The Netherlands Cancer Institute
Today’s research, for tomorrow’s cure
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The Netherlands Cancer Institute
## Contents

**CONTENTS**

<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>07</td>
<td>Foreword</td>
</tr>
<tr>
<td>08</td>
<td>Introduction</td>
</tr>
<tr>
<td>15</td>
<td>From basic research to clinical application</td>
</tr>
<tr>
<td>23</td>
<td>Basic research (research groups)</td>
</tr>
<tr>
<td>24</td>
<td>index research groups</td>
</tr>
<tr>
<td>69</td>
<td>Clinical research</td>
</tr>
<tr>
<td>70</td>
<td>Medical oncology</td>
</tr>
<tr>
<td>74</td>
<td>Surgical oncology</td>
</tr>
<tr>
<td>78</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>82</td>
<td>Diagnostic oncology</td>
</tr>
<tr>
<td>86</td>
<td>Facilities</td>
</tr>
<tr>
<td>92</td>
<td>Career and training</td>
</tr>
<tr>
<td>97</td>
<td>Contact</td>
</tr>
<tr>
<td>98</td>
<td>Colophon</td>
</tr>
</tbody>
</table>
Creating new opportunities for patient-tailored cancer treatments through basic, translational and clinical research
With great pleasure and pride I offer you this brochure on the research performed at The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital.

The Netherlands Cancer Institute (NKI) is an exciting and rewarding place to work, and has become an internationally recognized center of scientific excellence in many key areas relating to cancer. Recognition of our status is evident from our publication record, from the many invitations that staff receive to organize and speak at major conferences, from the prestigious prizes our staff receive, and from our ability to attract funding.

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 ‘flat’, non-hierarchical management style that gives our research staff the freedom to explore areas of interest and empowers them to pursue their goals and produce results. This makes it easy for group leaders to direct their work along exciting paths, with the expectation that they will make a valuable contribution to cancer research.

As a consequence, the NKI contains a wonderfully broad range of interests and experience. While some of the work has direct relevance to cancer, other projects are more fundamental in their nature. These fundamental projects, however, generate a foundation of knowledge and resources that the applied cancer research can build on.

The challenge is now clear. We know that research is the only way to improve cure rates and enhance the quality of life for cancer patients. But we know that scientific research only will make a difference when the acquired insights can be applied in a clinical setting. So having the Antoni van Leeuwenhoek Hospital as an integrated partner is essential.

As director, I have the privilege of overseeing this motivated group of investigators as they explore basic principles of cell and molecular biology and translate them into clinically useful tools and strategies. I am fascinated by the variety of approaches that we are advancing, ranging from genetic screening and imaging technologies to new drugs and therapeutic regimens.

A major outcome will be the ability to identify the best method of treating individual patients. My firm belief is that in the next decade we will see a move away from single forms of treatment for all patients in a broad category of cancer, to a much more patient-centered therapy where each patient receives treatment tailored to have maximum impact on his or her tumors, with the fewest side-effects.

This brochure shows many of the people who will make this happen, and gives a flavor of how today’s work will lead to tomorrow’s cure.

Prof. dr. Anton Berns,
Scientific Director.
Introduction

Improving cancer treatment depends heavily on a better scientific understanding of the function and development of normal cells and tumor cells. Through fundamental and clinical research we hope to contribute to better cancer treatment. Discoveries made in the laboratory are rapidly introduced into the clinic through our translational research program driven by close collaboration between our scientists and clinicians. The Netherlands Cancer Institute (NKI) aims to be a national and international center of excellence in cancer research. To reach our goals the NKI has a dedicated staff of internationally recognized scientists, highly motivated students and state of the art support facilities.

The NKI
The Netherlands Cancer Institute was established on October 10, 1916. The founders, Rotgans, professor of Surgery, the publisher De Bussy, and De Vries, professor of Pathology, wanted to build a cancer institute ‘where patients suffering from malignant growths could be treated adequately and where cancer and related diseases could be studied’. They bought a house on one of the canals in Amsterdam and named it the ‘Antoni van Leeuwenhoek Huis’, after the famous Dutch microscopist. The clinic had room for 17 patients, while the laboratory could accommodate 8 to 10 scientists.

Nowadays, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AVL) accommodates approximately 550 scientists and scientific support personnel, 53 medical specialists, 180 beds, an out-patients clinic that receives 24,000 new patients each year, five operating theaters and nine irradiation units. It is the only dedicated cancer center in the Netherlands and maintains an important role as a national and international center of scientific and clinical expertise, development and training.

The three major areas of research are fundamental, clinical and translational cancer research. A thorough understanding of the biological processes of normal cells is the basis for understanding cancerous cells. The laboratory covers all major areas of cancer research, with special emphasis on cell-based screens, mouse tumor models, cell biology, structural biology and epidemiology. The institute coordinates and participates in many clinical trials; most of these are phase 1, 2 or 3 studies of potential new treatments such as combinations of chemotherapeutics, radiotherapy and/or surgery. Results obtained from fundamental research are translated into clinical applications as part of our translation research program.

From its first inception the NKI-AVL saw close collaboration between scientists and clinicians as essential to fighting
cancer. Having a laboratory and hospital under one roof in a single independent organization with an open and collaborative atmosphere has led to many important discoveries and improved therapies.

**Organization**
Scientific research groups are organized into divisions: usually research groups on one floor form a division. Clinical research groups are also organized into scientific divisions according to the clinical departments of the cancer hospital. One of the group leaders is head of the division. He/she oversees the quality of the science within the division, promotes collaboration between division members, and conducts the general management of the division. The divisions and their group leaders are listed on the next page. For the clinical research divisions only the head of the division is indicated.

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 the board are frequently asked to advise the Director of Research on issues such as the appointment of faculty members, evaluation of research programs and policies. They also discuss the quality, significance, and main focus of individual NKI researcher’s projects.

The NKI-AVL Board of Directors consists of three members. The Board operates as a collective, although each member is responsible for his own field of expertise.

The Board of Governors 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. They advise and control the Board of Directors.
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From basic research to clinical application
Understanding cancer: 
basic research at the NKI

The basic research at the NKI aims for a more profound understanding of how cancer arises, and strives to translate this knowledge into new approaches to diagnosing and treating cancer. This encompasses many scientific themes in tumor-cell biology, notably how genetic mutations arise, in which genes, and with what consequences; how genes are organized in the nucleus and regulated, and how this regulation may go wrong in cancer; signaling in normal and cancer cells – at the cell surface and the transmission of signals to the cell interior; cell fate; cell division; programmed cell death (apoptosis); tumor growth and metastasis; and resistance to cancer treatment.

Cutting-edge technologies
Scientists at the NKI are among the world’s most talented inventors and users of new technologies. This can involve gadgets and reagents for use at the laboratory bench, the creation of new animal models, or new high-throughput devices in centralized facilities. The NKI has particular expertise in using and generating new animal models for cancer. These are helping to provide insights into many aspects of cancer biology, especially in understanding how the effects of different genetic mutations can combine to promote tumor development. They also serve as tools for the pre-clinical testing of new therapies and drugs. The NKI’s animal models are valuable not only to NKI researchers but also to other research institutes and pharmaceutical companies for the testing of new anticancer drugs.

Functional screens
NKI scientists use various techniques for altering the action of individual genes in both laboratory cell cultures and in animal models. This enables them to identify a gene’s normal function and to mimic the effects of mutation, which could either enhance or reduce a gene’s activity. To enhance the activity of a particular gene, for example, NKI scientists can use transgenic mouse technology to introduce molecular machinery to switch a gene on and maintain its activity at high levels, even at stages in development when it is normally switched off. Conversely, NKI scientists use a technique known as RNA interference (RNAi) to selectively switch off the activity of a particular gene, or genes, and examine the consequences for various cell processes. This involves introducing small pieces of RNA to effectively silence individual genes. The NKI has a collection of over 30,000 small RNA pieces, which in total correspond to all human genes. Besides contributing to understanding gene function, this technique also helps to identify new targets for drug development.

