>
</tr>
<tr>
<td>APRIL 13-17 AND</td>
<td>IN THE FOOTSTEPS OF ANTONI VAN LEEUWENHOEK, BASIC MICROSCOPY</td>
<td></td>
</tr>
<tr>
<td>OCTOBER 5-9</td>
<td>J Beliën, L Brocks, R Mebius, L Oomen, E Reits, T O’Toole, J Vallejo, N van der Wel (NKI-VU-AMC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 participants</td>
<td></td>
</tr>
<tr>
<td>MAY 11-22</td>
<td>EPIGENETICS AND NON-CODING RNA: FROM MECHANISM TO DISEASE</td>
<td>R Agami (NKI), F van Leeuwen (NKI)</td>
</tr>
<tr>
<td></td>
<td>25 participants</td>
<td></td>
</tr>
<tr>
<td>JUNE 8-12</td>
<td>MGC-OQA COURSE TRANSGENESIS, GENE TARGETING AND IN VIVO IMAGING</td>
<td>H te Riele (NKI), E Robanus-Maandag (LUMC), J Verbeek (LUMC)</td>
</tr>
<tr>
<td></td>
<td>24 participants</td>
<td></td>
</tr>
<tr>
<td>OCTOBER 13-15</td>
<td>PHD STUDENT RETREAT RENESSE</td>
<td>H te Riele (NKI), P Lagerweij (NKI), C Kapper (NKI)</td>
</tr>
<tr>
<td></td>
<td>159 participants from VUMC, AMC, NKI-AVL</td>
<td></td>
</tr>
<tr>
<td>NOVEMBER 2-6</td>
<td>BASIC MEDICAL STATISTICS</td>
<td>M Hauptmann (NKI), P Gradowska (NKI), K Jozwiak (NKI), W Heemsbergen (NKI)</td>
</tr>
<tr>
<td></td>
<td>52 participants</td>
<td></td>
</tr>
<tr>
<td>NOVEMBER 25</td>
<td>MEET THE EXPERT M PLATTEN AT VU</td>
<td>A Griffioen (VU)</td>
</tr>
<tr>
<td></td>
<td>6 participants</td>
<td></td>
</tr>
<tr>
<td>THROUGHOUT THE YEAR</td>
<td>LUNCH MEETINGS WITH NKI-AVL SEMINAR SPEAKERS</td>
<td>NKI seminar committee</td>
</tr>
<tr>
<td></td>
<td>150 participants</td>
<td></td>
</tr>
</tbody>
</table>
In 2015 the NKI-AVL hosted approximately 160 postdoctoral fellows, almost half of which are from abroad and with equal gender representation. The postdocs at the NKI are represented by a very active postdoc committee (postdocs@nki). Several special events were organized by the postdoc committee in the past year, including grant writing courses, a time management workshop, a tech transfer class, and an alumni career evening. Together with human resources and the postdoc dean, the postdoc committee is also actively involved in the career development program that is offered by the NKI to all its postdocs. This training program was initiated in 2014 and started with a basic three-day module for all the postdocs. In 2015, first-year NKI postdocs (20) were offered the mandatory basic module, while more senior postdocs could choose from one of four shorter workshops. Approximately 95 postdocs signed up for an advanced workshop. The goals of the program are to provide postdocs with the tools to take charge of their professional and personal development at the NKI, to promote maximum achievement of postdocs at the NKI, and to prepare postdocs for the next steps in their careers. The program, which is tailored to NKI postdocs, consists of special workshops given by professional trainers but with input and active participation of NKI group leaders. Topics covered in the career development program are communication skills, entrepreneurship and strategic thinking, time management, responsibilities beyond the laboratory, mentoring and being mentored, understanding the (inter)national funding process and getting funded, getting published, and networking and collaborating.
Clinical trials
<table>
<thead>
<tr>
<th>Type of cancer study</th>
<th>Title</th>
<th>Study coordinator in NKI-AVL</th>
<th>Phase</th>
<th>Activated (closed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14EMB</td>
<td>Prospective study on the treatment of unsuspected pulmonary embolism in cancer patients</td>
<td>Joke Baars other</td>
<td>1/7/2014</td>
<td></td>
</tr>
<tr>
<td>M09BGJ</td>
<td>A phase I open-label, multicenter, dose escalation study of oral BGJ398, a pan-FGF-R kinase inhibitor in adult patients with advanced solid malignancies</td>
<td>Jan Schellens I</td>
<td>12/10/2009</td>
<td></td>
</tr>
<tr>
<td>M09NIB</td>
<td>The NIB-Cohort study, therapeutic drug monitoring of tyrosine kinase inhibitors</td>
<td>Neeltje Steeghs other</td>
<td>6/9/2009</td>
<td></td>
</tr>
<tr>
<td>M1GAZD</td>
<td>A phase I open-label, multicenter study to assess the safety, tolerability, pharmacokinetics and preliminary anti-tumor activity of ascending doses of AZD5363 under adaptable dosing schedules in patients with advanced solid malignancies</td>
<td>Jan Schellens I</td>
<td>12/2/2010</td>
<td></td>
</tr>
<tr>
<td>M10FES</td>
<td>An open-label, dose escalation, pharmacodynamic, pharmacokinetic and effect of food phase I study of E7820 to determine the maximum tolerated dose following twice daily oral administration in subjects with unresectable solid tumours (LIFE-110)</td>
<td>Jan Schellens I</td>
<td>4/5/2011</td>
<td></td>
</tr>
<tr>
<td>M11EVE</td>
<td>A two parts, biomarker study to identify genetic aberrations predictive for response on Everolimus in solid tumors without regular treatment options (CPCT-03)</td>
<td>Neeltje Steeghs other</td>
<td>10/3/2012</td>
<td></td>
</tr>
<tr>
<td>M11PCT</td>
<td>Development of a platform for next-generation DNA sequencing based personalized treatment for cancer patients : protocol to obtain biopsies from patients with metastatic cancer (CPCT-02 biopsy protocol)</td>
<td>Neeltje Steeghs other</td>
<td>1/24/2012</td>
<td></td>
</tr>
<tr>
<td>M11RCE</td>
<td>Phase I open label multicenter dose-escalation study to evaluate safety, pharmacokinetics and activity of RO5479599, a glycoengineered antibody against HER3, administered as IV infusion either alone or in combination with Cetuximab or in combination with Erlotinib in patients with metastatic and/or locally advanced malignant HER3-positive solid tumors of epithelial cell origin (HuMAb HER3)</td>
<td>Jan Schellens I</td>
<td>17-11-2011 (3-11-2015)</td>
<td></td>
</tr>
<tr>
<td>M12CCA</td>
<td>A phase I study of CC-486 as a single agent and in combination with Carboplatin or ABI-007 in subjects with relapsed or refractory solid tumors</td>
<td>Jan Schellens I</td>
<td>4-2-2013 (9-1-2015)</td>
<td></td>
</tr>
<tr>
<td>M12DPP</td>
<td>Dexamethasone for the prevention of a pain flare after palliative radiotherapy for painful bone metastases: a multicenter double blind placebo-controlled randomized study (DEXA)</td>
<td>Marcel Verheij III</td>
<td>2/15/2013</td>
<td></td>
</tr>
<tr>
<td>M12SAR</td>
<td>A phase I study to assess the safety, tolerability, pharmacokinetics and biological activity of SAR405839 in patients with advanced cancer (TEQ12318)</td>
<td>Jan Schellens I</td>
<td>28-6-2012 (3-11-2015)</td>
<td></td>
</tr>
<tr>
<td>M12SEN</td>
<td>Observational study to evaluate pharmacokinetics and pharmacodynamics of docetaxel,paclitaxel, doxorubicine, gemcitabine, vinorelbine and capecitabine in elderly patients (senior)</td>
<td>Jan Schellens other</td>
<td>9/13/2012</td>
<td></td>
</tr>
<tr>
<td>M13AZF</td>
<td>A phase I, open-label, multicentre study to compare two dosage formulations of AZD5363 and to establish the effect of food on the pharmacokinetic exposure, safety and tolerability of AZD5363 in patients with advanced solid malignancies (DAK)</td>
<td>Jan Schellens I</td>
<td>13-12-2013 (19-1-2015)</td>
<td></td>
</tr>
<tr>
<td>M13BBY</td>
<td>A phase Ib, open-label study of oral BGJ398 in combination with oral BYL719 in adult patients with select advanced solid tumors</td>
<td>Jan Schellens I</td>
<td>11/8/2014</td>
<td></td>
</tr>
<tr>
<td>Type of cancer study</td>
<td>Title</td>
<td>Study coordinator in NKI-AVL</td>
<td>Phase</td>
<td>Activated (closed)</td>
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<tr>
<td>M13CEA</td>
<td>An open-label, multi-center, dose-escalation, phase I study with an expansion phase, to evaluate safety, pharmacokinetics and therapeutic activity of RO6895882, an immunocytokine, consisting of a variant of interleukin-2 (IL-2v) targeting carcinoembryonic.</td>
<td>Jan Schellens</td>
<td>I</td>
<td>2/13/2014</td>
</tr>
<tr>
<td>M13DOS</td>
<td>Dose-intensified Image-guided Fractionated Radiosurgery for Spinal Metastases (DOSIS)</td>
<td>José Belderbos</td>
<td>II</td>
<td>7-11-2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(9-7-2015)</td>
</tr>
<tr>
<td>M13INC</td>
<td>A phase I open-label dose escalation study with expansion to assess the safety and tolerability of RZC20 in patients with c-MET dependent advanced solid tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>6/12/2013</td>
</tr>
<tr>
<td>M13PRO</td>
<td>Protocol to obtain tumor biopsies from patients with locally advanced (incurable) or metastatic cancer to improve selection for clinical trials. (CPCT - IS biopsy protocol patient selection)</td>
<td>Neeltje Steeghs</td>
<td>other</td>
<td>3/12/2014</td>
</tr>
<tr>
<td>M13ROS</td>
<td>Rapid on-site evaluation (ROSE) vs. randomly collected samples from mediastinal and abdominal lymph nodes obtained by endoscopic ultrasound-guided fine-needle-aspiration</td>
<td>Monique van Leerdam</td>
<td>other</td>
<td>8/7/2014</td>
</tr>
<tr>
<td>M13SPI</td>
<td>A Phase 1 Study of Combination Therapy with SAR405838 and Pimasertib in Patients with Advanced Cancer</td>
<td>Jan Schellens</td>
<td>I</td>
<td>11/6/2013</td>
</tr>
<tr>
<td>M13SRP</td>
<td>Validity of the Steep Ramp Test to assess exercise capacity in patients with cancer undergoing chemotherapy (START)</td>
<td>Martijn Stuiver</td>
<td>other</td>
<td>8/1/2013</td>
</tr>
<tr>
<td>M14BEE</td>
<td>A phase Ib dose-finding study of BYL719 plus everolimus and BYL719 plus everolimus plus exemestane in patients with advanced solid tumors, with dose-expansion cohorts in renal cell cancer, pancreatic neuroendocrine tumors, and advanced breast cancer patients</td>
<td>Jan Schellens</td>
<td>I</td>
<td>10/30/2014</td>
</tr>
<tr>
<td>M14BIC</td>
<td>An open label phase I dose finding study of BI 853520 administered orally in a continuous dosing schedule in patients with various advanced or metastatic non-hematologic malignancies (1300.2)</td>
<td>Neeltje Steeghs</td>
<td>I</td>
<td>6/25/2014</td>
</tr>
<tr>
