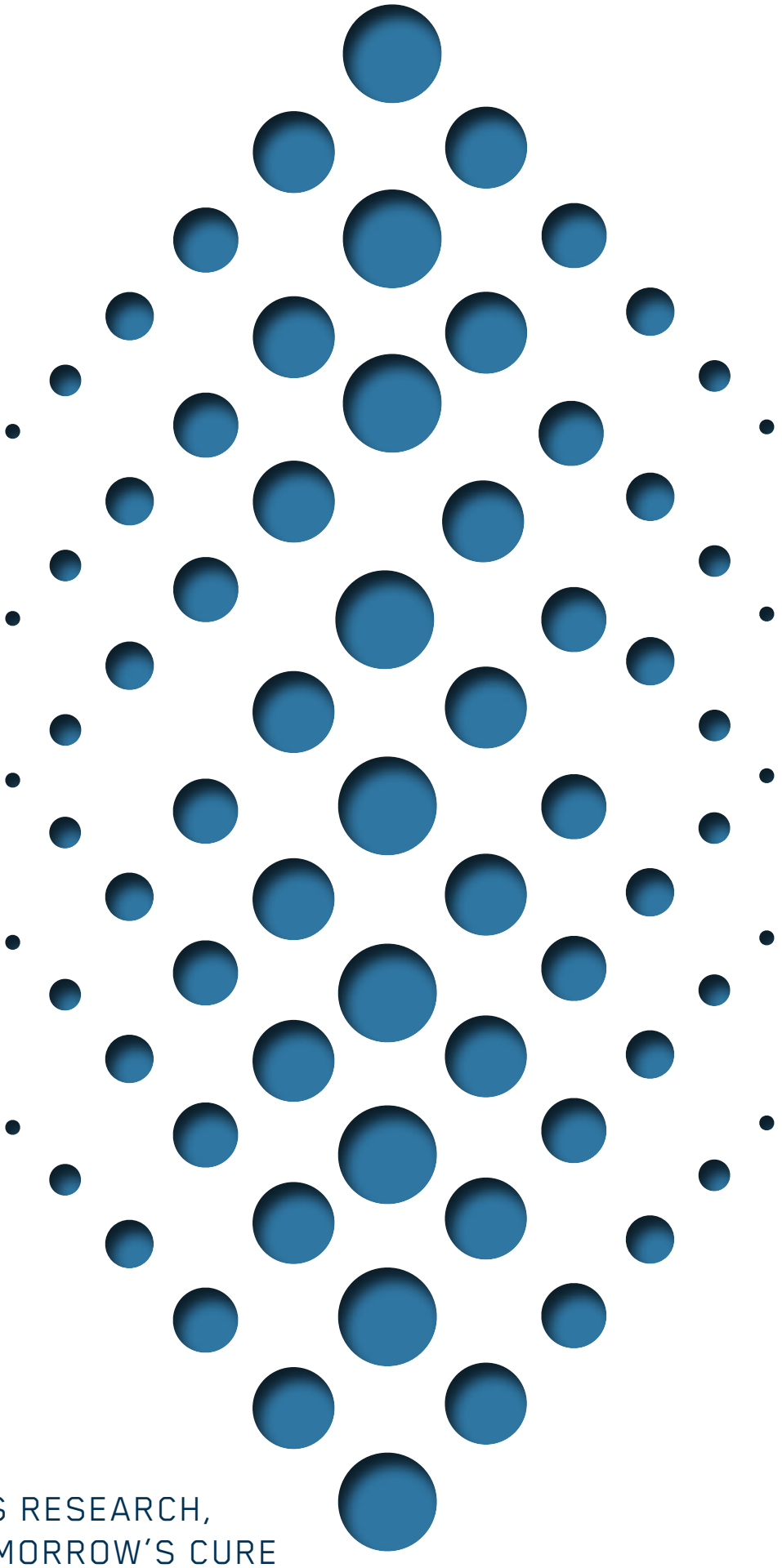

TODAY'S RESEARCH,
FOR TOMORROW'S CURE

The Netherlands Cancer Institute

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

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NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

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Todays's research for tomorrow's cure

With great pleasure and pride I offer you this brochure on the research performed at the Netherlands Cancer Institute.

Foreword

The Netherlands Cancer Institute (Nederlands Kanker Instituut, NKI) is an exciting and rewarding place to work. It is an internationally recognized center of scientific excellence in many key areas relating to cancer. Recognition of our status is evident from our substantive publication record, the many invitations our staff receive to speak at major conferences, the prestigious prices awarded to our employees, and from our ability to attract funding. The NKI also stands out as the only official Comprehensive Cancer Center in The Netherlands.

Our success arises largely from two key policies. First, we only hire ambitious researchers who wish to participate in a highly interactive community of scientists and doctors. Second, the institute has a non-hierarchical management style that gives our research staff freedom to pursue their goals and direct their work along exiting paths. Another invaluable characteristic of the NKI is the fact that it comprises both a research institute and a specialized cancer clinic, the Antoni van Leeuwenhoek Hospital. This combination ensures rapid translation of basic research into clinical applications and gives our patients the benefit of treatments based on the latest scientific insights.

The work presented here is result of the combined commitment and hard work of our scientists, clinicians, students, supporting staff and volunteers. Our work is possible due to many donations and other forms of support we received over the years, for which we are very thankful. Special thanks go to our major funding bodies: the Ministry of Health, Welfare and Sport and the Dutch Cancer Society.

As director, I have the privilege of overseeing this motivated group of investigators and their work. I am fascinated by the way we are advancing, ranging from genetic screening and imaging technologies to new drugs and therapeutic regimes. This brochure shows many of the people making this happen, and offers a taste of how today's work will lead to tomorrow's cure.

Prof. dr. Rene Medema
Director of Research

The Netherlands Cancer Institute was founded on October 10, 1913. From a small cancer center in one of Amsterdam's canal houses, it has grown to become a large, internationally acclaimed center of excellence and the only Comprehensive Cancer Center in The Netherlands.

Introduction

From its first inception the Netherlands Cancer Institute (in Dutch: Nederlands Kanker Instituut, abbreviation: NKI) saw close collaboration between scientists and clinicians as essential to fighting cancer. The NKI comprises a research institute as well as a dedicated cancer clinic, the Antoni van Leeuwenhoek Hospital. Through both fundamental and clinical research we aim to contribute to better cancer treatment. Discoveries made in the laboratory are rapidly introduced into the clinic through our translational research program. This ensures that patients within our hospital are treated based on the latest scientific findings. The research institute of the NKI accommodates approximately 750 scientists and scientific support personnel. The Antoni van Leeuwenhoek Hospital has 152 medical specialists, 180 beds, an out-patients clinic that receives 27,000 patients each year, 5 operating theaters and 11 radiotherapy units. These are the numbers for 2014; in the coming years the hospital will expand and treat more patients.

The scientific research at the NKI is made possible by structural funding from the Dutch Ministry of Health, Welfare and Sports and the Dutch Cancer Society, as well as individual project grants and generous donations by the public. The research groups at the NKI are organized into divisions. Usually research groups on the same floor form a division, which is headed by one of the group leaders. Clinical research groups are organized in divisions according to the clinical departments of the cancer hospital. The divisions and the group leaders are listed on the next page. For the clinical research divisions, only the head of the division is indicated.

The NKI is headed by a Board of Directors consisting of three members. The Board operates as a collective, although each member is responsible for his own field of expertise. In order to maintain the highest standards of research, the NKI has established a national and international Scientific Advisory Board consisting of internationally recognized scientists. Members of each board are frequently asked to advise the Director of Research on issues such as the appointment of faculty members and evaluation of research programs and policies. The NKI also has a Board of Governors, that is composed of knowledgeable and respected members of Dutch society. Governors monitor the Institute's operations to ensure that the organization not only fulfills its scientific and clinical mission, but also operates in a financially responsible manner and according to the highest ethical standards. The Governors advise and control the Board of Directors.

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Princess Margaret Hospital and University of Toronto,
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K Vousden
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Glasgow, United Kingdom

Situation on 1-1-2015

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Thijn Brummelkamp
Anastassis Perrakis

Division of Biological Stress Response
Reuven Agami (head)
Heinz Jacobs
Hein te Riele
Marcel Verheij

Division of Cell Biology I
Arnoud Sonnenberg (head)
Kees Jalink
René Medema
Wouter Moolenaar

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Elzo de Wit
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Karin de Visser

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Wilbert Zwart

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Neil Aaronson
Eveline Bleiker
Wim van Harten
Michael Hauptmann
Sanne Schagen

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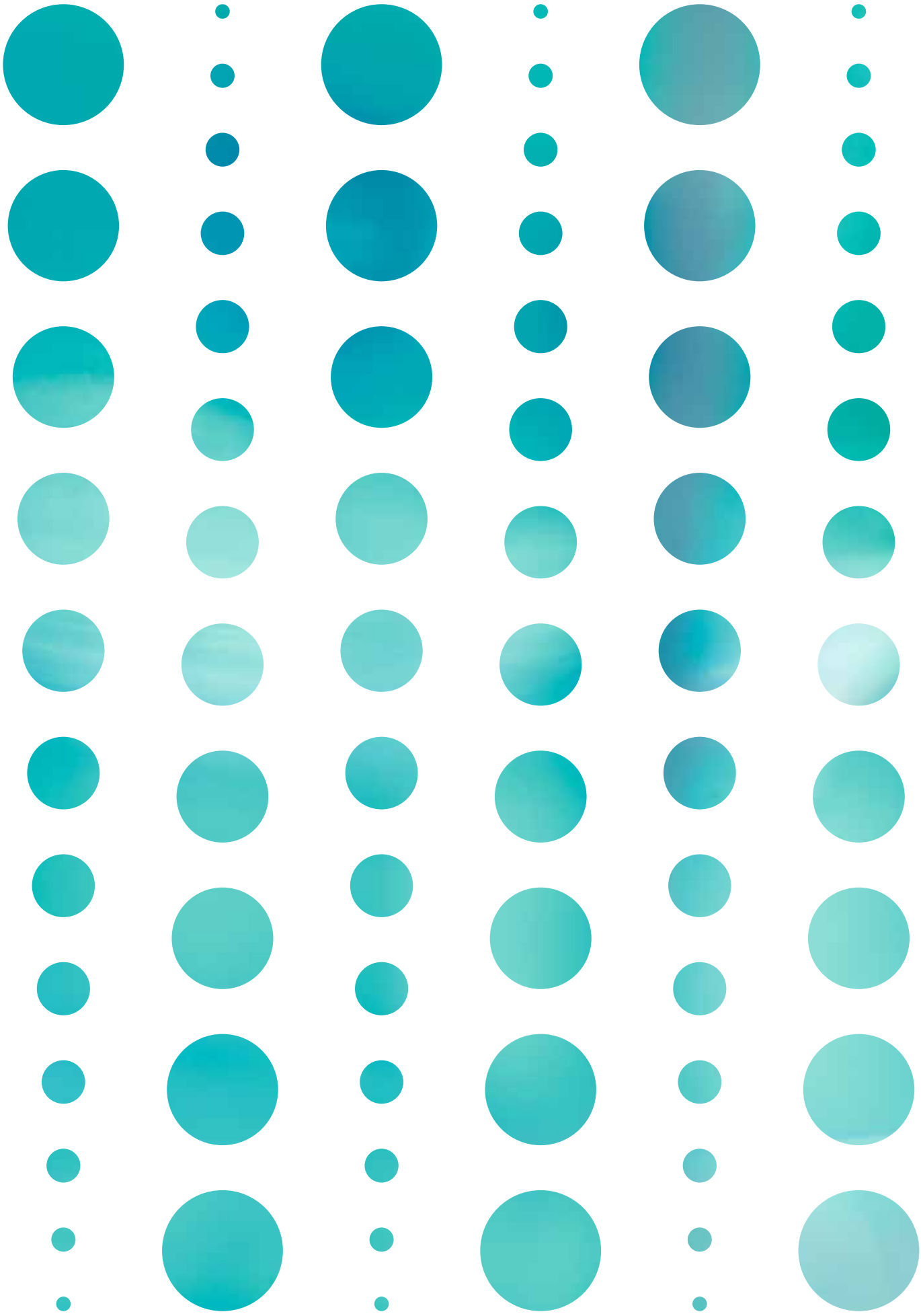
Marcel Stokkel (head)
Gerrit Meijer

Division of Radiation Oncology
Marcel Verheij (head)
Uulke van der Heide
Jan-Jakob Sonke

Division of Medical Oncology
John Haanen (head)

Division of Surgical Oncology
Theo Ruers (head)


FROM
BASIC
RESEARCH
TO
CLINICAL
APPLICATION



Basic research is the invaluable foundation on which our knowledge about cancer is built. The basic research at the NKI covers a broad range of topics, from gene regulation and cell signaling to tumor metastasis and treatment resistance. Our researchers also strive to translate this knowledge into new approaches for the diagnosis and treatment of cancer.

Understanding cancer: basic research at the NKI

Cutting-edge technologies

Scientists at the NKI are among the world's most talented inventors and users of new technologies. This can involve innovative tools and reagents for use at the laboratory bench, new genetic techniques or new high-throughput devices in the centralized facilities. The NKI also has exceptional expertise in using and generating new mouse models for cancer. Mouse models that reliably represent human cancers are extremely valuable for the pre-clinical testing of new therapies and drugs. Our scientists can also use transgenic mouse technology to selectively increase or decrease the activity of particular genes, in order to study their influence on tumor development. All our mice experiments are performed in a state-of-the-art new animal facility, with many treatment and imaging facilities.

Worldwide, one of the most important current focuses in cancer research is personalized medicine. The NKI is at the forefront of this type of research. In the past decades, the realization has dawned that cancer isn't a single type of disease. Nor is, for example, one breast tumor or melanoma the same as the next. Cancer is a collection of diseases, which can be caused by a multitude of genetic errors. A lot of the research at the NKI revolves around understanding the genetic mutations that lie at the heart of tumor formation. NKI scientists are using techniques like in vitro cell culture, functional screens, RNA interference, biochemistry and protein crystallography to investigate the effects of genetic mutations on processes that normally maintain cells in good health. Instead of treating tumors based on the affected organ, treatment should be tailored according to these underlying causes and Achilles heels of individual tumors.

New approaches

A related, important research topic is therapy resistance. How do tumors escape from therapies designed to kill them? NKI research into these 'escape routes' of tumors gave rise to a number of successful combination therapies and more are on their way. The aim of combination therapies is to block common escape routes of specific tumors, so they cannot use them to develop therapy resistance. In the long run, this approach could ideally turn cancer into a chronic disease. A number of NKI researchers also study how cancer cells leave their tissue of origin and move around the body to form new tumors, a process known as metastasis. Advances in the way tumors metastasize might one day lead to new approaches for cancer treatment.

Last but certainly not least, an important research focus at the NKI is that of immunology. The body's own immune system can, under certain circumstances, fight and even eradicate cancer. Recently the first effective immunotherapies against certain types of cancer have been developed. One such therapy is TIL, a therapy that strengthens the natural immune response and that was in part developed at the NKI. Our scientists are helping to understand why the natural immune response against cancer doesn't always take place, what happens when it does, and in which ways this immune response be enhanced.

One of the unique strengths of the NKI is that it comprises both a research institute and a specialized cancer hospital, the Antoni van Leeuwenhoek hospital. This combination helps support hundreds of clinical trials each year, aimed at both translating basic research findings into new clinical treatments as well as improving existing treatments.

Improving cancer treatment: clinical research

Personalized treatment

The NKI is proud of its position as one of the Netherlands' foremost biomedical research institutions, dedicated to improving the treatment and care of patients with cancer. Its clinical services operate to the highest international standards and continue to extend the boundaries of what can be done for patients. The NKI is at the forefront of personalized medicine, which aims to more finely tune the cancer treatment to the specific tumor and needs of the individual patient. At the NKI, personalized medicine means research into drugs that target the specific weak points of tumors or enhance the patient's own immune system. This program is supported by a renowned drug development program in which new drugs and concepts are tested in early clinical trials. It also means the development of high tech methods for more precise radiotherapy treatment, advanced image guided surgical technologies to more radically dissect tumors whilst sparing as much healthy tissue as possible, and improved diagnostic tools.

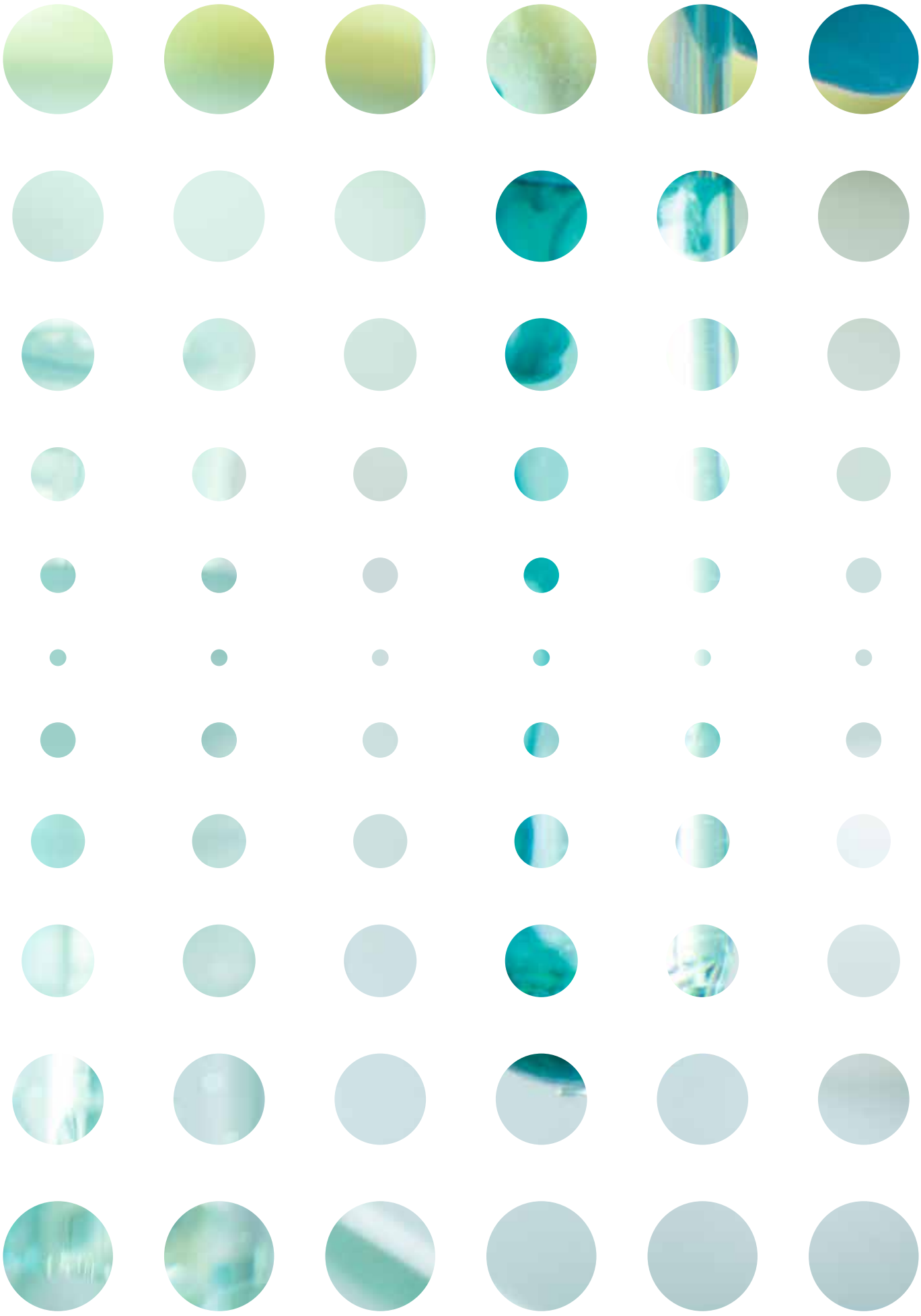
Over the past decades, with the help of the surgeons and pathologists at the hospital, the NKI has amassed an impressive bank of tumor material. Researchers are now using this material to develop genetic tests that can be used to predict the risk of metastasis and to predict how patients will respond to certain drugs. This way, the best treatment for individual patients can be chosen more easily. NKI researchers are also searching for new diagnostic biomarkers in biological fluids, mainly blood, urine and cerebrospinal fluid.

Combining therapies and monitoring outcomes

Another approach the NKI uses to make cancer treatments more effective is designing combination therapies. One strategy is to find and combine drugs that attack alternative weak spots in cancer cells and enhance the immune system, rendering the tumor more sensitive for treatment and possibly preventing or delaying drug resistance. A significant effort is made to grow individual tumors outside the patient to predict the treatment outcome before patients receive the treatment. The NKI also puts effort in improving ways to monitor treatment effects during treatment. This is for instance done by measuring drug levels and certain biomarkers within patients' blood, but also by imaging technologies such as state of the art MRI and PET technologies. Advanced imaging technologies allow monitoring of patients to see whether or not the treatment is killing and shrinking a tumor. All of the above is done to maximize treatment success as well as reduce side effects.

Being diagnosed with cancer can change one's whole life. Not just for the patient, but also for their relatives. In the 1990's the NKI founded a Family Cancer Clinic where family members of patients with an hereditary form of cancer can receive genetic counseling. The NKI also performs research into coping mechanisms. This has led to changes in the treatment and care for the benefit of patients, relatives, and people with a high cancer risk. Thus, the NKI's clinical research program ensures better care for patients, both during and after their cancer treatment.

BASIC RESEARCH



Index groupleaders

Division of Psychosocial Research and Epidemiology

Selected publications

Aaronson NK, Mattioli V, Minton O, Weis J, Johansen C, Dalton SO, Verdonck-de Leeuw IM, Stein KD, Alfano CM, Mehnert A, de Boer A, van de Poll-Franse LV. Beyond treatment – Psychosocial and behavioral issues in cancer survivorship research and practice. Eur J Cancer Suppl 2014;12:54-64

Duijts SFA, van Beurden M, Oldenburg HSA, Hunter MS, Kieffer JM, Stuiver MM, Gerritsma MA, Menke-Pluymers MBE, Plaisier PW, Rijna H, Lopes Cardozo AMF, Timmers GJ, van der Meij S, van der Veen H, Bijker N, de Widt-Levert LM, Geenen MM, Heuff G, van Dulken EJ, Boven E, Aaronson NK (2012). Efficacy of cognitive behavioral therapy and physical exercise in alleviating treatment-induced menopausal symptoms in patients with breast cancer: Results of a randomized controlled multicenter trial. J Clin Oncol 2012;30:4124-33

Eijzenga W, Aaronson NK, Hahn DEE, Sidharta GN, van der Kolk LE, Velthuizen ME, Ausems MGEM, Bleiker EMA. Effect of routine assessment of specific psychosocial problems on personalized communication, referrals and distress levels in cancer genetic counseling practice: A randomized controlled trial. J Clin Oncol 2014

Psychosocial oncology
NEIL AARONSON

With a background in clinical psychology and public health, Neil Aaronson has a keen interest in assessing how individuals adjust to their illness and medical treatment. For him, the NKI is an ideal place to do high-quality applied research that will make an immediate difference for patients and their families. It also provides a base to link to many international research groups. Over the past 25 years Aaronson has championed the development and use of standardized questionnaires for assessing the health-related quality of life (HRQOL) of patients with cancer, to help physicians and nurses provide a better quality of care.

Quality of life

"Our philosophy is that if you provide physicians and nurses with structured information about the health, symptom experience and quality of life of their patients, they will be better attuned to their patients' problems and limitations. And thus provide better care, either directly or through referral to the appropriate supportive care services. Although we initially developed questionnaires to assess these characteristics for use in research settings, they have now been implemented in daily clinical practice where patients can complete them on a touchscreen computer in-clinic, or via the internet at home. Their digital responses can then be integrated into an electronic medical record and used during clinical encounters to facilitate doctor-patient and nurse-patient communication. When aggregated across patients, these data can inform treatment policy and quality of care initiatives on a system-wide level."

Behavioral Interventions

"We are also developing and evaluating a range of behavioral interventions to relieve symptoms and enhance the physical and psychosocial wellbeing of our patients. For example, we recently completed a large scale, multicenter study in which we demonstrated that both counseling and physical exercise can effectively reduce the burden of endocrine symptoms experienced by young breast cancer patients who have gone into treatment-induced menopause. Similarly, we are investigating the efficacy of low and moderate intensity physical exercise as a means of minimizing fatigue and maintaining physical fitness among breast and colon cancer patients during adjuvant chemotherapy. We are also studying the usefulness of an internet-based sexual therapy program for women with breast cancer. All of our research is aimed at enhancing physical and psychosocial functioning and wellbeing, and therefore the overall quality of life of our patients and their families."

Division of Biological Stress Response

Selected publications

Rooijers K, Loayza-Puch F, Nijtmans LG, Agami R. Ribosome profiling reveals features of normal and disease-associated mitochondrial translation. Nat Commun 2013;4:2886

Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, Elkon R, Melo SA, Leveille N, Kalluri R, de Laat W, Agami R. eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell 2013;49:524-535

Loayza-Puch F, Drost J, Rooijers K, Lopes R, Elkon R, Agami R. p53 induces transcriptional and translational programs to suppress cell proliferation and growth. Genome Biol 2013;14:R32

Jenal M, Elkon R, Loayza-Puch F, van Haaften G, Kuhn U, Menzies FM, Oude Vrielink JA, Bos AJ, Drost J, Rooijers K, Rubinsztein DC, Agami R. The poly(A)-binding protein nuclear 1 suppresses alternative cleavage and polyadenylation sites. Cell 2012;149: 538-553

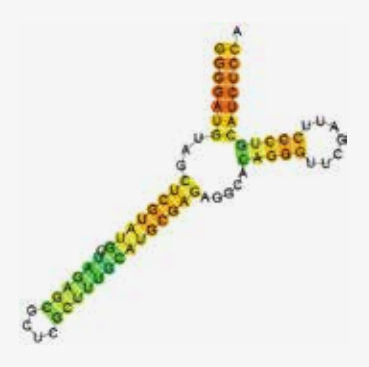
Coding and noncoding RNAs in Cancer

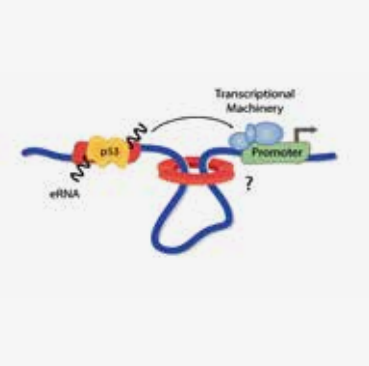
REUVEN AGAMI

The goal of Reuven Agami's research is to generate a better understanding of the molecular processes leading to human cancer. His strategy is to identify essential cancer genes and pathways, and he is particularly interested in the role of RNA. RNA is a versatile molecule and the human genome expresses many different types. Interestingly, only a small fraction of these encodes for proteins, while most regulate protein production and therefore control cell behavior. His group develops and utilizes novel RNA tools to interrogate and alter gene expression and thereby influence cancer cell fate. They hope to generate knowledge that can be used to develop more effective therapeutic strategies for treating cancer.

Enhancer-RNAs

"Much of our work is based on developing new genetic approaches to uncover molecular mechanisms involved in cancer. Recent discoveries have pinpointed the role of enhancers in cancer. Enhancers are genomic domains that regulate the expression of genes that are distantly located. How exactly this regulation occurs, and how it is involved in cancer, is yet unknown. Intriguingly, enhancers produce RNA termed enhancer-RNA (eRNA). We have shown that eRNAs are functionally involved in the process of cancer suppression by tumor suppressors that are enhancer factors.
Interestingly, genetic variations in enhancer sequences can generate high risk for cancer in the human population. We now use eRNA synthesis to quantify enhancer activity and pinpoint how genetic variations in enhancer domains increase cancer frequency."





Protein translation programs

"Ribosomes are the cellular machines that make protein from RNA, in a process termed translation. In recent years a genome-wide technique (Ribo-Seq) was developed to measure protein translation on a truly global manner. We used Ribo-Seq to discover novel translation networks underlying oncogenic and tumor-suppressive programs. We also study translation following adaptive cancer drug resistance. Furthermore, we adapted Ribo-Seq to measure protein synthesis in the mitochondria (an energy-producing organelle in cells) and studied the changes that occur in human patients with dysfunctional mitochondria. Therefore, global changes in ribosome positions can indicate changes in cellular metabolism. As cancer development involves dramatic changes in cellular metabolism, we use Ribo-Seq to examine metabolic changes in cancer in an attempt to discover novel therapeutics avenues."

Division of Molecular Carcinogenesis

Selected publications

Sun C, Wang L, Huang S, Heynen GJJE, Prahallad A, Robert C, Haanen J, Blank C, Wesseling J, Willems SM, Zecchin D, Hobor S, Bajpe PK, Lieftink C, Mateus C, Mateus C, Vagner S, Grernrum W, Hofland I, Schlicker A, Wessels L, Beijersbergen RL, Bardelli A, Di Nicolantonio, F, Eggermont AMM and Bernards R. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature 2014;508:118-122

Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100-3

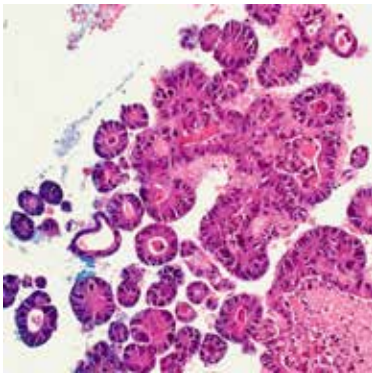
Nijwening JH, Geutjes EJ, Bernards R, Beijersbergen RL. The histone demethylase Jarid1b (Kdm5b) is a novel component of the Rb pathway and associates with E2f-target genes in MEFs during senescence. PLoS One 2011;6(9):e25235

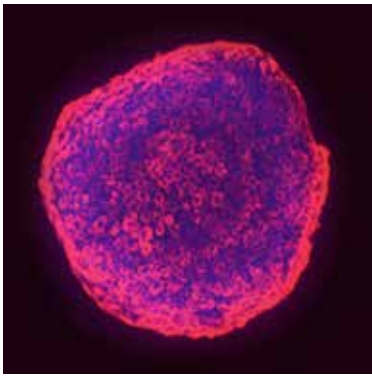
Signaling Networks in Cancer
RODERICK BEIJERSBERGEN

Many genetic mutations in tumor cells affect signaling pathways, ultimately leading to cancerous phenotypes. Components of these signaling networks are potential targets for cancer therapy. However, it remains challenging to select the right drug for each tumor and to avoid the development of drug resistance. Roderick Beijersbergen is interested in the regulation and dynamics of signaling networks in tumor cells. His group develops and applies functional genomics approaches to identify the critical components of signaling networks that are deregulated in cancer. They also investigate possible mechanisms of drug resistance and explore synthetic lethal interactions for developing novel anti-cancer drugs.

Unraveling Cancer Networks

"Cross talk between effector pathways and feedback inhibition are crucial regulatory components of signal transduction. The inhibition of individual components in signaling networks, which is the desired mechanism of action of targeted anti-cancer drugs, often disrupts these regulatory loops resulting in up-regulation of other pathway components or the activation of parallel signaling pathways. As consequence, a decrease or even absence of the desired effect of a targeted inhibitor is seen. To elucidate and understand the mechanisms underlying the response to pathway targeted therapeutics we use large scale functional genomic technologies including large scale RNAi screens, CRISPR screens and genome wide ORF collections. We use a phospho-proteomics approach to measure the activation state of individual proteins and to reveal the complex regulatory circuits involved. Such analyses are performed in large panels of cancer cell lines and primary tumor samples established as 3D organoid cultures. These tumoroid cultures more accurately reflect the heterogeneity of patient's tumors and can therefore improve the identification of the molecular determinants of therapy response. This approach allows for the identification of specific dependencies in the context of tumor- and patient-specific alterations and can ultimately provide predictive models for therapy response to pathway targeted therapeutics in cancer."





Data integration

"The complexity and heterogeneity of cancer poses an enormous challenge for the identification and selection of effective cancer therapies. By integrating our findings from these functional genomic screens with additional genomic analyses, transcription profiling and drug responses, we can develop algorithms to predict treatment response in individual cancers that are characterized by specific genetic alterations and aberrant signaling behavior. Our understanding of the complex dynamic circuitry of signaling pathways in the context of targeted inhibition is highly valuable for identifying biomarkers to stratify patients, and to enable the identification of more effective combination therapies."

Pharmacy Department

Selected publications

Gomez-Eerland R, Nuijen B, Heemskerk B, van Rooij N, van den Berg JH, Beijnen JH, Uckert W, Kvistborg P, Schumacher TN, Haanen JB, Jorritsma A. Manufacture of gene-modified human T-cells with a memory stem/central memory phenotype. Hum Gene Ther Methods 2014;25:277-87

Dubbelman AC, Rosing H, Jansen RS, Mergui-Roelvink M, Huitema ADR, Koetz B, Lymboura M, Reyderman L, Lopez-Anaya A, Schellens JHM, Beijnen JH. Mass Balance Study of 14C-eribulin in Patients with Advanced Solid Tumours. Drug Metab Dispos 2012;40:313-21

Damen CWN, Chen W, Chakraborty AB, van Oosterhout M, Mazzeo JR, Gebler JC, Schellens JH, Rosing H, Beijnen JH. Electrospray ionization quadrupole ion-mobility time-of-flight mass spectrometry as a tool to distinguish the lot-to-lot heterogeneity in N-glycosylation profile of the therapeutic monoclonal antibody trastuzumab. J Am Soc Mass Spectrom 2009;20:2021-33

Anticancer drug development
JOS BEIJNEN

Jos Beijnen oversees the NKI's pharmacy department, which not only serves the Antoni van Leeuwenhoek hospital but also runs a small pharmaceutical facility, supplying new drug formulations for research and treatment worldwide. The department also has a research line which focuses on monitoring drug effects and the development of models for response prediction.

GMP License

"Besides our standard pharmacy work, preparing and dispensing drugs for the hospital, and our research, we are very unusual for a pharmacy department in having a facility with an official GMP (good manufacturing practice) license from the Dutch health authorities to manufacture new investigational cytotoxic drugs. We make them for our own clinical research and for hospitals and biotech companies in Europe and the USA. As we're small, we can be quicker and more flexible than the pharmaceutical industry, and will supply just a handful of ampoules of a new drug for a clinical trial. We've just installed a 'biotherapeutics unit' that uses engineered Escherichia coli to produce DNA vaccines for clinical trials by the NKI's immunology division. The profits from our manufacturing are invested into the NKI."