Understanding mutations – the trigger for cancer
Genetic mutations lie at the heart of tumor formation. As mutations accumulate in genes that are critical to cell growth and signaling, cells stop producing key proteins, or produce abnormal versions, and begin to behave incorrectly.
They break free of normal constraints on cell growth and division and spread to other parts of the body. Mutations that result in cancer can either be spontaneous, appearing in a cell for the first time, or can be inherited by children from their parents, which increases the risk of developing a particular form of cancer, such as in the breast or colon.

On a basic level, NKI scientists are investigating the effects of genetic mutations on processes that normally maintain cells in good health, for example, the repair of damage or other alterations to DNA. When chemicals or radiation damage the nucleotide building blocks of DNA, a complex of proteins normally repairs the damage by replacing altered nucleotides with new ones. A similar repair process occurs when enzymes responsible for replicating DNA make an error and insert the wrong nucleotide into the DNA sequence, which can occur if mutations arise in one or more of the enzymes. Using in vitro cell culture, functional screens, biochemistry and protein crystallography, NKI scientists are investigating the processes leading to DNA damage or replication errors, and the effects of mutations on DNA repair proteins.

Cell signaling
Cells do not exist in isolation. Rather, they are in continuous communication with their surroundings, receiving and responding to signals from hormones, cytokines, and other cells. NKI scientists are investigating the mechanisms by which signals are relayed to the cell interior, and how these can go wrong in cancer cells. Research into abnormalities in the receptors on the surface of breast cancer cells has led to the creation of a new screening tool for patients which can determine how well they may respond to hormone treatment.

The body’s immune defenses, best known for their role in fighting infections, are also important in protecting against cancer. This too depends upon signaling at the surface of the immune system’s ‘soldiers’, the lymphocytes. These can ‘recognize’ tumor cells as abnormal and alert and attract other cells to kill the cancer cells. None of this would occur without extensive signals being exchanged between different cells, through receptors on their surfaces and the release of molecules called cytokines. NKI scientists are searching for ways to enhance these processes, including the design of novel cancer vaccines, which are currently being tested in animals before moving to clinical trials.

Tumor growth and metastasis
The ability of tumors to grow and ruthlessly destroy the body’s vital organs is the key feature that distinguishes them from normal tissues. NKI researchers are investigating how normal cells start to ignore the signals that would normally prevent a cell dividing too often. Alongside this, they are studying how cells start to move around the body and grow in new tissues, a process known as metastasis. Advances in understanding these processes may one day lead to new approaches to cancer treatment.
Improving cancer treatment: clinical research at the NKI

Clinical research aims to introduce new forms of treatment into the clinic and to improve existing ones. Highly trained clinical and auxiliary professionals, multidisciplinary patient reviews, specially trained oncology nurses, and excellent support facilities for clinical trials allow many clinical trials to be performed at the hospital.

**Improving diagnosis and prognosis**

Through collaboration with surgeons, the NKI-AVL’s pathology department has amassed an impressive bank of tumor tissue from breast cancer patients. This has allowed researchers to develop new tests for predicting metastasis using DNA microarray analysis, a method that is now widely used in the laboratory, but is only just beginning to have an impact in the clinic. This works by detecting patterns in gene activity that differ between tumor cells and normal tissues.

NKI researchers are now also applying microarray technology to predict the effects of different drugs on a tumor. These studies would not be possible without the institute’s bioinformaticians, who analyze the data and develop the computer algorithms needed for making confident predictions.

Meanwhile, NKI researchers are also searching for new diagnostic markers in biological fluids – mainly blood, urine and cerebrospinal fluid. The aim is to develop non-invasive ways of identifying the type of tumor a patient has, and the extent to which it has spread beyond its original site.

**Therapies of the future**

Cancer treatments of the future are likely to be more finely tuned to the needs of the individual patient than at present. Most existing anticancer drugs target rapidly dividing cells indiscriminately, which is why they can have severe side effects such as nausea, dizziness, loss of taste sensation, fatigue, and hair loss. Radiotherapy too can have long-term side effects on the tissues surrounding tumors, including causing secondary cancers and heart disease.

The NKI therefore places great emphasis on the goal of creating new approaches to cancer therapy that reduce side effects and enhance patient survival. One approach is to make existing treatments more effective by combining two or more different modalities, such as chemotherapy and radiotherapy. This allows smaller doses of either to be given to the patient, and may help to avoid long-term side effects.
Similarly, the use of photodynamic therapy, isolated limb perfusion, and hyperthermic intra peritoneal chemotherapy (HIPEC) enable chemotherapy to be given at high dose in more localized parts of the body, thus sparing other organs from exposure.

These treatments still fail, however, to distinguish between most normal tissues and tumors. NKI scientists are therefore exploring new ideas for targeting therapy specifically at cancer cells. These are tested in animal models first, to gauge safety and effectiveness, before entering clinical trials. One example is the use of DNA-based vaccines against cervical cancer and melanoma, to trigger the immune system to target tumors, combined with low doses of radiation to boost the influx of immune cells to a tumor site.

Overcoming resistance
Scientists at the NKI are working to enhance drug uptake into the body so as to enable patients to take drugs orally rather than by injection, and to raise the dose and extend the time that drugs are active in the body. They are also attempting to overcome the problem of drug resistance, which often arises through mutation in a patient’s tumor. One strategy is to find and design drugs that attack alternative weak spots in a cancer cell and render the tumor more sensitive to treatment.

Through imaging technology such as MRI and PET, patients can be monitored to see whether or not the drug is killing and shrinking a tumor, enabling clinicians to monitor the success of a particular treatment.

Protecting against cancer
The devastating effect of cancer sometimes leads patients or their relatives to volunteer as subjects for cancer research. Following the availability of new genetics tools during the late 1980s and 1990s, the NKI-AVL set up a Family Cancer Clinic where family members receive genetic counseling on their own risk of developing cancer. Molecular biologists and epidemiologists at the NKI are following families affected by cancer in order to identify new mutations associated with the disease, and to pinpoint how environmental factors such as diet, exercise and hormones may raise the risk of developing cancer, or serve as protection.

Coping with cancer
Being diagnosed for cancer changes one’s whole perspective on life. Not only the patient, but also relatives, have to cope with the diagnosis and treatment. Research into coping mechanisms has led to changes in treatment and care that help patients and their close relatives.

Forging ahead
The NKI-AVL 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, and helping healthy individuals to avoid cancer in future. Its clinical services operate to the highest international standards, and continue to extend the boundaries of what can be done for cancer patients. Its scientific achievements are celebrated internationally through awards and highly cited publications in peer-reviewed journals. With well-integrated clinical and basic research operations, the NKI-AVL aims to have a critical impact on cancer in the future.
Basic research
Index