<td>M14CDP</td>
<td>An open-label, multicenter, dose-escalation phase Ib study to investigate the safety, pharmacokinetics, pharmacodynamics, and therapeutic activity of RO7009789 (CD40 agonist) in combination with MPDL3280A (anti-PD-L1) in patients with locally advanced and/or metastatic solid tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>1/23/2015</td>
</tr>
<tr>
<td>M14CIP</td>
<td>Cancer in Pregnancy (CIP-study)</td>
<td>Christianne Lok</td>
<td>other</td>
<td>2/17/2015</td>
</tr>
<tr>
<td>M14COP</td>
<td>Safety, feasibility and cost-effectiveness of genotype- and phenotype-directed individualized dosing of fluoropyrimidines</td>
<td>Jan Schellens</td>
<td>other</td>
<td>3/31/2015</td>
</tr>
<tr>
<td>M14DTR</td>
<td>A phase II, open-label, study in patients with BRAF V600E-mutated rare cancers with several histologies to investigate the clinical efficacy and safety of the combination therapy of Dabrafinib and Trametinib</td>
<td>Jan Schellens</td>
<td>II</td>
<td>11/13/2014</td>
</tr>
<tr>
<td>M14EEP</td>
<td>Improving CNS penetration of radio-labeled TKI PET tracers through Pgp/BCRP inhibition</td>
<td>Neeltje Steeghs</td>
<td>other</td>
<td>6/11/2014</td>
</tr>
<tr>
<td>M14HOM</td>
<td>A phase I, open label, multicenter, dose-escalation study of oral HDM201 in adult patients with advanced solid and hematological tumors characterized by wild-type TPS3</td>
<td>Jan Schellens</td>
<td>I</td>
<td>12/18/2014</td>
</tr>
<tr>
<td>M14HEP</td>
<td>An Open-label, Non-randomised, Multicentre, Comparative, Phase I Study to Determine the pharmacokinetics, Safety and Tolerability of Daplapib following a Single Oral 300 mg Dose to Patients with Advanced Solid Tumours and Normal Hepatic Function or Mild or Moderate Hepatic Impairment</td>
<td>Jan Schellens</td>
<td>I</td>
<td>2/13/2014</td>
</tr>
<tr>
<td>Type of cancer study</td>
<td>Title</td>
<td>Study coordinator</td>
<td>Phase</td>
<td>Activated (closed)</td>
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</tr>
<tr>
<td>M14HER3</td>
<td>A phase I, first time in human, open-label, dose escalation study to investigate the safety, pharmacokinetics, and pharmacodynamics of anti-HER3 monoclonal antibody GSK2849330 in subjects with advanced HER3-positive solid tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>4/29/2015</td>
</tr>
<tr>
<td>M14HUM</td>
<td>Hubrecht Organoid Technology-Metastasis, a resource for functional studies on drug development for cancer treatment</td>
<td>Emile Voest</td>
<td>other</td>
<td>8/11/2014</td>
</tr>
<tr>
<td>M14HUP</td>
<td>Biobank Hubrecht Institute, a resource for functional studies on drug development for cancer treatment</td>
<td>Emile Voest</td>
<td>other</td>
<td>8/11/2014</td>
</tr>
<tr>
<td>M14MCL</td>
<td>A Phase I Study of MCLA-128, a Human IgG1 Bispecific Antibody Targeting HER2 and HER3, in Patients with Solid Tumours</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>3/11/2015</td>
</tr>
<tr>
<td>M14MKP</td>
<td>Phase Ib study of MK-3475 in subjects with select advanced solid tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>4/1/2014</td>
</tr>
<tr>
<td>M14MPD</td>
<td>A phase Ib study of the safety and pharmacology of MPDL3280A administered with ipilimumab or interferon-alpha in patients with locally advanced or metastatic solid tumors (RAPID)</td>
<td>Christian Blank</td>
<td>I</td>
<td>1/28/2015</td>
</tr>
<tr>
<td>M14MSA</td>
<td>A Multicenter, Open-Label, Dose-Escalating Phase I Trial of the DNA-PK Inhibitor MSC2490484A in Subjects With Advanced Solid Tumors or Chronic Lymphocytic Leukemia (EMR100036-001)</td>
<td>Jan Schellens</td>
<td>I</td>
<td>1/30/2015</td>
</tr>
<tr>
<td>M14PIF</td>
<td>Phase I study evaluating indomethacin in combination with platinum-based chemotherapy (PIFA-01)</td>
<td>Emile Voest</td>
<td>I</td>
<td>12/16/2014</td>
</tr>
<tr>
<td>M14REN</td>
<td>An Open-label, Non-randomised, Multicentre, Comparative, Phase I Study of the Pharmacokinetics, Safety and Tolerability of Olaparib Following a Single Oral 300 mg Dose to Patients with Advanced Solid Tumours and Normal Renal Function or Renal Impairment</td>
<td>Jan Schellens</td>
<td>I</td>
<td>2/13/2014</td>
</tr>
<tr>
<td>M14ROM</td>
<td>A Phase IB, open-label, multi-center, dose-escalation study of the safety, pharmacokinetics and therapeutic activity of RO6895882, an immunocytokine, which consists of a variant of Interleukin-2 (IL-2v), that targets carcinoembryonic antigen (CEA), and MPDL3280A, an antibody that targets programmed death ligand 1 (PD-L1), administered in combination intravenously, in patients with locally advanced and/or metastatic solid tumors</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>4/21/2015</td>
</tr>
<tr>
<td>M14TBA</td>
<td>An open-label, multicenter, dose-escalation phase I study to evaluate the safety, pharmacokinetics, and therapeutic activity of RO6958688, a novel T-cell bispecific antibody that targets the human carcinoembryonic antigen (CEA) tumor cells and CD3 on T-cells, administered intravenously in patients with locally advanced and/or metastatic solid tumors (BP29541)</td>
<td>Jan Schellens</td>
<td>I</td>
<td>1/14/2015</td>
</tr>
<tr>
<td>M14VID</td>
<td>Ventilator-Induced Diaphragm Dysfunction in ICU patients</td>
<td>Koen Hartenink</td>
<td>other</td>
<td>5/19/2014</td>
</tr>
<tr>
<td>M14VOS</td>
<td>A Phase 1, Open-label Clinical Study to Assess the Pharmacokinetics (Distribution, Metabolism, and Excretion) of 14C-Vosaroxin in Patients with Advanced Solid Tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>20-8-2014 (19-11-2015)</td>
</tr>
<tr>
<td>M15GLU</td>
<td>Glucose control for glucocorticoid induced hyperglycemia during chemotherapy (GLUCON)</td>
<td>Joke Baars</td>
<td></td>
<td>3/10/2015</td>
</tr>
<tr>
<td>M15MPA</td>
<td>An open-label, multicohort, phase II study of MPDL3280A in advanced solid tumors (Basket)</td>
<td>Cecile Grootscholten</td>
<td>II</td>
<td>7/3/2015</td>
</tr>
<tr>
<td>M15MSR</td>
<td>An Open Label, Phase Trial of the DNA-PK Inhibitor MSC2490484A in Combination with Radiotherapy in Patients with Advanced Solid Tumors</td>
<td>Jan Schellens</td>
<td>I</td>
<td>7/17/2015</td>
</tr>
<tr>
<td>M15OTD</td>
<td>Validation study of an assessment tool for Breakthrough Cancer Pain</td>
<td>Anne Lukas</td>
<td>other</td>
<td>8/5/2015</td>
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<tr>
<td>M15PDR</td>
<td>Open label multicenter Phase I/I study of the safety and efficacy of PDRD001 administered to patients with advanced malignancies</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>9/29/2015</td>
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<tr>
<td>Type of cancer study</td>
<td>Title</td>
<td>Study coordinator in NKI-AVL</td>
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<tr>
<td>M15SRB</td>
<td>Postoperative local stereotactic radiotherapy versus observation following complete resection of a single brain metastasis</td>
<td>Dieta Brandsma</td>
<td>III</td>
<td>9/9/2015</td>
</tr>
<tr>
<td>N07DOW</td>
<td>Weekly administration of oral Docetaxel in combination with Ritonavir</td>
<td>Serena Marchetti</td>
<td>I</td>
<td>11/14/2007</td>
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<tr>
<td>N10BOM</td>
<td>Weekly administration of (bi-1) daily Oral Docetaxel in combination with Ritonavir</td>
<td>Serena Marchetti</td>
<td>I</td>
<td>5/17/2010</td>
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<tr>
<td>N10CRC</td>
<td>Proof of principle and pharmacological phase 0 crossover study with controlled release capecitabine (MedrAcap001)</td>
<td>Serena Marchetti</td>
<td>I</td>
<td>11/17/2011</td>
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<tr>
<td>N10MOP</td>
<td>Development and clinical activity of low dose metronomic chemotherapy with oral paclitaxel</td>
<td>Jan Schellens</td>
<td>I</td>
<td>9/9/2010</td>
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<tr>
<td>N12MTG</td>
<td>Middle ear thiosulfate-gel protection against cisplatin-induced hearing loss in patients carrying a single nucleotide polymorphism in the TPMT, COMT or LRP2 gene</td>
<td>Jan Schellens</td>
<td>other</td>
<td>4/11/2013</td>
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<tr>
<td>N13OMA</td>
<td>An open-label study to investigate the pharmacokinetics (absorption, distribution and excretion) of Omacetaxine Mepesuccinate following subcutaneous administration (14CIDmepatexine Mepesuccinate in patients with relapsed and/or refractory hematologic malignancies or advanced solid tumors (C41443/1103)</td>
<td>Jan Schellens</td>
<td>I</td>
<td>23.8-2013 (3-11-2015)</td>
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<tr>
<td>N14CCT</td>
<td>Phase I pharmacological study of continuous and intermittent chronomodulated capecitabine therapy</td>
<td>Serena Marchetti</td>
<td>I</td>
<td>6/18/2014</td>
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<tr>
<td>N14CEC</td>
<td>Development of an assay for detection of Circulating Endothelial Cells (CEC) and Circulating Endothelial Progenitor cells (CEP) by fluorescence-activated cell sorting (FACS) in cancer patients and healthy individuals</td>
<td>Jan Schellens</td>
<td>other</td>
<td>7/22/2014</td>
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<tr>
<td>N14NIR</td>
<td>Absorption, metabolism, excretion, and the determination of absolute bioavailability of niraparib in subjects with cancer</td>
<td>Jan Schellens</td>
<td>I</td>
<td>1/20/2015</td>
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<tr>
<td>N14PLI</td>
<td>Mass balance Study of Plitidepsin Administered Intravenously Over 3 Hours to Patients with Advanced Cancer</td>
<td>Jan Schellens</td>
<td>I</td>
<td>2/16/2015</td>
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<tr>
<td>N15LDC</td>
<td>The effect of prehydration on the pharmacokinetics of low-dose Cisplatin</td>
<td>Wouter Vogel</td>
<td>other</td>
<td>11/6/2015</td>
</tr>
</tbody>
</table>