Drug effects

"Our research is mostly on cancer, although we also work on HIV and drug addiction. The pharmacology group, which Jan Schellens co-heads, has PhD students who work on the design of drug formulations, as well as analysis of the properties of drugs once they are given to patients. We also specialize in mathematical analysis and modeling of drug effects, which we use to predict how a patient might react to a drug, as well as determining its concentration in the body, and the risk of side effects. We are just beginning to explore two new avenues. First, we are looking for mutations that affect the distribution of drugs in tumors or tissues, and their side effects and activity. Armed with this information, it may be possible to lower drug doses for individual patients to minimize side effects. Second, our proteomics research aims to identify patterns of proteins in biological samples that are specific for different types of cancer and may predict a person's response to chemotherapy. This is in the preliminary phase so far, but we hope it will provide an alternative means of forecasting drug effects and a patient's progress."

Division of Molecular Genetics

Selected publications

Wissing MD, Coenen JL, van den Berg P, Westgeest HM, van den Eertwegh AJ, van Oort IM, Bos MM, Bergman AM, Hamberg P, Ten Tije AJ, Los M, Lolkema MP, de Wit R, Gelderblom H. CAST: A retrospective analysis of cabazitaxel and abiraterone acetate sequential treatment in patients with metastatic castrate-resistant prostate cancer previously treated with docetaxel. Int J Cancer. 2015;136: 760-772

Kwon ED, Drake CG, [...], Bergman AM, [...], Gagnier P, Liu D, Gerritsen WR. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15:700-712

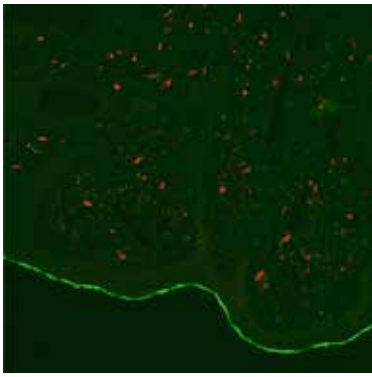
Badrising S, van der Noort V, van Oort IM, van den Berg HP, Los M, Hamberg P, Coenen JL, van den Eertwegh AJ, de Jong IJ, Kerver ED, van Tinteren H, Bergman AM. Clinical activity and tolerability of enzalutamide (MDV3100) in patients with metastatic, castration-resistant prostate cancer who progress after docetaxel and abiraterone treatment. Cancer. 2014;120:968-975

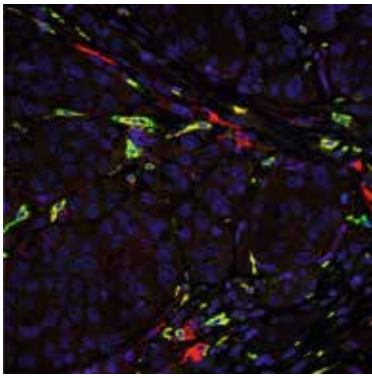
Prostate Cancer Development
ANDRE BERGMAN

Andre Bergman divides his time between treating cancer patients at the Antoni van Leeuwenhoek hospital and his research work at the adjacent NKI, where he studies the mechanisms underlying prostate cancer development. Prostate cancer develops through distinct stages. There is emerging evidence that the tumor microenvironment plays an important role in driving this progression. The Bergman group has been investigating the contribution of the microenvironment in prostate cancer development using both cancer models in the laboratory as well as clinical prostate cancer specimen. Their goal is to identify new targets that could be used to develop more effective therapeutic approaches.

The tumor microenvironment

"Prostate cancer is the most prevalent malignancy in men. It is a diverse disease, ranging from indolent, which is often left untreated, to aggressive with high morbidity and mortality. Prostate cancer progresses through distinct stages, beginning with disease that is sensitive to a decrease in testosterone levels, to a more advanced castrate-resistant disease. The tumor microenvironment is known to play an important role in the development of many cancers. Epidemiological, histopathological and molecular studies suggest that it is a critical factor in the initiation and progression of prostate cancer, particularly in the development of castrate-resistant disease. Our group has been studying the underlying molecular mechanisms involved using several mouse models, cell culture and human prostate cancer samples. We aim to delineate the role of different cell types and cellular processes, including the influx of immune cells, angiogenesis, and testosterone and growth factor signaling in prostate cancer development."





Immune system

"We recently discovered that the adaptive immune system plays a critical role in both the initiation and progression of prostate cancer, as well as in the development of castrate resistance. Our ultimate goal is to identify drug targets in these immune cells or in soluble mediators of the inflammatory response in the microenvironment. Targeted drugs could be used to prevent prostate cancer development in high-risk populations, or suppress disease progression in affected individuals, including those with castrate-resistant disease."

Division of Molecular Carcinogenesis

Selected publications

Sun C, Wang L, Huang S, Heynen GJJE, Prahallad A, Robert C, Haanen J, Blank C, Wesseling J, Willems SM, Zecchin D, Hobor S, Bajpe PK, Lieftink C, Mateus C, Mateus C, Vagner S, Grernrum W, Hofland I, Schlicker A, Wessels L, Beijersbergen RL, Bardelli A, Di Nicolantonio F, Eggermont AMM and Bernards R. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature 2014;508:118-122

Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, and Bernards R. Unresponsiveness to BRAF(V600E) inhibition of colon cancer through feedback activation of EGFR. Nature 2012;483:100-103

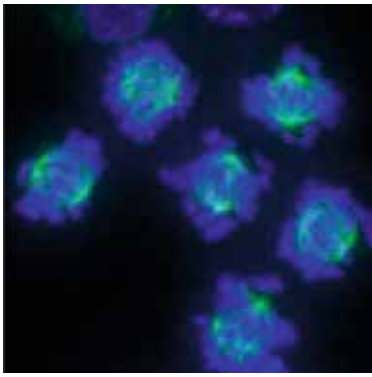
Huang S, Hölzel M, Knijnenburg T, Schlicker A, McDermott U, Garnett M, Grernrum W, Sun C, Prahallad A, Groenendijk FH, Nijkamp W, Beijersbergen RL, Wessels L and Bernards R. MED12 controls the response to multiple targeted cancer drugs through direct regulation of TGFβ receptor signaling. Cell 2012;151:937-950

Functional Cancer Genetics
RENÉ BERNARDS

There has been a trend in the treatment of cancer from an approach in which the tissue of origin and the histology were the guiding principles for the choice of therapy, towards a strategy in which knowledge of the oncogenic mutations is used to select patients for treatment with highly selective drugs. René Bernards contributes to this transition by studying the relationships between cancer genotypes and responses to cancer drugs. His group uses functional genetic approaches to identify genes that control drug responses, as well as to identify particularly effective drug combinations that can be used to treat certain cancers.

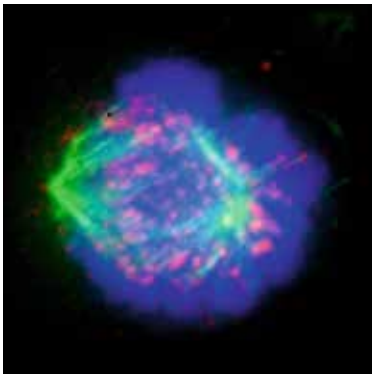
Biomarkers for Drug Response

"Unresponsiveness to therapy is a significant problem in the treatment of cancer. However, the underlying causes remain poorly understood. In my laboratory, we use functional genetic approaches to identify biomarkers that can predict responsiveness to targeted cancer therapeutics; drugs that specifically inhibit molecules or pathways that are often activated in cancer cells. We aim to elucidate the molecular pathways that contribute to this unresponsiveness. Understanding drug resistance will also help in the development of specific combination therapies that prevent drug resistance."



Resistance and lethality screens

"To identify biomarkers that control tumor cell responsiveness to cancer therapeutics, my group uses multiple complementary approaches. First, we use genome-wide loss-of-function genetic screens (with shRNA or CRISPR libraries) in cancer cells that are sensitive to a specific drug in order to identify genes whose down-regulation confers resistance to that drug. These are known as resistance screens. In addition, we use shRNA screens with a low dose of a specific drug to identify genes whose inhibition enhances the toxicity of that drug, known as enhancer or synthetic lethality screens. Together, these genetic screens identify rational and particularly powerful drug combinations. We work closely with NKI clinicians to test the drug combinations identified in the laboratory in our cancer clinic. In a final and distinct approach we perform high throughput sequencing of kinase-related genes of tumor samples to identify connections between cancer genotype and drug responses. Knowing the contribution of specific kinases to the oncogenic process can contribute to genotype-directed personalized cancer care."



Division of Molecular Genetics

Selected publications

Sutherland K, Song J-Y, Kwon M-C, Proost N, Zevenhoven J, and Berns A. Multiple cells-of-origin in K-RasG12D induced mouse lung adenocarcinoma. Proc. Natl. Acad. Sci. USA 2014;111:4952-4957

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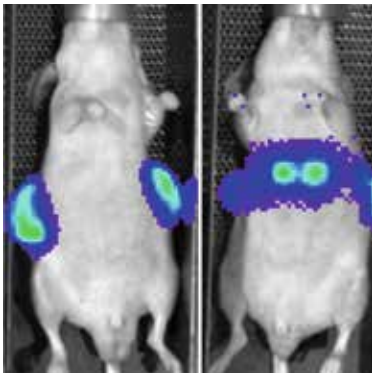
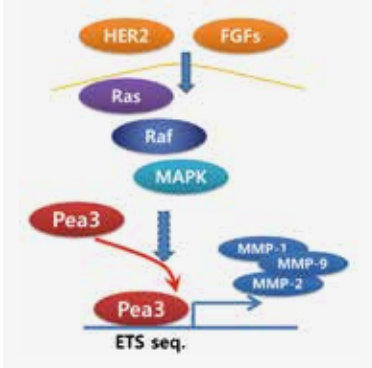
Mouse Models for Cancer
ANTON BERNS

Anton Berns served as director of the NKI from 1999-2011 whilst retaining a research group, and now continues as a full-time group leader. His aim is to develop new therapies faster using preclinical cancer models. His group uses mouse models, particularly for lung cancer and mesothelioma, two of the deadliest cancers. Using these models Berns wants to gain a better understanding of the mechanisms of cancer initiation and progression, with the hope of developing methods to cure it. Looking ahead, he hopes that once they can cure certain tumors in mice, a similar intervention can be applied in humans.

Improving models

"We are continuously trying to improve our mouse models to make them more versatile and therefore more valuable for studying cancer. We now have techniques that permit us to swiftly introduce additional genetic alterations in existing mouse models. This allows us to quickly test cancer-related alterations or mutations as found by sequencing the DNA from human tumors or identified in drug resistance screens. We also use mouse models to study tumor diversity. We know that mouse tumors, like tumors in man, vary greatly in their response to the therapies used in the clinic. By characterizing each tumor in detail, it becomes possible to design the best intervention and understand the escape strategies that it might employ to become refractory to particular drug regimens. In the mouse models we utilize this is much easier to achieve than in other models. We can subsequently test whether the principles found also apply to human tumors, that can then be grouped according to detailed genetic features. We hope this will contribute to making personalized medicine a reality."





Tumor heterogeneity

"A second focus of our research is the role of the cell-of-origin of tumors, as we suspect that this might be a determining factor in its phenotypic manifestation and behavior. A particular cell-of-origin might require distinct mutations to give rise to a tumor. Understanding the relationship between the cell-of-origin and the occurrence of specific mutations giving rise to distinct tumor characteristics can help us to design more rational intervention therapies. We are also intrigued by tumor heterogeneity, not as a reflection of genetic instability but as a functional feature in which genetically diverged cellular subclones of a tumor develop a symbiotic relationship with a diversification of tasks. We essentially view the tumor as evolving by Darwinian selection to contain multiple subclones that fulfill individual functions required for tumor progression."

Division of Immunology

Selected publications

Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A; KEYNOTE-006 investigators. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med. 2015;372(26):2521-32

Gadiot J, Hooijkaas AI, Kaiser AD, van Tinteren H, van Boven H, Blank C. Overall survival and PD-L1 expression in metastasized malignant melanoma. Cancer. 2011;117(10):2192-201

Blank C, Kuball J, Voelkl S, Wiendl H, Becker B, Walter B, Majdic O, Gajewski TF, Theobald M, Andreesen R, Mackensen A. Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in vitro. Int J Cancer. 2006;119(2):317-27

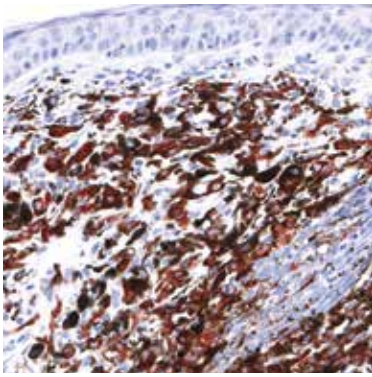
Blank C, Brown I, Peterson AC, Spiotto M, Iwai Y, Honjo T, Gajewski TF. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004 Feb 1;64(3):1140-5

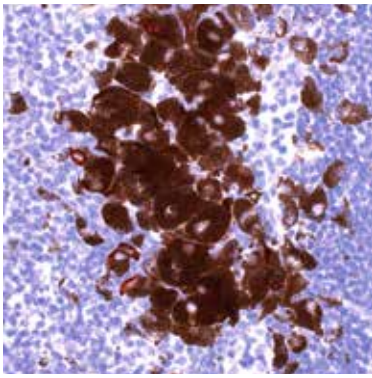
Immunotherapy and additional therapies
CHRISTIAN BLANK

Christian Blank is a medical oncologist who works half of his time in the hospital and the other half in the research department of the NKI. He believes that our immune system can be stimulated to attack cancer cells in all types of cancer. A major part of his career was dedicated to the characterization of a molecule called the programmed-death receptor 1 (PD-1). This belongs to the T cell checkpoint molecules, that are currently being tested with great success in clinical trials with melanoma and other cancers. At the moment, his main focus is modulation of the tumor environment by additional therapies like targeted therapies or depleting monoclonal antibodies.

Alterations of tumor microenvironment

"The aim of the additional therapies we study is to remove immune inhibitory cells from the tumor environment and to improve the efficacy of T cell checkpoint therapy. While targeted therapies are thought to not be toxic for immune cells, they alter the tumor microenvironment so that increased or decrease tumor immune infiltration can occur. Understanding mechanisms of this indirect immune modulation via targeted agents will provide insights for optimal combinations of these compounds. In melanoma mouse model we found that the combination of BRAF and MEK inhibition improved tumor control, while BRAF inhibitors impaired tumor immune infiltration and did not synergize with T cell checkpoint blockade by anti-PD-1, -PD-L1 and -CTLA-4 mAb. Current experiments aim at understanding the mechanism of BRAF inhibitor-mediated decrease of tumor immune infiltration."





Appropriate in vivo models

"It is crucial to test new therapeutic approaches in appropriate in vivo models that simulate the human cancer reality. Transplantable tumor models often do not mimic the complex interaction between the tumor cell and the tumor microenvironment and therefore may have little predictive value for the treatment of cancer patients. We have crossed an inducible melanoma mouse model that harbors mutations commonly found in human melanoma, has a fully intact immune system, and shows histological features of human melanoma. This model allows the simulation of targeted therapy combined with immunotherapy. The results from his work are directly translated into phase 1 trial proposal for in-house studies or trials together with pharmaceutical companies."

Division of Psychosocial Research and Epidemiology

Selected publications

Eijzenga W, Aaronson NK, Hahn DEE, Sidharta G, vd Kolk L, Velthuizen M, Ausems, MGE, Bleiker EMA. The effect of routine assessment of specific psychosocial problems on personalized communication, counselors' awareness, and distress levels in cancer genetic counseling practice: A randomized controlled trial. Journal of Clinical Oncology, 2014;32:2998-3004

Bleiker EMA, Esplen MJ, Meiser B, Petersen HV, Patenaude AF. 100 years Lynch Syndrome: What have we learned about psychosocial issues? Familial Cancer, 2013; 12:325-339

Lammens CRM, Aaronson NK, Wagner A, Sijmons RH, Ausems MGEM, Vriends AHJT, Ruijs MWG, Os TAM van, Spruijt L, Gómez García EB, Kluijt I, Nagtegaal T, Verhoef S, Bleiker EMA. Genetic testing in Li-Fraumeni Syndrome: Uptake and psychosocial consequences. Journal of Clinical Oncology 2010;28:3008-3014

Psychosocial Oncology in Genetics and Care
EVELINE BLEIKER

Eveline Bleiker has a background in medical psychology. She is eager to improve the quality of life and quality of care of those diagnosed with cancer, as well as those with a high risk to develop cancer because of a strong family history or a gene mutation. Since 2008, Eveline Bleiker is group leader within the division of Psychosocial Research and Epidemiology and is also associated with the Family Cancer Clinic of the Netherlands Cancer Institute. During the past years, her research program has focused on the psychosocial aspects of genetic counseling and testing in cancer. More recently, a second line of research was added, in which her group aims to evaluate psychosocial interventions to improve supportive care.

Clinical Genetics

"Genetic counseling and testing for cancer can be stressful. When someone is found to be a carrier of a cancer mutation, various decisions have to be made. Individuals at high risk may opt for prophylactic surgery (for example a mastectomy to reduce the risk of breast cancer) or may participate in a life-long surveillance program. Both options can have impact on the quality of life. Our studies show that approximately 20% of those counseled for hereditary forms of cancer, irrespective of their carrier status, exhibit clinically relevant levels of distress. Furthermore, we found that only one-third of these distressed individuals received psychosocial support; that psychosocial variables explain significantly more of the variability in distress levels than do sociodemographic and clinical variables; that partners suffer from at least as much distress as patients; and that in hereditary colorectal cancer, about 20% of the at-risk group is less than fully compliant with surveillance advice. These results have been used in guidelines to improve the care for this high risk group."

Supportive Care

"Another important aspect of the work of our group is to evaluate and promote the care provided to patients and their relatives. For example, within the family cancer clinic, we developed and tested a questionnaire, which aimed to improve the communication about specific problems experienced during the time of genetic counseling and testing. We found that the new tool (the PAHC questionnaire) was effective in reducing distress and cancer worries on the short term. A second example is a study in which we aimed to investigate the psychological, psychosexual and motivational experiences with breast and nipple reconstruction of women undergoing direct breast reconstruction after mastectomy. We found that a substantial group of women indicated to have missed psychosocial support and visual information on the various reconstructive options. Therefore, in 2015, a multicenter trial will start to improve (psycho-) education and balanced decision making for this group of patients."

Division of Immunology

Selected publications

Coquet JM, Middendorp S, van der Horst G, Kind J, Veraar EA, Xiao Y, Jacobs H, and Borst J. The CD27 and CD70 costimulatory pathway inhibits effector function of T helper 17 cells and attenuates associated autoimmunity. Immunity 2013;38:53-65

Coquet JM, Ribot JC, Bąbała N, Middendorp S, Van der Horst G, Xiao Y, Neves JF, Fonseca-Pereira D, Jacobs H, Pennington DP, Silva-Santos B, and Borst J. Epithelial and dendritic cells in the thymic medulla promote $CD4^+Foxp3^+$ regulatory T cell development via the CD27/CD70 pathway. J. Exp. Med. 2013;210:715-728

Peperzak V, Xiao Y, Veraar EAM, and Borst J. CD27 sustains survival of CTL in virus-infected non-lymphoid tissue in mice by inducing autocrine IL-2 production. J. Clin. Invest. 2010;120:168-178

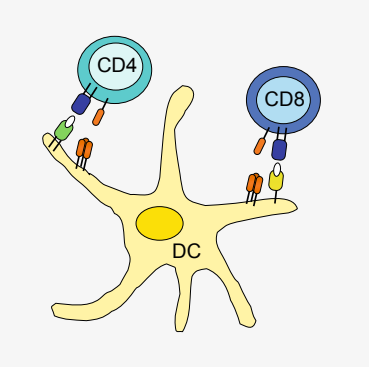
Promoting the T cell response to cancer

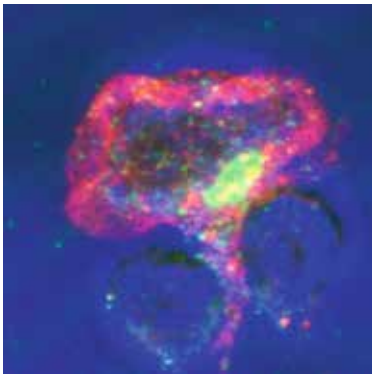
JANNIE BORST

The group of Jannie Borst studies T cells – key cells of the immune system that fight infection, but can also destroy cancer cells. To kick-start T cells, another cell type is important: the dendritic cell. This cell is the messenger that tells T cells to react. To do this properly, the dendritic cells need to be activated. Viruses and other microbes are good at activating dendritic cells, but cancer cells are not, because they look too much like normal cells. For this reason, the dendritic cells largely ignore the cancer cells and the T cells don't react. However, T cells can be stimulated to react against cancer cells by deliberately providing 'costimulation' signals.

Antibodies to stimulate the T cell response

"In the absence of dendritic cell activation, one can help the T cells to attack cancer cells by purposely engaging their receptors with antibodies. This is the basis of cancer immunotherapy. At the moment, there are very successful antibodies in the clinic that help kick-start the T cells by removing the break from their responsiveness. This is the current breakthrough in cancer immunotherapy. These antibodies target inhibitory receptors. However, the response can be further improved by pressing the gass pedal, i.e. stimulating the costimulatory receptors. We obtain and apply insights to improve cancer immunotherapy with immunostimulatory antibodies, but also with vaccination strategies.
We have discovered and extensively studied a costimulatory receptor that is now a prime target in cancer immunotherapy. We work together with biotech companies and the pharmaceutical industry to bring the targeting of this receptor to the clinic. Our work in mouse models predicts that an immunostimulatory antibody to this receptor will act synergistically with currently applied antibodies that target inhibitory receptors."





Cell death to stimulate the T cell response

"We combine this work on cancer immunotherapy with work on the mechanisms of cell death. Novel data indicate that tumor cells are not ignored by the immune system when they are killed in specific ways. We are exploring the pathways of this so-called 'immunogenic' cell death, with the aim to trigger it deliberately and to accomplish dendritic cell and T cell activation. In this work, we have a direct link with the divisions of Radiotherapy and Medical Oncology. In our radio-immunotherapy approach, we aim to translate the local tumor-destructive action of radiation into a systemic immune response, able to eliminate metastatic disease. Our knowledge on immunogenic cell death and the rules of T cell activation are expected to make this a reality."

Division of Molecular Oncology

Selected publications

Van Luenen H, Farris C, Jan S, Genest P-A, Tripathi P, Velds A, Kerkhoven RM, Nieuwland M, Haydock A, Ramasamy G, et al. Glucosylated hydroxymethyluracil (DNA base J) prevents transcriptional read-through in Leishmania. Cell 2012;150:909-921

Rottenberg S, Vollebergh MA, de Hoon B, de Ronde JJ, Schouten PC, Kersbergen A, Zander SA, Pajic M, Jaspers JE, Jonkers M, et al. Impact of intertumoral heterogeneity on predicting chemotherapy response of BRCA1-deficient mammary tumors. Cancer Res 2012;72:2350-2361

Jansen RS, Duijst S, Mahakena S, Sommer D, Szeri F, Váradi A, Plomp A, Bergen AA, Oude Elferink RP, Borst P, van de Wetering K. ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report. Arterioscler Thromb Vasc Biol. 2014;34:1985-9

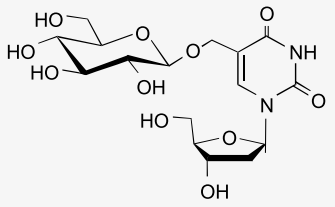
Drug transporters and DNA base J

PIET BORST

Piet Borst's early success as a biochemist led him to a professorship at the University of Amsterdam at the age of 30, and in 1983 he became director of the NKI. He handed over the directorship to Anton Berns in 1999 but retained his laboratory at the institute. A focus of his research has been on the human parasite *Trypanosoma*, which is common in Africa and can cause fatal sleeping sickness. He has also been investigating the mechanisms of drug resistance in cancer, a major problem in the clinic. This project has led to a more detailed investigation of the function of human drug transporters.

Base J

"One of our longest running research lines began in 1993 with the discovery of the first hypermodified DNA base in eukaryotes, called base J, in my lab. Base J is unique to trypanosomes and related parasites, making it an attractive target for developing new anti-parasitic drugs. Over the years we uncovered many characteristics of base J. This work has culminated in the discovery of its function in *Leishmania* parasites, where base J stops RNA polymerase II. If base J is removed from the DNA, this enzyme can no longer terminate transcription resulting in the death of the organism. With this discovery, the base J project has been terminated at the NKI, but will be continued elsewhere with our support."



New tools to fight cancer

"The main focus of our team has long been on the characterization of the molecular pumps found in cancer cell membranes, which expel drugs and lead to drug resistance. For this project various tools were generated, including cell lines that overproduce these pumps and 'knockout' mice lacking one or more pumps. As a spin-off from the drug resistance project, we recently studied *Pseudoxanthoma elasticum* (PXE), an inborn error of blood calcium regulation, which leads to damage of eyes and blood vessels due to calcium precipitation. Other investigators had shown that the disease is due to a defect in a transport protein ABCC6 (MRP6) that secretes an unknown compound X from the liver into the circulation. Experiments by Robert Jansen and Koen van de Wetering from our group recently showed that the mysterious compound X is ATP. In the circulation ATP is rapidly converted into pyrophosphate, a known metabolite that helps keep calcium in solution. These results provide new ideas to develop a treatment for PXE, which has thus far been proven hard to treat."

Division of Biochemistry

Selected publications

Jae LT, Raaben M, Herbert AS, Kuehne AI, Wirchnianski AS, Soh TK, Stubbs SH, Janssen H, Damme M, Saftig P, Whelan SP, Dye JM and Brummelkamp TR. Lassa virus entry requires a trigger-induced receptor switch. Science. 2014;344:1506-1510

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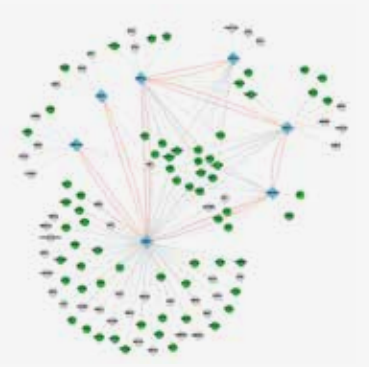
Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Dal Cin P, Dye JM, Whelan SP, Chandran K and Brummelkamp TR. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature. 2011;477:340-343

Biomedical Genetics
THIJN BRUMMELKAMP

Thijn Brummelkamp is interested in developing genetic tools to gain unique insights into complex biological systems. Arguably the most powerful and direct way to do this is to remove individual protein components and observe the phenotypic consequences. However, in human cell cultures, this approach has remained complicated due to the human diploid genome. To address this, Bummelkamp's group developed a novel genetic model system based on haploid human cells to enable the removal of most human genes. They use this model to search for cancer cell vulnerabilities and to identify host factors required for pathogen infection, to aid in the development of new treatments.

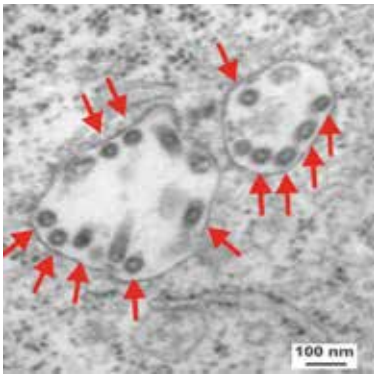
Identifying genes using haploid cells

"Our group recently developed an entirely novel genetic model system based on insertional mutagenesis in haploid and near-haploid human cells, which can be used to generate knockouts for most human genes. This tool is highly versatile and enables the identification of complex networks of genes underlying nearly any selectable cell trait. We have been using it to identify genes that are involved in phenotypes related to various human diseases. For example, we have identified the cholesterol transporter NPC1 as the long sought intracellular entry receptor for Ebola virus. Recently we also unravelled an unexpected entry route for Lassa virus that involved binding to a new receptor inside the host cell."



Tissue size control mechanisms

"In parallel, we are also interested in understanding the mechanisms controlling organ size, and particularly how tissues know to stop growing upon reaching a certain size. This remains a mystery in biology and is likely relevant in tumorgenesis, where tumor cells are able to bypass normal growth control and continue to proliferate unabated. *Drosophila* genetics has increased our understanding of the biology of organ size control, and the so-called Hippo signalling pathway has emerged as a key component, regulating cell proliferation and death. Interestingly, all the components of the Hippo pathway are conserved in mammals and some have been implicated in cancer. We are now using genetic mouse models and biochemical methods to address how this signalling pathway regulates tissue size in mammals and how it contributes to tumorigenesis."



Division of Immunology

Selected publications

Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau C-S, Verstegen NJM, Ciampricotti M, Hawinkels LJAC, Jonkers J, de Visser KE. IL17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature 2015;552: 345-348

Doornebal CW, Klarenbeek S, Braumuller TM, Klijn CN, Ciampricotti M, Hau C-S, Hollmann MW, Jonkers J, de Visser KE. A preclinical mouse model of invasive lobular breast cancer metastasis. Cancer Research 2013;73:353-363

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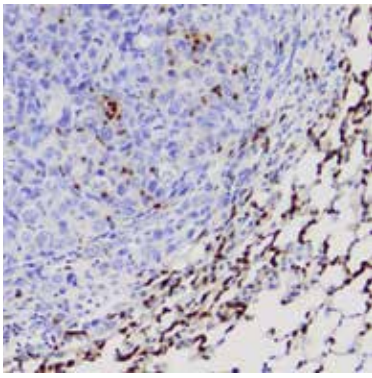
Ciampricotti M, Vrijland K, Hau C-S, Pemovska T, Doornebal CW, Speksnijder EN, Wartha K, Jonkers J, de Visser KE. Development of metastatic HER2(+) breast cancer is independent of the adaptive immune system. J. Pathol. 2011;224:56-66

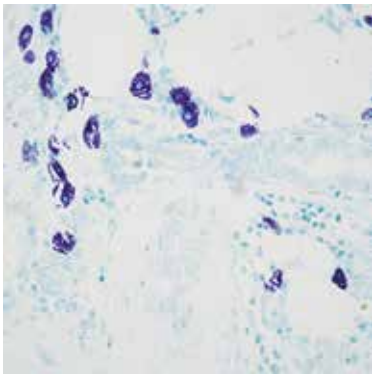
Inflammation and Cancer
KARIN DE VISSER

Karin de Visser is interested in the impact of the crosstalk between adaptive and innate immune cells on breast cancer behavior. Her group utilizes preclinical tumor models that faithfully represent human breast cancers to study how the inflammatory tumor microenvironment influences metastatic breast cancer and its response to conventional anti-cancer therapies such as chemo- and radiotherapy. The aim is to identify targets and to design strategies to inhibit the metastasis-promoting effects of the immune system, thus improving anti-cancer therapies in the clinic.

Pro-metastatic effects of the immune system

"Immune cells and their mediators are abundantly present in the microenvironment of cancer cells. Interactions between cancer cells and different components of the immune system influence tumor outgrowth, metastasis formation and the efficacy of anti-cancer therapy. The exact role of the immune system during these processes is controversial and poorly understood, as both tumor-protective and tumor-promoting properties have been reported. One major focus of our lab is to mechanistically understand how the immune system modulates breast cancer metastasis formation. To this end, we have established a novel pre-clinical model that recapitulates human metastatic breast cancer. By using biological, genetic and pharmacological approaches, we can target components of the immune system and analyze the effect on metastasis formation. We have recently discovered that breast tumors maximize their chance to metastasize by evoking a systemic inflammatory cascade involving certain T cells and neutrophils. In parallel, through collaborations with the clinic and the NKI Biobank, we study human breast cancer samples to obtain deeper insights into the prognostic and predictive power of immune parameters. This helps us to identify which type of immune cell or inflammatory mediator is an attractive candidate for development of novel anti-metastatic therapy."





Immune system modulates chemotherapy response

"In another project, we study how chemotherapy and radiotherapy influence the composition and function of the immune system, and how the immune system influences the anti-cancer efficacy of these therapies. We have found that macrophages counteract the anti-cancer efficacy of certain chemotherapeutic drugs. We are working on dissecting the mechanisms how these immune cells influence chemo-responsiveness and we are studying immune-mediated resistance mechanisms. We aim to utilize this knowledge to design novel immunomodulatory strategies to increase the efficacy of conventional anti-cancer therapies."

Division of Gene Regulation

Selected publications

De Vree PJ, De Wit E, Yilmaz M, Van de Heijning M, Klous P, Verstegen MJ, Wan Y, Teunissen H, Krijger PH, Geeven G, Eijk PP, Sie D, Ylstra B, Hulsman LO, Van Dooren MF, Van Zutven LJ, Van den Ouweland A, Verbeek S, Van Dijk KW, Cornelissen M, Das AT, Berkhout B, Sikkema-Raddatz B, Van den Berg E, Van der Vlies P, Weening D, Den Dunnen JT, Matusiak M, Lamkanfi M, Ligtenberg MJ, Ter Brugge P, Jonkers J, Foekens JA, Martens JW, Van der Luijt R, Van Amstel HK, Van Min M, Splinter E, De Laat W. Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping. Nat Biotechnol. 2014;32(10):1019-25

De Wit E, Bouwman BA, Zhu Y, Klous P, Splinter E, Verstegen MJ, Krijger PH, Festuccia N, Nora EP, Welling M, Heard E, Geijsen N, Poot RA, Chambers I, De Laat W. The pluripotent genome in three dimensions is shaped around pluripotency factors. Nature 2013;501(7466):227-31

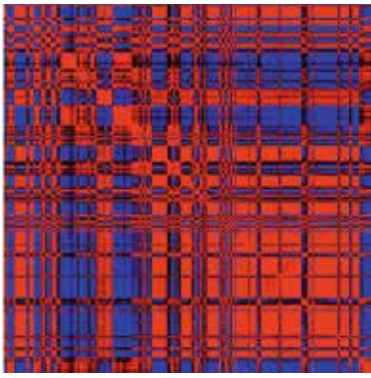
De Wit E, De Laat W. A decade of 3C technologies: insights into nuclear organization. Genes Dev. 2012;26(1):11-24

Genome Function and Dynamics
ELZO DE WIT

Technological advances in sequencing have made the €1000 genome a reality. This will usher in an era of personal genomics. Elzo de Wit is interested in how genetic variation leads to differences between individuals. With a background in computational biology he aims to integrate multiple sources of genomics data to make functional predictions about genetic basis of phenotypic traits and disease risks.