Aaronson, Neil  Psychosocial and behavioral oncology  p 25
Agami, Reuven  RNAi, microRNAs and cancer  p 26
Begg, Adrian  Individualization of radiotherapy  p 27
Beijersbergen, Roderick  The RNAi strategy in cancer research  p 28
Beijnen, Jos  Anticancer drug development  p 29
Bernards, René  Functional cancer genetics  p 30
Berns, Anton  Mouse models for cancer  p 31
Borst, Jannie  Cell survival and death pathways  p 32
Borst, Piet  Drug resistance and DNA base J  p 33
Collard, John  Tiam1-Rac signaling and cancer  p 34
Divecha, Nullin  Cancer cells: PIPed at the post  p 35
Fornerod, Maarten  Transactions at the nuclear envelope  p 36
Haanen, John  Attacking cancer with T-lymphocytes  p 37
Hilkens, John  Breast cancer genes  p 38
Jacobs, Heinz  Programmed mutagenesis  p 39
Jalink, Kees  Biophysics of cell signalling  p 40
Jonkers, Jos  Mouse models of breast cancer  p 41
Michalides, Rob  Estrogen receptor and breast cancer  p 42
Moelenaar, Wouter  LPA, a multifunctional growth factor  p 43
Neefjes, Jacques  Improving immune responses to tumors  p 44
Ovaa, Huib  Chemical tools for cancer research  p 45
Peepер, Daniel  Identifying novel cancer genes  p 46
Perrakis, Anastassis  Structural biology  p 47
Peters, Peter  Cryo-electron tomography in the cell  p 48
Roos, Ed  Metastasis: tumor cells on the move  p 49
Schellens, Jan  Pharmacology of anticancer drugs  p 50
Schinkel, Alfred  Improving anticancer drug efficacy  p 51
Schumacher, Ton  Immunotechnology & immunotherapy  p 52
Sixma, Titia  Structural biology  p 53
Sonnenberg, Arnoud  Cell-matrix adhesion  p 54
Stewart, Fiona  Vascular damage after radiotherapy  p 55
Te Riele, Hein  Gene modification: subtle is the oligo  p 56
Van Blitterswijk, Wim  Membrane rafts as gateways for drug  p 57
Van de Vijver, Marc  Genetic alterations in breast cancer  p 58
Van Leeuwen, Flora  Epidemiology of cancer  p 59
Van Leeuwen, Fred  Epigenetics in yeast  p 60
Van Lohuizen, Maarten  Cell fate control by Polycomb silencers  p 61
Van Steensel, Bas  Chromatin genomics  p 62
Van ’t Veer, Laura  Molecular profiles of breast cancer  p 63
Verheij, Marcel  Apoptosis modulation and radiotherapy  p 64
Wessels, Lodewyk  Bioinformatics  p 65
Neil Aaronson is certain that if you provide clear information about a patient’s quality of life, physicians and other carers become more attuned to the patient’s problems. Shortcomings in treatment and care are redressed, and the patients are more satisfied with their treatment.

With a background in clinical psychology, I have a keen interest in assessing how individuals adjust to illness and therapy. I’m also trained in public health and so have a good grasp of research based on large numbers of patients. For me, the NKI is the perfect place to do high-quality academic work in an applied setting. It also gives me a base from which I link to many international research groups.

Over the past 20 years I’ve championed the use of standardized questionnaires and structured interviews, trialing them on large groups of cancer patients. We’ve learned how cancer and its treatment affect patients and their families, and how survivors cope in the long term. The questionnaires were initially created for research, and it’s been exciting to see them move into the clinic. Patients can now complete the questionnaire on a touch-screen computer and take a printout to their consultation, which helps patient and clinician use their time together more productively.

In another line of research, my colleagues Frits van Dam and Sanne Schagen found that a small, but significant, group of people who have had chemotherapy find it harder to perform mental tasks. The research started after a chance conversation with a nurse over lunch one day, and led to a systematic assessment of the nature and scope of this problem. For patients with brain cancer, we are testing ways of helping them to recover cognitive skills or to learn to cope with their new limitations.

My group is also studying people’s responses to counseling and genetic testing for cancers that run in families, in particular colorectal and ovarian cancer. Led by Eveline Bleiker and myself, this research line investigates the emotional implications of undergoing testing, and examines how it affects the person’s anxiety about cancer, their family relationships, and their efforts to remain in good health.

SELECTED PUBLICATIONS


Reuven Agami came to the NKI in 1998 and develops tools that can reveal the functions of individual genes in the chain of events that transforms a healthy cell into a cancer cell.

Our most exciting work at present is developing the tiny RNAs called microRNAs to manipulate the expression of genes we suspect of playing a role in cancer. These 20-base RNAs are part of a cell’s normal controls on gene activity.

The potential of microRNAs as research tools is vast. On the one hand we can change gene regulation by introducing a microRNA that the cell does not produce; on the other we can introduce so-called antisense microRNAs that effectively wipe out their counterpart microRNAs in the cell.

We’ve made a unique set of viruses containing the genetic code for the 500 known human microRNAs; we use them to force cells to make a specific microRNA. A first use of our viruses was to identify cancer-causing microRNAs. Among these, we implicated two in the rare but curiously easily treatable type of testicular cancer suffered by cyclist Lance Armstrong in 1996. The vast majority of these tumor cells have a functional p53 tumor suppressor gene, so they should be protected from becoming cancerous. But we discovered they also contain two microRNAs that prevent p53 from working properly. Intriguingly, these microRNAs also make the tumor cells highly vulnerable to DNA damage, explaining why this cancer is easy to treat by therapies that attack DNA.

We’ve also discovered that these microRNAs are involved in helping some cancers resist therapy. This raises the exciting possibility of using antisense microRNAs medically to tackle this problem.

In previous work at the NKI on RNA interference — another way of silencing gene expression — I, and my colleagues devised a system, marketed as pSUPER RNAi™, that can silence almost any known gene in mammalian cells. This technology is now used in labs all over the world.

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**SELECTED PUBLICATIONS**


r.agami@nki.nl
Adrian Begg arrived at the NKI 22 years ago, drawn from the UK by an invitation to develop a site of excellence in radiobiology research. He stayed on as the institute grew and the work kept getting more interesting.

The main thrust of my work is predicting how tumors respond to radiotherapy, which is critical for improving cancer treatment. Our goal is to create a test that can accurately predict the ideal treatment for a patient before they begin therapy.

In the past, such assessments could only be made by drawing inferences from parameters such as tumor growth rate or oxygen levels, and was never very satisfactory. The arrival of DNA microarrays opened up new horizons for us. We can now determine which of the tumor cells’ genes are operating normally, which are overexpressed, and which are underrepresented. Having these genetic profiles is a huge step forward, and we should soon be able to link a tumor’s profile with particular therapeutic recommendations and more accurate prediction of outcome.

A valuable resource at the NKI is the tissue bank, where doctors have archived tumor tissue taken over many years. We use this tissue to compare gene profiles of tumors taken from patients who were treated five years ago with the outcome of their treatment. We have already found gene profiles that correlate with outcome — but we are still in an early stage. We’re also working on a more lab-based project on DNA repair. Ionizing radiation kills cells by damaging their DNA, and is most effective when the cell’s DNA repair mechanism is faulty — which is often the case for tumor cells. We want to understand the DNA repair mechanism well enough to find agents that can disable it, and to identify the genetic profile of cells in which DNA repair is weak.

The attraction of this approach is that cancer cells with mutations in one or more DNA repair pathways will be vulnerable to therapy that disables the remaining pathway, while normal cells with intact DNA repair will be unharmed. We hope that this approach will lead to a therapy that hits tumors hard, but causes few side effects.
The RNAi strategy in cancer research
Roderick Beijersbergen

After his post-doctoral position at the Whitehead Institute for Biomedical Research in the United States, Roderick Beijersbergen moved to the NKI in 1999. He now uses his imagination and innovative skills to develop and apply new research tools with the goal to identify key genes associated with cancer.

My main interest is to identify novel targets that can be used for the treatment of cancer. With the elucidation of the humane genome we can now examine the role of each individual gene in important cellular processes. We make use of the technique of RNA interference (RNAi) to silence gene expression and monitor their role in cell growth, survival and transformation. We have created large sets of so-called hairpin RNAs that can knock down specific human and mouse genes. By making use of our own robotic high throughput system to perform large scale automated cell based screens we can assess what happens when each of these genes is turned off in a cell. By doing so we can also study how anti-cancer drugs work and identify genes involved in resistance to cancer therapies.

A major challenge is to develop drugs that act only on tumor cells. We are using RNA interference to identify genes that upon inactivation only affect tumor cells and not normal cells. These genes are interesting candidates for the development of novel classes of more specific cancer therapies.