**BIOBANK**

| B15CTD               | Circulating tumor DNA in cancer patients: development of a clinical diagnostic tests and establishment of a biobank | Michiel van der Heijden    | Biobank | 10/7/2015          |
| B15HNC               | Analyse van weefsel van patienten met een tumor in het hoofd-halsgebied | Lotje Zuur                 | Biobank | 9/3/2015           |
| B15IMM               | Longitudinal tumor and blood sampling in patients with advanced stage urothelial cancer of the bladder for the analysis of mechanisms of response to immunotherapy | Michiel van der Heijden    | Biobank | 10/7/2015          |
| B15DES               | Tissue sampling of oesophagogastric cancer to enable tailored therapies (Together) | Johanna                    | Biobank | 6/17/2015          |

**BRAIN / CNS**

<p>| M14NRG               | A Randomized Phase 3 Open Label Study of Nivolumab versus Bevacizumab and a Safety Study of Nivolumab or Nivolumab in Combination with Ipilimumab in Adult Subjects with Recurrent Glioblastoma (GBM) (CheckMate 143: CHECKpoint pathway and nivolumab clinical Trial Evaluation 143) | Dieta Brandsma             | III   | 12/4/2014          |</p>
<table>
<thead>
<tr>
<th>Type of cancer study</th>
<th>Title</th>
<th>Study coordinator in NKI-AVL</th>
<th>Phase</th>
<th>Activated (closed)</th>
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<tbody>
<tr>
<td>M15NFM</td>
<td>A randomized placebo-controlled study in patients with a Gallium-68 DOTATATE PET/CT positive, clinically non-functioning pituitary macroadenoma (NFMA) of the effect of Lanreotide autosolution on Tumor (adenoma) size (GALANT)</td>
<td>Marcel Stokkel</td>
<td>III</td>
<td>10/27/2015</td>
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<tr>
<td>M07CBE</td>
<td>Late effects of chemotherapy on brain functioning in the elderly</td>
<td>Sanne Schagen</td>
<td>other</td>
<td>1-7-2008 (24-11-2015)</td>
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<tr>
<td>M08BCP</td>
<td>Prospective and Retrospective register study of the German Adjuvant Cancer Study Group (GABG) for diagnosis and treatment of breast cancer in pregnancy (BOOG 2003-04)</td>
<td>Sabine Linn</td>
<td>other</td>
<td>3/27/2008</td>
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<tr>
<td>M09TNM</td>
<td>Randomized phase II/III study of individualized neo-adjuvant chemotherapy in triple negative breast tumors (Neo-TN)</td>
<td>Sjoerd Rodenhuis</td>
<td>II/III</td>
<td>1/7/2010</td>
</tr>
<tr>
<td>M11FAM</td>
<td>Breast density as indicator for the use of mammography or MRI to screen women with familiar risk for breast cancer; a RCT (FaMRisc)</td>
<td>Emiel Rutgers</td>
<td>other</td>
<td>11/30/2011</td>
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<tr>
<td>M12BRC</td>
<td>BRCA mutations and ovarian ageing in normo-ovulatery women (BRAVA)</td>
<td>Lizet van der Kolk</td>
<td>other</td>
<td>20-9-2012 (13-2-2015)</td>
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<tr>
<td>M12DEN</td>
<td>Early detection of breast cancer in women with dense breasts (BENSE)</td>
<td>Claudette Loo</td>
<td>other</td>
<td>9/19/2012</td>
</tr>
<tr>
<td>M12LJM</td>
<td>A Multicenter, open-label, dose escalation, Phase I study of LJM716 administered intravenously in combination with trastuzumab in patients with HER2 overexpressing metastatic breast cancer or gastric cancer (CLM16X2102)</td>
<td>Jan Schellens</td>
<td>I</td>
<td>3-10-2012 (3-11-2015)</td>
</tr>
<tr>
<td>M12SSU</td>
<td>Detectie van onstekingsgeassocieerde eiwitprofielen in het serum, speeksel en urine van patiënten met mammatumoren</td>
<td>Emiel Rutgers</td>
<td>other</td>
<td>4/17/2012</td>
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<tr>
<td>M13BVG</td>
<td>Hereditary breast cancer and the clinical significance of variants within the BRCA1 and BRCA2 genes</td>
<td>Lizet van der Kolk</td>
<td>other</td>
<td>29-8-2013 (13-2-2015)</td>
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<tr>
<td>M13CPT</td>
<td>Early monitoring of trastuzumab +/- pertuzumab therapy with 18F-choline PET/CT in patients with advanced breastcancer</td>
<td>Wouter Vogel</td>
<td>Pilot</td>
<td>27-1-2014 (3-11-2015)</td>
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<tr>
<td>M13CYP</td>
<td>Phenotyping CYP2D6 in localized and in metastasized breast cancer patients using tamoxifen (metCYP study)</td>
<td>Frans Opdam</td>
<td>other</td>
<td>27-11-2013 (3-11-2015)</td>
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<tr>
<td>M13DDR</td>
<td>Breast cancer with low risk of local recurrence: partial and accelerated radiation with three-dimensional conformal radiotherapy (3DCRT) vs standard radiotherapy after conserving surgery (IRMA)</td>
<td>Nicola Russell</td>
<td>III</td>
<td>3/14/2014</td>
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<tr>
<td>M13MBC</td>
<td>Male Breast Cancer: prospective into perspective</td>
<td>Nicola Russell</td>
<td>other</td>
<td>4/10/2014</td>
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<tr>
<td>M13MUL</td>
<td>Multicenter study on the clinical relevance of one injection per tumor for sentinel node biopsy in patients with multiple breast tumors (multisent II)</td>
<td>Hester Oldenburg</td>
<td>other</td>
<td>4-6-2014 (16-11-2015)</td>
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<tr>
<td>M13SIB</td>
<td>A randomized study of an internet-based cognitive therapy program for sexuality and intimacy problems in women treated for breast cancer</td>
<td>Neil Aaronson</td>
<td>other</td>
<td>8/27/2013</td>
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<tr>
<td>M13TNB</td>
<td>Biomarker discovery randomized phase Ib trial with Carboplatin-Cyclophosphamide versus Paclitaxel with or without Bevacizumab as first-line treatment in advanced triple negative breast cancer (TRIPLE-B)</td>
<td>Sabine Linn</td>
<td>II</td>
<td>7/9/2013</td>
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<td>Type of cancer study</td>
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<td>M13TRT</td>
<td>Optimizing neoadjuvant systemic treatment in HER2 positive breast cancer - the TRAIN-2 study (BDOG 2012-03)</td>
<td>Gabe Sonke</td>
<td>III</td>
<td>12/9/2013</td>
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<tr>
<td>M13WEL</td>
<td>Downsides of being well-informed: tracking and preventing chemotherapy-related cognitive problems in breast cancer patients (CONTEXT)</td>
<td>Sanne Schagen</td>
<td>other</td>
<td>10/14/2013</td>
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<td>M14CAT</td>
<td>The value of completion axillary treatment in sentinel node positive breast cancer patients undergoing a mastectomy. A Dutch randomized controlled multicentre trial (BDOG 2013-07)</td>
<td>Jos van der Hage</td>
<td>III</td>
<td>7/24/2014</td>
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<tr>
<td>M14ECE</td>
<td>PI3K pathway analysis in tumor tumor tissue and circulating DNA to obtain further insight in the efficacy of everolimus when combined with exemestane. A side-study attached to standard treatment with everolimus and exemestane for postmenopausal patients with hormone receptor-positive advanced metastatic breast cancer, who have progressed on anastrozole or letrozol (BDOG 2013-06)</td>
<td>Sabine Linn</td>
<td>other</td>
<td>3/24/2015</td>
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<tr>
<td>M14HAR</td>
<td>Identifying subgroups with high cardiovascular risk in breast cancer survivors (HARBROR)</td>
<td>Floor van Leeuwen</td>
<td>other</td>
<td>4/13/2015</td>
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<tr>
<td>M14MON</td>
<td>A randomized double-blind, placebo-controlled study of LEESI 1 in combination with letrozole for the treatment of postmenopausal women with hormone receptor positive, HER2-negative, advanced breast cancer who received no prior therapy for advanced disease (MONALEESA-2)</td>
<td>Gabe Sonke</td>
<td>III</td>
<td>23-4-2014 (30-1-2015)</td>
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<tr>
<td>M14PER</td>
<td>A randomized phase II trial of pertuzumab in combination with trastuzumab with or without chemotherapy, both followed by T-DM1 in case of progression, in patients with HER2-positive metastatic breast cancer (Perneittta trial)</td>
<td>Carolina Smorenbure</td>
<td>II</td>
<td>5/7/2015</td>
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<tr>
<td>M14POS</td>
<td>Phase I/prospective randomized phase II trial Of the Safety and Efficacy of tamoxifen in combination with the Isoform selective PI3K inhibitor GDC-0032 compared with tamoxifen alone in hormone receptor positive, HER2 negative, metastatic breast cancer patients with prior endocrine treatment (POSEIDON trial)</td>
<td>Sabine Linn</td>
<td>I/II</td>
<td>31-10-2014 (9-11-2015)</td>
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<tr>
<td>M14REV</td>
<td>A phase I followed by a randomized phase II trial of two cycles carboplatin-dlaparib followed by olaparib monotherapy versus capecitabine in BRCA1 or -2 mutated Her2-negative advanced breast cancer as first line treatment (REVIVAL study)</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>4/21/2015</td>
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<tr>
<td>M14STG</td>
<td>An open randomized phase III study to compare 8 cycles of chemotherapy with 8 cycles of intermittent (2 times 4 cycles) chemotherapy in first line treatment, in combination with bevacizumab, and second line treatment of patients with HER2/neu negative, incurable, metastatic or unresectable locally advanced breast cancer (STOPEGG)</td>
<td>Sabine Linn</td>
<td>III</td>
<td>7/6/2015</td>
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<tr>
<td>M15OLY</td>
<td>A randomised double-blind parallel group placebo controlled multicenter phase III study to assess the efficacy and safety of olaparib versus placebo as adjuvant treatment in patients with germline BRCA1/2 mutations and high risk HER2 negative primary breast cancer who have completed definitive local treatment and neoadjuvant or adjuvant chemotherapy (OLYMPIAD) (BDOG 2014-03)</td>
<td>Gabe Sonke</td>
<td>III</td>
<td>6/3/2015</td>
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<tr>
<td>M15RIF</td>
<td>MONALEESA-3: A randomized double-blind, placebocontrolled study of ribociclib in combination with fulvestrant for the treatment of postmenopausal women with hormone receptor positive, HER2-negative, advanced breast cancer who have received no or only one (MONALEESA-3)</td>
<td>Gabe Sonke</td>
<td>III</td>
<td>9/17/2015</td>
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<tr>
<td>N07BOS</td>
<td>Genetic determinants of survival and second breast cancer development in premenopausal breast cancer patients (BOSDM)</td>
<td>Marjanka Schmidt</td>
<td>other</td>
<td>12/12/2007</td>
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<tr>
<td>N08AFT</td>
<td>A randomized prospective trial of 2-6 weeks pre-operative hormonal treatment for hormone receptor positive breast cancer: Anastrozole +/- fulvestrant or tamoxifen exposure - response in molecular profile (AFTER-study)</td>
<td>Sabine Linn</td>
<td>II</td>
<td>8/4/2008</td>
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<tr>
<td>Type of study</td>
<td>Title</td>
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<td>N08RMB</td>
<td>Tumorresponse monitoring in patients with breast cancer treated with primary systemic therapy: towards predicting response in both the primary tumor and in axillary lymph nodes</td>
<td>Marie Jeanne Vrancken Peeters</td>
<td>II</td>
<td>9/23/2008</td>
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<tr>
<td>N09PRF</td>
<td>Analgesia and nerve function following pulsed radiofrequency for postmastectomy pain (PRF4PMPS)</td>
<td>Anne Lukas</td>
<td>II</td>
<td>6/2/2010</td>
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<tr>
<td>N10RFS</td>
<td>Observational study into specific in vivo human discrimination between benign and malignant tissue using a combination of diffuse reflectance and fluorescence spectroscopy (DiSpect)</td>
<td>Theo Ruers</td>
<td>other</td>
<td>10/25/2010</td>
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<td>N11ISN</td>
<td>Monitoring of the &quot;healthy&quot; immune response in the sentinel lymph node of patients undergoing a prophylactic mastectomy</td>
<td>Emiel Rutgers</td>
<td>other</td>
<td>27-7-2011 (17-11-2015)</td>
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<tr>
<td>N12CLM</td>
<td>Determining the sensitivity and specificity of circulating tumor cells and cytology in cerebrospinal fluid of patients clinically suspected for leptomeningeal metastases</td>
<td>Dieta Brandsma</td>
<td>I</td>
<td>6/19/2012</td>
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<tr>
<td>N12OLG</td>
<td>High-dose alkylating chemotherapy in oligo-metastatic breast cancer harboring homologous recombination deficiency (OLIGO)</td>
<td>Gabe Sonke</td>
<td>III</td>
<td>7/3/2012</td>
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<tr>
<td>N12PRE</td>
<td>Okselklieroperatie zonder drain postoperatief en met behulp van het LigaSure Precise instrument tijdens de operatie (PRECISE)</td>
<td>Emiel Rutgers</td>
<td>II</td>
<td>6-6-2012 (3-11-2015)</td>
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<tr>
<td>N13ORB</td>
<td>Olaparib dose escalation combined with radiotherapy in patients with insurable breast cancer</td>
<td>Gabe Sonke</td>
<td>I</td>
<td>8/23/2013</td>
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<td>N14FHS</td>
<td>Feasibility study to assess the incremental value of DeclipSPECT during radioactive seed localisation in breast cancer surgery</td>
<td>Marie Jeanne Vrancken Peeters</td>
<td>other</td>
<td>12/15/2014</td>
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<td>N14MLS</td>
<td>Pilot for high-resolution SPECT imaging of breast cancer lumpectomy specimens for 3D identification and quantification of resection margins</td>
<td>Wouter Vogel</td>
<td>Pilot</td>
<td>7/24/2014</td>
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<tr>
<td>N14OPT</td>
<td>Optimizing Performance of afinitor by splitting Intake Moments and decreasing Adverse events whilst maintaining outcome quality (OPTIMAL study)</td>
<td>Neeltje Steeghs</td>
<td>other</td>
<td>5/27/2015</td>
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<td>N15CGC</td>
<td>A comparison of a hybrid compact gamma camera with planar lymphoscintigraphy to simplify the SN procedure (Xstrahl)</td>
<td>Marcel Stokkel</td>
<td>other</td>
<td>4/14/2015</td>
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<tr>
<td>N15MMC</td>
<td>Evaluation of a high-resolution dedicated hanging breast PET guided biopsy device for breast cancer diagnosis</td>
<td>Marcel Stokkel</td>
<td>other</td>
<td>9/8/2015</td>
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<tr>
<td>N15TON</td>
<td>Adaptive phase II randomized non-comparative trial of nivolumab after induction treatment in triple-negative breast cancer (TNBC) patients: TGNC-trialM14PRT</td>
<td>Marleen Kok</td>
<td>II</td>
<td>9/10/2015</td>
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</table>
GASTRO INTESTINAL

C14GIST
Prospective registratie GIST patienten
Neeltje Steeghs other 1/13/2014

E40091
Randomized phase II trial evaluating the efficacy of FOLFOX alone, FOLFOX plus bevacizumab and FOLFOX plus panitumumab as peroperative treatment in patients with resectable liver metastases from wild type KRAS colorectal cancer (BOSS/ BOS2)
Theo Ruers II 2/12/2014