Linking genotype to phenotype

"Years of genomics research has identified thousands of genetic variants that are associated with phenotypic traits or disease. Many of these genetic variants are found in the non-coding portion of the genome, where they likely affect gene expression. Understanding how non-coding variants affect gene expression will be an important part of understanding how our genome functions. This is complicated by the diploid nature of our genomes, meaning we have two copies of every chromosome, one from our mother and one from our father. We have developed tools to link genetic variants that are on the same chromosome (haplotype). By analyzing haplotypes we can much better predict the effect of mutations on gene expression. We will use this to study the effect of non-coding variation on the expression of cancer-risk genes."



3D organization of the genome

"When stretched out our genome measures 2 meters in length. But it has to fit into a nucleus that is one 100th of a millimeter in diameter. To achieve this the genome is very efficiently folded. It has become clear that the 3D organization of the genome plays an important role in the regulation of genes. We have been at the forefront of the development of tools that analyze how the genome is folded, such as 4C and Hi-C. We will continue to develop these tools to better understand the interplay between genome folding and gene expression."

Division of Immunology

Selected publications

Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, Joseph-Pietras D, Welters MJ, van der Burg S, Kapiteijn E, Michielin O, Romano E, Linnemann C, Speiser D, Blank C, Haanen JB, Schumacher TN. Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. Sci Transl Med. 2014;6:254ra128

Kvistborg P, Shu CJ, Heemskerk B, Fankhauser M, Thrue CA, Toebes M, van Rooij N, Linnemann C, van Buuren MM, Urbanus JH, et al. TIL therapy broadens the tumor-reactive CD8(+) T cell compartment in melanoma patients. Oncoimmunology 2012;1:409-418

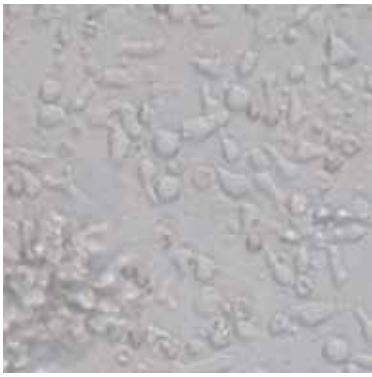
Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, Kaiser AD, Pouw N, Debets R, Kieback E, Uckert W, Song JY, Haanen JB, Schumacher TN. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med. 2010;16:565-70

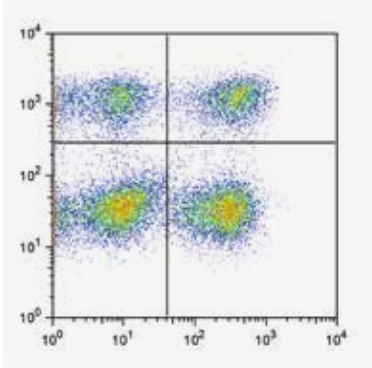
Cancer Immunotherapy
JOHN HAANEN

John Haanen is a medical oncologist and immunologist who spends half his time treating patients in the Antoni van Leeuwenhoek hospital (the cancer clinic of the NKI) and half doing research. His work is directed towards the development of novel therapies for the clinic that harness the inherent capability of the immune system to fight cancer. The immune system recognizes at least some tumors and mounts an immune response to help eradicate them. Haanen's group works on developing immunotherapies to strengthen this response, in particular for melanoma, which is the deadliest form of skin cancer.

Attacking cancer with T Cells

"My group focuses on developing approaches to effectively utilize the patients' own immune cells, known as T lymphocytes, for eradicating cancer. The T lymphocytes are first isolated from the patient, and can then be grown into large numbers in the laboratory for infusion back into the body. However, not all T cells will have anti-tumor capabilities. To enhance tumor eradication we have isolated a population of T cells derived from melanoma metastases (so-called tumor-infiltrating lymphocytes or TIL). These cells can already recognize the tumor but are not capable of destroying it. We hypothesized that expanding their numbers in vitro may create great killers in vivo. And indeed the clinical results with TIL therapy have so far been dramatic, with 5 out of 10 patients responding, and two durable complete responders in a first pilot study. This work is performed in collaboration with many people both from the lab and the clinic. Ton Schumacher's lab and the pharmacy of Jos Beijnen have been instrumental for the clinical application of these highly complex therapies. Together with Schumacher's lab we are now dissecting which T cells within the TIL are responsible for the anti-tumor effect in order to improve and simplify this therapy. We already found that TIL cells that recognize consequences of UV irradiation induced DNA damage seem superior killer T-lymphocytes."





Gene therapy

"We have also been utilizing gene therapy approaches to prime T cells against the tumor. Especially for tumor types from which TIL cannot easily be grown, this approach is interesting. It involves isolating T cells of any specificity from the patient's blood, and inserting DNA coding for protein receptors that are designed to recognize tumor antigens. We can then inject these modified T cells back into the patient, where they should be able to attack the tumor more strongly."

Division of Psychosocial Research and Epidemiology

Selected publications

Hauptmann M, Fossa SD, Stovall M, Van Leeuwen FE, Johannesen TB, Rajaraman P, Gilbert ES, Smith SA, Weathers RE, Aleman BM, Andersson M, Curtis RE, Dores GM, Fraumeni Jr JF, Hall P, Holowaty EJ, Joensuu H, Kaijser M, Kleinerman RA, Langmark F, Lynch CF, Pukkala E, Storm HH, Vaalavirta L, Van den Belt-Dusebout AW, Travis LB, Morton LM. Increased stomach cancer risk following radiotherapy for testicular cancer. Br J Cancer 2015;112(1):44-51

Meulepas JM, Ronckers CM, Smets AM, Nievelstein RA, Jahnen A, Lee C, Kieft M, Lameris JS, Van Herk M, Greuter MJ, Jeukens CR, Van Straten M, Visser O, Van Leeuwen FE, Hauptmann M. Leukemia and brain tumors among children after radiation exposure from CT scans: design and methodological opportunities of the Dutch Pediatric CT Study. Eur J Epidemiol 2014;29(4):293-301

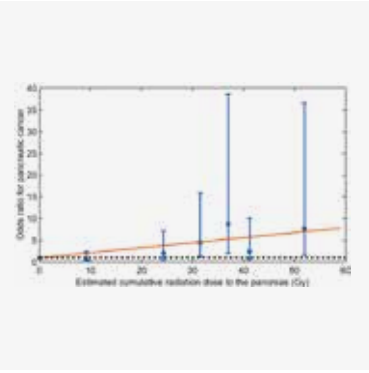
Cutter D, Schaapveld M, Darby SC, HauptmannM, Van Nimwegen FA, Krol SA, Janus C, Van Leeuwen FE, Aleman BM. Risk of valvular heart disease after radiotherapy for Hodgkin lymphoma. J Natl Cancer Inst 2015;107(4):djv008

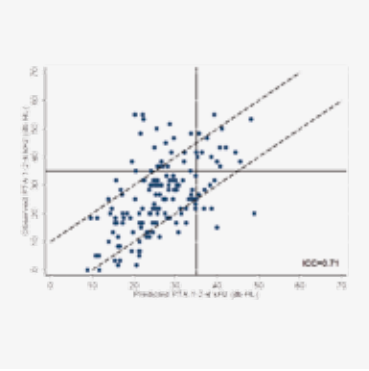
Biostatistics
MICHAEL HAUPTMANN

After statistical training in Germany, Michael Hauptmann spent several years at the prestigious US National Cancer Institute, before coming to the NKI as a senior statistician and head of the Biostatistics group. His team 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 researchers and clinicians from the Institute and the Antoni van Leeuwenhoek hospital on diverse topics, ranging from laboratory experiments to observational studies.

Low Dose Radiation Research

"The number of people exposed to radiation from medical sources, e.g., imaging and radiotherapy, is increasing every year. However, the health effects of low to medium doses of ionizing radiation exposure are currently not well understood because they are difficult to study in humans. Our group tries to better understand these exposures by conducting a cohort study of over 165,000 children exposed to radiation from computed tomography (CT) scans in the Netherlands. Based on those data, we will investigate by how much their cancer risk is increased. Our data will be pooled with 8 similar studies from other European countries, and we are leading the statistical analysis of this combined cohort of over one million children. Radiotherapy is another source of medical radiation exposure. Our group collaborates on several studies evaluating the late effects of radiation exposure during cancer treatment among cancer survivors, such as the risk of stomach cancer due to radiotherapy and chemotherapy for a previous testicular cancer."





Biostatistics Center

"We also provide state-of-the-art statistical expertise to researchers and doctors in the NKI and its hospital. This involves developing and implementing diverse statistical approaches to cover a wide range of topics, including the design and analysis of clinical trials, the identification of prognostic and predictive biomarkers, observational studies and risk prediction."

Division of Molecular Genetics

Selected publications

Klijn C, Koudijs MJ, Kool J, ten Hoeve J, Boer M, de Moes J, Akhtar W, van Miltenburg M, Vendel-Zwaagstra A, Reinders MJ, Adams DJ, van Lohuizen M, Hilkens J, Wessels LF, Jonkers J. Analysis of tumor heterogeneity and cancer gene networks using deep sequencing of MMTV-induced mouse mammary tumors. PLoS One 2013; e62113

Theodorou V, Kimm MA, Boer M, Wessels L, Theelen W, Jonkers J, Hilkens J. MMTV insertional mutagenesis identifies genes, gene families and pathways involved in mammary cancer. Nat. Genet. 2007;39:759-769

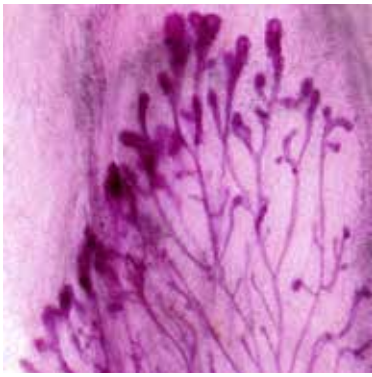
Hilkens J. Recent translational research: Oncogene discovery by insertional mutagenesis gets a new boost. Breast Cancer Res. 2006;8:102-105

Breast Cancer Genes
JOHN HILKENS

John Hilkens is interested in identifying and characterizing genes and oncogenic pathways that lead to breast cancer when they are deregulated. To find these genes, he uses a powerful technique called insertional mutagenesis in mouse models for human breast cancer. Once a cancer-causing gene is found, he works on uncovering its function in the cell, particularly the molecular pathway it controls. This may lead to the discovery of novel drug targets and treatment options, leading to more personalized cancer therapy for patients with breast cancer, and potentially with other types of cancers.

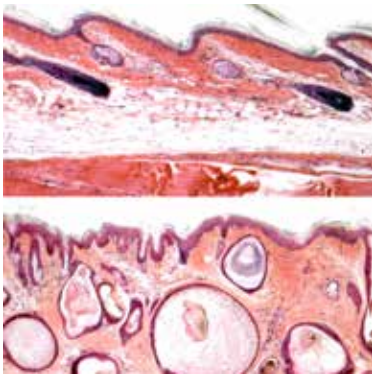
Newly Discovered Cancer Genes

"By using the insertional mutagenesis technology, our group has tracked down a series of genes that previously had not been associated with breast cancer. Some of these genes are being further studied using *in vitro* and *in vivo* methods to investigate their mechanism of action and their role in molecular pathways that can become deregulated in cancer. Among the genes under study are R-spondin genes, *Eras* and *Irs4*."



Studying Gene Function

"Transgenic mice have been made that overexpress one of those genes, known as R-spondin gene 3 (*Rspo3*). R-spondins are the ligands of the LGR receptors and act in the Wnt signaling pathway, which plays an important role in development, tumorigenesis and regulation of tissue stem cells. Indeed, we have shown that overexpression of *Rspo3* in the mouse mammary gland disturbs differentiation and development and causes mammary tumors. Moreover, when *Rspo3* is expressed in intestinal crypts or hair follicles it strongly affects the stem cell compartment in these tissues. Two other of our newly discovered cancer genes, *Eras* and *Irs4*, are not to be expressed in normal human mammary gland but appear highly transcribed in subsets of human breast cancer. Both genes were found to be active in the PI3-kinase pathway, which is central in oncogenesis and activated in almost every cancer. We demonstrated that *Eras* and *Irs4* closely collaborate with the *Her2* tyrosine kinase receptor, a gene overexpressed in 20% of human breast cancers. Both genes synergistically accelerate *Her2*-induced tumor growth in a tumor transplantation model in mice. We are also intrigued by the role these genes may have in the development of resistance in HER2 positive breast cancers treated with small molecule inhibitors or monoclonal antibodies as hyper activation of the PI3K/AKT-pathway frequently underlies the relapse of these cancers."



Division of Molecular Genetics

Selected publications

Isogai T, van der Kammen R, Innocenti M. SMIFH2 has effects on Formins and p53 that perturb the cell cytoskeleton. Sci Rep. 2015; 30(5):9802

Isogai T, van der Kammen R, Goerdayal SS, Heck AJ, Altelaar AF, Innocenti M. Proteomic Analyses Uncover a New Function and Mode of Action for Mouse Homolog of Diaphanous 2 (mDia2). Mol Cell Proteomics. 2015; 14(4):1064-78.

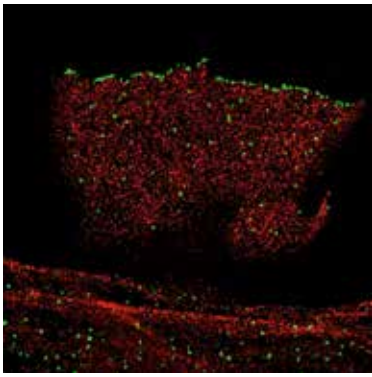
Galovic M, Xu D, Areces LB, van der Kammen R, Innocenti M. Interplay between N-WASP and CK2 optimizes clathrin-mediated endocytosis of EGFR. J Cell Sci. 2011; 124(12):2001-12.

Mechanisms of cell migration and cancer
METELLO INNOCENTI

The goal of Metello Innocenti's research is to achieve a better understanding of the mechanisms controlling migration of cancer cells. His approach is to identify key proteins and pathways that regulate actin, a main constituent of the cell's cytoskeleton. Actin is essential for life as its ability to form filaments is crucial for cells to remodel their shape, move and reshuffle internal components. His group develops new tools to study how the activities of conserved actin-regulatory proteins orchestrate actin dynamics and cell migration. They hope that elucidating these fundamental mechanisms will lead to new and more effective therapies to combat cancer metastasis.

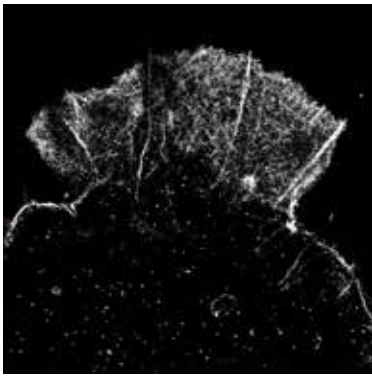
Actin-based protrusions and cell migration

"The assembly of actin into filaments controls the formation of different types of cellular protrusions regulating cell movement (figure 1). Yet, we do not understand how exactly different proteins team up to orchestrate actin-based protrusions and migration of normal and cancer cells. Thus, we developed a bottom-up biochemical approach to reconstitute the protein machinery that controls the formation of actin filaments *in vitro* and combine it with new gene-editing technologies and biosensors to monitor the activity of this machinery and membrane protrusion at the single-cell level."



Actin-regulatory proteins in cancer metastasis

"Cancer metastasis is a multistage process whereby tumour cells first leave the primary tumour, disseminate throughout the body and then colonize distant sites where they give rise to secondary tumours. Although metastasis causes the vast majority of all cancer deaths, we do no have yet any effective treatment to arrest it. As the actin cytoskeleton is instrumental in cell migration and viability, we couple mouse and molecular genetics with the study of cancer-cell movement in complex environments to understand how tractable pro-metastatic actin-regulatory proteins affect invasiveness and proliferation of tumour cells in an attempt to open new therapeutic avenues."



Division of Immunology

Selected publications

Hogenbirk MA, Velds A, Kerkhoven RM, Jacobs. Reassessing genomic targeting of the activation induced cytidine deaminase. Nat Immunol. 2012;13:797-798

Lutz J, Heideman M, Roth E, van den Berk P, Müller W, Raman C, Wabl M, Jacobs H, Jäck HM. Pro-B cells sense productive immunoglobulin heavy chain rearrangement irrespective of polypeptide production. Proc. Natl. Acad. Sci. USA 2011;108:10644-10649

Krijger PHL, Langerak P, van den Berk PCM, Jacobs H. Dependence of nucleotide substitutions on Ung2, Msh2, and PCNA-Ub during somatic hypermutation. J Exp Med. 2009;206:2603-2611

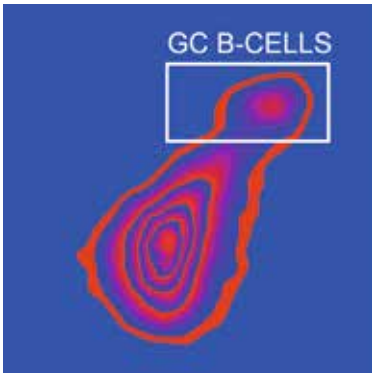
Langerak P, Nygren AO, Krijger PHL, van den Berk PC, Jacobs H. A/T mutagenesis in hypermutated immunoglobulin genes strongly depends on $PCNA^{K164}$ modification. J Exp Med. 2007;204:1989-1998

Programmed Mutagenesis
HEINZ JACOBS

Heinz Jacobs started as a graduate student at the NKI in 1987. Drawn by its excellent research environment he returned in 2002 to establish his own group. The Jacobs group studies how DNA is mutated during the process of programmed mutagenesis, which is part of the normal development of immune cells called B lymphocytes. B cells generate antibodies that recognize pathogens, and programmed mutagenesis, which involves DNA damage, is essential for the production of a diverse antibody arsenal. But DNA damage can also lead to mutations and cancer. Understanding these processes can provide valuable insights into the causes and treatment of cancer.

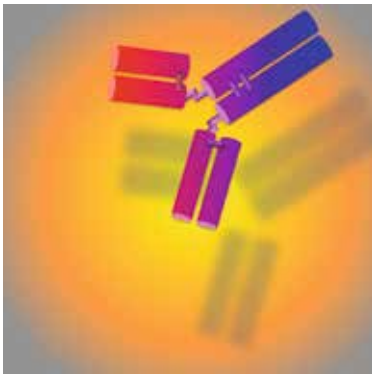
Origin of mutation clusters

"When B cells patrolling the body encounter an invading pathogen they undergo programmed mutagenesis, to generate specialized antibodies that help fight the disease. This involves first activating an enzyme called AID, which is a member of the pro-mutagenic APOBEC family of cytidine deaminases. AID damages DNA in antibody-encoding genes and inaccurate repair pathways lead to the production of a modified antibody protein, thereby tuning the immune response. B cells undergoing programmed mutagenesis are at risk of acquiring cancer-causing mutations. So one part of our research activities is focused on how AID affects the integrity of the genome. We are also interested in how DNA damage generated by AID is repaired by specific error-prone DNA polymerases, known as translesion synthesis (TLS) polymerases. As each TLS polymerase has a characteristic mutation signature, we can pinpoint which ones contribute to a given spectrum of DNA mutations. We are also investigating how these TLS polymerases are activated, as this may be involved in protecting cells against cancer-causing mutations."



Hypermutated DNA

"Mutations and cancer are intimately linked. Cancer cells display non-random clusters of hypermutated DNA, termed `kataegis', and members of the APOBEC gene family appear to be involved. The characteristics of kataegis are reminiscent of programmed mutagenesis in B cells. We are investigating whether the two phenomena are mechanistically related. Maintaining genomic integrity is a critical feature of all cells, and finding ways to artificially obstruct this process specifically in cancer cells may prove useful for developing more effective anti-cancer treatments."



Division of Molecular Oncology

Selected publications

Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van der Torre J, Wevers BA, Orthwein A, Durocher D and Jacobs JJL. MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5' end-resection. Nature 2015;521:537-540

Peuscher MH and Jacobs JJL. Posttranslational control of telomere maintenance and the telomere damage response. Cell Cycle 2012;11:1524-1534

Jacobs JJL. Fusing telomeres with RNF8. Nucleus 2012;3:143-149

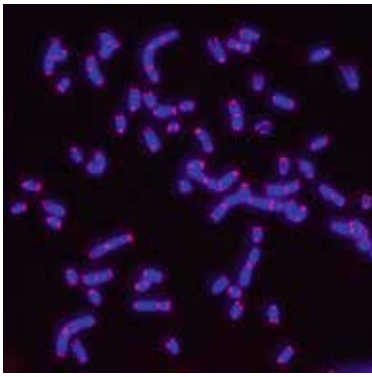
Peuscher MH and Jacobs JJL. DNA-damage response and repair activities at uncapped telomeres depend on RNF8. Nature Cell Biol 2011;13:1139-1145

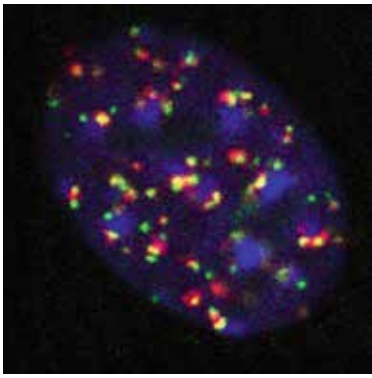
Telomere Damage and Cancer
JACQUELINE JACOBS

Jacqueline Jacobs began her research group at the NKI in 2008, and investigates the mechanisms that preserve cell viability and protect against cancer development. Cells with unstable genomes are at high risk of becoming cancerous, and her group is particularly interested in the detection and repair of DNA lesions, which maintain genome integrity. These mechanisms are prevented from operating at natural chromosome ends by unique nucleoprotein structures, called telomeres. However, telomeres shorten with every cell division, eventually compromising telomere protection and leading to cell death, senescence or genomic instability, which have important consequences for aging and the development of cancer.

Genomic Instability

"When telomeres become dysfunctional they limit the replicative lifespan of a cell, by activating a DNA damage response that forces it into a senescent state or to undergo cell death (apoptosis). While these both contribute to the aging process, they also act as a mechanism to inhibit cancer development. However, if the cell escapes senescence or death and divides, misplaced DNA repair at chromosome ends to form end-to-end chromosomal fusions can lead to extensive genome instability and ultimately to cancer. The aim of our work is to understand the precise nature of these responses and how they are regulated. To do this, we take both unbiased and candidate-driven approaches, alongside in-depth mechanistic studies, particularly focused on the molecular mechanisms controlling the DNA damage responses at telomeres and underlying telomere-driven genomic instability."





Underlying mechanisms

"We are utilizing genetic screens and proteomics-based approaches to identify proteins and post-translational modifications with critical roles in the cellular response to unprotected telomeres. We use well-controllable models such as the fast and reversible temperature-dependent inactivation of the telomere-capping protein TRF2, which allows us to investigate both short and long term effects associated with the activation of DNA damage responses at telomeres. These models also allow us to address how DNA damage responses are inhibited or terminated. Through subsequent functional studies on the newly identified factors and pathways, we aim to generate a comprehensive understanding of the mechanisms underlying telomere-dependent control of cancer development and aging."

Division of Cell Biology I

Selected publications

Jalink K. hiFRET: some tailwind for FRET resolves weak protein interactions. Nat Methods. 2013;10:947-948

Visser D, Langeslag M, Kedziora KM, Klarenbeek J, Kamermans A, Horgen FD, Fleig A, van Leeuwen FN, Jalink K. TRPM7 triggers Ca2+ sparks and invadosome formation in neuroblastoma cells. Cell Calcium. 2013;54:404-15

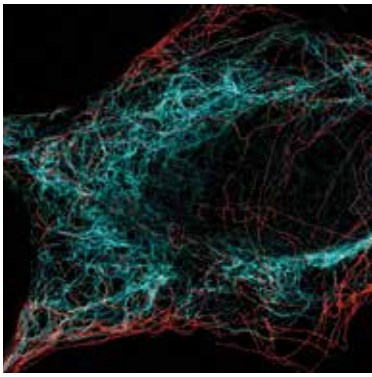
Middelbeek J, Kuipers AJ, Henneman L, Visser D, Eidhof I, van Horssen R, Wieringa B, Canisius SV, Zwart W, Wessels LF, van Leeuwen, FN, Jalink K. TRPM7 Is required for breast tumor cell metastasis. Cancer Res 2012;72:4250-4261

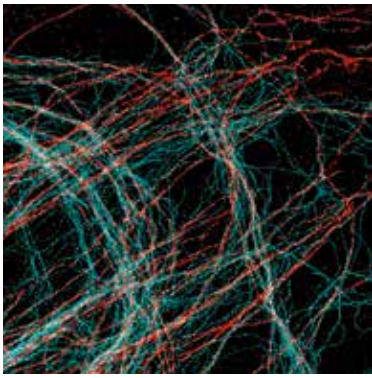
Biophysics of Cell Signaling
KEES JALINK

Kees Jalink is a biophysicist who is interested in designing and developing technologies for tackling diverse biological questions. He has brought many new technologies to the NKI and is an advisor to three companies on the creation of new devices. He often builds prototypes in the lab from individual pieces using sticky tape and then invites industry in to make them user-friendly. His group spends half of its time establishing new techniques and serving as the NKI biophysical center of expertise for those techniques, collaborating and publishing jointly with others. The other half of the time, they focus on their own research on cellular adhesion mechanisms involved in cancer.

Illuminating Proteins

"Our expertise is developing methods to follow individual cells by light microscopy. This allows us to see important events that are undetectable using biochemistry approaches, which average out results from over a hundred thousand cells. For over a century, the resolution of light microscopes has been limited to half the wavelength of light, which is approximately 250nm. Individual proteins are about 100 times smaller and thus invisible even to the best light microscopes. However, several revolutionary developments are now breaking the resolution barrier. One exciting new approach, called super-resolution localization microscopy (Nobel prize 2014), involves physically tagging a protein with a fluorescent dye so that the exact localization of the protein in the cell can be determined mathematically at least 30-fold more precisely than previously possible. To reveal interactions between proteins we employ another trick known as FRET (fluorescence resonance energy transfer), which involves tagging individual proteins with different fluorescing molecules. When the proteins come within 5 nm of each other (i.e., physically interact), energy transfers between the tags cause detectable changes in emitted light. We can put these tagged proteins into a single cell and determine if, when and where they interact. We also monitor the rate of intracellular reactions and can use these fluorophores to watch proteins change shape."





Cell adhesion and metastasis

"In our own research, we study the ion channel TRPM7, which is involved in cell adhesion and cancer metastasis. It contributes to the formation of invadopodia, which are extensions in the cell membrane used by metastatic cancer cells to escape the tumor and invade surrounding tissues. We also study how messenger molecules in the body bind to receptors present on the surface of cancer cells to influence cell locomotion and metastasis, which are both critical processes for cancer progression."

Division of Molecular Pathology

Selected publications

Jaspers J, Kersbergen A, Boon U, Sol W, van Deemter L, Zander SA, Drost R, Wientjens E, Ji J, Aly A, et al. Loss of 53BP1 causes PARP inhibitor resistance in BRCA1-mutated mouse mammary tumors. Cancer Discov. 2013;3:68-681

Bouwman P, and Jonkers J. The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat Rev Cancer 2012;12:587-598

Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, Klijn C, van der Heijden I, van der Gulden H, Wientjens E, et al. BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. Cancer Cell 2011;20:797-809

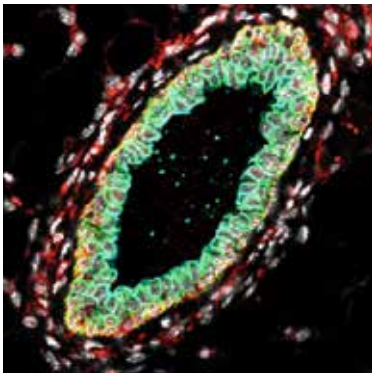
Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol. 2010;17:688-695

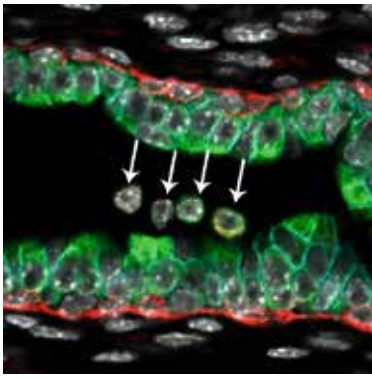
Modeling Breast Cancer
JOS JONKERS

Jos Jonkers began his scientific career at the NKI as a PhD student. He was drawn back in 2002 by the prospect of being able to rapidly translate basic research directly into clinical applications, and heads his own research group focusing on cancer. His group develops and utilizes sophisticated mouse models as critical tools to identify novel breast cancer genes and to learn how breast cancers grow and metastasize. They are also using them to test novel tumor intervention strategies that could improve treatment outcomes of breast cancer patients in the clinic.

Developing Mouse Models

"Here at the NKI we have created new strains of mice that have considerably improved laboratory studies of breast cancer. If mice carry too many genetic mutations, cell growth is affected and they are unable to breed, making experiments highly challenging. Therefore, our strategy was to mutate only a few carefully selected genes so that the mice would spontaneously develop tumors. To do this, we use a genetic recombination system called Cre-LoxP, which enables us to turn on or switch off defined sets of genes in just a few cells within a particular tissue. This mimics more closely the way that cancers arise in humans. Using this approach we have built up a unique colony of mice carrying different combinations of mutations in genes associated with breast cancer, such as BRCA1, BRCA2, E-cadherin, p53, and PTEN. We have also created a panel of patient-derived breast cancer xenograft models by propagating fresh human breast tumor tissue in immunodeficient mice. The genetic make-up of these mice is so well known that we no longer need to run tens of replicates for each experiment. This increases the pace at which we can work and reduces the numbers of animals needed to reach useful conclusions."





Mouse Cancer Clinic

"The mouse models are being used to study the molecular mechanisms underlying cancer progression and metastasis. We also use them to study response and resistance to anticancer therapies in our 'mouse cancer clinic', which lets us treat each mouse as if it were a human patient. We've already shown that platinum-based compounds and poly(ADP-ribose) polymerase (PARP) inhibitors are valuable chemotherapeutic drugs for treating BRCA1- and BRCA2-associated breast cancers in these mice. It will be exciting to see whether this holds true in human trials, which are currently ongoing."

Division of Molecular Pathology

Selected publications

Beelen K, Opdam M, Severson TM, Koornstra RHT, Vincent AD, Wesseling J, Muris JJ, Berns EMJJ, Vermorken JB, van Diest PJ, Linn SC. Phosphorylated p-70S6K predicts tamoxifen resistance in postmenopausal breast cancer patients randomized between adjuvant tamoxifen versus no systemic treatment. Breast Cancer Res 2014; 16:R6.

Vollebergh MA, Lips EH, Nederlof PM, Wessels LFA, […], van de Vijver MJ, van Tinteren H, de Bruin M, Hauptmann M, Rodenhuis S, Linn SC. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose, platinum-based, chemotherapy in HER2-negative breast cancer patients. Ann Oncol 2011; 22:1561-70.

Bueno-de-Mesquita JM, van Harten WH, Retel VP, van 't Veer LJ, […], van Krimpen C, Rodenhuis S, van de Vijver MJ, Linn SC. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). Lancet Oncol 2007; 8:1079-1087.

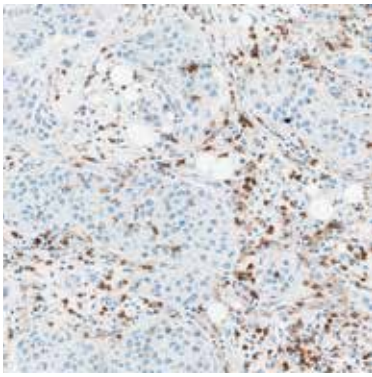
Tailoring therapy for breast cancer

SABINE LINN

During her residency in internal medicine Sabine Linn was struck by the uncertainty surrounding effectiveness of chemotherapy for an individual cancer patient. Medical oncologists appeared more confident about what side effects would occur during treatment than about its efficacy to eradicate the tumor. This observation motivated her to start her own research group at the NKI focusing on the development of prognostic and predictive tests to tailor systemic therapy for breast cancer patients. Her mission is to achieve a cure for every cancer patient through precision medicine.

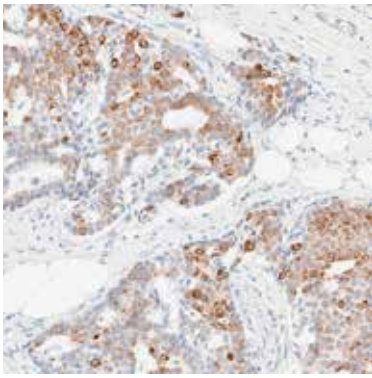
Prognostic tests to reduce overtreatment

"One of the first commercialized molecular prognostic tests in breast cancer has been developed at the Netherlands Cancer Institute and is called the MammaPrint® test. Several studies have demonstrated that this test can reduce overtreatment in node-negative breast cancer patients. The next step is to develop second-generation prognostic tests for defined subgroups of breast cancer, to further improve and refine tailored treatment. Several projects have been initiated."



Intensified alkylating chemotherapy

"While high-dose chemotherapy with autologous stem cell rescue for high-risk breast cancer was fashionable in the nineties and almost completely abandoned in the new millennium, at the NKI a small subgroup of breast cancer patients has been identified that derives substantial benefit from this treatment modality. Preclinical knowledge suggests that cancer cells with a damaged DNA repair system are extremely sensitive to drugs that induce severe DNA damage. As hypothesized, in three independent posthoc analyses it was shown that patients with breast cancer with a defective DNA repair system, defined as BRCA1-like, had five times less risk of a recurrence with intensified alkylating chemotherapy that induces massive DNA damage than with standard adjuvant chemotherapy. Prospective studies have been initiated to either confirm or reject these initial findings."



Endocrine therapy resistance

"One of the most important reasons for treatment failure in breast cancer is endocrine therapy resistance. We have initiated clinical trials with large translational programs using state-of-the-art genomic technologies that will provide new insights ultimately leading to improved therapeutic strategies."