Behind all my work is a fascination with the complexity of biological systems. You often start by working on a single gene, but soon you realize that the gene is part of an intricate network or pathway. Understanding the complex processes involved in these networks will allow us to develop better cancer drugs.

SELECTED PUBLICATIONS


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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.

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.

Our research is mostly on cancer, although we also work on HIV and drug addiction. The pharmacology group, which Jan Schellens also heads, has PhD students who work on the design of drug formulations, as well as analyzing 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.

SELECTED PUBLICATIONS


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René Bernards joined the NKI in 1992 to develop new molecular approaches to cancer diagnosis and treatment. He is co-founder of the spin-off company Agenda, and in 2005 he won the prestigious Spinoza prize from the Dutch scientific organization NWO.

Our group is using the latest genetic tools to systematically determine which of the 30,000 or so human genes can be involved in cancer. The popular approach in the past was to make a gene more active and see how this changed cell behavior. We’re now pioneering the opposite approach, using RNA interference (RNAi) to silence genes.

In one project we use RNAi to investigate resistance to anticancer drugs. A staggering two out of every three cancer patients don’t respond to the first drug they’re given. By the time the right drug is found, the tumor has often become too advanced for us to win the battle. We inactivate genes one at a time in tumor-cell cultures to see which ones, when they’re not working, render the cells resistant to a particular drug. If we find mutations in one of these genes in tumors of patients who are unresponsive to that drug, mutations in that gene become potential predictive biomarkers for unresponsiveness.

We are doing this for current and experimental drugs, and have already identified a biomarker in melanoma patients that predicts their response to a new type of drug called histone deacetylase inhibitors.

Our other big project is to identify new targets for so-called ‘smart drugs’ that aim to treat cancer more effectively than standard chemotherapy.

Our assumption is that tumor cells carrying certain mutations are likely to be more vulnerable than normal cells to further damage. Inactivating a second gene in the same pathway might kill the tumor cell, revealing a target for new drugs.

To find such genes, we use RNAi to inactivate genes one at a time in human tumor cells that have common mutations, such as p53. We have identified some candidates, and are about to test the concept in an animal model.

SELECTED PUBLICATIONS


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Mouse models for cancer
Anton Berns

Anton Berns became director of the NKI in 1999, but still maintains solid links with day-to-day research. His passion is to quicken the pace of basic research with the aim of getting the benefits to patients sooner.

I like to see primary data — it prevents me becoming detached from the actual research. My personal interest is in mouse models, particularly for lung cancer and mesothelioma, two of the deadliest cancers.

About eight years ago I set out to develop mice that mimicked human cancers. Our conditional knockout mice have since been a great success, and we have a number of strains that produce tumors that really resemble human disease. We can study the stem cells from which we believe tumors develop, and define the steps involved. We try to identify which pathways have to become defective for a healthy cell to become transformed into a tumor cell. Then we can start designing and testing rational interventions, and hopefully move rapidly to developing more effective treatments for these cancers in people.

Creating mouse models is time-consuming, however. Even if we have a mouse that closely resembles the human disease, as with our model of small-cell lung cancer, experiments can take a painfully long time. To get things moving faster, we are now culturing cells from the mouse tumors and putting them into the lungs of new mice, giving us a rapid way of studying underlying mechanisms and testing potential therapies.

Looking ahead, we hope that once we can cure a cancer in mice, we can apply similar approaches in humans. We know that mouse tumors vary greatly in their response to the therapies used medically. Each tumor has to be characterized in detail to design the best intervention. Undoubtedly, this needs also to be done for patients, who can then be grouped according to the genetic features of their tumors. Such a group will give more uniform results in clinical trials and fewer patients will be needed to provide statistically valid answers.

SELECTED PUBLICATIONS


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Jannie Borst has devoted her career to studying T cells – key cells of the immune system that fight infection and help destroy cancer cells. She studies the proteins that tell T cells to become fully operational, and is starting to be able to manipulate both these signals and the T cells in ways that should help the development of better vaccines and new cancer therapies.

We work on the ‘co-stimulation’ signals that kick-start T cells into action when they are needed to fight an infection or destroy cancer cells. The co-stimulatory molecules are proteins on the T-cell surface that dock onto partner proteins on other white blood cells. We know they’re important as we’ve shown that mice lacking co-stimulatory molecules are unable to react properly against the influenza virus.

This work involves elaborate technology and can only be done in an institute such as the NKI where there are good facilities and a lot of dedicated people with different expertise. We are excited about the prospect of applying our findings in the clinic, for example, by using antibodies to block co-stimulation and switch off the damaging action of T cells in autoimmune disease. Conversely, we are designing new types of cancer vaccines that provide strong co-stimulation, so that the patients’ T cells can destroy tumors more effectively.

We are also excited by another project that uses a protein called TRAIL to kill cancer cells by triggering cell suicide or apoptosis. TRAIL is attractive as it leaves most healthy cells unaffected. Other groups outside of NKI are testing the effects of TRAIL in clinical trials. Our group is taking another approach and combining TRAIL with radiotherapy. Animal studies show that injecting TRAIL into mice at the same time as irradiating the tumor kills far more tumor cells than either method alone. We believe this could be important in the clinic, particularly as the precision of radiotherapy enables the effect to be confined to the tumor site.

**SELECTED PUBLICATIONS**


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Drug resistance 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. In 1983 he became director of the NKI, introducing a streamlined style of management. He handed over to Anton Berns in 1999, but has retained his laboratory at the Institute.

I originally brought to the NKI my work on trypanosomes — parasites that can change their surface coat, enabling them to remain ‘invisible’ to antibodies and thus evade human immune defenses. During this work we discovered a previously unknown DNA base, base J, which is unique to trypanosomes and related parasites, and comprises around 0.2 per cent of their DNA. Now we are trying to understand how base J forms and how it functions. Its uniqueness also makes it a potential target for new anti-trypanosomal drugs.

Base J turns out to be derived from thymine through the actions of two hydroxylases. Discovering these enzymes has opened up new possibilities for interfering with base J formation as a possible means of attacking trypanosomes. In collaboration with Charles Weissmann at the Scripps Institute in Palm Beach, Florida, we are screening for potential drugs that inhibit the hydroxylases.

In an entirely different project at the NKI, my team has helped identify some of the molecular pumps in cancer-cell membranes that remove drugs from the cells, making them resistant to treatment. These are members of the ABC transporter family. We’ve made cell lines that overproduce the pumps and also ‘knockout’ mice lacking one or more pumps. The mice show us that the pumps often prevent the uptake of drugs from the gut and their penetration into the brain. These resources are being used by the pharmaceutical industry to screen for candidate drugs that do not interact with the pumps.

We are also very excited about the first proper animal model for drug resistance in an epithelial tumor. These mice, engineered by Jos Jonkers (see page 41), lack the BRCA1 protein and spontaneously develop breast cancer. The tumors respond to high doses of drugs, but eventually become resistant. How, we are now trying to find out.

SELECTED PUBLICATIONS


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Fifteen years ago, John Collard set out to find genes that influence the spread, or metastasis, of cancer in the body. He soon discovered a gene called Tiam1, and has since concentrated on revealing its complex functions in cancer cells.

Tiam1 is an intriguing gene. To start with, it is involved in making cells stick together in cell sheets and organs. Cells that carry mutant versions of the gene tend to break away from each other, because the adhesion protein cadherin, which binds cells together, cannot work properly without Tiam1. Cell detachment from primary tumors is a key part of metastasis, the process that allows cancer cells to migrate through the body and grow at sites far away from the original tumor.

To study the effects of Tiam1 we made ‘knockout’ mice that don’t express the gene. Generating these mice is intriguing in itself, but the real excitement comes from seeing what the Tiam1 protein can do, and unraveling its effects in various pathways leading to tumor initiation and progression.