M06CRI
A multicenter randomized phase III trial of neo-adjuvant chemotherapy followed by surgery and chemotherapy or by surgery and chemoradiotherapy in resectable gastric cancer (CRITICS-study: ChemoRadiotherapy after Induction chemo Therapy In Cancer of the Stomach)
Annemieke Cats III 11-1-2007 (17-4-2015)

M08ACL
Accelerated growth of synchronous colorectal liver metastases: effects of neo-adjuvant therapy (SILENT)
Theo Ruers II 2/21/2008

M08GPO
Genetic and protein profiling in patients with oesophageal cancer (PROFDEC)
Johanna van Sandick other 9/1/2008

M09OCB
A pilot evaluating response to induction chemotherapy with oxaliplatin, capecitabine and bevacizumab in patients with extensive peritoneal carcinomatosis of colorectal origin
Arend Aalbers Pilot 3/25/2010

M11BIO
Feasibility study of biomarker development for response prediction by large scale DNA mutational analysis of metastatic lesions (CPCT-01 trial)
Neeltje Steeghs other 12/7/2011

M12DEC
A randomized trial of dose escalation in definitive chemoradiotherapy for patients with oesophageal cancer (ART DEC0)
Berthe Aleman III 2/12/2013

M12LGX
A phase Ib/II multicenter, open label, dose escalation study of LGX818 and cetuximab or LGX818, BYL 719 and cetuximab in patients with BRAF mutant colorectal cancer
Jan Schellens I/II 11/15/2012

M12LJM
A Multicenter, open-label, dose escalation, Phase I study of LJM716 administered intravenously in combination with trastuzumab in patients with HER2 overexpressing metastatic breast cancer or gastric cancer (CLJM16X2102)
Jan Schellens I 3-10-2012 (3-11-2015)

M12RAP
Randomized multicentre phase III study of short course radiation therapy followed by prolonged pre-operative chemotherapy and surgery in primary high risk rectal cancer compared to standard chemoradiotherapy and surgery (RAPIDO study)
Annemieke Cats III 2/12/2013

M13DAP
Combination of dacomitinib and PD-325361 in advanced KRAS mutation positive colorectal, non-small cell lung and pancreatic cancer
Jan Schellens I 1/5/2014

M13DPT
An open-label, three-part, phase I/II study to investigate the safety, pharmacokinetics, pharmacodynamics, and clinical activity of the MEK inhibitor GSK1120212, B.R.A.F. inhibitor GSK2118436, and the anti-EGFR antibody Panitumumab in combination with BRAF-mutation V600E positive colorectal cancer
Jan Schellens I/II 9/23/2013

M13QRC
A randomized multicenter clinical trial for patients with multi-organ, colorectal cancer metastases comparing the combination of chemotherapy and maximal tumor debulking versus chemotherapy alone (ORCHESTRA)
Cecile Grootscholten III 6/8/2015

M13PRC
A phase Ib/II, open-label, multi-center, dose escalation study of MEK162 in combination with panitumumab in adult patients with mutant RAS or wild-type RAS metastatic colorectal cancer
Jan Schellens I/II 2/25/2014

M13REL
Radioembolization for colorectal liver metastasis after ablation: a prospective study (RELAPSE)
Warner Prevoo II 5/8/2014

M13SCO
Peritoneal dissemination in stomach cancer patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (PERISCOPE)
Johanna van Sandick I/II 1/15/2014
<table>
<thead>
<tr>
<th>Study ID</th>
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<th>Study Coordinator</th>
<th>Phase</th>
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<tr>
<td>M14AFS</td>
<td>Phase I/II study with the combination of afatinib and selumetinib in advanced K Ras mutant positive and PIK3CA wildtype colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>5/19/2015</td>
</tr>
<tr>
<td>M14CRS</td>
<td>Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases - a randomised phase 3 study of the Dutch Colorectal Cancer Group (DCCG) (CAIRO-5)</td>
<td>Cecile Grootscholten</td>
<td>III</td>
<td>6/9/2015</td>
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<tr>
<td>M14FMR</td>
<td>Pilot study evaluating the feasibility of endoscopy guided fiducial marker placement for rectal cancer</td>
<td>Monique van Leerdam</td>
<td>Pilot</td>
<td>5/8/2014</td>
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<tr>
<td>M14LTK</td>
<td>Phase I/II study with lapatinib plus trametinib in patients with metastatic K Ras mutant colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>8/4/2014</td>
</tr>
<tr>
<td>M14SAL</td>
<td>S-1 versus capecitabine in the first line treatment of metastatic colorectal cancer patients, the SALTO randomised phase III study of the Dutch Colorectal Cancer Group. A safety evaluation of oral fluoropyrimidines</td>
<td>Annemieke Cats</td>
<td>III</td>
<td>11-7-2014 (7-7-2015)</td>
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<tr>
<td>M14TLCD</td>
<td>A phase 3, randomized, placebo-controlled, multicenter, doubleblind study to evaluate the safety and efficacy of Telotristat Etiprate (LX1606) in patients with carcinoid syndrome (TELECAST)</td>
<td>Margot Tesselaar</td>
<td>III</td>
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<td>M14TRAP</td>
<td>Feasibility study of chemoradiation, TRAstuzumab and Pertuzumab in resectable HER2+ oesophageal carcinoma (TRAP)</td>
<td>Annemieke Cats</td>
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<td>M14TUM</td>
<td>Tumor organoids: feasibility to predict sensitivity to treatment in cancer patients (TUMOROID trial)</td>
<td>Emile Voest</td>
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<td>M14WAVE</td>
<td>Wallflex versus Egis stent for palliation of malignant dysphagia (WAVE)</td>
<td>Annemieke Cats</td>
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<td>M14WLC</td>
<td>A phase II/II multi-center, open label, dose escalation study of WNT1974, LGX818 and cetuximab in patients with BRAFV600-mutant KRAS wild-type metastatic colorectal cancer harboring Wnt pathway mutations</td>
<td>Jan Schellens</td>
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<td>M15COL</td>
<td>Adjuvant hyperthermic intraperitoneal chemotherapy in patients with colon cancer at high risk of peritoneal carcinomatosis, the COLOPEC randomized multicentre trial</td>
<td>Arend Aabers</td>
<td>other</td>
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<td>M15DIS</td>
<td>Implementation of Resect and Discard strategy for diminutive polyps amongst accredited endoscopists for the Dutch bowel cancer screening program, training and long-term quality assurance (NTR 4635). (DISCOUNT II)</td>
<td>Monique van Leerdam</td>
<td>other</td>
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<tr>
<td>M15LPR</td>
<td>Over het meten van frailty bij in opzet curative radiotherapie bij ouderen met borst-, long-, prostaat-, haof/hals-, endelbarm-, sigmoidkanker (LPMD-1)</td>
<td>Abraham Al-Mamgani</td>
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<td>M15RAI</td>
<td>A Randomized, Double-Blind, Placebo-Controlled Phase 3 Study of Capecitabine and Cisplatin With or Without Ramucirumab as First-line Therapy in Patients With Metastatic Gastric or Gastrooesophageal Junction Adenocarcinoma (RAINFALL)</td>
<td>Annemieke Cats</td>
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<td>M15SCA</td>
<td>The sensitivity of scar-biopsies for residual colorectal adenocarcinoma after endoscopic resection with uncertain radicality (SCAPURA)</td>
<td>Monique van Leerdam</td>
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<td>M15SOX</td>
<td>Feasibility study of adjuvant treatment with 5-1 and oxaliplatin in patients with resectable esophagael cancer (SDX)</td>
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<td>N05TP</td>
<td>Serum and tissue protein profiling and tumour genetic analysis in patients with potential premalignant conditions or colorectal cancer</td>
<td>Annemieke Cats</td>
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<td>N10RFS</td>
<td>Observational study into specific in vivo human discrimination between benign and malignant tissue using a combination of diffuse reflectance and fluorescence spectroscopy (OpSpect)</td>
<td>Theo Ruers</td>
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<td>N12CLM</td>
<td>Determining the sensitivity and specificity of circulating tumor cells and cytology in cerebrospinal fluid of patients clinically suspected for leptomeningeal metastases</td>
<td>Dieta Brandsma</td>
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<td>9/5/2012</td>
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<td>N13OME</td>
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<td>N15MSC</td>
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**GYNAECOLOGICAL**

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<td>M05PPO</td>
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<td>M05SNV</td>
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<td>M06OVH</td>
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<td>M07RCV</td>
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<td>M10MKD</td>
<td>II</td>
<td>7/8/2010</td>
<td>Jan Schellens</td>
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<td>M11CIR</td>
<td>Other</td>
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<td>Gemma Kenter</td>
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<td>M11LOC</td>
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<td>M12RTE</td>
<td>III</td>
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<td>Monique Bloemers</td>
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<td>M12SOC</td>
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<td>M13BI</td>
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<td>M13MAM</td>
<td>Long drainage of the groins after inguino-femoral lymphadenectomy (MAMBO 1A)</td>
<td>Henry Zijmанс</td>
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<td>M13DST</td>
<td>A Phase III, Randomised, Double Blind, Placebo Controlled, Multicentre Study of Diaparib Maintenance Monotherapy in Patients with BRCA Mutated Advanced (FIGO Stage III-IV) Duvian Cancer following First Line Platinum Based Chemotherapy (SOLD 1)</td>
<td>Gabe Sonke</td>
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<td>M14ISA</td>
<td>A multicenter, open label Phase I/Ii study to determine the safety and immune modulating effects of the therapeutic Human Papilloma Virus Type 16 (HPV16) E6/ E7 Synthetic Long Peptides Vaccine (ISA101) at different doses with or without interferon alpha as (CervISA)</td>
<td>Gemma Kenter</td>
<td>I/II</td>
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<td>M15ENS</td>
<td>Endometrial cancer SURvivors’ follow-up carE: Less is more? Randomized controlled trial to evaluate patient satisfaction and cost-effectiveness of a reduced follow-up schedule (ENSURE)</td>
<td>Hans Trum</td>
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<td>M15MAM</td>
<td>Short drainage of the groins after inguino-femoral lymphadenectomy in patients with vulvar cancer (MAMBO IB)</td>
<td>Henry Zijmанс</td>
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<td>M15PAG</td>
<td>Topical 5% imiquimood cream for vulvar Paget’s Disease: clinical efficacy, safety and immunological response (PAGET)</td>
<td>Marc van Beurden</td>
<td>other</td>
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<td>N12INT</td>
<td>Pilot study to evaluate the tumor-reactivity of infiltrating T cells in human malignancies</td>
<td>Sander Kelderman</td>
<td>Pilot</td>
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<td>N14HPV</td>
<td>Safety, toxicity and immunogenicity of HPV16 E7 DNA vaccination in HPV16+ vulvar intraepithelial neoplasia grade III a phase I study (SEVEN)</td>
<td>Gemma Kenter</td>
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**HEAD AND NECK**