Division of Cell Biology I

Selected publications

Krenning L, Feringa FM, Shaltiel IA, van den Berg J, and Medema RH. Transient activation of p53 in G2 phase is sufficient to induce senescence. Mol. Cell 2014;55:59-72

Janssen A, van der Burg M, Szuhai K, Kops GJ, and Medema RH. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. Science 2011;333:1895-1898

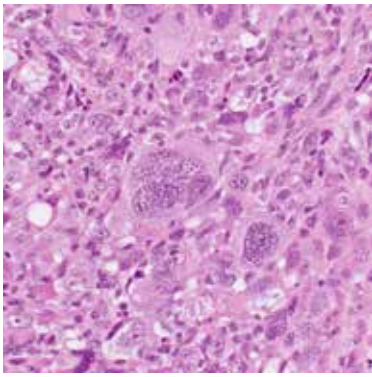
Macurek L, Lindqvist A, Lim D, Lampson MA, Klompmaker R, Freire R, Clouin C, Taylor SS, Yaffe MB, and Medema RH. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. Nature 2008;455:119-123

Cell Division and Cancer
RENÉ MEDEMA

René Medema became director of the NKI in 2012, and brought an established research group to the institute. He has extensive experience studying the mechanisms underlying cell division, particularly the molecular checkpoints that control progression of the cell cycle. Many classic anti-cancer drugs kill cells by targeting the cell cycle, for example by damaging DNA or by perturbing assembly of the mitotic spindle, which is required for cell division. René's group aims to gain a clearer understanding of the cellular responses to these drugs in order to better predict drug responses and experimentally test new and potentially more effective anti-cancer strategies.

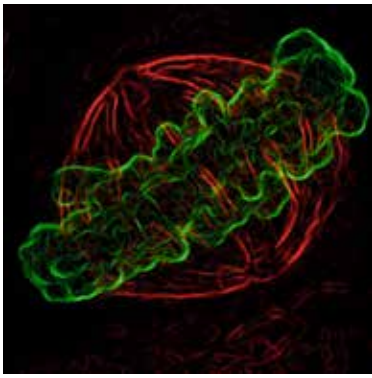
Cell Cycle Checkpoints

"Our group is interested in the molecular mechanisms of cell division. One major focus is to understand how cells recover from a DNA damaging insult, such as those caused by some anti-cancer drugs, which activates a checkpoint and triggers cell cycle arrest. Specifically, we are working on unravelling the mechanism that promotes cell cycle re-entry once the checkpoint is switched off and how this is coordinated with DNA damage repair. We have established a number of assays involving FRET-based biosensors and fluorescent markers to monitor the appearance and repair of double-stranded DNA breaks, as well as inactivation and reactivation of the cell cycle machinery, in a single living cell. Using these assays we have identified several protein kinases and phosphatases that control recovery and are studying how they coordinate this with ongoing DNA repair."



Chromosome Segregation

"We are also focusing on the mechanisms underlying bipolar spindle assembly, which is required to segregate chromosomes during cell division, particularly to understand how the correct balance of forces is established. For this we monitor spindle assembly and chromosome segregation in living cells. We have uncovered several novel roles for motor proteins and are working towards a global picture of motor-dependent control of spindle assembly. A secondary aim is to exploit chromosome segregation errors as a means to selectively target the fitness of cancer cells. We have discovered that lagging chromosomes in cancer cells can break during telophase and cytokinesis, which can lead to chromosome translocations in the next cell cycle. We are also working on characterizing how chromosome cohesion is established after DNA replication, and how it is subsequently removed to allow for chromosome segregation and cell division."



Division of Diagnostic Oncology

Selected publications

Sillars-Hardebol AH, Carvalho B, Tijssen M, Beliën JA, de Wit M, Delis-van Diemen PM, Pontén F, van de Wiel MA, Fijneman RJ, Meijer GA. TPX2 and AURKA promote 20q amplicon-driven colorectal adenoma to carcinoma progression. Gut. 2012;61(11):1568-75

de Wit M, Jimenez CR, Carvalho B, Belien JA, Delis-van Diemen PM, Mongera S, Piersma SR, Vikas M, Navani S, Pontén F, Meijer GA, Fijneman RJ. Cell surface proteomics identifies glucose transporter type 1 and prion protein as candidate biomarkers for colorectal adenoma-to-carcinoma progression. Gut. 2012;61(6):855-64

Haan JC, Labots M, Rausch C, Koopman M, Tol J, Mekenkamp LJM, van de Wiel MA, Israeli D, van Essen HF, van Grieken NCT, Voorham QJM, Bosch LJW, Qu X, Kabbarah O, Verheul HMW, Nagtegaal ID, Punt CJA, Ylstra B, Meijer GA. Genomic landscape of metastatic colorectal cancer, Nat Comm. 2014;5:5457

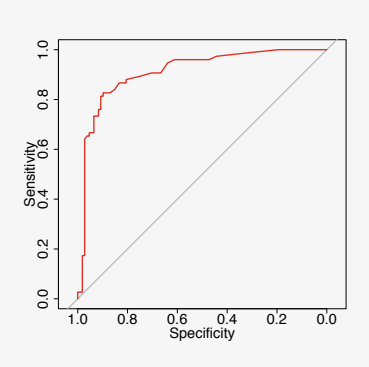
Pathology of gastrointestinal tumors

GERRIT MEIJER

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. Gerrit Meijer is a pathologist with a special interest in translational cancer research, focusing on the gastrointestinal tract. The work of his research group involves DNA-, RNA-, and protein-based 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. In addition, he is involved in several national and international research infrastructure programs.

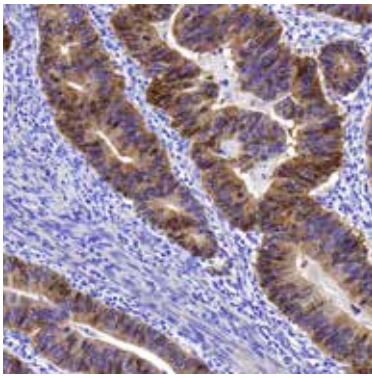
Early detection

"Patients can be cured from colorectal cancer (CRC) when the tumor is detected and removed in 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 tests 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."



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), which can be obtained more easily than tissue samples, are amenable to these assays."



Division of Cell Biology I

Selected publications

Perrakis A, Moolenaar WH. Autotaxin: structure-function and signaling. J Lipid Res 2014; 55:1010-18

Argenzio E, Margadant C, Leyton-Puig D, Janssen H, Jalink K, Sonnenberg A, Moolenaar WH. CLIC4 regulates cell adhesion and 1 integrin trafficking. J Cell Sci. 2014;127:5189-203

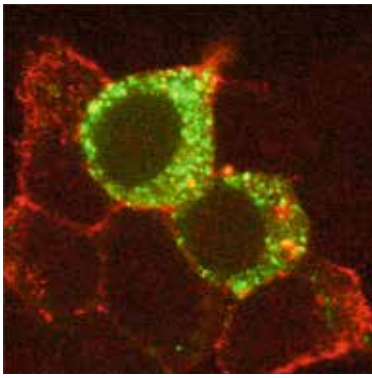
Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, Jansen S, Andries M, Hall T, Pegg LE, Benson TE, Kasiem M, Harlos K, Kooi CW, Smyth SS, Ovaa H, Bollen M, Morris AJ, Moolenaar WH, Perrakis A. Structural basis of substrate discrimination and integrin binding by autotaxin. Nat Struct Mol Biol. 2011;18:198-204

Lipid Growth Factors
WOUTER MOOLENAAR

Phospholipids are best known as the major building blocks of cell membranes, but some lipids play a signaling rather than structural role. Wouter Moolenaar is interested in nature's simplest phospholipid, lysophosphatidic acid (LPA), which he discovered as a lipid growth factor some 25 years ago. Since then, his group has made substantial progress in unraveling how LPA regulates numerous biological processes, including cell migration and proliferation. Their current work focuses on the LPA-producing enzyme autotaxin and other phospholipid-metabolizing enzymes, notably their mode of action and relevance to cancer. Their ultimate goal is to translate discoveries on phospholipid signaling into cancer treatments.

LPA Signaling and Cancer

"Our group focuses on characterizing the diverse functions of LPA, a serum phospholipid that acts through specific G protein-coupled receptors present on the surface of many normal and malignant cell types. LPA signaling is implicated in various physiological and pathological processes, ranging from embryonic development to tumor progression. LPA receptors stimulate cell migration and proliferation, while dramatically altering cell morphology. However, some receptors can mediate inhibitory responses, which renders the global LPA signaling picture increasingly complex. One challenge is to understand how distinct LPA receptors cooperate in a given cell type, which should allow us to predict the biological outcome of LPA stimulation. LPA is produced by autotaxin, a secreted enzyme that was originally discovered as a motility factor for tumor cells. We have been analyzing the function of autotaxin using multiple approaches, including structural studies and the development of mouse models and small-molecule inhibitors. The knockout mouse revealed a key function for autotaxin in vascular development, while its crystal structure uncovered novel and unexpected features. We are now addressing the inner workings of autotaxin, how it binds to the cell surface, and how its activity can be targeted to interfere with undue LPA production in the tumor microenvironment."



Stimulating cell differentiation through phospholipid attack

"GDE2 is an intriguing transmembrane ecto-enzyme implicated in phospholipid metabolism and embryonic neurogenesis. We find that elevated GDE2 expression strongly correlates with overall survival in neuroblastoma, a childhood cancer. GDE2 promotes cell adhesion and neurite outgrowth, while it suppresses cell motility and counteracts LPA receptor signaling. We are now examining the mode of action of GDE2 as a favorable prognostic factor, focusing on its capability to release certain phospholipid-anchored proteins from the cell surface. Pharmacological stimulation of GDE2 surface expression or enzymatic activity may be an attractive route for future therapy."

Division of Cell Biology II

Selected publications

Pang B, Qiao X, Janssen L, Velds A, Groothuis T, Kerkhoven R, Nieuwland M, Ovaa H, Rottenberg S, van Tellingen O, Janssen J, Huijgens P, Zwart W, Neefjes J. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nat Commun. 2013;4:1908

Neefjes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol. 2011;11:823-36

Paul P, van den Hoorn T, Jongsma ML, Bakker MJ, Hengeveld R, Janssen L, Cresswell P, Egan DA, van Ham M, Ten Brinke A, et al. A Genome-wide multidimensional RNAi screen reveals pathways controlling MHC class II antigen presentation. Cell 2011;145, 268-83

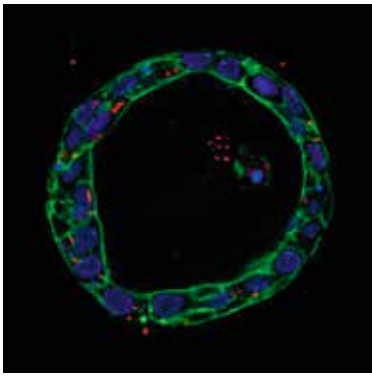
Chemical Immunology and Anticancer drugs

JACQUES NEEFJES

MHC class I and MHC class II molecules present fragments of cancer and viral antigens to the immune system, that can then launch an immune response to help eradicate disease. Jacques Neefjes is interested in the cell biology underlying this MHC antigen presentation in order to find ways to chemically control it. This could be used for the treatment of a host of diseases, including autoimmune diseases and cancer. In addition he is interested in determining how bacterial infections affect the development of cancer. Finally, his group has been applying novel techniques to identify new activities of existing anti-cancer compounds.

Manipulating Immunity

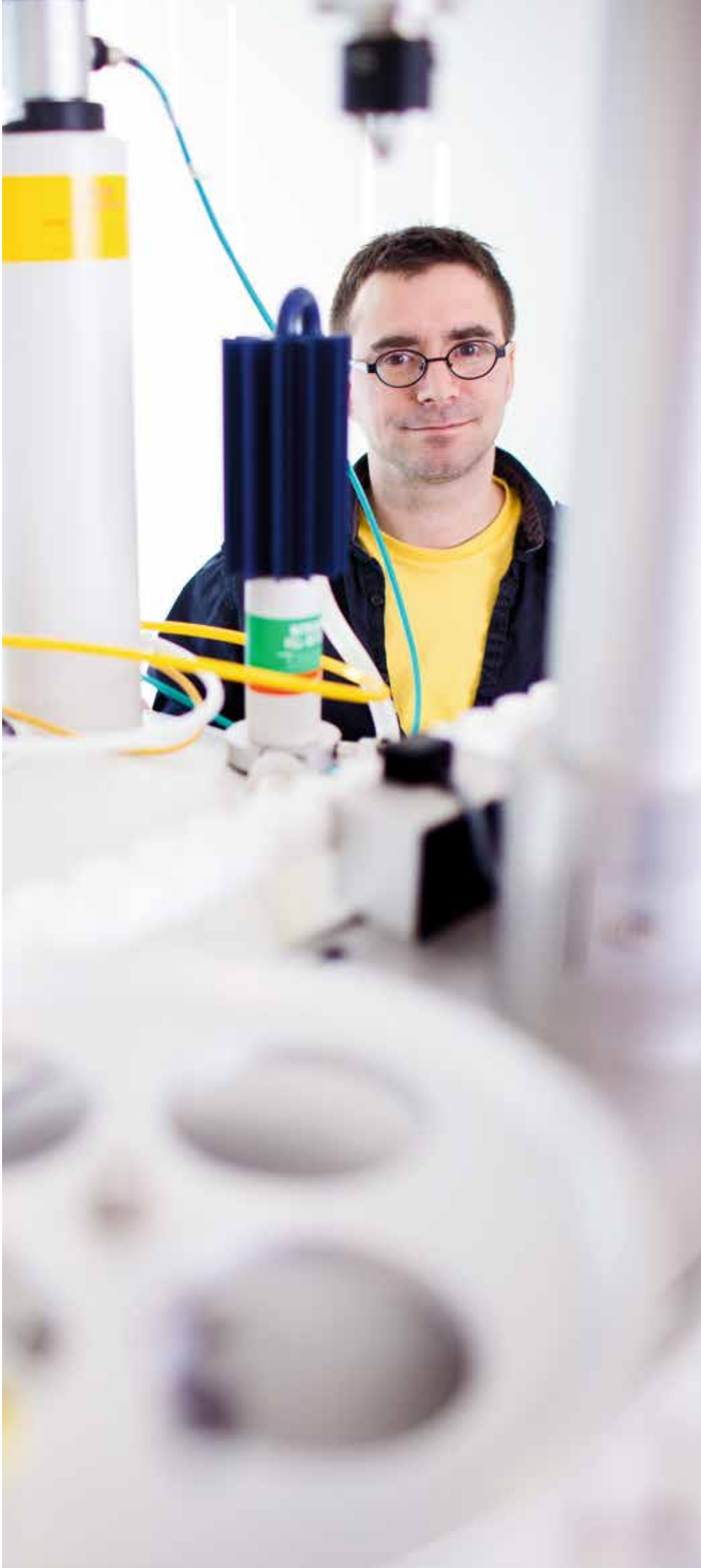
"Antigen presentation to the immune system by MHC class I and class II molecules involves a series of complex biological steps. Studying of the cell biology of this system allows for the translation into chemical manipulation of defined steps, to ultimately manipulate immune responses. We have applied high-throughput technologies to identify new proteins involved in MHC class II antigen presentation. These proteins are involved in the control of processes that were not recognized before, such as the control of export of the endosomal system by another intracellular compartment. In addition, we have identified novel compounds that manipulate new pathways in control of MHC class II antigen presentation and are now pursuing its development towards in vivo manipulation of immune responses."



Modifying Anti-Cancer Drugs

"We are also applying our knowledge in this area to two related topics: identifying and generating new functions for known anti-cancer compounds, and characterizing the role of bacteria in cancer development. We have already applied the modern cell biological toolbox to visualize new effects of an old but frequently used anti-cancer compound, doxorubicin. We discovered that doxorubicin appears to evict histones from particular areas of the genome, which is critical for its anti-cancer effects. We also identified a new compound that is very active on a series of tumors, including primary mesothelioma cells, and are now testing the activities and side effects on this new drug to build a portfolio required for introduction in the clinic. Following our studies of Salmonella infection in cells, we have been considering their potential contribution to cancer development. Using genetically modified mice and cell lines, we have shown that these bacteria can transform cells, which explains the association of gallbladder cancer with chronic typhoid carriers. Since other bacteria manipulate host cells in similar ways, we are now testing whether these can also contribute to cancer."

Division of Cell Biology II



Selected publications

Geurink PP, El Oualid F, Jonker A, Hameed DS, and Ovaa H. A general chemical ligation approach towards isopeptide-linked ubiquitin and ubiquitin-like assay reagents. Chembiochem 2012;13:293-297

El Oualid, F, Merkx R, Ekkebus R, Hameed DS, Smit JJ, de Jong A, Hilkmann H, Sixma TK, and Ovaa H. Chemical synthesis of ubiquitin, ubiquitin-based probes, and diubiquitin. Angew Chem Int Ed Engl 2010;49;10149-10153

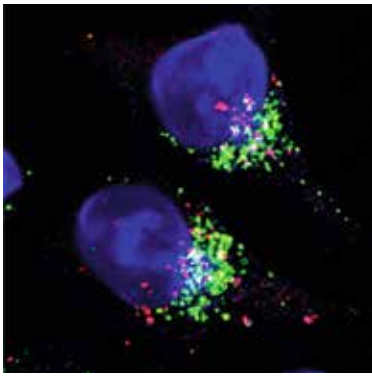
Celie PH, Toebes M, Rodenko B, Ovaa H, Perrakis A, and Schumacher TN. UV-induced ligand exchange in MHC class I protein crystals. J Am Chem Soc 2009;131:12298-12304

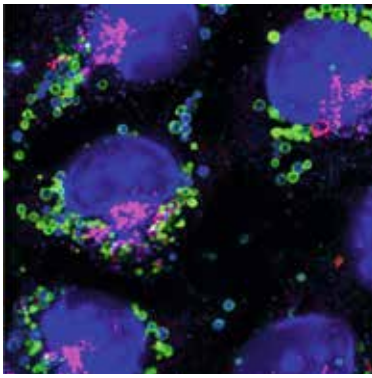
Chemical Tools for Research
HUIB OVAA

Following a PhD in organic chemical synthesis at Leiden University and three years at Harvard University, Huib Ovaa joined the NKI in 2004 to establish a chemical biology laboratory. Having built up a strong team of chemists within the institute, the group uses their amassed chemical skills to make new research tools that allow scientists to tackle previously intractable problems in cancer research. Their novel tools are used to study diverse biological processes, including post-translational protein modifications and the mechanisms of antigen presentation, which is a critical part of the immune response.

Customizing Molecules

"The ability to design and develop chemical tools is invaluable for investigating biochemical processes and can be tailored to the scientific field of interest. For example, together with Ton Schumacher's lab we looked at ways of detecting and characterizing T cells of the immune system. T cells are stimulated by small peptides known as antigens, which are produced from pathogens and tumor cells, and are presented by major histocompatibility complex (MHC) proteins to initiate an immune response. Tumor antigens may be used to develop cancer vaccines in order to target the immune system to destroy cancer cells. A major challenge has been to develop an efficient screening system to determine which antigens the T cells recognize. Specifically, we had to find a way to stabilize the MHC molecules so they could be stored and then easily combined with whatever peptide antigen was being tested. So, we generated MHC monomers carrying a temporary cargo of specially designed molecules, which made them stable. We also made the cargo molecules sensitive to UV light so that they can be easily replaced with the antigen of interest. This method is quick and simple, and is available for others to exploit in their research."





Chemical synthesis

"We also study how proteins are targeted for destruction by the proteasome through modification with a small peptide called ubiquitin. A major achievement in the lab has been developing a routine procedure for the total chemical synthesis of ubiquitin and many of its conjugates. This technique is being used to study in detail how proteins are destroyed, and how the resulting antigen fragments are presented to the immune system, which are important processes in many diseases including cancer."

Division of Molecular Oncology

Selected publications

Müller J, Krijgsman O, Tsoi J, Robert L, Hugo W, Song C, Kong X, Possik PA, Cornelissen-Steijger PD, Foppen MH, Kemper K, Goding CR, McDermott U, Blank C, Haanen J, Graeber TG, Ribas A, Lo RS, Peeper DS. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. Nat Commun. 2014;5:5712.

Possik PA, Müller J, Gerlach C, Kenski JC, Huang X, Shahrabi A, Krijgsman O, Song JY, Smit MA, Gerritsen B, Lieftink C, Kemper K, Michaut M, Beijersbergen RL, Wessels L, Schumacher TN, Peeper DS. Parallel In Vivo and In Vitro Melanoma RNAi Dropout Screens Reveal Synthetic Lethality between Hypoxia and DNA Damage Response Inhibition. Cell Rep. 2014; 9(4):1375-86.

Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, van der Burg SH, Verdegaal EM, Cascante M, Shlomi T, Gottlieb E, Peeper DS. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 2013; 498(7452):109-12.

Functional Oncogenomics
DANIEL PEEPER

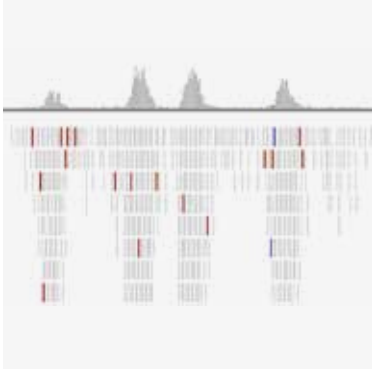
Peeper's research group develops and uses function-based oncogenomic approaches to ask clinically relevant questions. By systematic genetic perturbations they aim to dissect tumor-driving genetic networks for drug target and biomarker discovery. They have also begun building experimental systems to develop combinatorial targeted and immunotherapeutic therapies. Candidates are analyzed bioinformatically and functionally in a clinically relevant context, including patient-derived tumor xenografts. The ultimate goal is to develop new intervention strategies and to enhance the performance of newly available targeted drugs; two examples of these approaches will be discussed below.

New Intervention Strategies

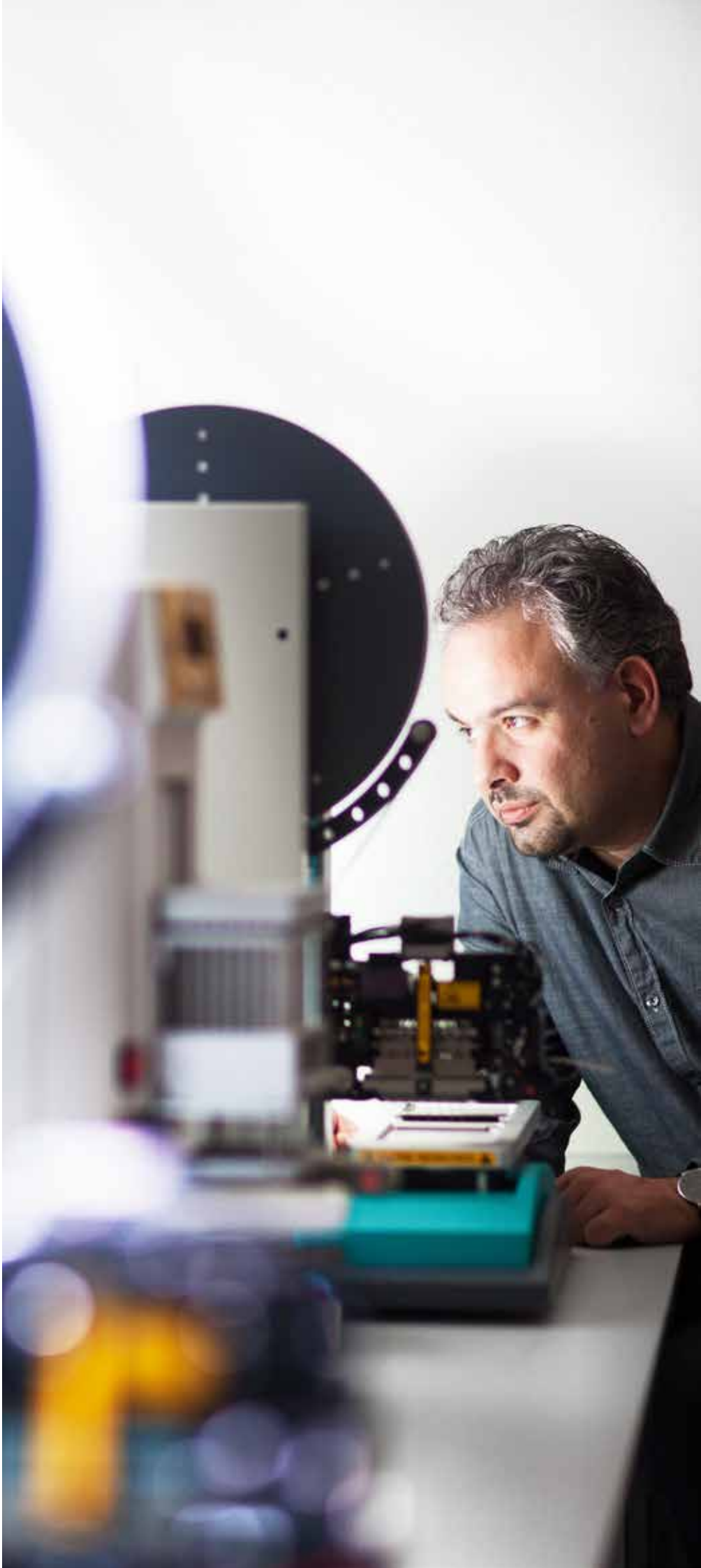
"We recently developed a new type of cancer drug target discovery screens to identify therapeutic targets for melanoma and breast cancer *in vivo*, using large-scale RNA interference libraries. To identify factors necessary for driving tumor expansion, we performed parallel *in vitro* and *in vivo* negative-selection screens. We found that several DNA damage response (DDR) kinases were essential for 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 a clinical BRAF inhibitor. 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."

Overcoming targeted drug resistance

"Increased expression of the key melanocytic transcription factor MITF is known to contribute to melanoma progression and resistance to BRAF pathway inhibition. We found that, in contrast, a lack of MITF was associated with more severe resistance to a range of targeted inhibitors, whereas its presence was required for robust drug responses. Both in primary and acquired resistance, MITF levels inversely correlated with the expression of several activated receptor tyrosine kinases, most frequently AXL. The MITF-low/AXL-high/drug-resistance phenotype was common among mutant BRAF and NRAS melanoma cell lines. The dichotomous behavior of MITF in drug response was corroborated in vemurafenib-resistant biopsies, including MITF-high and -low clones in a relapsed patient. Furthermore, drug cocktails containing AXL inhibitor enhanced melanoma cell elimination by BRAF pathway inhibition. These 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."



Division of Biochemistry



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Heidebrecht T, Fish A, von Castelmur E, Johnson KA, Zaccai G, Borst P, and Perrakis A. Binding of the J-binding protein to DNA containing glucosylated hmU (base J) or 5-hmC: evidence for a rapid conformational change upon DNA binding. J Am Chem Soc 2012;134:13357-13365

Hausmann J, Kamtekar S, Christodoulou E, Day JE, Wu T, Fulkerson Z, Albers HM, van Meeteren LA, Houben AJ, van Zeijl L, ... , Moolenaar WH, Perrakis A. Structural basis of substrate discrimination and integrin binding by autotaxin. Nat Struct Mol Biol 2011;18:198-204

Joosten RP, Joosten K, Cohen SX, Vriend G, and Perrakis A. Automatic rebuilding and optimization of crystallographic structures in the Protein Data Bank. Bioinformatics 2011;27:3392-3398

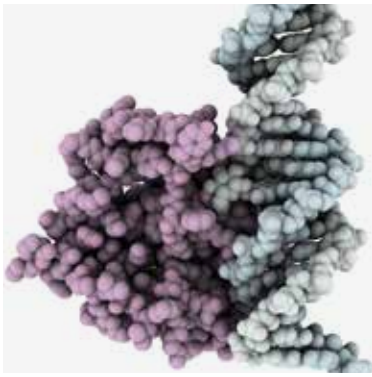
Hiruma Y, Sacristan C, Pachis ST, Adamopoulos A, Kuijt T, Ubbink M, von Castelmur E, Perrakis A, Kops GJ. Competition between MPS1 and microtubules at kinetochores regulates spindle checkpoint signaling. Science. 2015

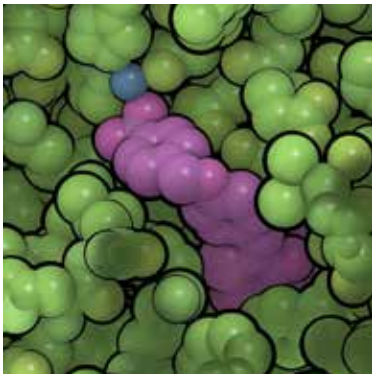
Macromolecular Structures
ANASTASSIS PERRAKIS

Macromolecular structures are critical for understanding the function of biological molecules and to evaluate and develop new drugs. While most proteins have a specific enzymatic activity that drives a chemical reaction necessary to fulfill their physiological function, multi-domain proteins often contain a smaller regulatory domain that directs their enzymatic activity in space and time. Tassos Perrakis is interested in understanding the spatiotemporal control that such small domains exert on the function of their 'host' protein at the level of the molecular structure. Concurrently, his group is developing methods to help the wider scientific community study macromolecules more efficiently by X-ray crystallography.

Structural Biology

"We are currently analyzing the regulatory mechanisms of a number of multi-domain enzymes involved in diverse biological processes, by studying their molecular structures and its relationship with their biophysical and biochemical properties. For example, autotaxin (ATX), which is a phosphodiesterase that produces the signaling lipid LPA, is a promising drug target in diseases such as fibrosis, arthritis, neuropathic pain and cancer. Our group has determined the structure of ATX, and together with other groups at the NKI we have helped develop highly specific and potent inhibitors. Now we study the regulatory domains of ATX that modulate its interaction with integrins, heparin, and LPA receptors at the cell surface, thereby regulating LPA signaling. We are also interested in the regulatory domains of mitotic kinases, which are critical components required for cell division, and ensure that kinase activity is located at the right place at the right time. Structural knowledge of these processes is important for developing small molecule inhibitors that can specifically block their function. Finally, we study the proteins responsible for maintaining the 'J-base', in the DNA of pathogenic parasites."





Structural Biology Methods

"A parallel interest is in developing automated methods to build macromolecular models more accurately and efficiently from X-ray crystallography data. Following a long-term commitment to the ARP/wARP software suite, originally developed for automated model building, we now focus to the PDB_REDO project, aiming to update existing models in the worldwide protein data bank (wwPDB), as well as helping scientists deposit better models. Specifically, we capitalize on our knowledge and experience in model-building tools to provide the crystallographic software and decision-making frameworks needed to rebuild and refine crystallographic macromolecular structures."

Division of Psychosocial Research and Epidemiology

Selected publications

Koppelmans V, Vernooij MW, Boogerd W, Seynaeve C, Ikram MA, Breteler MM, Schagen SB. Prevalence of cerebral small vessel disease in long-term breast cancer survivors exposed to both adjuvant radiotherapy and chemotherapy. J Clin Oncol 2015;33(6):588-93

Koppelmans V, Breteler MM, Boogerd W, Seynaeve C, Gundy C, and Schagen SB. Neuropsychological performance in survivors of breast cancer more than 20 years after adjuvant chemotherapy. J Clin Oncol 2012;30:1080-1086

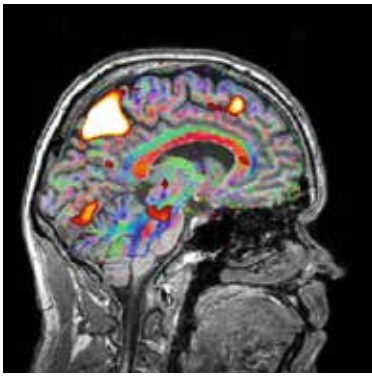
De Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, Douaud G, Lavini C, Linn SC, Boven E, van Dam FS, Schagen SB. Late effects of high-dose adjuvant chemotherapy on white and gray matter in breast cancer survivors: Converging results from multimodal magnetic resonance imaging. Hum Brain Mapp 2012;33:2971-2983

Cognition and Cancer
SANNE SCHAGEN

Compared to classical oncological outcome measures such as time to progression and survival, the importance of cognitive functioning in cancer patients has only recently been recognised. In patients with tumors either inside or outside the central nervous system (CNS), cognitive functioning is an important outcome measure. Sanne Schagen and her group study the effects of cancer and its treatment on cognitive and brain functioning using neuropsychological examinations, imaging techniques and animal models. The projects centre 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 or prevent cognitive symptoms.

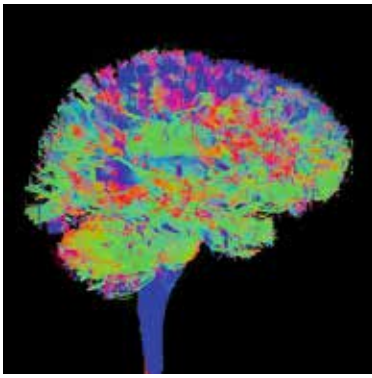
Side Effects of Therapy

"In the past years, our group has intensified its research on the potential effects of systemic therapy on cognitive functioning in non-CNS cancer patients. We want to learn more about the neural substrate and underlying mechanisms, to increase insight into the effects on the longer term and to develop interventions. We have also broadened our lines of research into the effects of cranial radiation on the brain, because with prolonged survival for subgroups of patients, we are increasingly facing late radiation induced CNS damage."