One of our very first experiments revealed that Tiam1 knockout mice were remarkably resistant to carcinogens. But while most did not get tumors at all, in the few that did, the tumors were small but highly aggressive and metastatic. So Tiam1 seems to have several functions in addition to controlling cell–cell adhesion.

Preventing programmed cell death, or apoptosis, appears to be one function of Tiam1. Cells lacking Tiam1 are more sensitive to apoptosis, so that even if they become cancerous, most of them die. The few that survive, however, are more aggressive and rapidly metastasize, as a result of the loss of Tiam1’s effects on cell adhesion and cell migration.

In tracking down Tiam1’s role we have knocked out its gene in mouse strains that already develop tumors spontaneously, a task that was made much easier because of the many such strains at NKI. These mice are providing a fast track to validate findings from cultured cells and to elucidate how Tiam1 expression could be influenced in order to treat cancer.
Cancer cells: PIPIped at the post
Nullin Divecha

With a PhD from Sheffield, UK, Nullin Divecha worked at the Babraham Institute in Cambridge, UK, before moving to the NKI in 1998. He currently studies the way that PI lipids in cell membranes transmit signals and thus help determine the cell’s immediate fate.

Cells frequently find they have a message-bearing molecule on one side of a membrane that needs to cause an effect on the other side. Many operate by influencing signaling systems within the membrane I study the phosphatidylinositol (PI)-based systems. These involve lipids that are present not only in the external membrane, but also in many different membrane compartments within cells.

Part of the task is seeing where in the cell these pathways are located, and what situations bring them into existence. We place fluorescently labeled probes inside the cell, in the cytosol, and observe them move to the PIs when they are generated in the membrane – you can watch that occur in real time. These probes are very popular because different probes can target different compartments within the cell. Our aim is to understand the orchestrated activity of all of the PIs in their various locations.

The relevance to cancer is that some PIs are part of the p53 cancer suppression system. If DNA is damaged, or the cell is under stress, then p53 activity usually increases and prevents the cell from dividing. Removing PI, which we can do by overexpressing an enzyme that uses it, reduces p53’s power to act. This is a real problem for the cell and can lead to the development of cancer. Interestingly, the enzyme that uses this PI is also overexpressed in human tumors.

One other piece in the puzzle is that when cells are about to undergo suicide they downregulate the amount of a different PI. As this PI is involved in regulating many cellular functions the cell is immediately in trouble. It would appear that downregulation of this PI is one of the very early stages in apoptosis. We have found that one of the enzymes responsible for making this lipid is upregulated in human cancers and may therefore points to an appealing target for cancer therapies.

SELECTED PUBLICATIONS


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Maarten Fornerod likes to operate at borders where, he believes, some of the most interesting things happen. His challenge is to understand how events at the border between the nucleus and cytoplasm regulate cell activity.

My group’s interest is the nuclear membrane, the boundary between the nucleus and the cytoplasm. It contains pores through which RNAs and proteins, including those that switch genes on and off, move into and out of the nucleus as the cell responds to different signals. As an analogy, I think of the old Roman border between Leiden and Nijmegen, where all sorts of exchanges took place: artifacts from all over the empire have been found there. So the border reflects the dynamics of the entire system, while at the same time remaining relatively stable.

In the same way, movement over the nucleus–cytoplasm boundary reflects what’s going on in the whole cell. Our biggest challenge is to find the most important interactions at this border, as these are often the most difficult to detect. We use a variety of techniques — biochemistry, confocal and electron microscopy, and DNA microarrays. Knockout mice that lack certain proteins also help us understand the relevance of the various players.

We have also collaborated with Bas van Steensel (see page 62) to use the DamID technique to track interactions between chromatin (DNA bound by histone proteins) and proteins called lamins that line the inside of the nuclear membrane. We find in particular that DNA that does not contain genes binds to this inner shell, implying that the DNA between genes, the so-called ‘junk’ DNA, is important in determining the arrangement of the genome within the nucleus.

These experiments are beginning to cast light on how the position of a gene in the nucleus is linked to its activity, which in turn may be crucial to ensuring that cells keep functioning correctly.

SELECTED PUBLICATIONS


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Attacking cancer with T-lymphocytes
John Haanen

John Haanen is a medical oncologist who devotes half his time to the hospital and half to research at the NKI. His dream is to see novel therapies in the clinic that harness the ability of the immune system to fight cancer.

The immune system recognizes at least some tumors as ‘foreign’ and mounts an immune response against them. My research group works on developing immunotherapies to strengthen this response, in particular for the skin cancer melanoma and the penile and cervical cancers caused by the human papilloma virus (HPV).

A therapeutic vaccine for cervical cancer will be different from the recently developed preventive cervical cancer vaccines, which are intended to prevent virus infection. They elicit antiviral antibodies that mop up HPV before it can infect its target sites and trigger cancer. In contrast, a therapeutic anticancer vaccine will need to activate the killer T cells of the immune system to attack the cancer cells themselves.

We collaborate closely with Ton Schumacher on two vaccine delivery strategies. One is a tattoo machine that we use to inject a DNA-based vaccine into the upper layers of the skin. It’s a simple device for a high-tech vaccine, but it is a great way of delivering up to 30,000 tiny shots all at once, to an animal or person, and spreading the exposure to antigen over a wider area of skin than would be achieved with a traditional needle.

The epidermis is packed with cells that help generate a strong immune response, including the dendritic cells that present tumor antigens to the immune system. Killer T cells with the appropriate receptors ‘see’ the tumor antigens on the surface of the dendritic cell, become activated, and then seek out and destroy the real tumor cells. We are producing clinical-grade DNA vaccines against cervical tumors and hope to begin phase 1 clinical trials in 2007.

The second delivery strategy modifies gene therapy procedures. The idea is to take T cells of any specificity from the patient’s blood, and insert DNA coding for receptors designed to latch tightly onto the tumor antigens. We would then inject the T cells back into the patients, where they should be able to attack the tumor more strongly.

SELECTED PUBLICATIONS


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Breast cancer genes
John Hilkens

Having arrived at the NKI in 1975, John Hilkens has extensive experience in cancer research. He uses the development of mammary tumors in mice to track down genes involved in the progression of breast cancer and its spread to other tissues.

The dream of cancer research is to find all the genetic pathways that make cells cancerous, and to find how they interact – then you can develop drugs to hit specific targets. But I’m a realist, and I know that we first need to find those genes that play outstanding roles in cancer. These will give us clues to the rest.

The NKI provides one of the best environments in the Netherlands to pursue this aim because of its excellent resources and strong research teams.

A powerful tool for revealing genetic pathways in cancer is a technique called insertional mutagenesis. Mice are infected with retroviruses, which randomly insert their DNA into the chromosomes of infected cells. Control regions in the retroviral DNA insert can turn on an adjacent mouse gene, and if that gene is an oncogene, the infected cell may give rise to a tumor. Finding where the viral DNA is inserted then points to the cancer-causing gene.

Our immediate goal is to use our observations in mice to identify equivalent genes in human breast cancer.

To discover genes involved in cancer spread, or metastasis, we have developed our own in vivo assay. For instance, we take lungs from a mouse that has a mammary tumor and inject the lung cells under the skin of a genetically similar mouse. If metastatic cells from the mammary tumor are present in the lungs they will give rise to a tumor at the site of injection. By comparing the genes expressed in the primary and the secondary tumors we discover genes involved in metastasis.

We’re now studying a newly discovered gene family called the R-spondins. There are four known members, two of which we have found to be involved in breast cancer. Now all we have to do is find out how they work!

SELECTED PUBLICATIONS


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Programmed mutagenesis
Heinz Jacobs

Heinz Jacobs began research at the NKI in 1987 as a graduate student, and drawn by its excellent research environment he returned to the NKI in 2002. He is now a group leader in molecular immunology and programmed mutagenesis.