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<td>M12PCA</td>
<td>An open-label, single arm, Phase II study to evaluate the safety and efficacy of PC-A11 with superficial and interstitial laser light application in patients with recurrent head and neck squamous cell carcinoma unsuitable for surgery and radiotherapy and without distant metastases</td>
<td>Karakulukcu, B.</td>
<td>II</td>
<td>17-7-2012 (8-7-2015)</td>
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<td>M13OPT</td>
<td>Klinische beoordeling van een nieuwe stomapleister voor het bevestigen van hulpmiddelen voor longrevalidatie na een totale laryngectomie: Provox Stabilibase Optiderm</td>
<td>Michel van den Brekel</td>
<td>IV</td>
<td>6-2-2014 (15-6-2015)</td>
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<td>M14PAR</td>
<td>TachoSil patch application as replacement of closed suction wound drainage by parotid gland surgery: a prospective study</td>
<td>Fons Balm</td>
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<td>M14PDC</td>
<td>Multicenter randomized 2x2 cross-over study for patient satisfaction and function of the ProTrach DualCare</td>
<td>Michiel van den Brekel</td>
<td>III</td>
<td>12/15/2014</td>
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<td>M14SEA</td>
<td>Phase-2 clinical trial on the treatment of chronic dysphagia in head and neck cancer patients with dedicated strengthening exercises using the Swallow Exercise Aid</td>
<td>Michiel van den Brekel</td>
<td>II</td>
<td>9/1/2015</td>
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<td>M14VOX</td>
<td>Clinical assessment of a new speaking valve for hands-free speech in laryngectomized subjects: Provox® FreeHands FlexiVoice</td>
<td>Michiel van den Brekel</td>
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<td>M15CRH</td>
<td>Dutch randomized multicenter trial Comparing two Palliative Radiation schemes for incurable head and neck cancer (COOPERATION)</td>
<td>Abraham Al-Mamgani</td>
<td>III</td>
<td>11/12/2015</td>
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<td>M15SEVR</td>
<td>Externe validatie van het risicopredictie model van patiënten met T3T4 larynxcancerinum</td>
<td>Michiel van den Brekel</td>
<td>other</td>
<td>11/13/2015</td>
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<td>M15LPR</td>
<td>Door het meten van frailty bij in opzet curatieve radiotherapie bij ouderen met borst-, long-, prostaat-, hoofd/hals-, endeldarm-, slokdarmkanker (LPRO-1)</td>
<td>Abraham Al-Mamgani</td>
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<td>4-8/2015 (30-10-2015)</td>
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<td>M15MKH</td>
<td>Phase 3 Randomized Trial of MK-3475 (Pembrolizumab) vs standard treatment in subjects with recurrent or metastatic head and neck cancer</td>
<td>Jan Paul de Boer</td>
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<td>M15PF0</td>
<td>A phase I, open-label, dose escalation study of PF-04518600 in patients with locally advanced or metastatic hepatocellular carcinoma (HCC), melanoma, clear cell renal cell carcinoma (RCC) or squamous cell head and neck cancer (SCCHN)</td>
<td>Jan Schellens</td>
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<td>N05HME</td>
<td>De korte termijn invloed van een Heat and Moisture Exchanger op de endotracheale temperatuur en luchtvochtigheid bij gelaryngectomeerden</td>
<td>Frans Hilgers</td>
<td>other</td>
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<td>N10OPT</td>
<td>Optimising photodynamic therapy for the treatment of head and neck cancer</td>
<td>Baris Karakulukcu</td>
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<td>N10VMO</td>
<td>Evaluation of tumor variability with MRI during radiotherapy treatment in patients with an oropharyngeal or oral cavity carcinoma</td>
<td>Olga Hamming-Vrieze</td>
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<td>N12MAC</td>
<td>Exploring the contribution of Macrophages in the microenvironment of HPV-induced squamous cell carcinoma of the head and neck (MSM)</td>
<td>Jan Paul de Boer</td>
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<td>8/31/2012</td>
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<td>N13QMM</td>
<td>Effects of radiation therapy on oral mucosal microcirculation in patients with malignant disease in the head and neck region</td>
<td>Ludi Snoeij</td>
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<td>N13ORH</td>
<td>Olaparib dose escalation trial in patients treated with radiotherapy for stage II-II laryngeal and stage II-III HPV-negative oropharyngeal squamous cell carcinoma</td>
<td>Marcel Verheij</td>
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<td>N14IMR</td>
<td>The immunological aspects of conventional therapies for the treatment of head and neck squamous cell carcinoma (HNSCC). An exploratory study to study the immunological effects of (chemo)radiotherapy in HNSCC patients (IMRAD)</td>
<td>Lotje Zuur</td>
<td>other</td>
<td>3/23/2015</td>
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<td>N14LMN</td>
<td>Lymphatic mapping of the neck in patients with oral cavity malignancies using ICG-nanocolloid</td>
<td>Martin Klop</td>
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<td>N14SUS</td>
<td>Sentinel node mapping Using SPECT to tailor highly-selective elective nodal irradiation in node-negative neck of patients with head and neck cancer (SUSPECT)</td>
<td>Abraham Al-Mamgani</td>
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<td>Protocol apopose regulatie van CLL</td>
<td>Joke Baars</td>
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<td>MPP treatment plan for patients with advanced or metastatic NSCLC that have progressed during or following therapy with an EGFR and/or ALK inhibitor (AUY922)</td>
<td>Egbert Smit</td>
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<td>Compassionate use program LDK378 (CERITINIB)</td>
<td>Michel van den Heuvel</td>
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<td>Maropitant (AZD9291) compassionate use program for metastasized EGFR T790M mutation positive NSCLC</td>
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<td>Compassionate use programma crizotinib voor patienten met een MET mutatie</td>
<td>Michel van den Heuvel</td>
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<td>C15NIV</td>
<td>Nivolumab EA program for metastasized NSCLC (NIVOLUMA)</td>
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<td>C15RET</td>
<td>Compassionate use programma sunitinib voor patienten met een RET mutatie (NKI-RETO03)</td>
<td>Michel van den Heuvel</td>
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<td>M09PBO</td>
<td>Dose escalation by boosting radiation dose within the primary tumor on the basis of a pre-treatment FDG-PET-CT scan in stage I, II and III NSCLC: a randomised phase II trial (PET-BOOST trial)</td>
<td>José Belderbos</td>
<td>II</td>
<td>11/26/2009</td>
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<td>M11LUN</td>
<td>A project of European Thoracic Oncology Platform (lungscape)</td>
<td>Paul Baas</td>
<td>other</td>
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<td>M11VOL</td>
<td>Treatment of larger tumor volumes or &gt; 2 lung tumors simultaneously in lung cancer patients using SBRT in a mean-lung dose escalation study (VOLUMES)</td>
<td>Heike Poulten</td>
<td>I/II</td>
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<td>M12PHA</td>
<td>Prophylactic Cranial Irradiation with or without hippocampal avoidance in SCLC: a randomized phase III study (HAPCI)</td>
<td>José Belderbos</td>
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<td>M13ABP</td>
<td>A randomized, double-blind,phase 3 study evaluating the efficacy and safety of ABP215 compared with Bevacizumab in subject with advanced non-small cell lung cancer</td>
<td>Sjaak Burgers</td>
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<td>M13DAP</td>
<td>Combination of dacomitinib and PD-0325901 in advanced KRAS mutation positive colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens</td>
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<td>M13LDK</td>
<td>A phase III multicenter, randomized study of oral LDK378 versus standard chemotherapy in previously untreated adult patients with ALK rearranged (ALK-positive), stage IIIIB or IV, non-squamous non-small cell lung cancer (Ascend-4)</td>
<td>Michel van den Heuvel</td>
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<td>M13MKD</td>
<td>A phase II/III randomized trial of two doses of MK-3475 (SCHR00375) versus docetaxel in previously treated subjects with non-small cell lung cancer</td>
<td>Paul Baas</td>
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<td>Iressa RE-challenge in advanced NSCLC EGFR mutated patients who responded to an EGFR-TKI used as first-line or previous treatment (NVALT16) (IRENE)</td>
<td>Sjaak Burgers II</td>
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<td>A phase II, double-blind, randomised, placebo-controlled study to assess the efficacy and safety of Selumetinib (AZD6244; ARRY-142886) (Hyd-Sulfate) in combination with Docetaxel, compared with placebo in combination with Docetaxel, in patients receiving second line treatment for locally advanced or metastatic non small cell lung cancer (Stage IIIB-IV) (SELECT-2)</td>
<td>Michel van den Heuvel II</td>
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<td>M13SEL</td>
<td>A phase III, double-blind, placebo-controlled study to assess the efficacy and safety of Selumetinib (AZD6244;ARRY-2142886)(Hyd-Sulfate) in combination with Docetaxel, in patients receiving second line treatment for KRAS mutation-positive loc (SELECT-1)</td>
<td>Michel van den Heuvel II</td>
<td>10/4/2013</td>
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<td>M13VSM</td>
<td>A phase II, randomized, double-blind, multicenter study of VS-6083 in subjects with malignant pleural mesothelioma who have not progressed on adequate front-line treatment with pemetrexed and platinum</td>
<td>Paul Baas II</td>
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<td>M14AFS</td>
<td>Phase I/Ii study with the combination of afatinib and selumetinib in advanced KRAS mutant positive and PIK3CA wt colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens I/I</td>
<td>5/19/2015</td>
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<tr>
<td>M14ALK</td>
<td>Non Small Cell Lung Cancer (NSCLC) ALK IHC positive study</td>
<td>Daphne de Jong</td>
<td>4/28/2014</td>
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<tr>
<td>M14APNI</td>
<td>A phase 2, multicenter, single-arm study of oral AP26113 in patients with ALK-positive, locally advanced or metastatic Non-small Cell Lung Cancer (NSCLC) who have previously been treated with crizotinib</td>
<td>Sjaak Burgers II</td>
<td>3-3-2015 (8-9-2015)</td>
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<tr>
<td>M14AUR</td>
<td>A Phase III, Open Label, Randomised Study of AZD9291 versus Platinum-based doublet chemotherapy for patients with locally advanced or metastatic NSCLC whose disease has progressed with previous epidermal growth factor receptor tyrosine kinase inhibitor therapy and whose tumours harbor a T790M mutation within the epidermal growth factor receptor gene (AURA3)</td>
<td>Willemijn Engelsman-Theelen III</td>
<td>17-10-2014 (27-8-2015)</td>
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<tr>
<td>M14AZI</td>
<td>A Phase I, Open-label, Non-randomised Study to Assess the Effect of Itracnazole (a CYP3A4 Inhibitor) on the Pharmacokinetics of a Single Oral Dose of AZD9291 in Patients with EGFRm Positive NSCLC Whose Disease has Progressed on an EGFR TKI (DS160C00012)</td>
<td>Jan Schellens I</td>
<td>1/23/2015</td>
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<td>M14AZR</td>
<td>A Phase I, Open-label, Non-Randomised, Multicentre Study to Assess the Effect of Rifampicin (a CYP3A4 inducer) on the Pharmacokinetics of AZD9291 in Patients with EGFRm Positive NSCLC whose disease has progressed on an EGFR TKI Sponsor: AstraZeneca (DS160C00013)</td>
<td>Jan Schellens I</td>
<td>1/23/2015</td>
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<td>M14CPR</td>
<td>Open-Label phase Ib/II multicenter study of the combination of ROS4795599 with Carboplatin and Paclitaxel in patients with advanced or metastatic Non-Small Cell Lung Cancer (NSCLC) of squamous histology who have not received prior chemotherapy or targeted Therapy for NSCLC.</td>
<td>Jan Schellens I/I</td>
<td>1/19/2015</td>
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<td>M14EN1</td>
<td>A phase II, multicenter, open-label study of EGFR16 in combination with Nivolumab in adult patients with EGFR mutated non-small cell lung cancer and of INC280 in combination with Nivolumab in adult patients with cMet positive non-small cell lung cancer</td>
<td>Willemijn Engelsman-Theelen II</td>
<td>6/9/2015</td>
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<td>M14FGF</td>
<td>Multi-arm, Non-randomized, Open-Label Phase III Study to Evaluate GS3052230 in Combination with Paclitaxel and Carboplatin, or Docetaxel or as Single Agent in Subjects with Solid Malignancies and Deregulated FGF Pathway Signaling (FGF117380)</td>
<td>Jan Schellens I</td>
<td>9/4/2014</td>
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<td>Type of cancer study</td>
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<td>Study coordinator in NKI-AVL</td>
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<tr>
<td>M14HAM</td>
<td>Determination of peripheral immune cell activity during treatment with either surgery or radiotherapy in patients with early stage non-small cell lung cancer (HAMLET)</td>
<td>Koen Hartemink</td>
<td>other</td>
<td>3/17/2015</td>
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<td>M14NG</td>
<td>A phase Ib/Ii, open-label, dose escalation and multicenter study of INC280 administered orally in combination with gefitinib in adult patients with EGFR mutated and c-MET amplified NSCLC who have progressed after EGFR inhibitor treatment</td>
<td>Willemijn Engelsman-Theelen</td>
<td>I/I</td>
<td>5/26/2015</td>
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<td>M14LTk</td>
<td>Phase I/Ii study with lapatinib plus trametinib in patients with metastatic KRAS mutant colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens</td>
<td>I/I</td>
<td>8/4/2014</td>
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<td>M14N15</td>
<td>Phase II study with oral fibroblast growth factor-1 inhibitor BIBF1120 as second line treatment in lung carcinoma patients harboring fibroblast growth factor receptor-1 gene amplification (NVALT-15)</td>
<td>Sjaak Burgers</td>
<td>II</td>
<td>9/12/2014</td>
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<tr>
<td>M14NIV</td>
<td>An open-label, randomized, phase 3 trial of Nivolumab versus Investigators’ Choice chemotherapy as first line for stage IV or recurrent PD-L1+ non-small cell lung cancer (NSCLC) (CheckMate 026:CHECKpoint pathway and nivliMA: TrialEvaluation 026)</td>
<td>Michel van den Heuvel</td>
<td>III</td>
<td>17-6-2014 (2-4-2015)</td>
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<tr>
<td>M14PAC</td>
<td>A phase II, randomised, double-blind, placebo-controlled, multi-centre, international study of MEDI4736 as sequential therapy in patients with locally advanced, unresectable non-small cell lung cancer (stage III) who have not progressed following definitive, platinum based, concurrent chemoradiation therapy (PACIFIC)</td>
<td>Michel van den Heuvel</td>
<td>III</td>
<td>6/9/2015</td>
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<tr>
<td>M14STA</td>
<td>Complete endosonographic intrathoracic nodal staging of lung cancer patients in whom SABR is considered (STAGE)</td>
<td>Wienieke Buijkhuisen</td>
<td>other</td>
<td>12/15/2014</td>
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<td>M14TIG</td>
<td>A phase 2, open-label, multicenter, safety and efficacy study of oral CD-1868 as 2nd line EGFR-directed TKI in patients with mutant EGFR non-small cell lung cancer (NSCLC) with the T790 resistance mutation (TIGER-2)</td>
<td>Michel van den Heuvel</td>
<td>II</td>
<td>2/16/2015</td>
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<td>M14TUM</td>
<td>Tumor organoids: feasibility to predict sensitivity to treatment in cancer patients (TUMOROID trial).</td>
<td>Emile Voest</td>
<td>other</td>
<td>7/22/2014</td>
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<td>M15CIN</td>
<td>A phase II, multicenter, three-cohort study of oral cMET inhibitor INC280 in adult patients with EGFR wild-type (wt), advanced non-small cell lung cancer (NSCLC) who have received one or two prior lines of systemic therapy for advanced/metastatic disease (CINC280A2201)</td>
<td>Egbert Smit</td>
<td>II</td>
<td>9/15/2015</td>
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<td>M15MYS</td>
<td>A phase III randomized, open-label, multi-center, global study of first-line MED14736 in combination with tremelimumab and MED14736 monotherapy versus standard of care platinum-based chemotherapy in first line treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) (MISTIC trial)</td>
<td>Michel van den Heuvel</td>
<td>III</td>
<td>10/14/2015</td>
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<tr>
<td>M15NIC</td>
<td>Afatinib in pretreated patients with advanced NSCLC harbouring HER2 exon 20 mutations. NICHE = afatinib in NSCLC with HER2 mutation (NICHE)</td>
<td>Egbert Smit</td>
<td>II</td>
<td>11/5/2015</td>
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<tr>
<td>M15MPI</td>
<td>An open-label/randomized phase 3 trial of nivolumab or nivolumab plus ipilimumab versus platinum doublet chemotherapy in subjects with chemotherapy-naive stage IV or recurrent non-small cell lung cancer (NSCLC) (CHECKMATE)</td>
<td>Michel van den Heuvel</td>
<td>III</td>
<td>11/12/2015</td>
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<tr>
<td>M15TID</td>
<td>A phase 3, open-label, multicenter, randomized study of oral rocitinib (CD-1686) monotherapy versus single-agent cytotoxic chemotherapy in patients with mutant EGFR non-small cell lung cancer (NSCLC) after failure of at least 1 previous EGFR-directed tyrosine kinase inhibitor (TKI) and platinum-doublet chemotherapy (TIGER-3)</td>
<td>Egbert Smit</td>
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<td>6/24/2015</td>
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<td>Type of cancer study</td>
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<td>N10ILM</td>
<td>Designing and testing new intervention therapies for lung cancer and mesothelioma</td>
<td>Paul Baas</td>
<td>other</td>
<td>30-8-2010 (12-11-2015)</td>
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<tr>
<td>N10RFS</td>
<td>Observational study into specific in vivo human discrimination between benign and malignant tissue using a combination of diffuse reflectance and fluorescence spectroscopy (OpSpect)</td>
<td>Theo Ruers</td>
<td>other</td>
<td>10/25/2010</td>
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<td>N11ORL</td>
<td>Olaparib dose escalating trial in patients treated with radiotherapy with or without daily dose cisplatin for locally advanced non-small lung cancer</td>
<td>Michel van den Houvel</td>
<td>I</td>
<td>2/21/2012</td>
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<tr>
<td>N12CLM</td>
<td>Determining the sensitivity and specificity of circulating tumor cells and cytology in cerebrospinal fluid of patients clinically suspected for leptomeningeal metastases</td>
<td>Dieta Brandsma</td>
<td>I</td>
<td>6/19/2012</td>
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<td>N12HYB</td>
<td>Phase I study: combined stereotactic radiotherapy and conventional fractionation in stage II and III non small cell lung cancer with peripheral tumor smaller than 5 cm (HYBRID)</td>
<td>Heike Poulén</td>
<td>I</td>
<td>7/12/2012</td>
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<tr>
<td>N12LON</td>
<td>Longitudinal analysis of lung cancer-specific immunity in stage III and IV lung cancer patients</td>
<td>Michel van den Houvel</td>
<td>other</td>
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<tr>
<td>N12LPR</td>
<td>Early FDG-PET/CT response evaluation of lung cancer during chemoradiation</td>
<td>Wouter Vogel</td>
<td>other</td>
<td>12/10/2012</td>
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<td>N12PRO</td>
<td>Pharmacogenomic profiling of short-term cultures of malignant pleural mesothelioma</td>
<td>Josine Quispel</td>
<td>other</td>
<td>9/21/2012</td>
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<tr>
<td>N13FPB</td>
<td>Fluid phase biopsy (circulating tumour DNA and serum tumour markers) in patients with non-small cell lung cancer</td>
<td>Wim Groen</td>
<td>other</td>
<td>12/17/2013</td>
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<td>N13POR</td>
<td>Evaluating feasibility and preliminary effects of a patient portal on empowerment of breast and lung cancer patients ans survivors (A-CaRe)</td>
<td>Floor van Leeuwen</td>
<td>other</td>
<td>12/5/2013</td>
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<td>N14TO</td>
<td>Immunogenicity of Tumor Organoids, a feasibility study</td>
<td>Emile Voest</td>
<td>other</td>
<td>7/22/2014</td>
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<tr>
<td>N14MPN</td>
<td>A single arm phase II study of Nivolumab in patients with recurrent malignant pleural mesothelioma: interim biopsy analysis to determine efficacy (NivoMos)</td>
<td>Paul Baas</td>
<td>II</td>
<td>7/3/2015</td>
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<tr>
<td>N14PLU</td>
<td>Personalized treatment with combination therapy for patients with pleural effusion due to malignant pleural mesothelioma or lung cancer in second or third line. An open label phase II study (PROOF)</td>
<td>Paul Baas</td>
<td>II</td>
<td>10/3/2014</td>
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**LYMPHOMA - HODGKIN’S DISEASE**