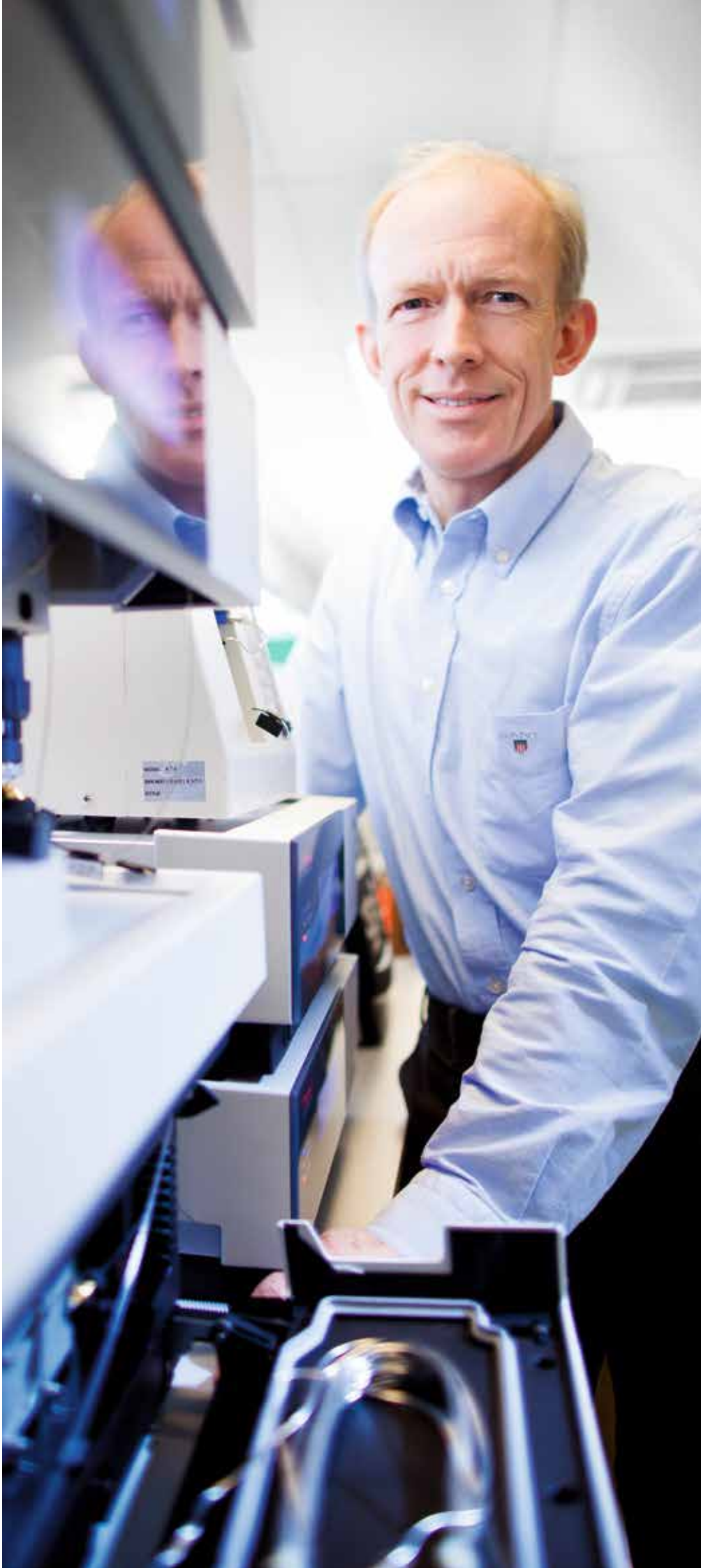


Avoiding Sensitive Areas

"One approach to reduce cognitive effects of brain irradiation is to avoid sensitive regions of the brain. Prophylactic cranial irradiation (PCI) is standard treatment in patients with all stages of small cell lung cancer (SCLC) who have achieved a remission following primary treatment. Memory dysfunction is a major side effect of PCI. New radiotherapy techniques enable hippocampal sparing, while obtaining high-dose coverage of about 97% of the brain. In close collaboration with the Division of Radiation Oncology (J. Belderbos) we designed an international clinical trial in which SCLC patients eligible for PCI will be randomized to standard PCI or PCI with hippocampus avoidance (HA) by using IMRT or VMAT. In the coming years we will complete the data collection and conduct the analyses. The primary outcome of the trial is memory function, but it has many secondary endpoints, including other cognitive functions, patient related outcomes on quality of life and fatigue, neurotoxicity, financial costs, incidence and location of brain metastases, and overall survival. A specific translational part is dedicated to structural and functional brain abnormalities using MR imaging and to Placental Growth Factor as a possible predictive biomarker for brain metastases."



Division of Molecular Pathology



Selected publications

Deenen MJ, Meulendijks D, Cats A, Sechterberger MK, Severens JL, Boot H, Smits PHM, Rosing H, Mandigers CMPW, Soesan M, Beijnen JH, Schellens JHM. Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. J Clin Oncol 2015

Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009;361:123-34

Marchetti S, de Vries NA, [...] Mazzanti R, van Tellingen O, Schellens JH. Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva) disposition in in vitro and in vivo pharmacokinetic studies employing Bcrp1-/-/Mdr1a/1b-/-(triple-knockout) and wild-type mice. Mol Cancer Ther 2008;7:2280-87

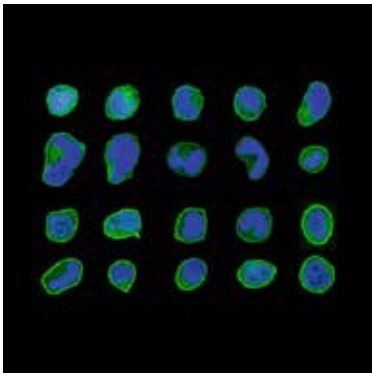
Kruijtzer CM, Beijnen JH, [...] Paul EM, Schellens JH. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002; 20:2943-50

Personalized Cancer Treatment
JAN SCHELLENS

Jan Schellens is interested in the clinical implementation of personalized cancer treatment. His group develops and validates biomarkers and assays to predict antitumor activity and toxicity of novel and existing anticancer drugs. In addition, Jan's work as a clinical pharmacologist is focused on increasing the efficacy of anticancer drugs for testing in clinical trials, on improving patient safety by implementing pharmacogenetics and on development of oral taxane formulations.

Novel concepts

"My group promotes personalized cancer treatment using several different approaches. We use basic research findings to identify novel concepts for reversal of tumor resistance that can be directly translated to treatment strategies in patients. Our current focus is on combining BRAF and EGFR inhibitors for treating advanced colorectal cancer patients carrying BRAF V600 mutations. This approach is based on the finding that crosstalk between the two signaling pathways targeted by these inhibitors (i.e. MAPK and EGFR signaling, respectively) results in resistance of these tumors to the BRAF inhibitor. This can likely only be counteracted by the proposed combined treatment, which could lead to a new standard of care for these patients who make up around 10% of all colorectal cancer patients. Another strategy employs a combination of MEK inhibitor and pan-HER inhibitor to reverse unresponsiveness of KRAS mutant cancers."



Anticancer drug development

"We also develop strategies to support clinical trials of new anticancer drugs. For example, an improved oral controlled-release formulation of capecitabine (ModraCape001) has enabled us to apply so-called chronotherapy whereby capecitabine is taken only once daily by patients in the evening at around 22.00 h. This schedule is markedly different from standard bidaily dosing and is based on our results that showed circadian variation in two key enzymes that determine antitumor activity and inactivation of the metabolite 5-FU, respectively. Our group also develops and validates assays to aid the implementation and assessment of this and other clinical trials, including those involving oral metronomic paclitaxel, which is a widely used chemotherapeutic. We develop oral taxanes, prototype ModraDoc 006, to improve patient safety and convenience."

Division of Molecular Oncology

Selected publications

Tang SC, Sparidans RW, Cheung KL, Fukami T, Durmus S, Wagenaar E, Yokoi T, van Vlijmen BJ, Beijnen JH, Schinkel AH. P-glycoprotein, CYP3A and plasma carboxylesterase determine brain and blood disposition of the mTOR inhibitor everolimus (Afinitor) in mice. Clin. Cancer Res. 2014;20:3133-45

Van de Steeg E, Stránecký V, Hartmannová H, Nosková L, Hřebíček M, Wagenaar E, van Esch A, de Waart DR, Oude Elferink RP, Kenworthy KE, et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. J. Clin. Invest. 2012;122:519-28

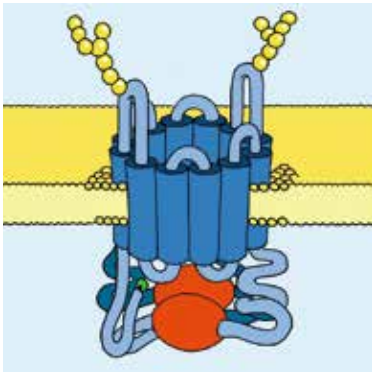
Van de Steeg E, Wagenaar E, van der Kruijssen CMM, Burggraaf JEC, de Waart DR, Oude Elferink RPJ, Kenworthy KE, Schinkel AH. Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids and drugs. J. Clin. Invest. 2010;120:2942-52

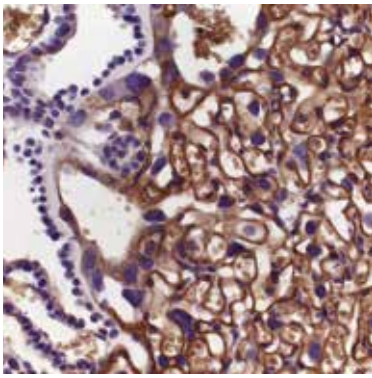
Improving Drug Efficacy
ALFRED SCHINKEL

Alfred Schinkel is interested in the proteins responsible for susceptibility and resistance to anticancer drugs. Drug resistance is one of the major barriers to effective cancer treatment, and is often due to reduced uptake or increased efflux of the drug in tumor cells. Other problems include the toxicity of anticancer drugs to normal tissues, and the variable tissue and tumor distribution found in patients. Moreover, how drugs move around the body also determines how efficiently they can tackle the disease. Using knockout and transgenic mouse models, his group aims to better understand the way the body handles drugs, thereby supporting the optimization of clinical chemotherapy and pharmacotherapy.

Studying Detoxifying Proteins

"We have been addressing the problem of drug resistance by focusing our efforts on three main questions. First, how effectively is a drug taken up into the blood circulation after oral administration, and how fast is it subsequently degraded and eliminated? Second, how efficiently does a drug reach a certain tissue or compartment in the body where a tumor (or other drug target) resides? And finally, how effectively does a drug enter tumor cells, and to what extent is it subsequently pumped out or degraded? All of these processes directly determine the clinical effectiveness of anticancer drugs, and they are all predominantly regulated by a family of so-called detoxifying proteins. We are studying three classes of these detoxifying proteins, namely drug efflux transporters, drug uptake transporters and drug-metabolizing enzymes. Each of these classes can profoundly influence oral uptake and elimination, tissue distribution, and tumor distribution of many different drugs. By knocking out or overexpressing genes encoding these detoxifying proteins in mouse models we can precisely determine their pharmacological and physiological functions. Also a number of endogenous compounds and metabolites, which are potentially toxic to the organism, are detoxified by the same proteins that affect the behavior of drugs in the body. This represents an important physiological function of these proteins."





Predicting Consequences of Drug Treatment

"Using the basic insights obtained through this work we aim to predict the consequences of drug treatment and potentially modulate this response in a controlled fashion by administration of certain inhibitors. Our ultimate aim is to facilitate personalized medicine by enabling the optimization of drug treatment for each patient, in terms of drug type and dosing schedule, as well as identifying possible inhibitor combinations that best fit the properties of the tumor."

Division of Molecular Pathology

Selected publications

Garcia-Closas M*, Couch FJ*, Lindstrom S*, Michailidou K*, Schmidt MK* et al Genome-wide association studies identify four ER negative-specific breast cancer risk loci. Nat Genet. 2013;45(4):392-8, 398e1-2 (* shared first authorship)

Fasching PA*, Pharoah PD*, Cox A*, Nevanlinna H, Bojesen SE, [...], Schmidt MK. The role of genetic breast cancer susceptibility variants as prognostic factors. Hum Mol Genet. 2012;21(17):3926-39 (* shared first authorship)

Mook S, Van 't Veer LJ, Rutgers EJ, Ravdin PM, van de Velde AO, van Leeuwen FE, Visser O, Schmidt MK. Independent prognostic value of screen-detection in invasive breast cancer. J Natl Cancer Inst. 2011;103(7):585-97

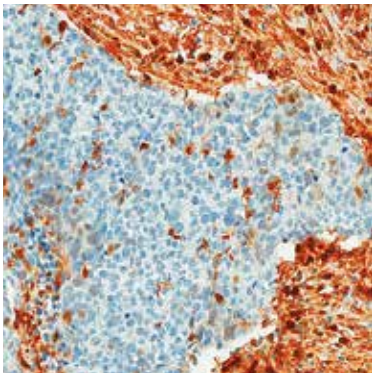
Breast Cancer Risk and Prognosis

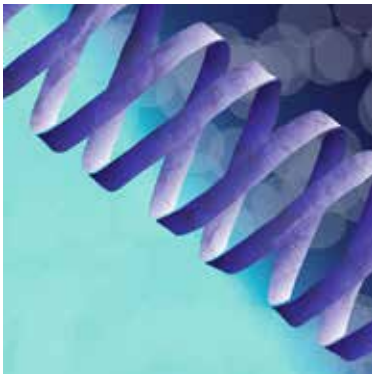
MARJANKA SCHMIDT

Marjanka Schmidt's group was established in 2010 and focuses on the impact of hereditary genetic variants, tumor profiles and treatment response on the development of contralateral breast cancer and prognosis of breast cancer. The group also works on the etiology of the development of specific breast cancer subtypes. Findings may eventually lead to inclusion of genetic information in guidelines and prediction tools for improved disease management, or in the pre-selection of women for breast cancer screening programs. A second research line focuses on patient information and consent procedures, and return of results from research using human materials.

Genetic Determinants

"The Breast Cancer Association consortium identified many new breast cancer susceptibility loci over the last years. However, the genetic determinants of breast cancer prognosis are still largely unexplained. The BCAC clinico-pathological database, that includes tumor characteristics, clinical diagnostic and treatment information as well as follow-up from 65 studies comprising ~110,000 patients is maintained by our group. We aim to identify germline variants that are important in the etiology of breast cancer subtypes as well as variants that affect prognosis through treatment response or other mechanisms. We are specifically interested in outcome of young breast cancer patients. In our nationwide consecutive breast cancer cohort of women diagnosed with breast cancer <50 years of age, we are investigating prognosis and long-term outcome of *BRCA1/2* mutation carriers using FFPE tissue. We are also 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."





Biobanking: Consent Procedures

"In the Netherlands, the use of residual tissues for research is regulated by an 'opt-out' procedure. The quality of information for patients about this use differs widely across Dutch hospitals. Our previous research showed that cancer patients are unsatisfied with the current information procedure: they are mostly unaware of residual tissue storage for their own clinical benefit and possible use in research, although only a minority wishes to refuse this. We are surveying patients to investigate different consent procedures for the secondary use of human materials in clinical research, including satisfaction and preference for different procedures. Another line of research concerns the return of results and optimal ways to inform donors about research results."

Division of Immunology

Selected publications

Gerlach C, Rohr JC, Perie L, van Rooij N, van Heijst JW, Velds A, Urbanus J, Naik SH, Jacobs H, Beltman JB, et al. Heterogeneous differentiation patterns of individual CD8+ T cells. Science 2013;340:635-639

Van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, van Dijk LJ, Behjati S, Hilkmann H, El Atmioui D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. Journal of Clinical Oncology 2013;31:e439-442

Linnemann C, van Buuren M, Bies L, Verdegaal EM, Schotte R, Calis J, Behjati S, Velds A, Hilkmann H, El Atmioui D, et al. (2015). High throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T-cells in human melanoma. Nat Med 2015;21:81-85

Cancer Immunology
TON SCHUMACHER

The human immune system has the capacity to destroy cancer cells in at least some patients, but the rules of this interaction are not completely understood. Ton Schumacher incorporates the development of new technologies into his research, to harness the power of the immune system for treating cancer. His group studies a special group of white blood cells known as T lymphocytes, which play a key role in fighting infection and cancer. Using this experimental approach, his research group reveals how T lymphocytes attack human tumors, and how such T cell attacks can be optimized.

T Cell Responses

"One part of our research is centered around the development of novel technologies to understand the basic mechanisms behind T cell-mediated immune responses. T cells are composed of functionally distinct subtypes. One focus in our group is to determine the fate of these different subtypes during and after an immune response, which is essential for understanding cancer immunity. To do this, we have developed new approaches in which individual cells are labeled with unique genetic tags. This 'paternity test' at the cellular level allows us to follow the progeny of individual T cells in vivo. We can then, for instance, identify which T cell subtype gives rise to the long-lived memory T cells that provide long-term immune protection."

Tumor Recognition

"In a second set of projects, we aim to understand how T cells recognize tumor cells in patients that respond to cancer immunotherapies. T cells carry a clone-specific T cell receptor (TCR) on their cell surface that detects specific (tumor) antigens presented by major histocompatibility complexes (MHC) on the surface of cancer cells. We have developed novel monitoring technologies that use soluble multimeric pMHC complexes to measure many different antigen-specific T cell populations in clinical samples. This enables us to study how cancer immunotherapy alters immune responses against cancer-associated antigens. On the basis of this knowledge we develop methods to further enhance T cell attack. These analyses have for instance made it possible to reveal the role of patient-specific tumor mutations in T cell control. In addition, we are using these and complementary tools to determine which T cells in human tumors do or do not contribute to cancer cell killing."

Division of Biochemistry

Selected publications

Sahtoe, D.D., van Dijk, W.J., El Oualid, F., Ekkebus, R., Ovaa, H., Sixma, T.K. Mechanism of UCH-L5 activation and inhibition by DEUBAD domains in RPN13 and INO80G Mol. Cell 2015:57:887-900.

Mattiroli, F., Vissers, J.H., van Dijk, W.J., Ikpa, P., Citterio, E., Vermeulen, W., Marteijn, J.A., Sixma, T.K. RNF168 Ubiquitinates K13-15 on H2A/H2AX to Drive DNA Damage Signaling. Cell 2012; 150:1182-95.

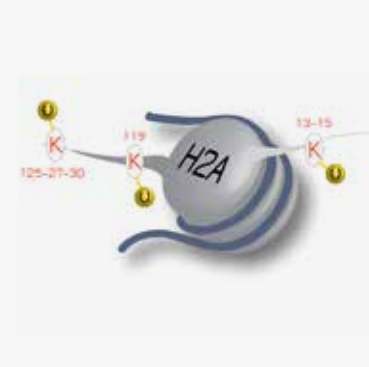
Faesen, A.C., Dirac, A.M., Shanmugham, A., Ovaa, H., Perrakis, A., Sixma, T.K. Mechanism of USP7/HAUSP activation by its C-terminal ubiquitin-like domain and allosteric regulation by GMP-synthetase. Mol Cell 2011; 44:147-59.

Structural Biology
TITIA SIXMA

Titia Sixma is interested in the working mechanism of proteins. She uses crystallography to obtain snapshots of proteins in atomic detail, which are then used to uncover functional attributes, such as how one protein activates another. Combining protein structure with biochemical analyses is a powerful approach for gaining unique insights into protein action and her group is particularly interested in the processes that regulate DNA function. They study proteins that affect DNA repair and the access to DNA, many of which have been linked with cancer. Understanding the relationship between protein structure and function can be valuable for designing new and effective anti-cancer drugs.

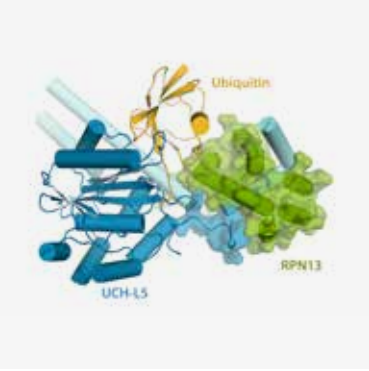
DNA Regulation

"All biological processes in a cell are carefully regulated in order to maintain homeostasis and protect the organism against damage and disease. This regulation occurs on a number of different levels, and our group studies the mechanisms of protein regulation, primarily by analyzing their three dimensional structures. We focus on basic processes in DNA regulation, which are particularly relevant to diseases such as cancer. One project involves understanding how base mismatches are recognized and repaired when DNA is inaccurately replicated during cell division. This process is highly complex, and involves the regulation and orchestration of a conserved set of enzymes. Since the intermediate steps are all highly transient, it has been difficult to decipher the molecular mechanisms involved. Only by combining structural studies with kinetic data have we been able to reveal the critical steps in this pathway."



Ubiquitin

"The other major interest of the lab centers on the role of ubiquitin in DNA regulation. Ubiquitin is a small protein that can be covalently attached to a target protein to change its fate. Ubiquitination regulates diverse biological processes and the mechanisms of ubiquitin conjugation and deconjugation are therefore carefully controlled. Our studies are focused on specific enzymes in this process, the E3 ligase and deubiquitinase enzymes. We study their mechanisms of action and how these are regulated by a combination of in vitro reconstitution and structural studies. In this process we generate separation-of-function mutations that make it possible to study the role of these proteins at the cellular and organismal level, to provide better understanding of associated diseases including cancer."



Division of Radiation Oncology

Selected publications

Schaake EE, Rossi MMG, Buikhuisen WA, Burgers JA, Smit AAJ, Belderbos JSA, Sonke J-J. Differential Motion Between Mediastinal Lymph Nodes and Primary Tumor in Radically Irradiated Lung Cancer Patients, Int J Radiat Oncol Biol Phys. 2014; 90(4):959-66

van Kranen S, Mencarelli A, van Beek S, Rasch C, van Herk M, Sonke J-J. Adaptive radiotherapy with an average anatomy model: Evaluation and quantification of residual deformations in head and neck cancer patients, Radiother Oncol. 2013; 109(3):463-8.

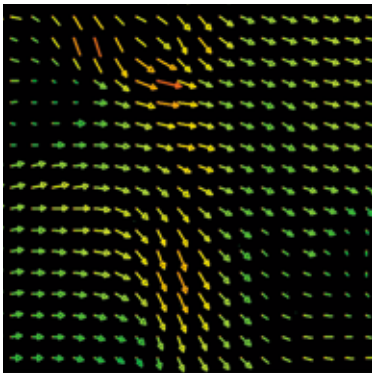
Nijkamp J, Rossi M, Lebesque J, Belderbos J, van den Heuvel M, Kwint M, Uyterlinde W, Vogel W, Sonke J-J. Relating acute esophagitis to radiotherapy dose using FDG-PET in concurrent chemo-radiotherapy for locally advanced non-small cell lung cancer, Radiother Oncol. 2013; 106(1):118-23.

Adaptive Radiotherapy
JAN-JAKOB SONKE

With a background in engineering and image processing Jan-Jakob Sonke joined The Netherlands Cancer Institute to develop innovative solutions to improve radiation therapy. Over the course of radiation therapy, patients undergo continuous changes in posture, anatomy and biology due to both physiology and treatment response. Consequently, the actually delivered dose typically deviates from the planned dose. Jan-Jakob's group therefore focusses on strategies to monitor these changes through an image feedback loop and adapt the treatment to optimize the treatment outcome in the presence of anatomical and functional changes.

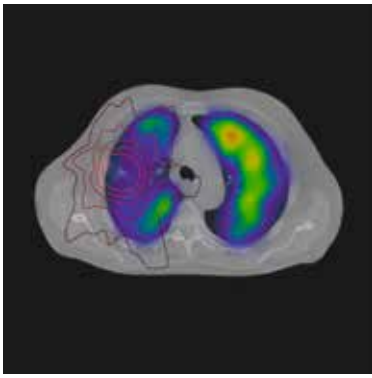
High precision radiotherapy

"Traditionally, medical images of a patient scheduled for radiotherapy are acquired only once during the treatment preparation phase, one or more weeks before the start of treatment delivery. These images are subsequently used for target definition and treatment plan optimization but only represent a snapshot of the patient's anatomy. To increase the precision of radiotherapy to the continuously changing anatomy, cone-beam CT (CBCT) scanners have been integrated with the treatment machine to capture the patient's anatomy just prior to irradiation. We are investigating both hardware and software solutions to improve the CBCT image quality."



Repeated imaging and Correction algorithms

"To quantify anatomical changes, (deformable) image registration algorithms are optimized facilitating both couch corrections to align the target to the planned position as well as contour propagation and dose accumulation to adapt the treatment plan. Similarly, repeat functional imaging is utilized to monitor and model radiation response of target and organs at risk. This adaptive radiation therapy framework is also prototyped pre-clinically using a dedicated small animal irradiator. Recently, we started to develop adaptive radiotherapy techniques using an MRI integrated treatment machine providing superior soft tissue and real time imaging."



Division of Cell Biology I

Selected publications

Sachs N, Secades P, van Hulst L, Song JY, and Sonnenberg A. Reduced susceptibility to twostage skin carcinogenesis in mice with epidermis-specific deletion of CD151. J. Invest. Dermatol. 2014;134, 221-228

Frijns E, Kuikman I, Litjens S, Raspe M, Jalink K, Ports M, Wilhelmsen K, and Sonnenberg A. Phosphorylation of threonine 1736 in the C-terminal tail of integrin β4 contributes to hemidesmosome disassembly. Mol. Biol Cell. 2012;23:1475-1485

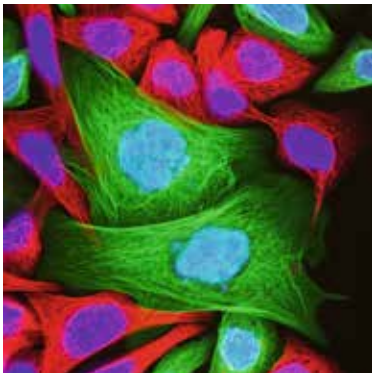
Sachs N, Secades P, van Hulst L, Kreft M, Song J-Y, and Sonnenberg A. Loss of integrin α3 prevents skin tumor formation by promoting epidermal turnover and depletion of slow-cycling cells. Proc. Natl. Acad. Sci. USA. 2012;109, 21468-21473

Cell-Matrix Adhesion
ARNOUD SONNENBERG

To understand how cells reorganize and respond to changes in their environment, Arnoud Sonnenberg studies the structure, function and physiology of integrins. These are proteins that span the cell's plasma membrane. His group main interests are two integrins, α3β1 and α6β4, which are strongly expressed in the epidermis. Additionally, α3β1 is the main β1 integrin in the glomerulus of the kidney. Using mouse models, his group has discovered that these proteins play important roles in skin cancer, wound healing, and in maintaining a healthy, functioning skin and kidney. Sonnenberg also works on the extensive protein networks physically connected to these integrins, in order to identify their function and potential malfunction in disease.

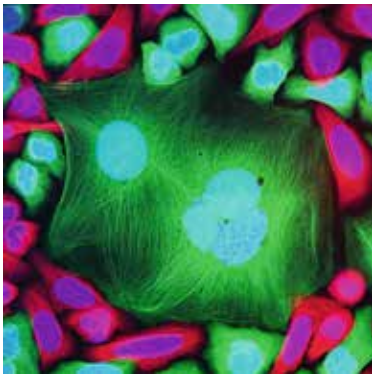
Roles of laminin-binding integrins in tumorigenesis

"Integrin α3β1, a major laminin receptor, forms on cells a high stoichiometric and stable complex with the tetraspaninin CD151 and is intimately linked to the actin-based cytoskeleton by a variety of actin-binding proteins. We are studying the role of the α3β1/CD151 ternary complex in a chemically induced model for skin carcinogenesis and have identified an important function of this complex in the development and progression of papillomas, by controlling the migration of epidermal stem cells from their primary niche in the hair follicle bulge. We hypothesize that cancer-initiating cells (cancer stem cells), lacking the α3β1/CD151 complex and present in the bulge region of the hair follicle, exit their compartment and terminally differentiate before they can acquire additional mutations that would lead to the onset of cancer. Our current research focuses on providing further support for this hypothesis and understanding the underlying mechanism(s)."



Hemidesmosomes and cell migration

"In contrast to α3β1, integrin α6β4 (the other major laminin receptor on keratinocytes) is concentrated in so-called hemidesmosomes and is connected via the cytoskeletal linker protein plectin to the intracellular keratin cytoskeleton. Hemidesmosomes play a critical role in maintaining epithelial integrity and mutations in the genes encoding α6, β4 or plectin can cause severe blistering of the skin. The disassembly of hemidesmosomes is a prerequisite for keratinocyte migration and squamous cell carcinoma invasion. To understand how the disassembly of hemidesmosomes is regulated, we study the mechanism(s) by which EGF-induced signals dissolve hemidesmosomes through phosphorylation-dependent dissociation of the complex between β4 and plectin."



Division of Biological Stress Response

Selected publications

Wielders EAL, Hettinger J, Dekker R, Kets CM, Ligtenberg MJL, Mensenkamp AR, Van den Ouweland AMW, Prins J, Wagner A, Dinjens WNM, Dubbink HJ, Van Hest L, Menko F, Hogervorst F, Verhoef S, Te Riele H. Functional analysis of Msh2 unclassified variants found in suspected Lynch syndrome patients reveals pathogenicity due to attenuated mismatch repair. J Med Genet 2014;51:245-53

Vormer TL, Wojciechowicz K, Dekker M, de Vries S, van der Wal A, Delzenne-Goette E, Naik SH, Song JY, Dannenberg JH, Hansen JB, Te Riele H. RB Family Tumor Suppressor Activity May Not Relate to Active Silencing of E2F Target Genes. Cancer Res 2014; 74:5266-76

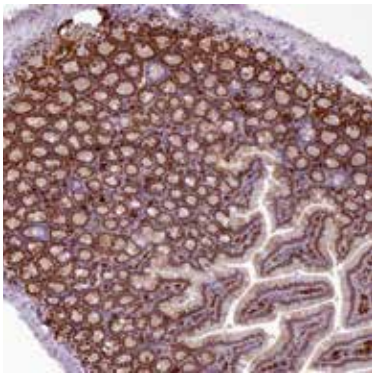
Wojciechowicz K, Cantelli E, Van Gerwen B, Plug M, Van Der Wal A, Delzenne-Goette E, Song JY, De Vries S, Dekker M, Te Riele H. Temozolomide increases the number of mismatch repair-deficient intestinal crypts and accelerates tumorigenesis in a mouse model of Lynch syndrome. Gastroenterology 2014;147(5):1064-72

Missing Mismatch Repair
HEIN TE RIELE

Over the 20 years Hein te Riele has worked as a group leader in the NKI, he has been intrigued by mechanisms cells use to avoid changes in their genetic code that may lead to cancer. His team works on a specific error-checking system, DNA mismatch repair (MMR), which supervises the process of DNA replication: wrongly incorporated bases that have escaped proofreading by polymerases are recognized and removed by MMR to make the error rate as small as one per cell division.

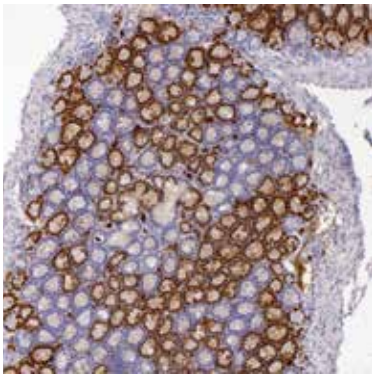
Lynch syndrome

"How important removal of erroneously incorporated bases is, became clear in the early nineties when inherited defects in MMR were found to underlie a cancer syndrome, called Lynch syndrome (LS). This exciting discovery was made possible by decades of research on MMR in bacteria and yeast. Fundamental research does pay off!
MMR gene defects that fully abrogate gene function can easily be assigned as the cause of disease and carriers need regular screening for tumor development.
We have generated a novel LS mouse model in order to identify environmental and genetic risk factors for tumor development and to test prophylactic therapies to suppress cancer risk."



Variants of uncertain clinical significance

"With the spectacular advance of modern sequencing, many variants of disease-related genes, including MMR genes, are identified. Often only a single base pair has changed affecting a single amino acid. Is it disease causing? We have developed a protocol to find out whether mutations in MMR genes are deleterious and hence pathogenic in humans. We introduce suspected mutations in a suitable cell type by using short single-stranded DNA fragments. The method is not that efficient, but if it causes cells to loose MMR capacity, we can easily pick them up.
It's a powerful technique. In selected cases we generate mice to see if the mutation we've made predisposes to cancer. Furthermore, we can now tease apart the individual roles of domains in mismatch repair proteins."



Division of Radiation Oncology

Selected publications

Houweling AC, Wolf AL, Vogel WV, Hamming-Vrieze O, van Vliet-Vroegindeweij C, van de Kamer JB, van der Heide UA. FDG-PET and diffusion-weighted MRI in head-and-neck cancer patients: implications for dose painting. Radiother Oncol. 2013;106(2):250-4

Van der Heide UA, Houweling AC, Groenendaal G, Beets-Tan RG, Lambin P. Functional MRI for radiotherapy dose painting. Magn Reson Imaging. 2012;30(9):1216-23

Groenendaal G, Borren A, Moman MR, Monninkhof E, van Diest PJ, Philippens ME, van Vulpen M, van der Heide UA. Pathologic validation of a model based on diffusion-weighted imaging and dynamic contrast-enhanced magnetic resonance imaging for tumor delineation in the prostate peripheral zone. Int J Radiat Oncol Biol Phys. 2012;82(3):e537-44

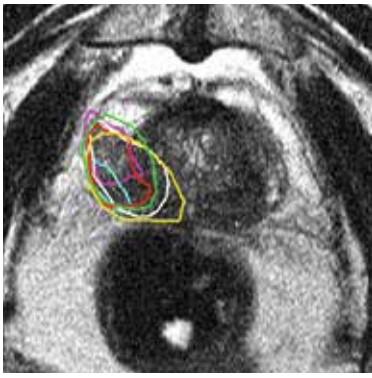
Multiparametric MRI for radiotherapy guidance

UULKE VAN DER HEIDE

Uulke van der Heide works as a medical physicist in the radiotherapy department of the Netherlands Cancer Institute. He investigates the use of magnetic resonance imaging (MRI) techniques to improve the treatment with radiotherapy. The focus of research in his group is the improvement of target definition in radiotherapy by application of MRI and the development and validation of quantitative imaging methods for tumor characterization. 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.

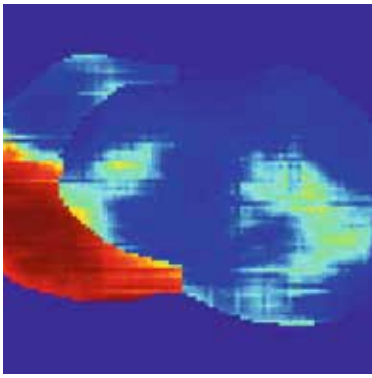
Identifying optimal imaging modalities for dose painting

"Radiotherapy has the capacity for differential treatment, in which a high dose can be delivered to the visible tumor, whereas tissue holding microscopic disease is irradiated with a lower dose. In this way, tumor cells are eradicated while the underlying healthy tissue is allowed to recover. This capacity sets it apart from all-or-nothing therapies, such as surgery. For dose painting to be effective, imaging is required to identify the most radioresistant part of the tumor. For head-neck cancer and cervical cancer we investigate which imaging modality is optimal to guide decisions on dose levels."