My group is interested in programmed mutagenesis and its causal relationship to cancer development. Programmed mutagenesis is part of the normal development of specialized immune-cells known as B lymphocytes. However, B cells can give rise to cancers known as lymphomas, and we suspect a link between the natural mutation-creating process and lymphoma development.

B cells produce antibodies, the proteins that latch onto and help destroy invading bacteria, viruses and parasites. B cells stimulated by these invaders activate an enzyme called AID, which creates lesions in the genes that code for antibodies. This damage is repaired, but errors are introduced, which results in B cells that produce antibodies slightly different to the previous ones. Some of these bind more strongly to the invader, so that the immune response becomes more efficient over time. The downside to this beneficial process, we believe, is that the AID enzyme sometimes targets other genes, and so B cells undergoing this programmed mutagenesis are at risk of acquiring potentially cancer-causing mutations.

We are using the DamID technique (see Bas van Steensel page 62) to identify all genes that AID can bind to and damage. The special error-prone DNA polymerases called TLS polymerases that repair the AID damage are recruited to the lesions in DNA. We are trying to understand what regulates when these DNA polymerases act, and when other, more precise, repair processes act instead to restore the exact original DNA sequence. We think that this regulation is what keeps the balance between permitting mutations to persist in antibody genes and eliminating lesions in other genes that might lead to cancer.

We hope that eventually the insights we gain into lymphoma development will provide us with markers that can be used in the clinic to predict the course of a lymphoma and to select the best treatments.

SELECTED PUBLICATIONS


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Biophysics of cell signaling
Kees Jalink

Kees Jalink is a biophysicist who loves to tinker. He brings new technology to the NKI, and is an advisor to three companies on the creation of new devices. He often builds prototypes in the lab using individual pieces and sticky tape and then invites industry in to make them user-friendly.

My group spends half of its time getting techniques running and serving as a biophysical nerve center for the whole of the NKI, collaborating and publishing jointly with others. With the other half of our time we focus on our own research.

Our expertise is in following cells individually by light microscopy. This allows us to see important events that would otherwise be missed by biochemistry on, say, 10,000 cells. A big challenge is overcoming the resolution of light microscopes, in which we can only see things bigger than a quarter of a micrometer across. Individual proteins, for example, being 50 times smaller, are invisible to us. One option is to tag a protein with a fluorescent dye which indicates where a protein is.

It does not, however, reveal interactions between proteins. To do this we employ another trick known as FRET (fluorescence resonance energy transfer). In this we tag different proteins with different fluorescing molecules. When the proteins come within 5 nanometers of each other, energy transfers between the tags. This causes changes in the light that each molecule is emitting. We can put these into a single cell, watch both proteins and see whether they are interacting.

We also monitor the rate of intracellular reactions and can use these fluorophores to watch proteins change shape. Collaborating with Rob Michalides, for example, has revealed that resistance to tamoxifen treatment in some breast cancer patients is linked to a lack of conformational change by the estrogen receptor in their tumors. In our own research, we study the ion channel TRPM7 that is involved in cell adhesion and metastasis. This sits in the membrane of extended processes, invadopodia, that are used by metastatic cancer cells to squeeze out between the cells lining blood vessels and into surrounding tissues.

SELECTED PUBLICATIONS


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Mouse models of breast cancer

Jos Jonkers

After working at the NKI as a PhD student and postdoc, Jos Jonkers spent six months in the Sanger Centre in Cambridge, UK. In 2002, he was drawn back to the dynamic research base at the NKI by the prospect of being able to rapidly translate basic research into clinical tools for diagnosis, prognosis or therapies.

Here at the NKI we have created new strains of mice that will improve laboratory studies of breast cancer considerably. The overall aim was to alter a few carefully selected genes so that the mice spontaneously generate tumors that resemble those seen in humans.

If mice carry too many mutations, however, they cannot breed, and cells don’t grow properly. To just mutate the genes in the cells we are interested in, we use a genetic recombination system called Cre-LoxP recombination, which enables us to turn off defined sets of genes in just a few cells within a particular tissue. This mimics much better the way that cancer arises in humans, and we now have a world-beating colony of mice with different combinations of mutations in genes associated with breast cancer, such as BRCA1, BRCA2, P53, and E-cadherin.

We are using these mice to identify novel cancer genes and to learn how breast cancers grow and metastasise. We are also using them to test novel tumor intervention strategies. 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. These mice have huge potential, and it is made even greater by our new ‘mouse clinic’, which lets us treat each mouse as if it were a human patient. We’ve already shown in these mice that platinum-based drugs have distinct promise as chemotherapeutic drugs for BRCA1-associated breast cancers. It will be exciting to see whether this holds true in human trials.

I am fascinated by the intellectual challenge of working in cancer research. Our task now is to speed up the process of translating understanding of the science into therapies, and our mouse models will be important links in that chain of discovery.
Estrogen receptor and breast cancer
Rob Michalides

Since arriving at the NKI in 1975, Rob Michalides has pursued many lines of cancer research. He now has resistance to the cancer drug tamoxifen in his sights, and is focusing his efforts on understanding tamoxifen’s target — the estrogen receptor.

Estrogen hormones often stimulate the growth of breast cancers, and so anything that prevents this is a potential therapy. The drug tamoxifen is a good example — it works by inhibiting the estrogen receptor on breast cancer cells. This receptor only becomes activated when both estrogen and other molecules (cofactors) bind. Tamoxifen works by blocking both estrogen and cofactor binding.

However, tamoxifen prevents only half of the recurrences in breast cancer. The recurrences that do occur are either resistant or the tamoxifen makes their cancer grow faster. They would be better served by, for instance, fulvestrant, a drug that blocks the estrogen receptor in a different way. So we want to find out two things: what is going on at the estrogen receptor to cause these effects, and can we determine beforehand who will benefit from tamoxifen?

We’ve found that a cellular enzyme called protein kinase A (PKA) can promote tamoxifen resistance. When PKA is active, the cofactor-binding pocket remains open even when tamoxifen binds to the receptor. If cofactor binds, the receptor is activated and sends a growth signal to the cell. To confirm this we engineered estrogen-dependent tumor cells that also overproduce PKA. True to expectation, they continued to grow in the presence of tamoxifen.

To tackle our second question, we’ve worked with the American company Upstate to develop an antibody that sticks only to PKA-modified receptors. At the hospital we are screening a panel of 200 breast cancer patients with these antibodies to see whether the tumors with PKA-modified receptors are also the ones that do not respond to tamoxifen.

If so, we’ll have a valuable new test to guide therapy.

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LPA, a multifunctional growth factor
Wouter Moolenaar

In the early 1990s, Wouter Moolenaar’s discovery of a lipid called LPA set the course of his career. Intrigued by its potential role in cancer, he has devoted his energies to defining its actions and discovering potential anticancer drugs that inhibit its formation.

Most people think of lipids as just the boring building blocks of cell membranes, but lysophosphatidic acid (LPA) is far from boring. It’s made outside cells and transported throughout the body in the bloodstream and, unusually for a lipid, it stimulates cell growth, proliferation and migration.

As this activity is important in both normal development and cancer formation, we decided to look at how LPA alters a cell’s behavior in these ways. We’ve found that LPA switches on activities such as cell proliferation and migration through a variety of molecular switches that stimulate intracellular signaling pathways. We also discovered that LPA is produced following injury and blood clotting, which points to an important role in wound healing.

In collaboration with the NKI clinicians, we have found that levels of LPA are extremely high in peritoneal fluid from patients with ovarian cancer, suggesting a role for LPA in the spread of ovarian cancer to the peritoneum.

Our current focus is on an enzyme called autotaxin, which makes LPA. To investigate its action, we’ve made mice that lack autotaxin, and so cannot make LPA. These mice have shown us that one role of LPA is in the formation of blood vessels during development. That gives us its link to cancer, as tumors need to make new blood vessels if they are to grow.