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<thead>
<tr>
<th>Type of cancer study</th>
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<th>Study coordinator in NKI-AVL</th>
<th>Phase</th>
<th>Activated (closed)</th>
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<tbody>
<tr>
<td>M13SOP</td>
<td>Study of Menopause in ex-patients with Hodgkin Lymphoma: influence on long-term adverse events (SOPHIA)</td>
<td>Floor van Leeuwen</td>
<td>other</td>
<td>1/17/2014</td>
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<td>M14CHL</td>
<td>Diagnostic yield of screening colonoscopy in Hodgkin lymphoma survivors (IDICHOS)</td>
<td>Monique van Leeuwen</td>
<td>other</td>
<td>10/21/2014</td>
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<tr>
<td>M15MKL</td>
<td>A phase II clinical trial of MK3475 in subjects with relapsed or refractory (R/R) classical Hodgkin Lymphoma (cHL)</td>
<td>Jan Paul de Boer</td>
<td>II</td>
<td>10/27/2015</td>
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<tr>
<td>Type of cancer study</td>
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<td><strong>LYMPHOMA - NON-HODGKIN’S</strong></td>
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<td>M12H11D</td>
<td>A randomized Phase I/II trial of lenalidomide and rituximab with or without bendamustine in patients &gt; 18 years with a relapsed follicular lymphoma. (RoBel/ HDVON110)</td>
<td>Joke Baars</td>
<td>II</td>
<td>6/12/2012</td>
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<tr>
<td>M14NRL</td>
<td>A Single-Arm, Open-Label, Phase 2 Study of Nivolumab (BMS-936558) in Subjects with Relapsed or Refractory Diffuse Large B-Cell Lymphoma (DLBCL) After Failure of Autologous Stem Cell Transplant (ASCT) or After Failure of At Least Two Prior Multi-Agent Chemotherapy Regimens in Subjects Who Are Not Candidates for ASCT (Checkmate 139)</td>
<td>Jan Paul de Boer</td>
<td>II</td>
<td>6-11-2014 (13-7-2015)</td>
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<td>M15ROB</td>
<td>Phase 3 randomized, double-blind, placebo controlled, multicenter study to compare the efficacy and safety of Lenalidomide (CC-5013) plus R-CHOP chemotherapy (R2-CHOP) versus placebo plus R-CHOP chemotherapy in subjects with previously untreated activated B-cell type diffuse B-cell lymphoma (ROBUST)</td>
<td>Joke Baars</td>
<td>III</td>
<td>11/10/2015</td>
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<td><strong>MELANOMA / SKIN</strong></td>
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<td>C13DTV</td>
<td>Compassionate use NPP dabrafenib and trametinib (MEK117341)</td>
<td>John Haanen</td>
<td>other</td>
<td>10-7-2013 (3-11-2015)</td>
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<td>C13MME</td>
<td>Compassionate use programma oral MEK162 (M11MME)</td>
<td>Christian Blank</td>
<td>other</td>
<td>30-5-2013 (3-11-2015)</td>
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<td>C14POI</td>
<td>Expanded access program MK3475</td>
<td>Christian Blank</td>
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<td>E120BMS</td>
<td>Minitub: Prospective registry of Sentinel Node (SN) positive melanoma patients with minimal SN tumor burden who undergo Completion Lymph Node Dissection (CLND) or Nodal Observation (NCT01942603)</td>
<td>Alexander van Akkooi</td>
<td>other</td>
<td>4/23/2015</td>
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<td>E1325</td>
<td>Adjuvant immunotherapy with anti-PD-1 monoclonal antibody Pembrolizumab (MK-3475) versus placebo after complete resection of high-risk Stage III melanoma: A randomized, double-blind Phase III trial of the EORTC Melanoma Group</td>
<td>Christian Blank</td>
<td>III</td>
<td>11/12/2015</td>
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<tr>
<td>E180B1</td>
<td>Adjuvant peginterferon alfa-2b for 2 years vs observation in patients with an ulcerated primary cutaneous melanoma with T12-4(RM2M0D): a randomized phase III trial of the EORTC Melanoma Group</td>
<td>Alexander van Akkooi</td>
<td>III</td>
<td>5/27/2015</td>
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<tr>
<td>M11TCR</td>
<td>Feasibility study using T-cell receptor gene therapy in metastatic melanoma</td>
<td>John Haanen</td>
<td>II</td>
<td>4/17/2012</td>
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<td>M12TFU</td>
<td>Prospective randomized trial for the evaluation of a theoretical follow-up schedule in cutaneous melanoma (MELOD)</td>
<td>Michel Wouters</td>
<td>other</td>
<td>8-6-2012 (13-2-2015)</td>
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<td>M13DTA</td>
<td>A phase III randomized double blind study of dabrafenib (GSK2118436) in combination with trametinib (GSK1120212) versus two placebos in the Adjuvant treatment of high-risk BRAF V600 mutation-positive melanoma after surgical resection (COMBI-AD)</td>
<td>John Haanen</td>
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<td>M13NEM</td>
<td>A randomized phase III, open label, multicenter, two-arm study comparing the efficacy of MEK162 versus dacarbazine in patients with advanced unresectable or metastatic NRAS mutation-positive melanoma (NEMO)</td>
<td>Christian Blank</td>
<td>III</td>
<td>23-7-2013 (20-3-2015)</td>
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<td>M14BNI</td>
<td>An exploratory study of the biological effects of Nivolumab and Ipilimumab monotherapy and Nivolumab in combination with Ipilimumab treatment in subjects with advanced melanoma (unresectable or metastatic) (CA209-038)</td>
<td>John Haanen</td>
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<td>M14LOG</td>
<td>A phase II, multi-center, open-label study of sequential LGX818/MEK162 combination followed by a rational combination with targeted agents after progression, to overcome resistance in adult patients with locally advanced or metastatic BRAF V600 melanoma (Logic-2)</td>
<td>Christian Blank</td>
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<td>M14NNI</td>
<td>A Single-Arm, Open-Label, Multicenter Clinical Trial with Nivolumab (BMS-936558) for Subjects with Histologically Confirmed Stage III (unresectable) or Stage IV Melanoma Progressing Post Prior Treatment Containing an Anti-CTLA-4 Monoclonal Antibody Disease (CHECKMATE 172)</td>
<td>John Haanen</td>
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<td>M14OSS</td>
<td>Treatment of basal cell carcinoma using a one-stop-shop with reflectance confocal microscopy: a randomized controlled multi-center trial</td>
<td>Bijana Zupan-Kajcovski</td>
<td>other</td>
<td>2/5/2015</td>
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<td>M14REP</td>
<td>A Phase II, Open-Label, Multicenter Study of Vemurafenib plus Cobimetinib (GDC-0973) in Unresectable Stage IIIc or Metastatic Melanoma -Response Prediction with Positron Emission Tomography and Tumor Characteristics- (REPOSIT)</td>
<td>Bernies van der Hiel</td>
<td>II</td>
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<td>M14TIL</td>
<td>Randomized phase III study comparing a non-myeloablative lymphocyte depleting regimen of chemotherapy followed by infusion of tumor infiltrating lymphocytes and interleukin-2 to standard ipilimumab treatment in metastatic melanoma</td>
<td>John Haanen</td>
<td>III</td>
<td>8/8/2014</td>
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<tr>
<td>M15PFO</td>
<td>A phase I, open-label, dose escalation study of PF-04518800 in patients with locally advanced or metastatic hepatocellular carcinoma (HCC), melanoma, clear cell renal cell carcinoma (RCC) or squamous cell head and neck cancer (SCCHN)</td>
<td>Jan Schellens</td>
<td>I</td>
<td>9/9/2015</td>
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<td>N03LAM</td>
<td>Longitudinal analysis of melanoma-specific immunity in stage III and IV melanoma patients</td>
<td>John Haanen</td>
<td>other</td>
<td>8/22/2003</td>
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<td>N06TIS</td>
<td>Integrated analyses of melanoma-T cell interactions; relevance for immunotherapy</td>
<td>John Haanen</td>
<td>other</td>
<td>8/29/2006</td>
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<td>N10MMI</td>
<td>Local immunotherapy by the synergism of monobenzone and imiquimod cream (MI) for cutaneous metastases in stage III-IV melanoma patients</td>
<td>J.P.J.W.</td>
<td>II</td>
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<td>N10MSN</td>
<td>Pilot study on the use of fluorescence imaging of lymph nodes during melanoma sentinel node procedure, using indocyanine green</td>
<td>Michel Wouters</td>
<td>Pilot</td>
<td>12/20/2010</td>
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<tr>
<td>N10TIL</td>
<td>Randomized phase II study using a non-myeloablative lymphocyte depleting regimen of chemotherapy followed by infusion of tumor infiltrating lymphocytes and interleukin-2 in metastatic melanoma (TIL)</td>
<td>John Haanen</td>
<td>II</td>
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<td>N11RFA</td>
<td>Phase Ib/Ii study exploring safety and efficacy of the combination of ipilimumab with radiofrequency ablation (RFA) in patients with unresectable uveal melanoma liver metastases (SECRA-UM)</td>
<td>Christian Blank</td>
<td>I/II</td>
<td>4/17/2012</td>
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<tr>
<td>N12CLM</td>
<td>Determining the sensitivity and specificity of circulating tumor cells and cytology in cerebrospinal fluid of patients clinically suspected for leptomeningeal metastases</td>
<td>Dieta Brandsma</td>
<td>I</td>
<td>6/19/2012</td>
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<td>N12SER</td>
<td>Prevention of seroma by TachoSil application after inguinal lymphnode dissection; a prospective registry</td>
<td>Jos van der Hage</td>
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<td>12-12-2013</td>
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<td>N12VDT</td>
<td>Therapeutic drug monitoring of BRAF- and MEK-inhibitors in a “Real Life” Cohort of Melanoma Patients</td>
<td>Jan Schellens</td>
<td>other</td>
<td>6/18/2013</td>
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<td>N13GEN</td>
<td>Regulation of skin tumorgenesis by integrin alpha3beta1</td>
<td>Arnoud Sonnenberg</td>
<td>other</td>
<td>11/27/2013</td>
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<td>N13ICG</td>
<td>99mTc-Senti-Scint vs ICG-99mTc-nanocolloid for sentinel node biopsy of malignant melanoma of the trunk, of an extremity or in the head and neck region</td>
<td>Jos van der Hage</td>
<td>other</td>
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<td>Type of cancer study</td>
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<td>N13NDT</td>
<td>Cytoreductive treatment of dabrafenib combined with trametinib to allow complete surgical resection in patients with BRAF mutated, prior unresectable stage III or IV melanoma (REDuCTOR)</td>
<td>John Haanen</td>
<td>II</td>
<td>12/6/2013</td>
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<td>N14OPC</td>
<td>Feasibility Study to Identify the Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN)</td>
<td>Christian Blank</td>
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<td>4/3/2015</td>
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<tr>
<td>N15HCL</td>
<td>Human Cerenkov Luminescence Imaging of Superficial In-Vivo Tumours after Administration of 18F-FDG</td>
<td>Theo Ruers</td>
<td>Pilot</td>
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**MISCELLANEOUS**