Multi-parametric MRI for radiotherapy of prostate cancer

"To improve the outcome of radiotherapy for prostate cancer and reduce treatment-related toxicity, we investigate strategies to differentiate the radiation dose between the tumor and the rest of the prostate gland. This is currently tested in the FLAME trial, a multi-center randomized trial for intermediate and high-risk prostate cancer. To delineate the tumors, multi-parametric MRI is used. Such an exam consists of a T2-weighted MRI reflecting anatomy, as well as functional MRI techniques reflecting diffusion (Apparent Diffusion Coefficient ADC) and the perfusion and permeability of the microvasculature of the tissue (K^{trans}). Our group develops methods for computer-aided delineation of tumors. A detailed comparison with histology is ongoing to identify the characteristics of those tumors that are not detected with MRI, as compared to those that are visible."



Division of Molecular Carcinogenesis

Selected publications

Van der Heijden MS, van Rhijn BW. The Molecular Background of Urothelial Cancer: Ready for Action? Eur Urol. 2014

Brody JR, Hucl T, Costantino CL, Eshleman JR, Gallmeier E, Zhu H, van der Heijden MS, Winter JM, Wikiewicz AK, Yeo CJ, Kern SE. Limits to thymidylate synthase and TP53 genes as predictive determinants for fluoropyrimidine sensitivity and further evidence for RNA-based toxicity as a major influence. Cancer Res. 2009;69(3):984-91

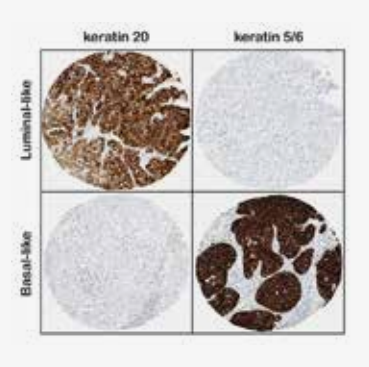
Van der Heijden MS, Brody JR, Dezentje DA, Gallmeier E, Cunningham SC, Swartz MJ, DeMarzo AM, Offerhaus GJ, Isacoff WH, Hruban RH, Kern SE. In vivo therapeutic responses contingent on Fanconi anemia/ BRCA2 status of the tumor. Clin Cancer Research 2005; 15:7508-15

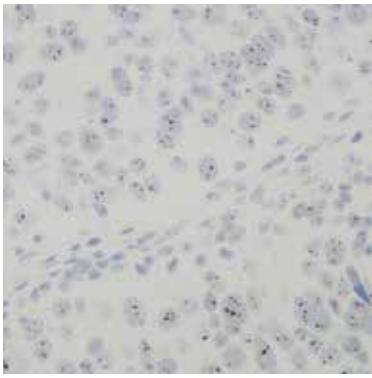
Targeted Cancer Therapy
MICHIEL VAN DER HEIJDEN

Molecular pathways that are specifically activated in cancer cells provide targets for new and more effective cancer treatments, so-called targeted therapies. Michiel van der Heijden aims to advance the development of targeted therapies for treating bladder cancer, which is the fifth most common cancer worldwide. For patients with advanced disease, new therapeutic approaches are urgently needed. Michiel and his group are searching for novel molecular targets, as well as mechanisms of drug resistance and biomarkers that can guide therapy. Through the large number of bladder cancer patients at the Antoni van Leeuwenhoek Hospital, the clinic of the NKI, their discoveries can be rapidly translated into practice.

Circulating Tumor DNA

"Therapeutic options for metastatic cancers are often very limited; therefore identifying molecular targets in metastases that can be used for treatment is vital. However, this is challenging because metastases can be hard to biopsy due to proximity to vital structures, and molecular targets need to be present in the bulk of metastatic locations for treatment to be effective. Also, genotyping is often done on archive tissue whereas subsequent treatment could alter the molecular profile. To overcome these challenges we are exploiting the presence of cell-free tumor DNA that circulates in the plasma of cancer patients. Circulating tumor DNA (ctDNA) contains information on tumor mutations and may also reflect metastatic burden. Perhaps more importantly, specific mutations in ctDNA could reflect changes in abundance of specific cancer cell clones, making it possible to adapt molecularly targeted therapies to dominant clones. In our liquid biopsy project, we aim to measure the spectrum of cancer mutations in the blood and urine of bladder cancer patients to better guide treatment."





Personalized Medicine

"In the last decade, revolutionary advancements in the analysis of cancer genomes have been made, and the number of available cancer therapeutics that target a specific genetic alteration has rapidly expanded. This has led to great optimism that treatment of most cancers based on the genetic profile of the malignant cells is within reach. Indeed, several successful examples of 'personalized treatment' using drugs that target specific activating mutations have been implemented in the clinic. We have prioritized the implementation of precision medicine / targeted therapies at the NKI by developing a next-generation sequencing assay that can reliably and rapidly test relevant cancer genes for mutations that can be clinically targeted. In addition, this assay will test for genetic events that are likely to be relevant for cancer therapy in the near future."

Division of Psychosocial Research and Epidemiology

Selected publications

Retèl VP, Joore MA, Drukker CA, Bueno-de-Mesquita JM, Knauer M, van Tinteren H, Linn SC, van Harten WH. Prospective cost-effectiveness analysis of genomic profiling in breast cancer. Eur J Cancer. 2013;49(18):3773-9

Kuijpers W, Groen WG, Aaronson NK, van Harten WH. A systematic review of web-based interventions for patient empowerment and physical activity in chronic diseases: relevance for cancer survivors. J Med Internet Res. 2013;15(2):e37

Mewes JC, Steuten LM, IJzerman MJ, van Harten WH. Effectiveness of multidimensional cancer survivor rehabilitation and cost-effectiveness of cancer rehabilitation in general: a systematic review. Oncologist. 2012;17(12):1581-93

Technologies and Services in Oncology
WIM VAN HARTEN

Wim van Harten works on improving the clinical management and outcome of cancer patients by studying health technologies and services. His group conducts assessments of novel technologies developed in the laboratory, such as sequencing or immunotherapy, in terms of efficacy, feasibility and cost for application in the clinic. They also develop new solutions for better managing patient treatments in the hospital, and review the effectiveness of ICT-supported rehabilitation programs designed to reduce the disease- and treatment-based morbidities suffered by many cancer survivors. These topics cover the entire range from basic research through to clinical application, and are critical elements of all cancer hospital programs.

Technology Assessment

"My group has been conducting early-stage assessments on a variety of different technologies to improve the efficacy of cancer treatment. For example, we conducted a technology assessment study on the introduction of a 70-gene microarray test as a prognostic tool in the treatment of node negative breast cancer. As the diffusion of this technology was in an early stage and the course of development not easy to predict, we chose an early stage evaluation approach known as constructive technology assessment (CTA), which takes the technology dynamics into account. We also performed a prospective cost effectiveness analysis of this prognostic tool. Additionally, we have initiated early stage technology assessments on the application of diagnostic/prognostic markers in neo-adjuvant breast cancer treatment, which is part of the Center for Translational Molecular Medicine (CTMM) program BREASTCARE, as well as on tumor infiltrating lymphocyte (TIL)-transfer technology for treating advanced melanoma, in cooperation with the University of Twente."

Operations Improvement and rehabilitation

"In a number of other collaborative projects, we also focus on operations improvements in oncology. For example, we have been using mathematical analyses to improve the efficiency and capacity of treatments such as radiotherapy. We are also leading the development of a European benchmarking system as a powerful tool to inform management on improvement options and patients on the quality of services. Other projects relate to the added value of peer review- and accreditation systems in oncology and to the identification of excellent translational research programs in oncology. Another important part of cancer treatment that we work on is survivorship care and rehabilitation, with a particular focus on patient empowerment, physical exercise and promoting return to work. These projects are supported by innovative IT, and we also analyze the cost effectiveness and budget impact of such multidisciplinary/multifaceted rehabilitation interventions."

Division of Psychosocial Research and Epidemiology

Selected publications

Van Nimwegen FA, Schaapveld M, Janus CM, Krol AD, Petersen EJ, Raemaekers JM, Kok WE, Aleman BM, van Leeuwen FE. Cardiovascular disease after hodgkin lymphoma treatment: 40-year disease risk. JAMA Int Med. 2015;175(6):1007

Morton LM, Dores GM, Curtis RE, Lynch CF, Stovall M, Hall P, Gilbert ES, Hodgson DC, Storm HH, Børge Johannesen T, Smith SA, Weathers RE, Andersson M, Fossa SD, Hauptmann M, Holowaty EJ, Joensuu H, Kaijser M, Kleinerman RA, Langmark F, Pukkala E, Vaalavirta L, Van den Belt-Dusebout AW, Fraumeni JF, Travis LB, Aleman BM, van Leeuwen FE. Stomach cancer risk after treatment for Hodgkin lymphoma. J Clin Oncol 2013;31:3369-3377

Pijpe A, Andrieu N, Easton DF, Kesminiene A, Cardis E, Noguès C, Gauthier-Villars M, Lasset C, Fricker JP, Peock S, Frost D [...] Ausems MG, Meijers-Heijboer H, Thierry-Chef I, Hauptmann M, Goldgar D, Rookus MA, van Leeuwen FE; GENEPSO; EMBRACE; HEBON. Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations: retrospective cohort study (GENE-RAD-RISK). BMJ 2012;345:e5660

Epidemiology of cancer
FLORA VAN LEEUWEN

Since arriving at the NKI in 1981, Flora van Leeuwen has studied the epidemiology of cancer, identifying risk factors from large-scale studies in the population. Her group focuses on two topics. One topic concerns the late adverse effects of cancer treatment. Unfortunately radio- and/or chemotherapy can cause various health problems in the long run. Her other research focuses on the role of hormone-related risk factors for breast cancer and ovarian cancer.

Late adverse effects of cancer treatment

"As more people survive cancer, it is increasingly important to evaluate whether radiotherapy and systemic treatment have late adverse health consequences. We are now realizing that 20 or 30 years after receiving treatment, cancer survivors often are at increased risk of developing new tumors and cardiovascular disease. One study we are doing in this field is following 10.000 patients treated for Hodgkin's lymphoma in the 1960s through 2010. We have found that the risk of heart disease is strongly increased in these patients, both after receiving radiotherapy and chemotherapy. Also, female survivors are at a high risk of developing breast cancer. Other studies include large groups of patients with testicular cancer or breast cancer. Furthermore, for lymphoma patients we are setting up a nationwide infrastructure for survivorship care. Patients who were treated in the past will be recalled under medical surveillance and invited to participate in screening to detect adverse treatment effects early, to enable timely interventions."

Risk factors for female hormone-related cancers

"For our breast cancer studies, we have set up a nationwide cohort of 40,000 women tested for BRCA mutations. Thus far we discovered that for women carrying a mutation in the BRCA genes, not just the gene but also oral contraceptive use, physical activity and low radiation doses affect breast cancer risk. We also study the long-term effects of ovarian stimulation for in vitro fertilization on the risks of breast and ovarian cancer; for this nationwide study we are following 38,000 IVF-treated women. Recently, we also became interested in the potential effects of disruption of the day/night rhythm on breast cancer. For this study, we have identified a cohort of 60,000 female nurses, many of whom have done shift work for a long time."

Hebon

Hebon-onderzoek
Vragenlijst

Division of Gene Regulation

Selected publications

Vlaming H, Van Welsem T, De Graaf EL, Ontoso D, Altelaar AFM, San-Segundo P, Heck AJ, and Van Leeuwen F. Flexibility in crosstalk between H2B ubiquitination and H3 methylation in vivo. EMBO Rep. 2014;15:1077-1084

Radman-Livaja M, Verzijlbergen KF, Weiner A, van Welsem T, Friedman N, Rando OJ, and van Leeuwen F. Patterns and mechanisms of ancestral histone protein inheritance in budding yeast. PLoS Biol 2011;9:e1001075

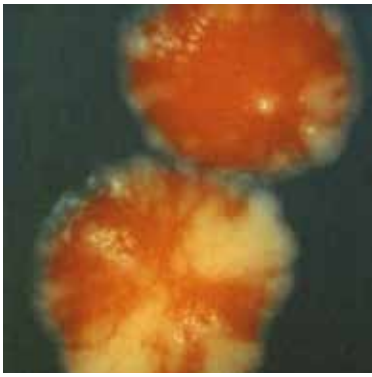
Frederiks F, Tzouros M, Oudgenoeg G, van Welsem T, Fornerod M, Krijgsveld J, and van Leeuwen F. Nonprocessive methylation by Dot1 leads to functional redundancy of histone H3K79 methylation states. Nat Struct Mol Biol 2008;15:550-557

Chromatin Dynamics
FRED VAN LEEUWEN

Beginning his scientific career at the NKI working on African trypanosomes, Fred van Leeuwen developed an interest in epigenetics – the process by which genes are stably switched on or off. He returned to the institute in 2003 to establish his own research group on the topic. Although an identical set of genes is found in every cell in the body, they are not all in the same on or off state, which allows each cell to function differently. Fred's group is interested in how cells maintain their identity and pass on the memory of gene activity to daughter cells, which can lead to insights into the development of cancer.

Epigenetic Memory

"Switching genes on or off and keeping them in that state involves 'packaging' the DNA in the nucleus to form chromatin, by wrapping it around proteins called histones. Histones carry different chemical modifications that affect the packaging of chromatin. This in turn affects the ease with which the cellular machinery can access the DNA sequence and regulate gene expression. One of the goals of my lab is to investigate if and how these accessibility instructions are involved in epigenetic memory. By taking advantage of budding yeast as a model system, my lab is developing new tools to investigate the basic principles of chromatin-based gene regulation. Using these tools we measure the inheritance of ancestral histones as cells replicate their DNA, which will help us to understand how histones can pass on information after cell division. We also monitor the stability of chromatin in non-replicating cells. Our results, and those of others, show that individual histones are not permanently packaged into chromatin, but can in fact be replaced. Since this is expected to lead to changes in DNA accessibility and thereby gene expression, we are working on identifying the molecular mechanisms involved in histone dynamics."



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. Together, our studies are aimed at providing a deeper understanding of the inheritance of protein-based information in dividing cells, a fundamental process not only relevant for cancer, but also for other diseases, evolution, and aging."

Division of Molecular Genetics

Selected publications

Akhtar W, de Jong J, Pindyurin AV, Pagie L, Meuleman W, de Ridder J, Berns A, Wessels LF, van Lohuizen M, van Steensel B. Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell. 2013;154(4):914-27

Gargiulo G, Cesaroni M, Serresi M, de Vries N, Hulsman D, Bruggeman SW, Lancini C, van Lohuizen M. In vivo RNAi screen for BMI1 targets identifies TGF-β/BMP-ER stress pathways as keyregulators of neural- and malignant glioma-stem cell homeostasis. Cancer Cell. 2013;23(5):660-76

Nacerddine K, Beaudry J-B, Ginjala V, Westerman B, Mattiroli F, Song J-Y, van der Poel H, Balague Ponz O, Pritchard C, Cornelissen-Steijger P, Zevenhoven J, Tanger E, Sixma T, Ganesan S, van Lohuizen M. Synergy between PI3K/Akt and Bmi1 in mouse prostate carcinogenesis reveals a novel role for Bmi1 phosphorylation in its oncogenic potential, E3 ligase activity and DNA damage repair. J. Clin Invest 2012;122(5):1920-32

Cell Fate and Cancer
MAARTEN VAN LOHUIZEN

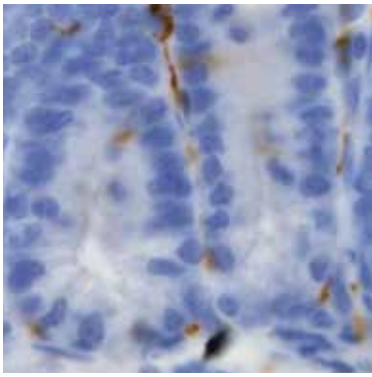
Maarten van Lohuizen gained his PhD at the NKI in 1992 studying oncogenes with Anton Berns. After a postdoc at the University of California, San Francisco, he returned to the NKI in 1995 and now heads the Division of Molecular Genetics. He works on the master switches that control cell and tissue development, and how these go wrong in cancer. His group is working on one set of these switches, known as the Polycomb group proteins. These proteins are known to be involved in tumor formation, and the group has been working out how, which could lead to the development of more effective drugs for treating cancer.

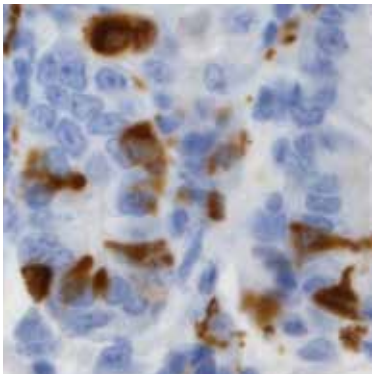
Gene Silencing by Polycomb

"The role of Polycomb proteins is to keep genes that control cell differentiation switched on or off as appropriate. If Polycomb proteins fail to work, tumors can develop. My group is focused on understanding this tumorigenic process in molecular detail. This could lead to the development of entirely new types of cancer drugs that act in more precise ways than simply killing rapidly dividing cells, like many chemotherapeutics do. We are focusing in particular on how Polycomb proteins control the fate of stem cells, such as those that continually renew the skin and the lining of the gut. We suspect that many cancers are the result of stem cells losing their ability to respond to environmental signals and continuing to divide when they should instead be starting to differentiate into a specific cell type. At the beginning of this process, however, these cells may divide too slowly to be killed by many of the current cancer drugs, which only act on rapidly proliferating cells. This leaves the cells free to continue contributing to tumor development, and the patient refractory to treatment."

Large-scale Screens

"In collaboration with Anton Berns and the Sanger Institute in the UK, we are using large-scale screens for mutations caused by retrovirus insertion to systematically search for genes and pathways involved in cancer. With these screens we have already identified one Polycomb gene, BMI1, which plays an important role in tumor formation and stem cell regulation. These screens are also uncovering many possible targets for a new generation of anticancer drugs, and are providing insight into which combinations of mutated oncogenes and tumor suppressor genes act together to cause cancer. In addition, we collaborate with the Van Steensel and Wessels labs in using novel transposon reporter screening to investigate chromatin function."





Division of Gene Regulation

Selected publications

Akhtar W, de Jong J, Pindyurin AV, Pagie L, Meuleman W, de Ridder J, Berns A, Wessels LF, van Lohuizen M, van Steensel B. Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell 2013;154:914-927

Kind J, Pagie L, Ortabozkoyun H, Boyle S, de Vries SS, Janssen H, Amendola M, Nolen LD, Bickmore WA, van Steensel B. Single-cell dynamics of genome-nuclear lamina interactions. Cell 2013;153:178-92

Bickmore WA, van Steensel B. Genome architecture: domain organization of interphase chromosomes. Cell 2013;152:1270-1284

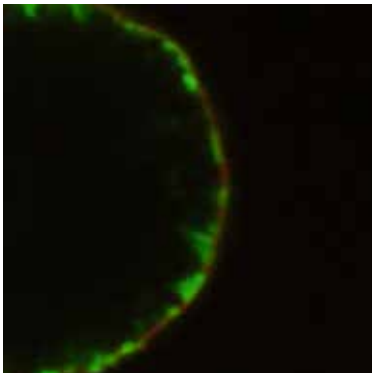
Chromatin Genomics
BAS VAN STEENSEL

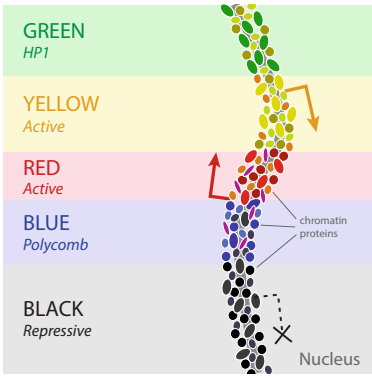
Cancer often involves defects in gene expression. To detect and correct these defects, we first need to understand how thousands of genes in our cells are normally controlled. Bas van Steensel's group studies these fundamental principles of gene regulation. In particular, they are interested in the regulation of gene activity by the packaging of DNA into chromatin, and by the spatial folding of chromosomes in the nucleus. To achieve this, they develop and use new genomics techniques that enable them to study gene regulation in its full complexity, and to uncover the underlying basic mechanisms. These insights will help understand how gene expression can go awry in cancer.

Technology to Map the Genome

"To study the 'big picture' of chromatin and gene regulation, we need methods that provide a genome-wide perspective. We previously developed the DamID method to map protein-DNA interactions with high sensitivity, and are now using it to dissect the interplay between chromatin proteins. We have also adapted DamID to visualize protein-DNA contacts by microscopy in living cells. Furthermore, we developed a new genomics method, employing thousands of parallel reporter integrations, to study the effects of various chromatin environments on gene regulation. All of these experimental methods necessitate the parallel development of new bioinformatics tools by our group, in order to extract biologically meaningful information from the resulting rich datasets.

For another line of research we used DamID to systematically map the location of over 100 chromatin proteins along the entire genome of fruit fly cells. This revealed that the genome is segmented into five principal chromatin types that consist of unique combinations of proteins and form domains that can extend over 100 kb. We are currently investigating how the chromatin proteins work together to form these different chromatin types, and how they regulate gene expression."





Spatial Organization

"Another important aspect is the spatial organization of chromosomes inside the cell nucleus. To investigate this, we have constructed detailed maps of the interaction sites of the entire genome with the nuclear lamina, which lines the nuclear envelope. We found that genome-lamina interactions occur through more than 1,000 sharply defined large domains named LADs, which contain genes that are generally silent. Currently, we aim to understand the single-cell dynamics of LADs, the molecular mechanisms that drive the interactions between LADs and the lamina, and the role of these interactions in the spatial organization of chromosomes and the regulation of gene expression."

Division of Radiation Oncology

Selected publications

Rooswinkel RW, van de Kooij B, de Vries E, Paauwe M, Braster R, Verheij M, Borst J. Antiapoptotic potency of Bcl-2 proteins primarily relies on their stability, not binding selectivity. Blood. 2014;123:2806-15

Van Hell AJ, Melo MN, van Blitterswijk WJ, Gueth DM, Braumuller TM, Pedrosa LR, Song JY, Marrink SJ, Koning GA, Jonkers J, Verheij M. Defined lipid analogues induce transient channels to facilitate drug-membrane traversal and circumvent cancer therapy resistance. Sci Rep. 2013;3:1949

Alderliesten MC, Klarenbeek JB, van der Luit AH, van Lummel M, Jones DR, Zerp S, Divecha N, Verheij M, van Blitterswijk, WJ. Phosphoinositide phosphatase SHIP-1 regulates apoptosis induced by edelfosine, Fas ligation and DNA damage in mouse lymphoma cells. Biochem J. 2011;440:127-35

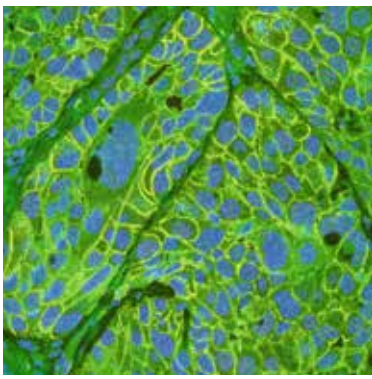
Verheij M, Vens C, van Triest B. Novel therapeutics in combination with radiotherapy to improve cancer treatment: rationale, mechanisms of action and clinical perspective. Drug Resist Updat. 2010;13:29-43

Targeted Radiosensitization
MARCEL VERHEIJ

Marcel Verheij combines his activities as a radiotherapy specialist at the Antoni van Leeuwenhoek Hospital, the clinic of the NKI, with translational research. This helps him keep his laboratory work focused on clinical need, and his clinical work scientifically up to date. A two-year fellowship at the Memorial Sloan-Kettering Cancer Center in New York during his residency-PhD program sparked an interest in apoptosis, or programmed cell death. This led to his current line of research investigating the mechanisms of radiation-induced cell death, with an aim to design more effective treatment strategies by identifying targeted agents that increase the cytotoxic effect of radiation.

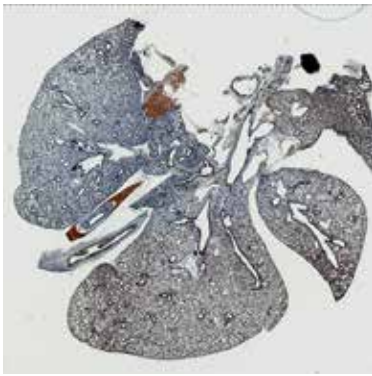
Combination Therapies

"Many tumors don't respond well to single modality treatments. Instead, combinations of therapies that target different cancer-specific characteristics may be more effective. The primary aim of our research is to make tumor cells more sensitive to radiation therapy, which would be a powerful weapon against cancer. Cancer cells are known to have a reduced capacity to repair DNA damage, which is induced by radiation. To exploit this weakness, in collaboration with Conchita Vens we have been using inhibitors of the DNA repair enzyme PARP to increase the cytotoxic effect of radiation and/or chemotherapy in preclinical models, as well as in clinical settings. Combinations of chemotherapy and radiotherapy are known to improve survival in an increasing number of patients with solid tumors. The juxtaposition of the NKI laboratories with the Antoni van Leeuwenhoek Hospital enables us to make great advances with new combinations of conventional and targeted therapies."



Apoptosis Signaling

"Apoptosis is strictly controlled by complex signaling pathways involving many molecular components. In collaboration with Jannie Borst, we are also exploring promising therapeutic combinations of radiation with drugs that target specific molecules involved in apoptosis signaling, including death receptor proteins and Bcl-2 family members. Another focus has been on a group of lipids that can induce apoptosis. We have been investigating the underlying mechanisms involved, as well as their ability to sensitize cancer cells to radiation, which have advanced these anti-cancer lipids towards clinical use. Highly organized clusters of cell membrane constituents appear to mediate the cytotoxic effects of anti-cancer lipids and of other agents. Our group is interested in how these lipid microdomains sense pro-apoptotic signals and induce cell death, and how co-delivery of short chain sphingolipids promotes drug uptake by cancer cells."



Division of Molecular Oncology

Selected publications

Houthuijzen JM, Daenen LGM, Roodhart JML, Oosterom I, van Jaarsveld MTM, Govaert KM, Smith ME, Sadatmand SJ, Rosing H, Kruse F, Nijkamp MW, Helms BJ, van Rooijen N, Beijnen JH, Haribabu B, van de Lest CHA, Voest EE. Lysophospholipids secreted by splenic macrophages induce chemotherapy resistance via interference with the DNA damage response. Nature Communications, 2014; 5:5275

Lolkema MP, Gadellaa-van Hooijdonk CG, Bredenoord AL, Kapitein P, Roach N, Cuppen E, Knoers NV, Voest EE. Ethical, legal and counselling challenges surrounding the return of genetic results in oncology. J Clin Oncol 2013;31:1842-8

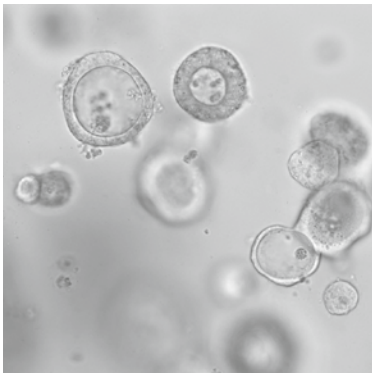
Roodhart JML, Daenen LGM, Stigter ECA, Prins H-J, Gerrits J, Houthuijzen JM, Gerritsen MG, Schipper HS, Backer MJG, Amersfoort M, Vermaat JSP, Moerer P, Ishihara K, Kalkhoven E, Beijnen JH, Derksen PWB, Medema RH, Martens AC, Brenkman AB, Voest EE. Mesenchymal Stem Cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell, 2011;20:370-383

Host responses and Personalized Medicine
EMILE VOEST

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

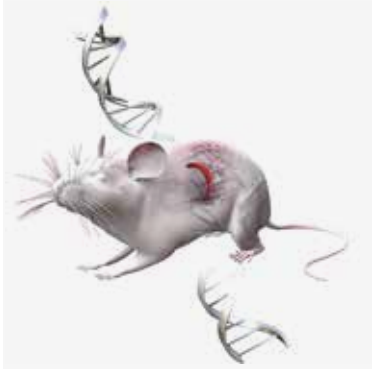
Host Responses

"My group focuses on two different approaches to improve treatment outcome. First, we are investigating the impact of chemotherapy on normal cells with emphasis on bone marrow derived stem cells, mesenchymal stem cells and macrophages. These cells become activated when the host is exposed to specific chemotherapies and migrate to tumors or release very active fatty acids that render tumor cells resistant to DNA damage. The field of fatty acids is novel and many mechanistic questions remain presently unanswered."



Organoids and Genomics

"Second, we are increasingly capable of culturing tumor cells from individual patients which potentially has the power to develop an in vitro assay that will predict treatment outcome. Tumor organoids and organoids from normal epithelial cells may be used to determine responsiveness of individual tumors to chemotherapy and determine an individual toxicity profile on normal tissue. Together with Hans Clevers from the Hubrecht Institute, we perform both clinical and preclinical studies to further develop this technology. In line with this approach an extensive genomics initiative (the Center for Personalized Cancer Treatment) is currently being rolled out in the Netherlands. Patients at the start of their treatment are undergoing biopsies for genetic testing and treatment outcome is monitored. This allows us to generate a database that will facilitate research at multiple levels: bioinformatics, systems biology, biology, clinical trials. The translational studies are truly teamwork with many scientists and clinicians at the institute and beyond."



Division of Molecular Pathology

Selected publications

Elshof LE, Tryfonidis K, Slaets L, van Leeuwen-Stok AE, Skinner VP, Dif N, Pijnappel RM, Bijker N, Rutgers EJT, Wesseling J. Feasibility of a prospective, randomised, open-label, international multicentre, phase III, non-inferiority trial to assess the safety of active surveillance for low risk ductal carcinoma in situ - The LORD study. Eur J Cancer 2015;51:1497-1510

Mascini NE, Eijkel GB, Brugge Ter P, Jonkers J, Wesseling J, Heeren RMA. The use of mass spectrometry imaging to predict treatment response of patient-derived xenograft models of triple-negative breast cancer. J Proteome Res. 2015; 14: 1069–75.

Lips EH, Mulder L, de Ronde JJ, Mandjes IA, Koolen BB, Wessels LF, Rodenhuis S, Wesseling J. Breast cancer subtyping by immunohistochemistry and histological grade outperforms breast cancer intrinsic subtypes in predicting neoadjuvant chemotherapy response. Breast Cancer Res Treat. 2013 Jul;140(1):63-71.

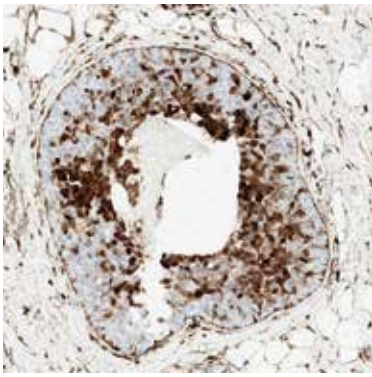
Breast Cancer Biomarkers
JELLE WESSELING

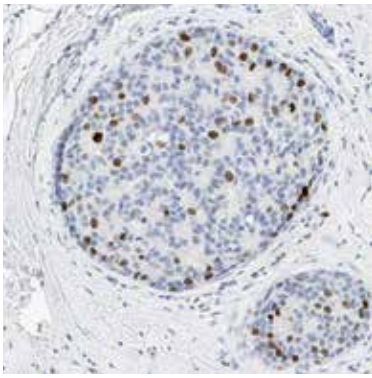
Breast cancer is a highly heterogeneous disease. This makes it a challenge to identify the most effective drug for each individual patient. Jelle Wesseling's group focuses on optimizing personalized diagnosis and treatment of breast cancer, by searching for novel features predicting treatment sensitivity. His group uses a combination of pathology, molecular analyses, and epidemiology. To predict disease outcome and effect of treatment, they analyze the genetics and the molecular mechanisms underlying disease progression and treatment resistance in patient samples and animal models. Ultimately, they aim to develop routine prognostic and predictive tests to tailor treatment to the individual cancer patient.

Treatment response prediction

"Our first research line focuses on the molecular mechanisms involved in drug response and disease progression. The aim is to improve outcome of patients for the three main subtypes of breast cancer: triple negative breast cancer, luminal breast cancer, and HER2-positive breast cancer. Using patient-derived xenografts of triple negative breast tumors in mice, which we treated with anti-cancer drugs such as DNA cross-linking agents or the PARP inhibitor olaparib, we have shown that tumors that are BRCA1-deficient are initially sensitive to these agents but inevitably develop resistance. Using molecular tools we could show that this resistance was caused by epigenetic changes and complex genetic rearrangements that restore BRCA1 function.

Luminal breast cancer responds poorly to neoadjuvant chemotherapy and there is an urgent need for accurate predictions of response to systemic treatment. Therefore, we have developed and validated a novel computational approach to identify the genes involved in treatment resistance. In addition, we have also been developing a test for BRCA2-like luminal breast cancer, which is known to respond better to therapy. This may aid improved tailored treatment choice in the near future."





Preventing overtreatment

"The second research line focuses on Ductal Carcinoma In Situ (DCIS), a very common non-invasive precursor of breast cancer. The majority of DCIS lesions is detected by breast cancer screening due to the presence of calcifications seen on the mammogram. DCIS lesions range from being non-hazardous and low-grade to more aggressive high-grade lesions. The high-grade lesions are much more likely to become invasive. For the low grade lesions, substantial overdiagnosis and hence overtreatment exists. We aim to identify biomarkers helping to avoid overtreatment of the non-hazardous DCIS by discriminating these from the aggressive ones. Ultimately, this will help to save women with low risk DCIS intensive treatment while giving the high risk lesions all necessary treatment"

Division of Molecular Carcinogenesis

Selected publications

Lee E, de Ridder J, Kool J, Wessels LFA, Bussemaker HJ. Identifying regulatory mechanisms underlying tumorigenesis using locus expression signature analysis. Proc Natl Acad Sci U S A. 2014;111:5747-52

Farazi TA, Ten Hoeve JJ, Brown M, Mihailovic A, Horlings HM, van de Vijver MJ, Tuschl T, Wessels LFA. Identification of distinct miRNA target regulation between breast cancer molecular subtypes using AGO2-PAR-CLIP and patient datasets. Genome Biol. 2014;15:R9

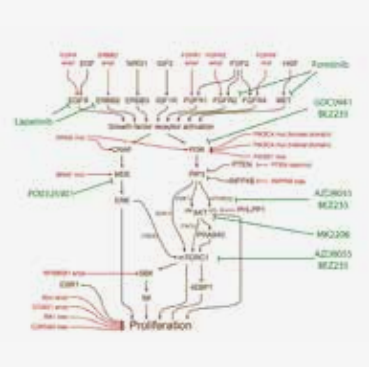
Akhtar W, de Jong J, Pindyurin AV, Pagie L, Meuleman W, de Ridder J, Berns A, Wessels LFA, van Lohuizen M, van Steensel B. Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell. 2013;154:914-27

Computational Biology
LODEWYK WESSELS

Lodewyk Wessels was trained as an electrical engineer and worked in machine learning and control systems before joining the NKI in 2006 as a computational biologist. In his first project at the NKI he applied machine learning algorithms to predict the BRCA1 mutation status of breast cancers. These classifiers are now used in the clinic to screen patients and to select those that will best respond to specific types of chemotherapy. Since then he has established computational biology at the NKI and heads the Computational Cancer Biology group. This group actively pursues a number of computational biology research themes.