Our great motivation is that patients will benefit from our discoveries. Autotaxin is an ideal drug target as it is found outside cells and is easily accessible to drugs in the bloodstream: several pharmaceutical companies are looking at it as a drug target. At the NKI we are screening more than 20,000 chemical compounds to identify autotaxin inhibitors, which we’ll then use to test our ideas about how autotaxin and LPA work in the body. Compounds that block angiogenesis by blocking autotaxin might be the route to new, more specific, cancer therapies.

SELECTED PUBLICATIONS


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Improving immune responses to tumors
Jacques Neefjes

Jacques Neefjes trained in chemistry at the Free University Amsterdam and did his PhD at the NKI on the cell biology of antigen presentation in the immune system. After a postdoc at the DKFZ in Heidelberg, Germany, he joined the NKI in 1993, and in 1999 became head of the Division of Tumor Biology.

We aim to understand how the immune system recognizes and responds to cancer cells in general, and then find ways of encouraging a patient’s immune system to attack their own cancer. Immunotherapy might be used by itself, or it could supplement treatments such as radiotherapy.

These therapies will involve the white blood cells known as T lymphocytes. We know that T cells can recognize tumor cells as abnormal and eliminate them, but at some point, tumors develop the ability to evade them. Our task is to find a way round this.

We are exploring two main strategies. One is to render tumors more visible to the immune system. We’ve found that low doses of radiation increase the production of abnormal proteins by tumor cells, which sends a stronger signal to the immune system, and we are currently testing the effects of this treatment in mice.

The second strategy is to increase the numbers and range of T cells that can attack the tumor. We can grow a patient’s T cells outside the body to expand their numbers and then inject them back into the patient. We’re also developing ways of stimulating T-cell expansion inside the body, using bacteria as the stimulant to boost the specific immune response to tumor proteins. We would then use radiotherapy to increase the tumor signal and so direct large numbers of T cells to the tumor. We want to test whether this sort of treatment will be sufficient to enable the immune system to recognize and eliminate metastases. If successful, it will give us a new combination therapy to be introduced into the clinic.

We hope to try out these ideas first in patients with melanoma or kidney cancer, for which we already have encouraging evidence that tumors can spontaneously shrink and disappear – which may be due to immune attack.

SELECTED PUBLICATIONS


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Chemical tools for cancer research
Huib Ovaa

After a PhD in organic chemical synthesis at Leiden University and three years at Harvard University, Huib Ovaa arrived at the NKI in 2005. Although one of the newest members of staff, he has already built up a strong team of chemists within the Institute.

My aim is to use my chemical skills to make new research tools that will allow others to tackle previously intractable problems in cancer research.

My group’s collaboration with Ton Schumacher’s lab (see page 52) is a good example of how we work. Together we’re looking at ways of detecting and characterizing the T cells of the immune system, which are the cells that cancer vaccines will need to stimulate. We characterize T cells by finding out which antigen they recognize — which is always a small peptide of some kind. We mix the T cells with ‘tetrarmers’ of four MHC proteins complexed with a given peptide — and see if they bind.

But there was one big problem. To make this screening system really practical we needed to be able to ‘mass-produce’ the MHC tetrarmers on their own, so that they could be stored, and then combined with whatever peptide antigen was needed. But we couldn’t do that at first, because MHC proteins only assemble and remain stable if they’ve already got a peptide to bind.

This is where my group’s chemical expertise came in. We’ve made MHC tetrarmers carrying a ‘temporary’ cargo of specially designed molecules. These tetrarmers are stable and can be stored. The trick is that we made this cargo sensitive to UV light. When we want to use the tetrarmers, we put them into a solution containing the required peptides. UV illumination breaks the old cargo in shorter fragments: they drift free and the peptide takes their place — it really works!

Now that we’ve shown how quick and simple this method is, it’s available for others to exploit in their research.

SELECTED PUBLICATIONS


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Identifying novel cancer genes

Daniel Peeper

Molecular biologist Daniel Peeper came to the NKI in 1995, after a PhD in Leiden and a postdoc in Boston. He is fascinated by how deregulated genes cooperate to cause cancer, with the aim of finding new drugs that block this process.

My group is identifying novel genes involved in cancer, some of which might correspond to specific targets for new therapy. We use functional genomics, which entails screening the 30,000 genes in the human genome for specific, cancer-relevant functions. Our focus is on defining genes involved in two phenomena that protect us against cancer: ‘oncogene-induced senescence’ (premature cell aging) and ‘anoikis’ (a form of cell death). We aim to identify the genes which, when mutated, interfere with these processes and cause cancer.

It may seem paradoxical that activation of an oncogene can cause cells to undergo senescence, as oncogenes were defined by their ability to contribute to tumor formation. What happens, however, is that ‘tumor suppressor’ genes become activated and serve as protective brakes on proliferation. Only in the presence of additional mutations do cells start proliferating. We have discovered several senescence genes that play a role in cancer. But senescence is more than just a powerful tool: we found that human melanocytic naevi (‘moles’) are in a state of active senescence. They express a mutant oncogene, have activated tumor suppressors and are in a stable state of growth arrest. Thus, senescence protects us against cancer; only rarely does the senescence program become deficient, allowing malignant melanomas to emerge. Our second major research theme corresponds to the identification of genes causing tumor cells to spread, a major cause for the death of cancer patients. Upon detachment from their natural environment cells die by anoikis. Thus, tumor cells must resist anoikis when they metastasize to other parts of the body.

In a genomic screen for anoikis resistance genes we recently identified the neurotrophic receptor TrkB, which we demonstrated to act as an enzyme capable of rendering cells anoikis-resistant, giving rise to highly metastatic tumors. We are currently exploiting the intriguing possibility that TrkB represents a novel target in cancer therapy.

SELECTED PUBLICATIONS

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Anastassis Perrakis received his PhD in 1996. For the next two years he was an EMBO post-doctoral fellow at the NKI. Following a three-year appointment as staff scientist at the EMBL Grenoble outstation, in 2000 he moved back to the NKI to set up a new research team in structural biology.

My interests are split two ways. On the one hand I develop ways of moving rapidly from X-ray crystallography data to a detailed knowledge of a protein’s 3D atomic structure. The data is in the form of diffraction patterns, which then have to be interpreted to determine the protein structure. In the early days of crystallography, researchers built real models with metal sticks, nuts and bolts, and colored ribbons. More recently, they’ve had to sit for hours in front of the computer screen manipulating their structure. But we’ve developed software, called ARP/wAPP, where you simply enter the X-ray data and the protein’s amino-acid sequence, and then press ‘run’. A couple of hours later, you have the structure! Around 90 per cent of X-ray crystallographers now use this software, and it has featured in some 2,500 scientific papers. We are now exploring further concepts in statistical pattern recognition to make our software work even better.

My other interest is in structural biology itself. In our team we are trying to sort out the relationship of structure to function of proteins involved in critical control points in the cell-division cycle. One of the proteins we are trying to understand is geminin, which controls both cell proliferation and differentiation — two opposing cell fates with enormous implications in the development of cancer. We also study the Polo kinases, enzymes that are often referred to as the ‘choreographers of the cell cycle’.

Our constant aim is to understand how the chemical structures of complicated macromolecules enable them to do their job, and what has gone wrong at this level when they stop doing it properly. Others can then use this information to develop better cancer therapies.
Cryo-electron tomography in the cell
Peter Peters

With a passion for invention, Peter Peters has worked in a range of jobs from technician to professor. His ingenuity is driving developments literally at the cutting edge of electron microscopy.

I want to see how molecular machines operate inside cells. Some cells have pits in their membranes that accept growth factors, for example, and when these structures get altered it can lead to cancer. One can guess how all the proteins in the pit work together by looking at the structures of individual proteins determined by X-ray crystallography, but being able to see how the proteins really fit together in the cell would remove a lot of uncertainty.