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<tbody>
<tr>
<td>M11COM</td>
<td>Phase III randomized double blind cross-over trial of supersaturated calcium-phosphate rinse (Caphosol) versus NACL 0.9% in the relief of oral mucositis in renal cell carcinoma, hepatocellular carcinoma and gastrointestinal stromal tumor patients receiving Targeted Therapy (CDMTT)</td>
<td>Neeltje Steeghs</td>
<td>III</td>
<td>12-12-2011 (18-6-2015)</td>
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<tr>
<td>M13LXC</td>
<td>Een fase 3, gerandomiseerd, placebo gecontroleerd, in parallele groepen uitgevoerd, dubbel blind multicenteronderzoek ter beoordeling van de werkzaamheid en veiligheid van telotristat etipraat (LX1606) bij patiënten die niet reageren op de behandeling van hun carcinoid syndroom (CS) met somatostatine-analoga (SSA) (teletar)</td>
<td>Margot Tesselaar</td>
<td>III</td>
<td>11/11/2013</td>
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<tr>
<td>M14AFS</td>
<td>Phase I/II study with the combination of afatinib and selumetinib in advanced KRAS mutant positive and PIK3CA wildtype colorectal, non-small cell lung and pancreatic cancer</td>
<td>Jan Schellens</td>
<td>I/II</td>
<td>5/19/2015</td>
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<tr>
<td>M14FAS</td>
<td>Fast-track thyroidectomy and radioiodine ablation therapy in patients with differentiated thyroid cancer, a multicenter randomized controlled trial (FASTHYNA)</td>
<td>Jos van der Hage</td>
<td>other</td>
<td>1/28/2015</td>
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<td>M15GRA</td>
<td>Registration study. Prospective registration study on growth behavior of aggressive fibromatoses without therapeutic intervention (GRAFTITI)</td>
<td>Frits van Coeverden</td>
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<tr>
<td>M15PFQ</td>
<td>A phase I, open-label, dose escalation study of PF-04518600 in patients with locally advanced or metastatic hepatocellular carcinoma (HCC), melanoma, clear cell renal cell carcinoma (RCC) or squamous cell head and neck cancer (SCCHN)</td>
<td>Jan Schellens</td>
<td>I</td>
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<tr>
<td>M15TLP</td>
<td>A multicenter, long-term extension study to further evaluate the safety and tolerability of Telotristat Etiprate (LX1606). TELEPATH (Telotristat Etiprate expanded treatment for patients with carcinoid syndrome)</td>
<td>Margot Tesselaar</td>
<td>III</td>
<td>10/16/2015</td>
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<tr>
<td>N12OST</td>
<td>Discrimination of benign and malignant human tissue during percutaneous interventions using optical spectroscopy techniques (PercuSpect)</td>
<td>Theo Ruers</td>
<td>other</td>
<td>9/13/2012</td>
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<tr>
<td>N14ONP</td>
<td>A prototype opto-nuclear probe for combined radio- and fluorescence tracing of the sentinel node</td>
<td>Henk van der Poel</td>
<td>other</td>
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<td>N14SRO</td>
<td>Somatostatin receptor expression and occupancy during lanreotide therapy</td>
<td>Marcel Stokkel</td>
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**SOFT TISSUE / OSTEOSARCOMA**

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<tr>
<td>E62092</td>
<td>A phase III randomized study of preoperative radiotherapy plus surgery versus surgery alone for patients with retroperitoneal sarcoma (RPS) (STRASS)</td>
<td>Rick Haass</td>
<td>III</td>
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<td>Type of cancer study</td>
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<td>M11COM</td>
<td>Phase III randomized double blind cross-over trial of supersaturated calcium-phosphate rinse (Caphosol) versus NaCl 0.9% in the relief of oral mucositis in renal cell carcinoma, hepatocellular carcinoma and gastrointestinal stromal tumor patients receiving Targeted Therapy (COMTT)</td>
<td>Neeltje Steeghs</td>
<td>III</td>
<td>12-12-2011 (18-6-2015)</td>
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<tr>
<td>M13MIG</td>
<td>A prospective, multicenter, randomized, open-label, active-controlled, 2-parallel group, Phase III study to compare efficacy and safety of AB1010 at 7.5 mg/kg/day to imatinib at 400 or 600 mg in treatment of patients with gastrointestinal stromal tumor in first line medical treatment (AB04030)</td>
<td>Neeltje Steeghs</td>
<td>III</td>
<td>9/5/2013</td>
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<tr>
<td>M13MSG</td>
<td>A prospective, multicenter, randomized, open label, active controlled phase 3 study to compare the efficacy and safety of Masitinib to Sunitinib in patients with gastrointestinal stromal tumor after progression with Imatinib at 400 mg as first line treatment (AB11002)</td>
<td>Neeltje Steeghs</td>
<td>III</td>
<td>9/5/2013</td>
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<tr>
<td>M15GCD</td>
<td>Gastrointestinal stromal tumors (GIST): assessment of mutation in tumors and in circulating tumor DNA and measurement of TKI plasma exposure to optimize treatment (GALLOP)</td>
<td>Neeltje Steeghs</td>
<td>other</td>
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<tr>
<td>N10DMY</td>
<td>Dose reduction of neoadjuvant radiotherapy in Myxoid liposarcomas (DOREMY)</td>
<td>Rick Haas</td>
<td>II</td>
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<tr>
<td>N15HCL</td>
<td>Human Cerenkov Luminescence Imaging of Superficial In-Vivo Tumours after Administration of 18F-FDG</td>
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<td>N15MSC</td>
<td>The Mesenchymal Stem Cell biomarker study (MSC biomarker)</td>
<td>Emile Voest</td>
<td>other</td>
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**URO-GENITAL**