Classifying cancer to select the best therapy

"The BRCA1 classifier is a typical example of one of the themes we work on: building predictors of disease outcome and response to specific therapies. To do this we combine various genome-wide data sources that reflect, for example, the genetic makeup and gene and protein activity in a tumor sample. We also focus on developing interpretable computational models in order to facilitate our understanding of the associated biology, and to enable the formulation of new hypotheses and the design of new experiments to test these hypotheses. To this end we develop approaches to identify genes that collaborate or can substitute for each other in the development of cancer and in the development of drug resistance. In these approaches we exploit the fact that collaborating genes are frequently simultaneously mutated in cancer whereas genes that are functionally redundant will seldom be mutated together."



Cancer Systems Biology Center

"To tailor treatments to specific cancer types, we have established the Cancer Systems Biology Center. In this center we focus on signaling pathways that are frequently associated with many cancer types with the goal of finding novel therapeutic strategies to block them. We employ cell lines, mouse models, and high throughput screening combined with computational modeling to build accurate models of cancer-associated signaling pathways. We use these models to develop strategies to block the activity of the pathway with single drugs or combinations, in order to induce selective cancer cell death. In collaboration with the Wellcome Trust Sanger Institute in Cambridge we also perform data mining on a panel of 1000 cell lines exposed to 400 anti-cancer drugs to discover molecular characteristics (biomarkers) that predict sensitivity to single and combination therapies. These biomarkers and targeted strategies could be used for personalized medicine, to more effectively treat individual cancer patients in the clinic."

Division of Cell Biology II

Selected publications

CL Zuur, AJ Dohmen, MWM van den Brekel, XJ Wang and SP Malkoski. Preclinical Models of Head and Neck Squamous Cell Carcinoma. Chapter 12 in "Head and Neck Cancer: Multimodality Management", 2nd Edition 2015 in press, editor Dr. Jacques Bernier, Springer Science.

A new grading system for ototoxicity in adults. Theunissen EA, Dreschler WA, Latenstein MN, Rasch CR, van der Baan S, de Boer JP, Balm AJ, Zuur CL. Ann Otol Rhinol Laryngol. 2014 Oct;123(10):711-8.

Head and Neck Cancer treatment
LOTJE ZUUR

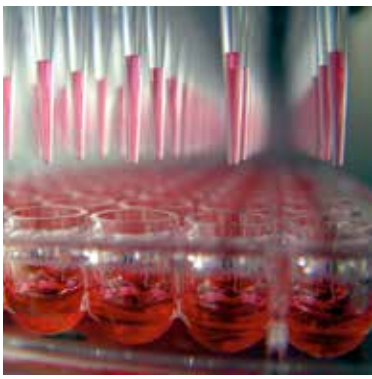
Lotje Zuur is a head and neck surgeon who divides her time between clinical patient care in the Antoni van Leeuwenhoek Hospital and her research at the departments of Cell Biology and Immunology in the Netherlands Cancer Institute. As a clinician she is taking care of head and neck cancer patients who suffer relatively limited survival and substantial toxicity from therapy. Therefore, her research group focusses on exploring personalized novel treatment options for these patients with improved cure rates and better quality of life.

Novel radio-sensitizing compounds

"A majority of patients with head and neck cancer (HNC) presents with advanced stage disease and have a 5-year overall survival of 40% or less. The mainstay of treatment is extensive surgery and/or the addition of high-dose cisplatin to radiation therapy (RT) followed by severe toxicity in at least 70% of patients. The research group of Zuur exerts a strong collaboration with the Neefjes and Ovaa laboratories to, first of all, focus on finding alternatives for cisplatin to optimize cure rates and personalize treatment, while reducing toxicity. Automated drug screens are employed to identify novel radio-sensitizing compounds targeting tumor specific genetic pathways. Different chemical libraries are tested for their biological effects on different in-vitro head and neck cancer models. Thereafter, to improve lead structures, several novel analogue chemical structures are generated. These studies potentially will improve radiotherapy outcome (also after surgery) for HNC patients."

Immunotherapy

"Secondly, the research group has started a collaboration with the Schumacher laboratory to explore the role of immunotherapy in patients treated with surgery for head and neck cancer. Again, Zuur focusses on patients in the highest need for improvement of prognosis and quality of life after treatment. Tumor tissues of patients with advanced Human Papilloma Virus (HPV) negative and positive disease will be investigated in-vitro to assess immunogenicity and the influx of immune effector cells (tumor-specific T cells) to provide a rationale for the actual clinical use of specific immunotherapies in well-defined subgroups of HNC patients. Clinical trials will be initiated."



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45493	49499	41793	23119
49460	58041	44633	14992
71342	53288	42208	12789
51099	29691	43533	9226
73964	46128	58833	6704
53051	24604	52756	5829
4786	4645	4864	4749

Division of Molecular Pathology

Selected publications

Rosell M, Nevedomskaya E, Stelloo S, Nautiyal J, Poliandri A, Steel JH, Wessels LF, Carroll JS, Parker MG, and Zwart W. Complex Formation and Function of Estrogen Receptor alpha in Transcription Requires RIP140. Cancer Res, 2014;74(19): 5469-5479

Oosterkamp HM, Hijmans EM, Brummelkamp TR, Canisius S, Wessels LF, Zwart W, and Bernards R. USP9X Downregulation Renders Breast Cancer Cells Resistant to Tamoxifen. Cancer Res, 2014;74(14):3810-3820

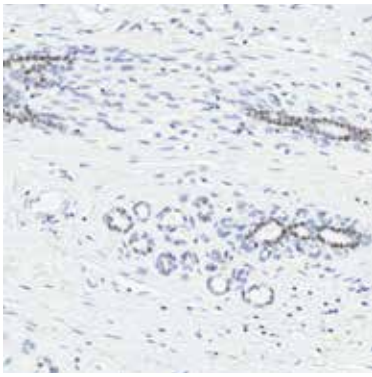
Jansen MP, Knijnenburg T, Reijm EA, Simon I, Kerkhoven R, Droog M, Velds A, van Laere S, Dirix L, Alexi X, Foekens JA, Wessels L, Linn SC, Berns EM, and Zwart W. Hallmarks of aromatase inhibitor drug resistance revealed by epigenetic profiling in breast cancer. Cancer Res, 2013;73(22):6632-6641

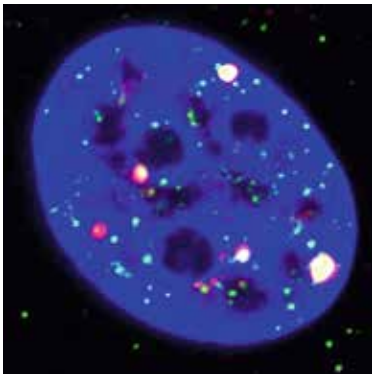
Hormone-Associated Cancers
WILBERT ZWART

Wilbert Zwart joined the NKI as a junior group leader in 2011. He is interested in the biology of hormonal tumors, including breast cancer, endometrial cancer and prostate cancer, and how this links with patient response to treatment in the clinic. Hormone-related cancer types share one crucial characteristic: tumor induction and/or tumor cell proliferation is dependent on hormonal regulation. Drug treatment is often directed at inhibiting hormonal action, but resistance is common. His group combines genomics, molecular pathology and molecular endocrinology, which are directly correlated with clinical analyses, to determine which patients would benefit the most from a specific treatment.

Matching Therapy to Patients

"Hormonal regulation plays a major role in cancer. Breast cancer is the most frequently diagnosed malignancy among women, with 1.4 million newly diagnosed cases each year. Around 75% of all breast tumors are thought to depend on the activity of the Estrogen Receptor α (ERα). Inhibition of this transcription factor by hormonal therapies is therefore a major treatment modality of these tumors. Still, resistance to treatment is common. We are interested in identifying predictive markers that can be used to select the most effective hormonal treatment for individual patients, as well as elucidating the underlying processes driving treatment response. My group is also studying prostate cancer, which is one of the most prevalent cancers in males, with 1 million newly diagnosed cases each year. When the disease is confined to the prostate, patients can be treated with a curative intent. However, the disease will recur in 30% of patients, most frequently with macro-metastasized disease, which cannot be cured. Therefore, prevention of recurrences and metastatic outgrowth is one of the biggest challenges in prostate cancer research. Previous studies suggest that hormonal therapy can eradicate micrometastases, and we aim to develop predictive biomarkers that can identify the patients who would benefit from such adjuvant hormonal therapy."

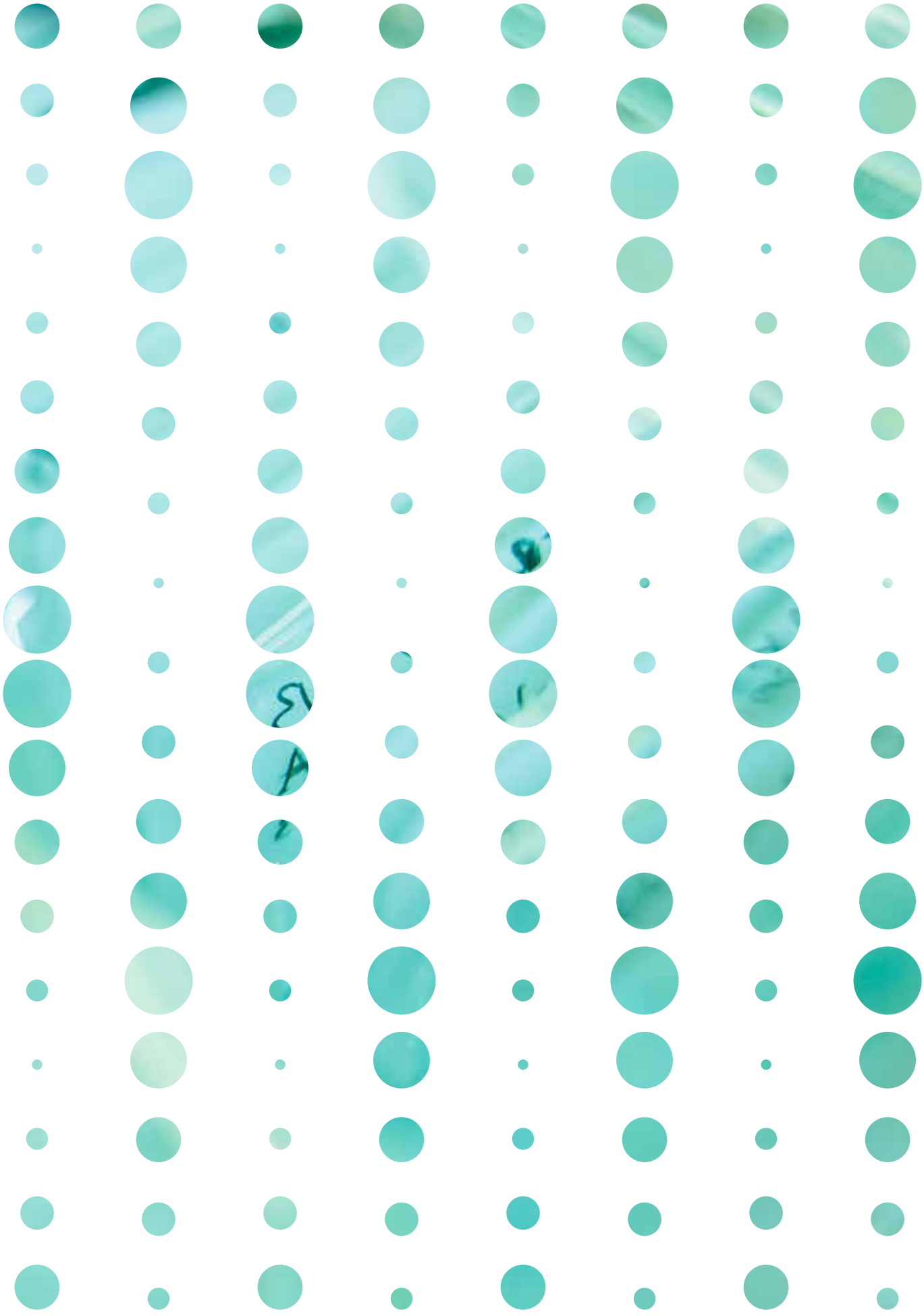




Novel Applications for Existing Drugs

"While most hormonal therapies are designed to treat a specific disease, their targets are seldom tissue exclusive, nor are the drugs truly tumor type specific. This lack of tissue exclusivity of drug target expression generates multiple opportunities of translatability of medications between tumor types. We are currently systematically assessing sensitivity to all hormonal therapies for a number of tumor types, for which no targeted therapy is currently available. With this, we aim to not only identify novel applications of currently available drugs, but also to enable an efficient and swift introduction of our findings in the clinical setting."

CLINICAL RESEARCH



Diagnostic Oncology

The division of Diagnostic Oncology is at the hub of interactions between clinical departments and research departments. It comprises of the departments of Radiology, Nuclear Medicine, Pathology, Clinical Chemistry, Clinical Physics and the Family Cancer Clinic. Diverse as these departments are, they have a common focus: to improve the diagnostics and thereby treatment as well as follow-up of different types of cancer, using the latest technologies. The main research focus lies on personalized medicine and image guided therapy.

Cancer is a heterogeneous disease, requiring an individualized approach for optimal diagnosis and treatment. The role of the departments involved in diagnosing and staging disease has evolved into a leading position in this respect, as cancer treatment and prognosis are based on a combination of diagnostic test results. The diagnostic departments focus their research on two main topics defined in the Antoni van Leeuwenhoek hospital: image guided surgery and personalized medicine. For this purpose, a strong collaboration between the departments is a prerequisite for success.

Pathology

The department of Pathology is at the crossroads between the research from bench to bedside at the NKI and performs both routine diagnosis and translational research on human tissues with the patient's consent. Over the past 45 years, the department has amassed a considerable bank of paraffin embedded and frozen tumor tissue, which supports research across all departments of the NKI. Currently, most research questions that concern the Pathology department relate to finding, validating, and implementing prognostic and predictive biomarkers, combined with tumor classification issues. For this, molecular techniques have become increasingly important. Better biomarkers can lead to better, personalized treatment. A clinically relevant example is the 70-gene signature test or 'Mammaprint' predicting for a substantial group of breast cancer patients the risk on development of metastases. This helps to decide whether additional chemotherapy is indicated. Likewise, a molecular test was developed in our department detecting a cellular defect (DNA repair) making these tumors more sensitive to particular types of treatment.

Clinical Chemistry

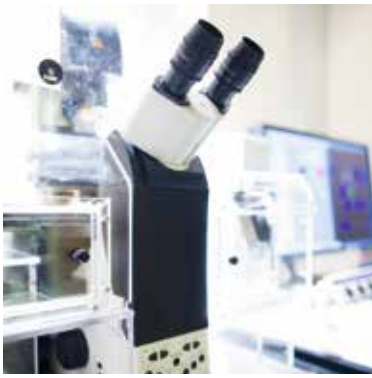
The main focusses of the department of Clinical Chemistry are, besides the analysis of clinical samples, the development and clinical validation of biomarkers and the support of many clinical trials. This department focuses on biomarkers in fluids, including proteins, circulating tumor DNA and miRNA's in serum or blood. Worldwide the knowledge about associations between specific biomarkers, genetic mutations and treatment options are rapidly increasing within research settings. The department of Clinical Chemistry aims to establish the clinical relevance of such markers. Ongoing projects are for example focused at HE4, a biomarker with a good specificity and sensitivity in predicting ovarian cancer. The evaluation of analytical and clinical aspects of circulating tumor DNA, improvement of current assays (testosterone, estradiol), the development of new assays (serotonin, steroid panels) using LCMSMS and investigation of new clinical applications.

Biobanks

To ensure adequate tissue and body fluid banking and subsequently proper use of the samples, the Core Facility Molecular Pathology & Biobanking was founded in 2011. This facility registers, coordinates, assists and facilitates research involving archived patient material. The archived material includes the formalin fixed paraffin embedded block archive and the fresh frozen tissue bank from the Pathology department. The biobanks of the department of Clinical Chemistry contain whole blood and other components such as plasma or platelets, but also other body fluids such as urine or cerebrospinal fluid.

Family cancer clinic

Some methods for DNA testing have been



around for a while. In 1995 the Family Cancer Clinic was opened at the NKI. In the past few years, the number of families who have sought genetic testing and counseling through our hospital has risen to 1400 patients a year. Most families are referred because of a possible genetic predisposition for breast or ovarian cancer. Other indications include Lynch syndrome, familial renal cancers, pancreatic cancer, stomach cancer and melanoma.
The Family Cancer Clinic also contributes data to several international and national research projects, like the GEO-HEBON study (Gene-Environment interactions in Hereditary Breast and Ovarian cancer). This long-term, nation-wide study is looking at how genetic and other factors, such as lifestyle, diet and hormonal changes during breastfeeding may alter the risk of breast or ovarian cancer in people with a family history of these diseases.

Radiology

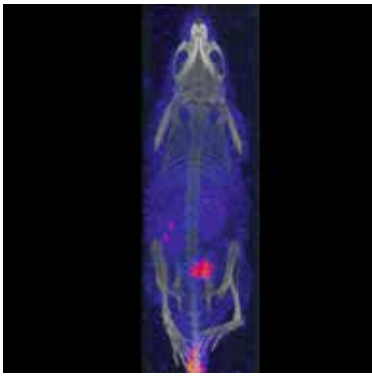
Not all modern diagnostic tools concern molecular and genetic techniques. Two departments within the Division of Diagnostic Oncology are at the forefront of cancer diagnosis and monitoring using radiological and nuclear medicine imaging techniques, which provides important information about the tumor without being invasive. Their research activities are fully supported by the Clinical Physics Department. At the moment, the focus of the department of Radiology lies on the use of magnetic resonance imaging (MRI) to diagnose prostate and breast tumors and to measure the effect of the different treatment regimens. Shortly after initiating chemotherapy, patients can already be identified as responder or non-responder by using MRI, providing the option to switch cancer therapy when required in order to give the best treatment and to avoid side-effects. Also the introduction of image guided tissue sampling, for example in prostate cancer, has facilitated early diagnostic and response measurement studies. Finally, Interventional Radiology as a sub-specialization is highly involved in image-guided cancer treatment. Radio Frequency Ablation, chemo-embolization and radioembolization are typical examples of techniques that have been introduced over the past years in clinical practice and in research setting. Their role as alternative for surgical intervention will further increase in the upcoming years.

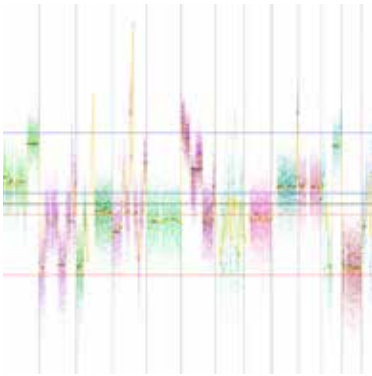
Nuclear Medicine

The Department of Nuclear Medicine also focuses on improvement of imaging techniques, but with a focus on the innovation and implementation of radiolabelled diagnostic and therapeutic agents. In close collaboration with the department of Radiology, new tracers are studied in the treatment of liver metastases from colorectal cancer and neuroendocrine tumors. Another topic of interest is the use of PET scans for guided radiotherapy, in close collaboration with the Division of Radiotherapy. Respiratory-gated PET/CT is used to improve delineation of RT margins in lung and esophageal cancer.
Personalized medicine, as an important overlying research focus at the NKI, is found within the department of Nuclear Medicine in the form of an emphasis on the introduction of new tracers and strategies. The introduction of new PET tracers for diagnosing and follow-up of cancer has currently an important application in melanoma, prostate cancer and neuro-endocrine tumors, but it is also applied in other tumors. In this respect, immunoPET is an important focus of research in which several radiolabelled antibodies or its fragments are part of many studies. By labelling new anti-cancer drugs with radionuclides, such as Trastuzumab, pre-treatment imaging can be used to assess whether an expensive pharmaceutical will be effective or not. In the next years, this Theranostics concept will be extended to other anti-cancer drugs.

Preclinical imaging facility

The completion of the new Mouse Cancer Clinic will also result in the formation of a new preclinical imaging facility specifically designed for translational research. The availability of advanced imaging modalities, such as PET/CT, SPECT/CT, IVIS and high-field MRI, enables the evaluation of processes like tumor growth, metabolism, receptor expression and therapy response at (sub)millimeter level in mouse models. The first preclinical study launched from within the department of Nuclear Medicine is Cisplatin SPECT, which started in 2013. Cisplatin is often used in combination with radiotherapy for the treatment of several types of cancer. An optimal imaging protocol for mice to study cisplatin-radiotherapy regimes has been developed. The ultimate goal of this pre-clinical study is to predict survival and toxicity of patients who receive cisplatin-based therapies.





Medical Oncology

The division of Medical Oncology is at the clinical end of the NKI's emphasis on translating laboratory results into clinical practice. The staff is devoted to improving cancer diagnosis and making treatment more effective for the individual patient.

Developing new therapies and procedures is a two-way process. Many new concepts and tools originate in the NKI's laboratories, but their clinical usefulness is uncertain until tested in clinical trials. At the same time, questions arise in clinical practice that inspire new laboratory studies. To improve protocols, for example, or to find new disease markers and drugs. The success of the division's research is due to extensive collaborations both within the NKI and with other institutions.

The NKI is one of the major referral centers for breast cancer patients in The Netherlands. Consequently, there is a large population of patients with breast cancer to which the NKI has access for research. The NKI is also the main Dutch referral center for patients with metastasized melanoma. Other types of cancer in which the NKI is specialized are head and neck tumors, gastrointestinal tumors and urological tumors. Many hundreds of patients a year take part in one of the approximately 200 clinical trials that are open at a given time.

Immunotherapy

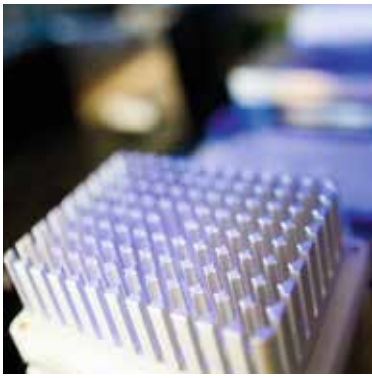
The head of the division of Medical Oncology, John Haanen, has a particular interest in immunology. Under his supervision the department has in recent years developed a focus on various forms of immunotherapy. The journal *Science* hailed immunotherapy as 'scientific breakthrough of 2013'. After decades of research, it is finally becoming clear how the body's immune system can be trained to recognize and kill cancer cells and how suppression of the immune system by the tumor can be overcome. One of the successes within this division is the development of a DNA vaccine for the treatment of high-risk HPV associated cancers. This vaccine will in the coming years be tested in a phase I/II clinical trial, in patients with cancer of the head and neck area, the penis and the cervix. There are also ongoing tests with T-cell receptor gene therapy in melanoma patients.

Another successful form of immunotherapy that was tested within the division is the TIL-study (TIL stands for Tumor Infiltrating Lymphocytes). This study was a collaboration between John Haanen and Ton Schumacher from the research division of Immunology and was carried out between 2011 and 2013. In patients with metastasized melanoma, T-cells that were active against cancer cells were isolated from the tumors. These T-cells were then cultured in the laboratory, until massive amounts of them could be given back to the patient, in combination with chemotherapy and a high dose of IL-2. The idea behind this was that flooding the tumors with these cells could strengthen the immune response that was already present but before was too weak to kill the cancer cells. The response rate in the ten treated patients was about fifty percent. Two patients showed complete remission. In September 2014 a phase III clinical trial has started in which TIL therapy is compared to treatment with the monoclonal antibody Ipilimumab, also a form of immunotherapy, as first or second line treatment for patients with stage IV melanoma.

Melanoma patients have thus far benefited most from immunotherapy. But immune system based therapies for other types of cancer are currently being developed. Within the NKI division of Medical Oncology, immunotherapy is also investigated as an option for patients with renal cancer, bladder cancer and lung cancer.

Personalized Medicine

Next to immunotherapy, another field of research and treatment that has gained importance in the past few years is personalized medicine. As genetic techniques improved over time it has become clear that tumors should not primarily be classified based on the



affected organ. The reason why cells become cancerous lies within their DNA. It turns out that there are hundreds of different mutations that can cause cells to divide uncontrollably and become a tumor. Instead of treating tumors with a broad chemotherapeutic agent that affects all fast dividing cells, the focus has shifted to developing drugs that are designed to target the specific mutated genes or faulty proteins within individual tumors. This has led to the development of drugs like vemurafenib for melanoma patients with a BRAF mutation. Patients with other types of cancer that harbor a BRAF mutation might also benefit from this drug.

In collaboration with other research departments within the NKI, the division of Medical Oncology focuses on finding new drugable targets with the use of genomics and deep sequencing. New types of drugs against specific mutations as well as combinations of these drugs are tested in clinical trials. A nice example is the collaboration between Jan Schellens from the division of Medical Oncology and René Bernards from the research division Molecular Genetics. Together they have developed combination therapies with targeted drugs for patients with colon cancer. Another example is the collaboration with Jacques Neefjes from the research division Cell Biology II. For this project, cell cultures taken from the pleural fluid of patients with malignant mesothelioma are subjected to a large number of different enzymes and drugs to see which might benefit the individual patient. Promising new drugs are tested in animal models.

The NKI has also joined forces with two other major Dutch cancer centers, the Erasmus MC and the UMC Utrecht, in the Center for Personalized Cancer Treatment (CPCT). The aim of this organization is to offer patients tailor-made medical care, by analyzing the genetic material of the tumor at the start of the course of treatment and base the treatment on the outcome of this analysis.

Response prediction

Genetic techniques can also be used for other purposes than identifying drugable targets. Genetic profiles, which are like DNA fingerprints, can for instance be used to predict how likely it is that a patient will develop metastases or that the patient's tumor will respond to certain existing treatments. Sabine Linn and Gabe Sonke focus on predicting the response and prognosis of breast cancer patients who are given hormonal therapy or chemotherapy. There is also a large multidisciplinary program that focuses on response prediction in women with different subtypes of breast cancer who are given preoperative chemotherapy. At the end of 2013, data and tissues of approximately 750 patients were collected for this program.

Within the subdivision of urologic oncology, there is an ongoing study that focuses on finding predictive biomarkers and RNA fingerprints to determine the outcome of prostate cancer progression.

Therapeutic drug monitoring

A vital part of assessing a new treatment strategy with either existing or new drugs is monitoring their effects in patients. The subdepartment of Pharmacy and Pharmacology, led by Jan Schellens and Jos Beijnen, monitors patients during clinical trials to determine (for example) which doses are most effective and how to minimize side effects. Within some projects, patients are also screened for genetic differences that could indicate how long a drug persists in the body. Ultimately, this kind of therapeutic drug monitoring could help to establish tailored dose regimes for each individual.

The pharmacology department is also involved in the different phases of development of new drugs and in finding the best way to administer a drug. A nice example of the latter is the development of an oral administration form of taxanes, a class of commonly used chemo therapeutics. Traditionally taxanes are administered intravenously, because when they are given orally they don't reach the blood stream. Research within the NKI showed that certain proteins within the colon prevent uptake of the drugs. But these proteins can safely be inhibited for some time. Based on these results the pharmacology department developed an oral form of two taxanes that are administered in combination with the protein inhibitor. This method has proven to be safe and efficient in 350 patients.

Surgical Oncology

The Division of Surgical Oncology is a large and diverse department, with 64 academic staff members, 16 medical residents and more than 20 research students. The division's research interests are for a large part focused on bringing modern technologies into the operating room. But the research also reaches beyond surgery itself, into areas such as immunotherapy.

The main research interest within the division is image guided surgery. It often isn't easy to differentiate between tumor tissue and healthy tissue with the naked eye. To minimize the risk of tumor tissue staying behind, surgeons remove tumors with a broad margin, taking out surrounding healthy tissue as well. The downside of this is that it can affect organ function or induce morbidity. Division head Theo Ruers is specialized in translating modern technology to applications in operating rooms. New imaging techniques and surgical guidance procedures should lead to more radical resections while sparing normal tissue and organ function. To develop such new techniques, the NKI works in close collaboration with the Technical University of Twente and various industrial partners.

One of the prime new techniques that's currently being developed is the surgical equivalent of Google Earth. The research group of Ruers is working on an innovative solution for bringing navigational equipment into the OR. Using pre-operative, high-resolution anatomical images, a sophisticated 3D roadmap of the tumor and surrounding tissue is fabricated. Surgical instruments can be tracked and superimposed on this 3D map in real time, using an augmented reality setting. This way, surgeons can get a better view of the tumor and vital surrounding tissues, and better recognize the right dissection planes. The new operating complex of the clinic, which is being built in 2014, will contain a hybrid OR with an incorporated radiology unit that has all the equipment necessary for this navigational technology.

Optical tools

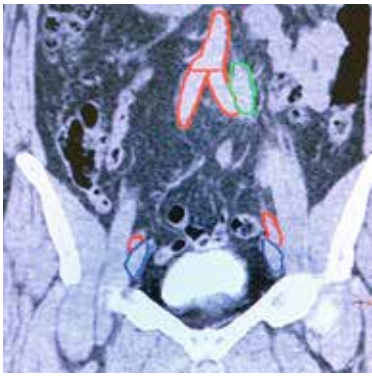
Another prime example of a new image guided surgical technique is the optical needle that is being developed together with an industrial partner. By means of spectroscopy and fluorescence, this biopsy needle can differentiate between healthy tissue and tumor tissue, and thus improve the quality of biopsies. In tissue samples of a hundred patients the needle proved to be 90 percent accurate at recognizing tumor tissue. This result was validated by extensive in vivo testing in over a hundred patients with breast cancer, liver metastases and lung tumors. In 2013 the Percupsect study was started, in which the new optical biopsy tool will be tested in the radiological work flow. It is also currently being incorporated into smart surgical practice to allow accurate tissue differentiation during surgery.

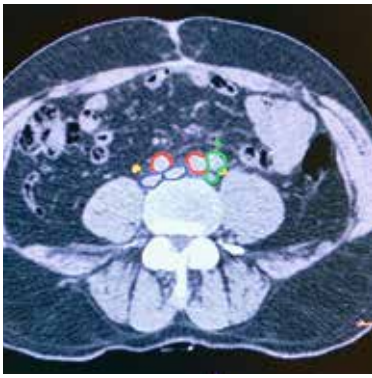
Da Vinci Robot

Optical techniques can also be used to detect lymph nodes.Urologist dr. Henk van der Poel focuses on detecting lymph node metastases using fluorescence techniques. The new operating robot Da Vinci Si®, that came in use in 2014, is expected to be of great help for this. The robot is equipped with a camera head that can detect near red fluorescence. It is the second operating robot within the surgical department. Its predecessor, an older type of the Da Vinci, was used for surgical procedures in prostate cancer, bladder cancer, kidney cancer, head-neck tumors and gynecological cancers. The new robot will also be used to remove tumors from the rectum, lungs and liver. It is expected that it will be even better at precision surgery than the older model, due to the near infrared camera and other imaging improvements.

Radioactive seeds

Neoadjuvant chemotherapy has become the standard treatment for a subset of breast cancer patients in the clinic of the NKI. Locating the tumor and determining the lymph node status after this pre-operative form of chemotherapy can be hard. Marie-Jeanne Vrancken Peters started





a project in which radioactive iodine seeds are implanted before the administration of the chemotherapy. Her study showed that the use of these seeds is a valuable tool for fine-tuning breast-conserving surgery after neoadjuvant chemotherapy. She expects that a review of the results of the first 1000 implanted seeds will be published in 2014.

In recent years, it has become more and more recognized that not all breast cancer patients need adjuvant chemotherapy. For patients with a low risk of recurrence, chemotherapy may be unnecessarily damaging. Almost a decade ago, the so-called 70-gene signature test or MammaPrint was developed at the NKI. This test was developed to evaluate the aggressiveness of the tumor and determine the risk of distant metastases. Prof. dr. Emiel Rutgers supervised multiple trials that evaluated the clinical use of the MammaPrint. The first prospective data became available in 2011, and the 5-year follow up results of the RASTER study showed an excellent 100 percent recurrence free rate in patients who did not receive adjuvant chemotherapy based on their low risk 70-gene signature result.

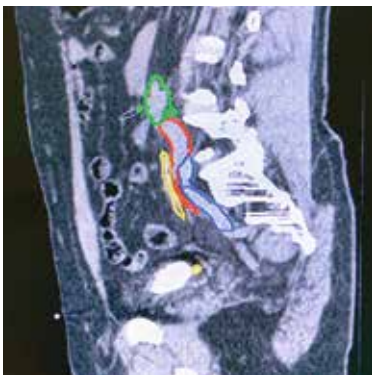
Maintaining function

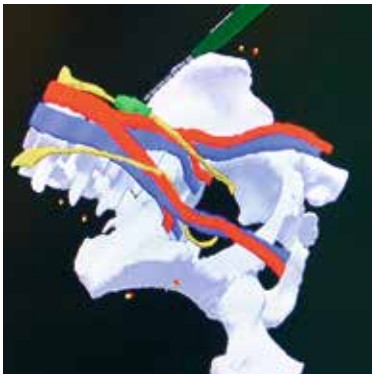
Cancer of the head and neck is particularly problematic to treat because it is difficult to remove tumors in this region without damaging vital tissues and organs. The division is involved in multiple projects that aim to spare healthy tissue and organ function. One of these innovative projects is led by Frans Balm of the Head and Neck department. Together with the University of Twente he develops digital tools that can predict function loss after surgery. Using state of the art imaging techniques and modeling tools he will develop 3D models of the head and neck region of patients, which can be used to accurately predict how, for instance, speech will be affected by the surgery.

Immunotherapy

Immunotherapy is a recent but important research focus within the NKI. Within the division of Surgical Oncology, it is applied by the gynecology and dermatology department. Gemma Kenter focuses on the possibilities for immunotherapy in HPV related tumors. A phase I/II trial with a DNA vaccination for patients with HPV related squamous cell carcinomas of the anogenital or the head and neck region will start in 2014. This trial is part of a European project to study the molecular pathways and potentials for innovative drugs in advanced cervical carcinoma.

Melanoma is a good candidate for treatment with immunotherapy, during which vitiligo development (discoloration of the skin) is a good sign. In collaboration with the dermatology department of the AMC, a new therapy against melanoma was developed, based on the potent vitiligo-inducing effect of monobenzone combined with the immunostimulatory adjuvant imiquimod. This treatment has shown to effectively eradicate melanoma in mice. Based on this data, a phase II clinical trial was started with this new therapy for stage III-IV melanoma patients within the NKI in 2011. At the moment of writing, this trial is still in effect.





Radiation Oncology

The NKI enjoys the privilege of being linked with one of the top radiotherapy departments in the world. Not only does it have the latest commercially available equipment, it also has an integrated team of technology experts, scientific researchers and healthcare providers who are constantly developing new ways of making radiation treatments more effective and less toxic. This is done by personalizing treatment to better fit individual patients, as well as improving the technology and inventing new strategies.

The work within the division of Radiation Oncology builds on more than a century of innovation using ionizing radiation to treat cancer. Soon after German physicist Wilhelm Conrad Röntgen discovered X-rays in 1895, doctors started experimenting with radiation to combat cancer. By 1920 X-rays had become a major weapon in their armory. Although other types of treatment are now available, radiation therapy is still the major therapy for more than half of the cancer patients. The history of radiotherapy in the Netherlands Cancer Institute, which was founded in 1913, can be viewed on the website historad.nl, launched to celebrate the 100th anniversary of the NKI.

Nowadays the NKI radiotherapy department focuses on two major scientific research themes: targeted radiosensitization and image guided adaptive therapy. The aim of both themes is making radiotherapy more effective at killing cancer cells while leaving healthy cells as unscathed as possible. Targeted radiosensitization involves the search for chemicals and biologicals that, when combined with radiation, massively increase its impact. The trick is to find compounds that selectively sensitize cancer cells but not healthy cells. Image guided adaptive therapy is the field of research that revolves around improving imaging techniques to better pinpoint the location of the tumor at the moment of treatment, adapt the radiation treatment accordingly, and closely monitor the effect of the radiation. This minimizes the damage to healthy tissue and thus side effects of the treatment.

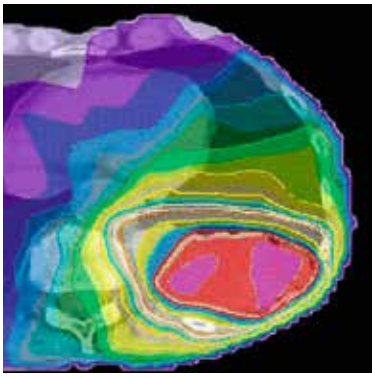
Combination therapies

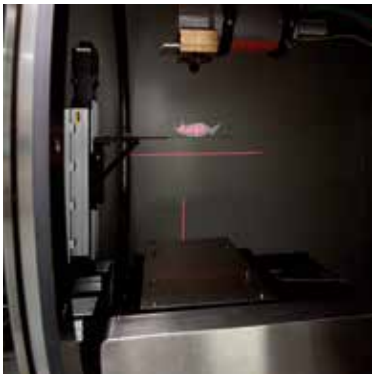
Division head Marcel Verheij has been involved in the search for new combination therapies to improve the effect of radiotherapy for years. Back in 1992, researchers of the NKI published a pioneering paper that showed that adding the cytotoxic drug cisplatin to radiotherapy dramatically improved therapy outcome. Cisplatin makes cancer cells even more sensitive to the radiation. Today, nearly all solid tumors that are treated with radiation therapy receive combination therapy. Sometimes with cisplatin, but also with other, more tumor-specific drugs.

At the moment, Verheij and his colleagues focus on drugs that affect DNA repair mechanisms as candidates for combination therapy. One of the compounds that are currently under interest are so-called PARP-inhibitors. When given as a single agent, PARP-inhibitors selectively kill cancer cells that are not able to repair double strand breaks in their DNA. Laboratory studies have showed that PARP inhibitors increase the response to (chemo) radiotherapy. Phase I and II studies to test this double or triple combination therapy in patients with locally advanced breast cancer, non-small cell lung cancer and head and neck cancer are currently taking place.

Response prediction

The research group of Verheij is also interested in predicting sensitivity to radiotherapy. For this, the group uses RNA screens and developed software scripts to analyze combinations of different types of RNA. Their technique was recently used in a pilot study which analyzed RNA sequence data of patients with larynx carcinomas, treated with radiotherapy in the NKI over the last ten years. They have also performed siRNA lethality screens on prostate cancer cells to identify molecular determinants of radiation susceptibility.





The NKI hosts an extensive mouse facility, including a specialized mouse clinic. One of the most outstanding pieces of equipment in this clinic is a mouse sized image-guided irradiator. This irradiator is basically a miniature version of the ones that are used to treat human patients, including a radiation source and an integrated cone-beam CT scanner. This equipment has proven to be a very valuable tool for evaluating new tumor-specific radiosensitization strategies. With the mouse irradiator and the advanced mouse models of the NKI, researchers from the division of Radiation Oncology are able to closely mimic human clinical practice, in terms of treatment set-up, scheduling and determining normal tissue toxicity.

Adaptive therapy

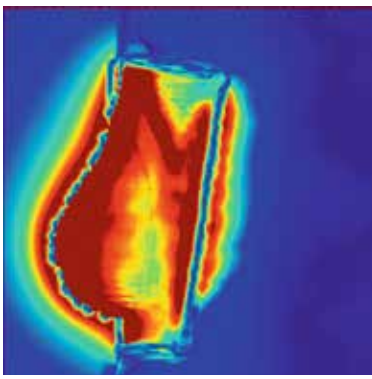
Personalized medicine is an important focus within the NKI. The term is most often used within the context of tumor specific drugs, but adaptive radiotherapy also is a type of personalized medicine. Tumors are seldom located in a fixed place. They shift due to the constant alteration of the intestines caused by breathing, the beating of the heart and movements of gas. Adaptive radiotherapy is the image guided feedback loop in which patients are continuously scanned, irradiated, and scanned again, after which (depending on the feedback) the treatment plan can be adapted.

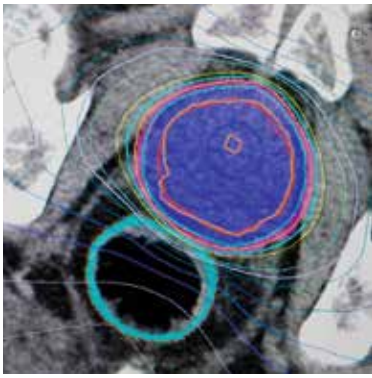
In the past decade, a cone beam CT that can be integrated with a linear accelerator was co-developed at the NKI. This enables checking patients right before treatment, when they are already positioned to receive the radiation. This technique is now the standard procedure in the NKI as well as other hospitals around the world. But the NKI research groups of Jan-Jakob Sonke and Uulke van der Heide are still trying to further improve imaging and guidance techniques. For instance by improving the software that analyses the Cone Beam CT images, by integrating anatomical and functional MRI scans and PET in radiotherapy and by improving image quality in free-breathing conditions.

Another important focus within the field of adaptive radiotherapy is in vivo dosimetry. The linear accelerators with an integrated cone beam CT contain a detector panel that was originally used for image guidance. Now that this role has been taken over by the CT, the panel can be used to measure the amount of radiation after passing through the patient, and thus the actual dose that was delivered. This is compared to the prescribed dose, and if there is a significant deviation, the treatment can be adapted. Clinically relevant deviations between the prescribed and received dose of radiation turn out to appear approximately once in every 300 treatments. They can arise due to unexpected anatomical changes as well as software errors.

ARTFORCE

One of the high-impact clinical studies currently taking place within the division of Radiotion Oncology is the ARTFORCE study. This study, which is a European collaboration, combines the two research themes of the department. Part of the study revolves around treating patients with head and neck cancer with the radiosensitizing drug cisplatin versus the drug Cetuximab. For the other part of the study, these drugs are combined with either standard radiation treatment or radiation treatment with dose redistribution. In the second type of treatment, the most resistant parts of the tumor receive a higher dose of radiation than other parts. This double design will hopefully lead to better ways to treat patients with these difficult types of tumors.





FACILITIES

Facilities

Modern day biomedical research depends on expensive equipment and on techniques that can take years of practice to do well. Individual researchers need to use a wide range of techniques in their work, and it is impossible for anyone to master them all or be given the money to buy all the equipment they are likely to need. The NKI has resolved this problem and used its funding in the most efficient way by creating dedicated centralized technology facilities that serve the whole institute. All NKI scientists have direct access to these facilities. Periodic review of the facilities ensures that they maintain a high standard. For a complete and detailed overview of all NKI research facilities and the contact details, please visit the NKI website (www.nki.nl).

Animal facility

Much of NKI's research is carried out on cultured mammalian cells and tissues, but cells act very differently in culture to the way they behave in the body. Consequently, cancer research can only progress by using animals for some experiments. Hundreds of mouse strains have been bred with particular genetic traits that make them ideal for research into cancer genetics as well as efficient models for testing new treatments.

At any one time the NKI houses approximately 25,000 mice, from almost 700 specially bred strains. Some mice are used for experiments, while others are held for breeding. The mice are housed in a new, state-of-the-art animal facility with much better living conditions for the mice and working conditions for the researchers. The animals' living conditions are carefully controlled and the welfare of every mouse is monitored daily. In the design of all animal experiments we incorporate the 3 R's (reduction, refinement and replacement). A system of tags and barcodes combined with web-based communication enables staff to alert a researcher immediately if an issue arises with an individual animal. The NKI further houses a very small colony of *Xenopus*.

Along with day-to-day care of the animals, animal facility staff help researchers carry out their experiments. This can range from weighing and routine sampling to performing complex operations and detailed tissue analyses at the end of the experiment.

Mouse cancer clinic

A new development is the 'mouse clinic'. This is intended to house mice with mutations that influence their responses to cancer and cancer therapy. The facility consists of an imaging unit equipped with radiotherapy equipment and advanced imaging systems, an intervention unit which enables scientists to treat and study the mice in ways comparable to treating humans in a clinic, a pre-clinical pharmacology unit, and a mouse model transgenesis unit. The mouse clinic is part of the Mouse Clinic for Cancer and Aging Research (MCCA, www.mccanet.nl) which received a major grant from the Netherlands Organization for Scientific Research (NWO).

Intervention unit

In the preclinical intervention unit, advanced mouse models are used as surrogate cancer patients to identify and validate targets that can be exploited by anti-cancer therapy. In close collaboration with our scientists, and sometimes in collaboration with scientists from pharmaceutical companies or other institutes, an intervention experiment is designed. The staff of the intervention unit will treat and monitor the mice carefully during the whole experiment. Via this strategy several classical and novel anti-cancer agents (chemotherapy, targeted inhibitors, immunotherapy, radiotherapy or combinations) that are eligible for clinical trials have been or are being tested.

Imaging unit

The preclinical imaging facility houses a 1 and a 7 Tesla MRI, a PET/CT, a USPECT/

CT, and an IVIS system. The diverse selection of both functional and anatomical imaging enables researchers to monitor and examine tumors in animal models. Anatomical imaging using MRI and CT allows researchers to monitor internal growth of tumors and enable longitudinal studies using a smaller number of animals. Functional imaging helps researchers examine tumor characteristics by imaging high glucose uptake with ^{18}F-FDG PET or by imaging hypoxia, perfusion, and diffusion with MRI. The combination of imaging modalities such as PET/CT, SPECT/CT, PET/MRI, or even PET/SPECT/CT/MRI gives researchers the ability to acquire comprehensive information about the physiological states of their test animals.

Pre-clinical Pharmacokinetics & Pharmacology unit

The work in the mouse cancer clinic involves intervention studies with (combinations of) agents, including many of the novel targeted agents that are already registered or still under clinical evaluation. The PK/PD unit manages the stock of these agents and provides advice on their formulation and use.

An important component of any intervention study involving novel agents is to acquire knowledge about the disposition of the compound(s) that is/are under investigation. To this end, the unit is equipped with dedicated HPLC-MS/MS and HPLC-UV/FD systems, whereas metal (*e.g.* platinum) containing drugs can also be analyzed using flameless atomic absorption. Within the PK/PD unit there is ample experience in setting up bio-analytical assay to support preclinical PK/PD studies.

The PK/PD unit is located at the General Clinical Laboratory (GCL) and it will also coordinate the measurement of hematological parameters (Hb, WBC, platelets) in blood samples from animals as this is being done using the Hematology analyzers of the GCL.

Transgenesis unit

Generating genetically engineered mice is a complex process that requires a good strategy and detailed planning. In consultation with the researcher we provide advice on the strategy and construct design for generating the mouse strain tailor-made to answer a biological question. Depending on the complexity and workload a practical plan is devised and in some case the researcher participates in cloning and/or embryonic stem cell (ESC) culture.

We typically employ two techniques to generate mice, 1) the Crispr/Cas9 technology in zygotes and 2) our in-house developed GEMM-ESC strategy. Crispr/Cas9 is ideal to create simple knockout or point-mutant strains with short development and production time. The GEMM-ESC strategy is perfectly suited to introduce targeted modifications in existing mouse models. The approach is based on the re-derivation of ESCs from existing genetically engineered mouse models (GEMMs). The re-derived ESCs, referred to as GEMM-ESCs, contain all the modified alleles present in the original strain and can be used for further genetic engineering, either by classic targeting, genome editing or recombinase-mediated transgene integration. Our unique archive of GEMM-ESCs currently consists of more than 50 unique genotypes and includes models for melanoma, breast- and lung cancer.

Besides mouse model development, the transgenic facility also offers cryopreservation of mouse strains. Depending on the complexity of the genetic background of the mouse model, sperm or embryos are frozen and stored at NKI's cryogenic storage facility. We also offer re-derivation of mouse strains by in vitro fertilization (IVF).

Animal pathology

The animal pathology facility has two pathologists and four technicians. They process tens of thousands of tissue samples per year. The technicians perform all histological techniques such as histochemistry and immunohistochemistry, including the optimization of new protocols. The pathologists offer the analysis of animals and tissues from experiments. They can be consulted during the whole scientific process from designing experiments to the writing of manuscripts. They are also involved in monitoring the health of the animal colony. In this way, the facility contributes to optimal collection and visualization of results, as well as correct scientific interpretation of in vivo experiments.

Genomics facility

Wet lab specialists and bio-informaticians are working together in a single facility to assist users from start to finish with deep sequencing projects. The Genomics Core Facility handles hundreds of projects

and thousands of samples annually. Users hand-in tumor tissue, cells, DNA or RNA as starting material, but also ready to sequence libraries prepared by users themselves. The origin of the material is often fresh (frozen) material but also paraffin blocks with formalin fixed tumors can be used. Users collaborate with the facility on library design, novel applications and improved analysis methods.

The NGS libraries are prepared by the facility in a pre-PCR environment and sequenced post-PCR on machines from Illumina: HiSeq2500 (sequence power and flexibility) and MiSeq (sequence speed). The facility sequences genomic DNA for CNV-Seq, ChIP-seq, FAIRE-seq, MatePair-seq and they perform targeted Enrichment Analysis (Agilent Sureselect Capture and Haloplex, and Nimblegen SeqCap-EZ). Among these are Exome Capture, custom gene panels and Methylome Capture. Total RNA is frequently extracted by the core to study gene expression profiles using stranded RNA-seq and smallRNA-seq. The degradation observed when RNA and/or DNA is isolated from paraffin blocks is no objection for starting library preparation. Even stronger: this (clinical) material is the basis of a diagnostic application that is being set-up at the NKI.

Several users have developed procedures to hand-in custom prepared sequence libraries for the read-out of functional screening tests for essential genes (HaploScreens), sh-RNA screens (TRC/Mission), mutagenesis screens (CRISPR) or nuclear localization screens (DAM-Id).

The facility has developed a user-friendly database where users can register their samples via a web-based interface and get cost estimates of the experimental procedures beforehand. Progress of samples can be traced and (semi-) automated bioinformatics pipelines are triggered to analyze the sequence reads upon completion of the sequence runs. The bioinformatics support ranges from providing raw data, the generations of plots of genome wide copy number distributions, the production of cohorts of gene expression fingerprints, to the extensive analysis of mutation spectra in tumor-normal sample pairs to find targets for immunotherapy.

Microscopy facility

The hospital associated with the NKI is named after Antoni van Leeuwenhoek, the 17th-century Dutch scientific pioneer who was one of the first people to use microscopes. It is fitting, then, that the NKI has an impressive microscopy facility, with nine digital microscopes, four of them capable of confocal microscopy. The facility trains researchers to use these complex instruments and maintains them.

Confocal microscopes produce amazingly high-definition images at high magnifications and have revolutionized microscopy. By directing laser light through a series of lenses, mirrors, and diaphragms, confocal microscopes take a thin 'optical slice' through the sample. They can also make a series of optical slices within an object, feeding the data to a computer to construct a 3D image.

The nine systems are tailored to different needs, from simple fluorescence imaging of fixed cells and tissues to 3D analysis of living cells and organs. For instance, researchers at the NKI have started using confocal microscopes to look at the skin of living mice. By bringing the mouse back on successive days, they can follow the growth, development and movement of cells within individual tumors. "Simpler" digital microscopes are used for monitoring changes in cells growing in multi-well plates. These fully motorized microscopes can take images of cells at set time intervals over a period of up to more than a week.

NKI researchers can also make use of the electron microscopy facilities present at the institute. Dedicated staff will operate the microscope and deliver high quality images.

Proteomics

Proteins are sometimes referred to as the "work-horses of the cell" as they are involved in almost every cellular process. To study the function of a particular protein or protein complex, the first step often is over-expression of the protein of interest followed by purification. The NKI Protein Facility hosts multiple cell culture incubators and shakers for small- and large-scale protein expression in bacteria, insect cells (baculovirus system) and mammalian cells. The facility is equipped with automated column chromatography

systems to isolate proteins to more than 90% purity. In addition, NKI researchers can request the expression and purification of monoclonal antibodies, selected from our in-house hybridoma bank.
The facility also provides access to a variety of biophysical technologies to explore protein stability, activity and molecular interactions, including - but not limited to – Light Scattering, Fluorescence Polarization, Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC). Experiments are performed under supervision of a biophysical expert.

The NKI also provides support for proteomics and metabolomics experiments. A dedicated unit with a high end mass spectrometer equipment and trained operators help research plan and perform their experiments.

For researchers that want to obtain a three-dimensional crystal structure of a protein, a high-throughput protein crystallization platform comprising nanoliter-dispensing robots and automated imagers, is available for screening crystallization conditions. Crystallographic analysis of the crystals is beyond the scope of the facility, however suitable conditions and/or protein crystals can be used to solve the crystal structure in collaboration with crystallography groups, selected by the user.

Peptides

Since 1988 the chemical synthesis of peptides and small proteins has become indispensable in our research. Synthetic peptides find wide use within the NKI.
The peptide facility makes use of two Syroll robots and a Prelude synthesizer. On one of the Syro's, 48 peptides can be synthesized simultaneously. The other Syro robot can produce up to 288 peptides (2-4 µmol) in one run. This is ideally for screening purposes. The Prelude is a handy tool for large-scale peptide production.
The peptide facility provides custom Peptide Synthesis, Long Peptide Synthesis (Ubiquitin, SUMO, ect), Large Scale Peptides up to 1 gram and Peptide Modifications.
The quality and identity of all peptides is monitored by HPLC and LCMS. Peptides can be purified by preparative HPLC to meet the highest-quality standards.

Robotics and Screening Center

During the last decade, new technologies have been developed that allow for the perturbation of expression of individual genes. To be able to make use of these new functional genomic technologies, the NKI has built a technology platform that is used for the development, production and application of reagents to perform functional genomic screens. The Robotic and Screening Center provides researchers with support, expertise and tools for large-scale screening. The facility provides access to highly flexible, high-throughput liquid handling systems designed to accommodate several different experimental set-ups to support the many different research lines pursued in the institute, ranging from high throughput cell-based screens to small-molecule drug screens. In addition the facility offers researchers support for the analysis and interpretation of large-scale screening projects.

Flow Cytometry Facility

A flow cytometer is able to measure the size, complexity and molecular characteristics of individual cells; thousands of cells can be analyzed per second. Fluorescent labels mark specific components on the cell surface or can be coupled to particles inside the cell, thus elucidating their functional characteristics.
The Flow Cytometry Facility accommodates six bench-top analyzers and two multicolor high-speed sorters. We have multi-laser equipped flow cytometers that can analyze up to 18 fluorescent labels simultaneously, depending on the number of detectors.
The high-speed sorters have the ability to physically sort cells or particles of interest. Both our sorters can make sorting decisions at a speed of 20,000 cells per second, creating very pure cell populations that can be used for further study. In our facility, we can simultaneously sort up to 6 different cell populations based on maximally 15 fluorescence parameters. We collect cells in a variety of plate formats, tubes or slides. If required we sort bio-safety class II level specimens.

The facility provides instrumentation and technical assistance for performing flow cytometry analyses and sorting. Every researcher who is interested in flow cytometry analysis is offered an instructional session on flow cytometry basics and special applications, and on the operation of the analyzers.

Molecular pathology and Biobanking

To ensure human material is used properly and efficiently, especially in the case of scarce, valuable samples, the NKI has a facility for issue and use of NKI biobank materials; the Core Facility Molecular Pathology & Biobanking (CFMPB). The CFMPB registers, coordinates, assists and facilitates research involving biobank material: archived human/patient material. The facility provides professional expertise, appropriate samples and tissue based experimentation in the context of optimally controlled medical-ethical and legal issues.

All study applications can be submitted online using the Application & Request tool ART for Registration & Review by our Translational Research Board. 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 we have the Discovery Ultra (Ventana, Roche) automated stainer, adaptable to a broad array of tissue testing capabilities. 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. To guarantee standard quality all DNA and RNA isolations from NKI Biobank material (FFPE, FF, serum, and blood) are performed by, or under supervision of, the CFMPB technicians. All samples will be handled and stored in the facility (pre-PCR, QC conditions) under supervision of the coordinating technicians according to standard protocols modified and adapted to the specific requirements per project.

Technology Transfer

The Technology Transfer Office (TTO) supports the NKI by managing the protection and exploitation of intellectual property created by the institute's scientists. This helps to advance the institute's mission to improve cancer diagnosis and treatment for the benefit of cancer patients by transferring basic research discoveries to academia for further research and to the marketplace for commercial development and broad implementation.

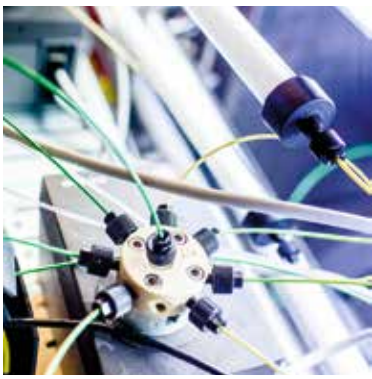
The TTO is equipped to identify and protect the institute's intellectual property without impeding scientific research. It manages the institute's patent portfolio and is responsible for its commercialization through licensing or spin-off development. NKI has - over the years - also developed a sizeable portfolio of valuable research materials and distribution of such materials to other academic research organizations via Material Transfer Agreements as well as licensing to commercial entities is arranged through the TTO. A sizeable effort is involved in the negotiation of research-related contracts such as collaboration agreements on behalf of the institute. TTO monitors the obligations of NKI and those of its contractual partners under all agreements that it concludes.

Research IT

Research and clinical data collected in the past and at present is invaluable for current and future research, and ultimately for clinical practice. However, many of these data-sets are not secured and are not accessible. It is therefore that Research IT aims (1) to implement central storage and high performance compute facilities for backup, archiving and processing of data, (2) to implement a data governance structure and tools to allow for search and access to data in concordance with ownership, law and privacy regulations, (3) to implement a data warehouse in order to link data from subjects (i.e. a patient, mouse or tumor) registered in multiple databases and systems and to track their history (e.g. their diagnoses, treatments and experimental data) over time, and (4) to introduce data exploration tools and medical intelligence systems to support research and, ultimately, clinical decision making.

Aside from this effort we want to realize institute-wide data stewardship. Research IT also delivers dedicated support to research groups and (multi-center) research projects with expertise and tools such as collaboration platforms and publication websites.

To achieve its goals Research IT works in close collaboration with the central IT department and various departments within the NKI and takes part in local, national and international IT infrastructure projects. The Research IT team consists of bioinformaticians, scientific programmers, data managers, integration specialists, web



developers and system administrators. We execute our projects using SCRUM methodology.

Biometrics

The NKI takes part in many clinical trials and other patient related clinical research projects; some of which are NKI initiatives. 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. Support for central administration, patient registration, and data collection of clinical trials is provided through the Trial Office of the department. The department also maintains a Tumor Register database containing information on patients with benign tumors, pre-malignant, and malignant tumors seen in the Institute since 1977. This database is a valuable resource for research and currently contains more than 225,000 registrations.

Biostatistics

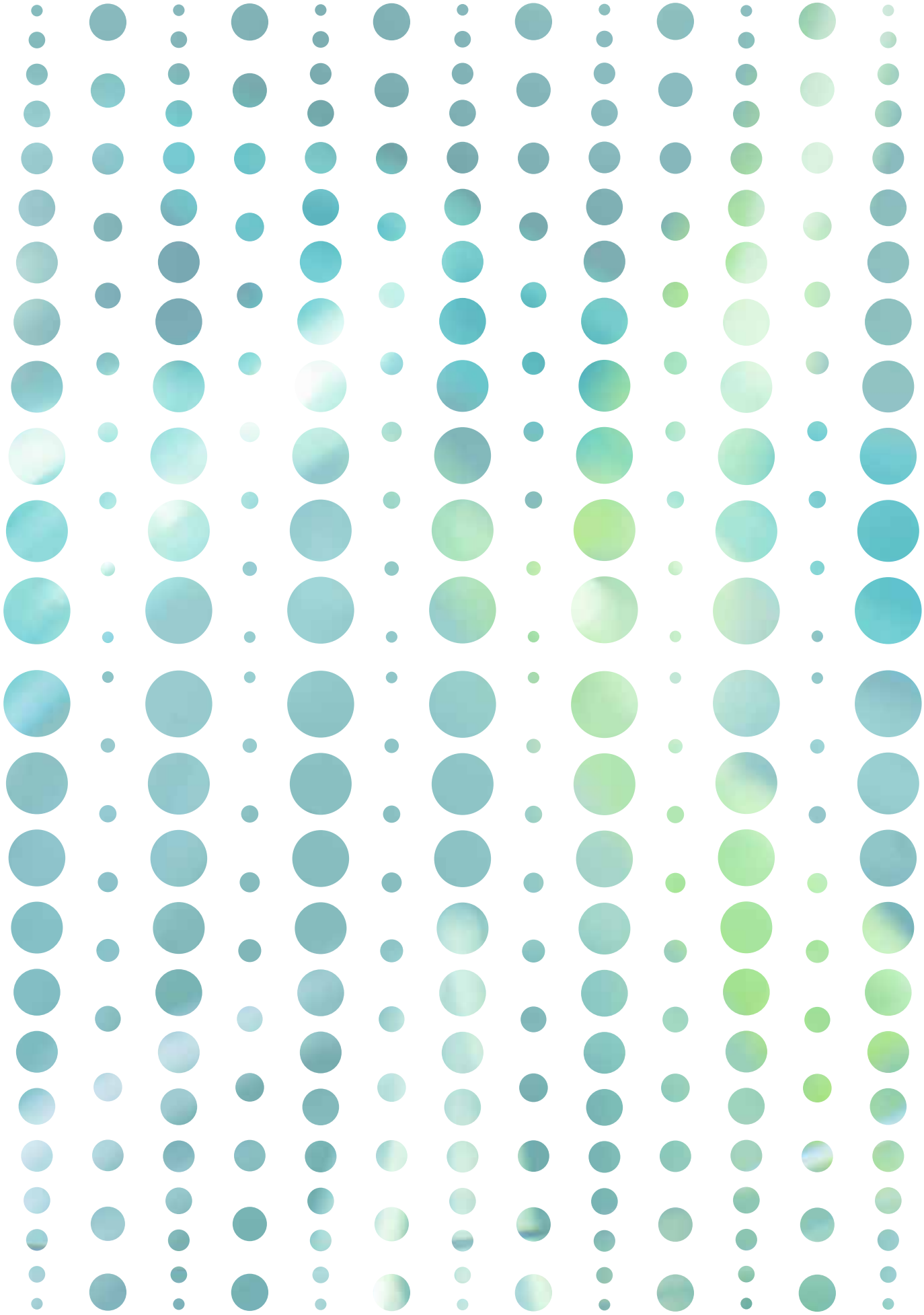
Biostatistics is a key component in planning, conducting and analyzing studies in biomedical cancer research, including epidemiology. The Biostatistics Center collaborates with investigators and doctors from the Institute and the hospital to ensure that state-of-the-art statistical techniques are used in the design and analysis of scientific studies. Statistical expertise is available within the Center on diverse topics from all areas of observational and experimental biomedical cancer research. Collaboration involves development and implementation of various methods to cover a wide range of topics such as the design and analysis of epidemiologic studies and clinical trials, the identification of prognostic and predictive biomarkers, sample size calculation, risk prediction, as well as animal and in vitro experiments. Members of the Center are involved at all phases of the research including grant application, set-up of trials, as well as analysis and interpretation of collected data. Moreover, the Biostatistics Center teaches the popular annual Basic Medical Statistics Course.

Other facilities

Other NKI facilities include the central ICT department that provides general IT support for all research groups, as well as specific 'site-wide' services (such as email facilities) for all personnel. Tasks include management of routers, Ethernet switches, file servers, job-and-database servers, storage, back-up and archiving of data, data security, configuration of network software on different types of devises using different operation systems of end users and, if necessary, the development and maintenance of custom server and/or client-side software.
The Cancer Library serves the research, clinical, nursing, and paramedical departments of the institute. In recent years site licenses for a wide range of electronic publications and services have been obtained, giving full-text, electronic access to key publications over the intranet.
The Sequence Facility offers a service for DNA sequence and fragment analyses to the research departments and the clinical DNA-diagnostics laboratory. The facility makes use of two sequencers, the 3730 DNA analyzer and the 3500xL Genetic Analyzer.
The institute has a central radioactivity facility. The staff offers help and advice on all aspects of work involving radioactivity. The department is equipped with up-to-date gamma and scintillation counters, a gamma analyzer and HPLC apparatus. There are also separate facilities for animal experiments with radioactive tracers. Radiation safety courses are regularly organized for students and scientists.
The institute also has dedicated labs for working with biological reagents, such as viruses, at several levels of containment, a dedicated lab for working with carcinogens, a central cryogenic storage facility, a glassware kitchen, and an electro- and technical workshop.

Finally, scientists at the NKI are supported by personnel, financial and general facilities departments, relieving group leaders of considerable administrative work.

CAREER AND TRAINING



Career and training

Faculty

The NKI provides a unique and challenging environment with state-of-the-art facilities for young and established researchers. Although the NKI is an independent research institute, many staff scientists have professorships at Dutch universities.

On a regular basis we seek assistant professors. These junior group leaders are given the opportunity to start their own group, building on their proven excellence as a postdoctoral fellow and having access to the NKI's full range of facilities, and financial support in the initial phase to appoint personnel.

Established researchers and candidates for a junior group leader position are invited to contact the director of research.

Postdoctoral fellows

About 150 postdoctoral fellows are employed in the different research groups at the NKI. The institute helps postdocs to obtain their own funding and reach a more independent position that prepares them for a faculty position.

The NKI has a very active postdoc committee (see the NKI website) which aims to make the institute an excellent place to work for postdocs and to help them develop their careers. One of its main tasks is to organize special events for postdocs, covering topics such as grant writing, management, ethics, tech transfer, and alumni careers. The postdoc committee is also actively involved in a career development program that is offered by the NKI to all its postdocs. This training program topics include communication skills, setting personal expectations, 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.

Any postdoctoral fellows interested at training at the NKI are invited to contact one of the group leaders or look at our website. For general information about postdocs please contact the postdoc committee (postdocinfo@nki.nl) or the postdoc dean Dr. Fred van Leeuwen (fred.v.leeuwen@nki.nl).

Graduate students

Besides working in a stimulating and challenging research environment, graduate students are participating in the PhD program of the Oncology Graduate School Amsterdam (OOA), an alliance of the oncology researchers of the NKI and the two Amsterdam universities. The OOA offers technical courses (statistics, microscopy, bio-informatics, English writing, animal handling) as well as in-depth scientific courses that are often organized around a symposium. During lunch meetings, PhD students can meet with renowned seminar speakers who are invited in the context of the NKI seminar program. Another great opportunity for OOA graduate students to interact is the three-day annual retreat, where each student presents his/her work to fellow students.

Each graduate student has a yearly meeting with a supervising committee consisting of NKI faculty members who are selected for their capacity to evaluate the quality and progress of the project. During the meeting, the performance of both the supervisor and the student is discussed. All research divisions have a representative in the PhD student committee that regularly meets with the dean of the OOA.

Masters of Science are welcome to check for positions that are regularly advertised on our website, or to contact our group leaders or the dean of the graduate students, Prof. dr. Hein te Riele (h.t.riele@nki.nl).

Master students

Master students from universities and students from HLO schools also have the opportunity to take part in cutting-edge research and to enjoy its associated excitement and challenges. The NKI is happy

to receive them for rotation projects. University students may undertake projects in the institute provided they have obtained a Bachelor's degree with a major in biology, bioinformatics, computational biology, medical biology, medicine, pharmacy, chemistry, or a closely related subject. In addition, the Division of Psychosocial Research and Epidemiology takes students with relevant theoretical backgrounds. Students with a background in informatics and/or physics can also join the research facilities or the Division of Radiotherapy. English language is the teaching medium in daily practice. Every Master student who does a project in the institute in the area of Biology/Medicine for more than 4 months is obliged to pursue a course and examination in experimental oncology.

Master students interested in rotation projects are invited to make direct contact with a research group at the NKI, or contact the dean of Master Education (onderwijscoördinator), Prof. Dr. Jannie Borst (j.borst@nki.nl). HLO students, please contact Dr. John Hilkens (j.hilkens@nki.nl).

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