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<tbody>
<tr>
<td>M10PCM</td>
<td>Prostate cancer molecular medicine (PCMM)</td>
<td>Henk van der Poel</td>
<td>other</td>
<td>2/17/2011</td>
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<tr>
<td>M11COM</td>
<td>Phase III randomized double blind cross-over trial of supersaturated calcium-phosphate rinse (Caphosol) versus NaCl 0.9% in the relief of oral mucositis in renal cell carcinoma, hepatocellular carcinoma and gastrointestinal stromal tumor patients receiving Targeted Therapy (COMTT)</td>
<td>Neeltje Steeghs</td>
<td>III</td>
<td>12-12-2011 (18-6-2015)</td>
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<tr>
<td>M11FLA</td>
<td>Single Blind Randomized Phase III Trial to Investigate the Benefit of a Focal Lesion Ablative Microboost in Prostate Cancer (FLAME)</td>
<td>Floris Pos</td>
<td>III</td>
<td>5-4-2012 (5-2-2015)</td>
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<tr>
<td>M11PRC</td>
<td>Impact of new approaches to pharmacological management of patients with renal cell carcinoma: a population-based study of process outcomes in The Netherlands (PERCEPTION)</td>
<td>Simon Horenblas</td>
<td>other</td>
<td>8/18/2011</td>
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<tr>
<td>M12CBP</td>
<td>A phase II study in mCRPC on the pharmacodynamic effects of budesonide on cabazitaxel (Jevtana®): a randomised, open-label multicenter study (CABARESC)</td>
<td>André Bergman</td>
<td>II</td>
<td>8-5-2012 (2-11-2015)</td>
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<tr>
<td>M12MAG</td>
<td>A randomized, double blind, placebo controlled phase II trial to evaluate the safety and efficacy of recMAG-A3 + AS15 ASCI in patients with MAGE-A3 positive muscle invasive bladder cancer after cystectomy (MAGNOLIA)</td>
<td>Martijn Kerst</td>
<td>II</td>
<td>2-2-2012 (3-11-2015)</td>
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<td>M13ASP</td>
<td>A Randomized, Double blind, Phase 3 Efficacy Trial of Prostvac-VF ± GM-CSF in Men With Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer (PROSTVAC)</td>
<td>André Bergman</td>
<td>III</td>
<td>2/14/2014</td>
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<td>Type of cancer study</td>
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<td>M13CVU</td>
<td>A randomised Phase II/III study of cabazitaxel versus vinflunine in metastatic or locally advanced transitional cell carcinoma of the urothelium (secavin12)</td>
<td>Martijn Kerst</td>
<td>II/II</td>
<td>6-9-2013 (30-1-2015)</td>
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<td>M13PMR</td>
<td>Validation of multiparametric MRI with histopathology for prostate cancer</td>
<td>Floris Pos</td>
<td>other</td>
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<td>M13PSN</td>
<td>Prospective randomized multicenter comparison of indocyanine green (ICG)-99mTc-nanocolloid vs. 99mTcnanocolloid plus an intraoperative injection of ICG for the detection and surgical resection of the sentinel nodes in patients with prostate cancer</td>
<td>Henk van der Poel</td>
<td>II</td>
<td>4/17/2014</td>
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<td>M14HSN</td>
<td>Sentinel node biopsy for bladder cancer using the hybrid tracer</td>
<td>Bas van Rhijn</td>
<td>other</td>
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<td>M14LET</td>
<td>A case-cohort study to identify risk factors for cardiovascular disease in testicular cancer survivors TACKle-study Tackling Adverse Chemotherapy- associated Late Effects</td>
<td>Martijn Kerst</td>
<td>other</td>
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<td>M14MCB</td>
<td>A phase II, open-label, multicenter, randomized study to investigate the efficacy and safety of MPDL3280A (anti-PDL-1 antibody) compared with chemotherapy in patients with locally advanced or metastatic urothelial bladder cancer after failure with platin (GO29294)</td>
<td>Michiel van der Heijden</td>
<td>III</td>
<td>5/18/2015</td>
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<tr>
<td>M14MSU</td>
<td>A phase II, multicenter, single-arm study of MPDL3280A in patients with locally advanced or metastatic urothelial bladder cancer (GO29293)</td>
<td>Michiel van der Heijden</td>
<td>II</td>
<td>26-8-2014 (30-3-2015)</td>
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<td>M14RAC</td>
<td>Relapsing patients after adjuvant Carboplatin for seminoma stage I – characteristics of treatment and outcome</td>
<td>Martijn Kerst</td>
<td>other</td>
<td>10/31/2014</td>
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<td>M14ROT</td>
<td>Registry of Treatment Outcomes in a non-study population of Symptomatic Metastasized Castration Resistant Prostate Cancer (mCRPC) Patients Treated with Radium-223 (RDTOR-registry). non WMO-protocol</td>
<td>André Bergman</td>
<td>other</td>
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<td>M14SID</td>
<td>A Randomized, Double-Blind, Placebo-Controlled, Phase IIIb Study of the Efficacy and Safety of Continuing Enzalutamide in Chemotherapy Naive Metastatic Castration Resistant Prostate Cancer Patients Treated with Docetaxel plus Prednisolone Who Have Progressed on Enzalutamide Alone. (PRESIDE)</td>
<td>André Bergman</td>
<td>III</td>
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<tr>
<td>M15CRB</td>
<td>Prospective trial evaluating the outcome of induction chemotherapy followed by extended lymph node dissection and chemoradiation for high risk invasive bladder cancer (CHEMORDAD-trial)</td>
<td>Simon Horenblas</td>
<td>II</td>
<td>10/30/2015</td>
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<tr>
<td>M15MDP</td>
<td>A phase III, open-label, multicenter, randomized study of MPDL3280A (anti-PDL-1 antibody) versus observation as adjuvant therapy in patients with PD-L1 selected, high-risk muscle-invasive bladder cancer after cystectomy</td>
<td>Michiel van der Heijden</td>
<td>III</td>
<td>11/16/2015</td>
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<tr>
<td>M15PFO</td>
<td>A phase I, open-label, dose escalation study of PF-04518600 in patients with locally advanced or metastatic hepatocellular carcinoma (HCC), melanoma, clear cell renal cell carcinoma (RCC) or squamous cell head and neck cancer (SCCHN)</td>
<td>Jan Schellens</td>
<td>I</td>
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<tr>
<td>M15RAM</td>
<td>A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Ramucirumab plus Docetaxel Versus Placebo plus Docetaxel in Patients with Locally Advanced or Unresectable or Metastatic Urothelial Carcinoma Who Progressed on or After Platinum-Based Therapy</td>
<td>Michiel van der Heijden</td>
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<td>M15RTO</td>
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<td>André Bergman</td>
<td>Registry of Treatment Outcomes in a non-study population of Symptomatic Metastasized Castration Resistant Prostate Cancer (mCRPC) Patients Treated with Radium-223 (RDTOR-registry). WMO-protocol.</td>
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<td>M945AL</td>
<td>II</td>
<td>Sjoerd Rodenhuis</td>
<td>Salvage regimen incorporating repeated ablative chemotherapy with autologous PSCT, a phase II study</td>
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<td>N08SNR</td>
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<td>Axel Bex</td>
<td>Site and distribution of sentinel lymph nodes in renal cell carcinoma, a phase II study</td>
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<td>N10BPA</td>
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<td>André Bergman</td>
<td>Phase I trial evaluating combined radiotherapy with Panitumumab (Vectibix) in patients with muscle invasive transitional cell carcinoma of the bladder</td>
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<td>N12IGP</td>
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<td>Henk van der Poel</td>
<td>The use of indocyanine green for accurate sentinel node detection and removal in a group of high-risk nodal metastasis prostate cancer patients</td>
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<td>N12ITC</td>
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<td>Floris Pos</td>
<td>Influence of injection time of contrast agent on quantitative Dynamic Contrast-Enhanced (DCE) MRI in patients with prostate cancer</td>
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<td>N12LAR</td>
<td>12/14/2012</td>
<td>Christian Blank</td>
<td>Longitudinal analysis of RCC-specific immunity in renal cell carcinoma patients</td>
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<td>N13CCCI</td>
<td>12/18/2013</td>
<td>Wouter Vogel</td>
<td>Confirming the pharmacological interaction between colchicine and 18F-choline PET</td>
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<td>N13END</td>
<td>3/17/2014</td>
<td>Henk van der Poel</td>
<td>Laat de met de Endopat gemeten endothelium functie na een chemische castratie voor prostaatcarcinoom veranderingen zien en zo ja op welke termijn (Endopat 2)</td>
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<td>N13KCM</td>
<td>1/24/2014</td>
<td>Michiel van der Heijden</td>
<td>Longitudinal kinetics of cancer mutations in the plasma, urine and tumor of patients with urothelial cancer treated with chemotherapy</td>
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<td>N14DAR</td>
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<td>Henk van der Poel</td>
<td>Dynamics of Androgen Receptor genomics and transcriptomics after neoadjuvant androgen ablation (DARANA)</td>
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<td>N14ITO</td>
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<td>Emile Voest</td>
<td>Immunogenicity of Tumor Organoids, a feasibility study</td>
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<td>N14MRS</td>
<td>23-7-2014 (29-5-2015)</td>
<td>Floris Pos</td>
<td>Polyamines detection with MR spectroscopy to increase diagnostic specificity in prostate cancer</td>
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<td>N15CMR</td>
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<td>Floris Pos</td>
<td>Investigation of the signature of recurrence and radiation effects after External-Beam radiotherapy on multi-parametric MRI</td>
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<td>N15MSC</td>
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<td>Emile Voest</td>
<td>The Mesenchymal Stem Cell biomarker study (MSC biomarker)</td>
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<tr>
<td>N15PEN</td>
<td>8/31/2015</td>
<td>Floris Pos</td>
<td>Chemoradiation in the treatment of loco-regionally advanced Penile Cancer</td>
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Marjanka Schmidt, Amsterdam, The Netherlands
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Christopher Vakoc, Cold Spring Harbor, USA
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Victor Velculescu, Baltimore, USA
The bacterial DNA replication machine caught in the act by cryo-EM

Andrea Ventura, New York, USA
Non coding RNAs: from biology to technology

Karin de Visser, Amsterdam, The Netherlands
The immune system: a double-edged sword in metastatic breast cancer

Lars Zender, Tübingen, Germany
In vivo RNAi screening for accelerated cancer gene discovery in gastrointestinal tumors
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<td>Agami, Reuven</td>
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<td>Al-Mamgani, Abraham</td>
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<td>Beets, Geerard</td>
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<td>Belderbos, Jose</td>
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<td>Bernards, Rene</td>
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<td>Finding genetic dependencies in cancer: the missing link in personalized medicine.</td>
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<td>Bleiker, Eveline</td>
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<td>Choices in breast surgery and reconstruction: implementation and testing of a web-based psycho-educational intervention to facilitate decision making.</td>
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<td>Boekhout, Annelies</td>
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<td>Borst, Jannie</td>
<td>NKI 2012-5397</td>
<td>Defining the nature of CD4 T-cell help for the CTL response to optimize immunotherapy of cancer</td>
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<td>Borst, Jannie</td>
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<td>Enhancing the anti-tumor efficacy of immunotherapy by localized radiotherapy.</td>
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<td>Brummelkamp, Thijn</td>
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<td>A mutation-based approach to examine the principles of synthetic lethality in human cells.</td>
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<td>Haanen, John</td>
<td>2013-5924</td>
<td>Feasibility study using T-cell receptor gene therapy in metastatic melanoma</td>
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<td>Haanen, John</td>
<td>NKI 2012-5726</td>
<td>A randomized controlled phase II/III trial comparing treatment with tumor-infiltrating lymphocytes to standard of care ipilimumab as 2nd line treatment for metastatic melanoma.</td>
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<td>Haas, Rick</td>
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<td>Dose Reduction of preoperative radiotherapy in Myxoid liposarcomas.</td>
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<td>Jacobs, Heinz</td>
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<td>How ubiquitination controls telomere-induced genomic instability</td>
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<td>Jonkers, Jos</td>
<td>NKI 2011-5220</td>
<td>Resistance to Parp inhibitors-association with DNA damage response alterations</td>
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<td>Jonkers, Jos</td>
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<td>Jonkers, Jos</td>
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<td>Cohesin loading and stripping: two fundamental principles for the maintenance of genomic stability</td>
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<td>Meijer, Gerrit</td>
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<td>Schmidt, Marjanka</td>
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<td>Tan, Bing</td>
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<td>Genetic causes of resistance to new androgen receptor signaling inhibitors in circulating tumor DNA of metastasized castration resistant prostate cancer patients.</td>
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<td>NKI 2006-4176</td>
<td>Phase III randomized clinical trial for stage III ovarian carcinoma randomizing between secondary debulking surgery with or without hyperthermic intraperitoneal chemotherapy (OVHPEC-1).</td>
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<td>van Harten, Wim</td>
<td>NKI 2010-4854</td>
<td>A-Care 2; ICT supported patient empowerment, return-to-work, tele rehabilitation and implementation of Cancer Rehabilitation Programs</td>
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<td>NKI 2015-7804</td>
<td>A randomized controlled trial of an internet-based tailored physical activity support program in breast and prostate cancer survivors</td>
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<td>van Leeuwen, Floor</td>
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<td>A nationwide survivorship care program for adult (non-Hodgkin lymphoma survivors.</td>
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<td>Role of the histone methyltransferase Dot1 in gene expression and leukemic transformation</td>
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<td>Epigenetic Pathways in Cancer Development and Treatment: Crosstalk between Conserved Histone Modifiers in T-cell Lymphoma.</td>
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<td>van Lohuizen, Maarten</td>
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<td>Functional identification of novel genes implicated in Glioblastoma Multiforme.</td>
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<td>Advanced Logistics Optimization of the Radiotherapy Treatment (ALORT)</td>
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<td>Tumor-specific radio sensitization by exploiting base excision repair deficiencies.</td>
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<td>Platinum-induced fatty acids (PIFA) as mediators of systemic resistance to chemotherapy in cancer patients.</td>
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<td>Tumor organoids: feasibility to predict sensitivity to treatment in cancer patients (TUMOROID trial)</td>
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<td>Vogel, Wouter</td>
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<td>Recurrent differentiated thyroid cancer: personalized treatment based on evaluation of tumor characteristics with PET (THYROPET).</td>
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<td>Wesseling, Jelle</td>
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<td>Management of low grade ductal carcinoma in situ: active surveillance or not? A randomized, non-inferiority phase III trial (LORD study) (BDOG).</td>
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<td>Prediction of response to neoadjuvant chemotherapy in luminalER-positive/HER2-negative breast cancer.</td>
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<td>Drugging steroid hormone receptors in novel tumor types: new applications of existing drugs. Bas Mulder Award.</td>
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Research projects supported by other organisations
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<td>A recombinant mouse strain with human CD27 knocked into the mouse CD27 gene locus will become available at the NKI in Q1 2015. In vitro, human CD27 expression on specific tissues and its functionality will be tested</td>
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