This is where electron microscopy (EM) comes in. After 25 years experience I’ve developed ideas and scientific contacts that could make this dream come true. My aim is to devise a way of doing cryo-electron tomography. This will give us a 3D view of a cell’s internal structure. The principle is rather like a CAT scan, but at the nanoscale and at very low temperature — it’s not going to be easy.

The low temperature overcomes one problem, distortion. In cryo-EM, cells are flash frozen to −180°C. This vitrifies them — the cells become a ‘solid’ liquid (like glass). Another obstacle is getting thin enough slices. Working with the Swiss company Diatom we’ve developed a diamond knife with a tip just 2 Ångstroms across (two ten-millionths of a millimeter), which cuts slices 20–150 nanometers thick (a nanometer is a millionth of a millimeter) at −160°C.

For cryo-electron tomography we take 100 images, slowly tilting the specimen through a 140° arc. We do this twice, along three axes. The result is a 3D map of the electron density within the specimen. The electron density is the raw data from which protein structures are deduced, so our map potentially shows the locations of all the proteins within a slice. By putting a series of slices together we can get a 3D view right through the cell. The next step will be to identify the proteins by matching their electron densities to those determined by X-ray crystallography. The end result will be a tool that could revolutionize cell biology.

SELECTED PUBLICATIONS


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Metastasis: tumor cells on the move
Ed Roos

When Ed Roos embarked on the study of metastasis in 1975 it was “a crazy time” with few technical tools - no monoclonal antibodies, no recombinant DNA technology, and no concept of oncogenes. Inspired by what he could see under the microscope, he set out to understand the processes by which tumor cells invade tissues and grow in new environments.

My group is trying to find out why some tumors spread easily - a process known as metastasis - and others do not. For a long time we focused on lymphoma because it’s a highly invasive cell type: the tumor cells travel via the blood, then squeeze out between the cells that line blood vessels into the surrounding tissues. This is similar to how white blood cells normally behave when they patrol the body looking for infection. We study this invasion process in cell culture with mixtures of normal cells and tumor cells.

In particular, we are investigating two groups of proteins that we believe are important. One group is the adhesion receptors that enable cells to stick to the vessel wall. The other comprises the chemokines. These can attract cells to move when they bind to their receptors on the cell surface. We have recently made progress on a molecule called synaptotagmin, which controls the levels of some chemokine receptors on lymphoma cells. Chemokine receptors are present on a wide variety of tumors, including the common carcinomas. We found that if we block the receptor CXCR4 on carcinoma cells, they are unable to metastasize in animals. Without the action of CXCR4 and other chemokine receptors, the tumors cannot grow beyond a certain size. Currently, we are investigating the hypothesis that the blocked cells are unable to adapt to the lack of oxygen and nutrients within these tumors.

SELECTED PUBLICATIONS


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Pharmacology of anticancer drugs
Jan Schellens

Jan Schellens is qualified in both medical oncology and clinical pharmacology, enabling him not only to diagnose and treat cancer, but also to do research into making anticancer drugs more effective.

The NKI is probably the largest center in Europe conducting phase 1 and phase 2 clinical trials of anticancer drugs, with around 120 trials in progress. We test very new drugs, and different combinations of existing ones. These trials serve to monitor a drug’s side effects, measure how much gets into, and stays in the body for a period of time (pharmacokinetics), and assess its effectiveness against tumors (pharmacodynamics).

The trials involve close collaborations between the NKI’s divisions of medical oncology and experimental therapy, and the pharmacy department, and a trial office that coordinates the gathering of clinical data. We also conduct large-scale phase 3 clinical trials. A current one is comparing two drugs’ abilities to relieve physical and emotional side effects of patients (most often with breast cancer) whose treatment has brought on an early menopause.

A key goal of my own research is to improve the body’s uptake of drugs taken by mouth — up to now most anticancer drugs have to be injected intravenously. We have to find ways of overcoming the gut’s natural defenses against poisons in food, as these hamper the passage of anticancer agents across the gut wall. Uptake from the gut is often prevented by the actions of drug pumps, the ABC transporters, in the membranes of cells lining the gut. We are testing compounds that can temporarily block these transporters, enabling larger amounts of anticancer drugs to get into the body. Using these inhibitors, we can increase the availability to the internal tissues of the ovarium cancer drug topotecan from 30 to 100%, and paclitaxel from 5 to approximately 50%. This raises the possibility of giving drugs more frequently but at lower doses, which could make for safer and more active therapy.

SELECTED PUBLICATIONS


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Improving anticancer drug efficacy
Alfred Schinkel

Alfred Schinkel has a broad interest in the genes and proteins responsible for resistance to anti-cancer drugs. Using knockout and transgenic mouse models his team is providing a broader understanding of drug resistance and the way the body handles drugs, thus supporting optimization of clinical chemotherapy.

We study how drugs move around the body, which is a very important aspect of how efficiently they treat disease. One of the main problems in the cancer field is the resistance that arises in tumors to drugs. Once this has occurred in a patient, the tumor is often resistant to many different drugs, including ones to which the patient has not yet been exposed.

It was found that this can be due to overexpression of molecules named multidrug transporters that can occur in the membranes of tumor cells. These proteins can actively pump large numbers of drugs from the cell. We and other researchers discovered that they are present in normal tissues too, for example in the gut.

A typical example, P-glycoprotein, is present in the small blood vessels of the brain where it prevents toxins and other substances crossing from the blood into brain tissue. Consequently P-glycoprotein will normally prevent drugs from getting to small cancer metastases in the brain. In the gut lining, P-glycoprotein and other pumps protect the body against food toxins. This can prevent certain drugs from being absorbed at high enough concentrations to work against tumors, and forces them to be administered intravenously.

Blocking these pumps might thus improve chemotherapy in various ways. Drugs that block these pumps have been developed, and our collaborators, Jan Schellens (page 50) and Jos Beijnen (page 29), are now testing them in clinical trials to see if these can safely allow anti-cancer drugs such as topotecan and paclitaxel to enter the body via the oral route.

We are currently extending our research to drug-uptake and drug-metabolizing systems, as these may be equally important for drug efficacy, and can be inhibited as well.

SELECTED PUBLICATIONS


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Immunotechnology & immunotherapy
Ton Schumacher

Ton Schumacher’s early career included a period as a postdoc among chemists and physicists. This enabled him to become an inventor of new technologies, which he’s now using to answer biological questions.

Our lab’s activities all revolve around measuring or manipulating immune responses that involve T lymphocytes, or T cells. These are cells with a key role in fighting infection and — in some cases — cancer. In one project we insert unique DNA barcodes into individual T cells, using retrovirus carriers, so that we can follow T-cell differentiation during and after an immune response. This will help us find out, for example, precisely what type of T cell gives rise to the memory cells that linger after an immune response and provide long-term immunity.

In collaboration with Huib Ovaa (page 45), we’ve developed a novel technology for high-throughput detection of T cells, as an aid to analyzing T-cell responses. This involved creating large ‘libraries’ of tetramers of MHC proteins that bind a vast range of peptide antigens. These libraries can be used to detect T cells in a wide range of situations, including the evaluation of vaccine trials.

One day we hope our MHC tetramers will benefit patients undergoing bone marrow transplants for leukemia or lymphoma. At present, the transplant is accompanied by an injection of donor white blood cells, including T cells, in the hope that these will attack any remaining tumor cells. But it’s a haphazard procedure, as there’s no way of predicting what tissues the cells might attack. Our MHC tetramers could be used to select donor T cells specific for tumor-cell antigens that aren’t shared with other tissues. We could then inject the purified T cells into patients. If we are unable to select T cells with a useful reactivity we aim to create them by gene therapy, engineering patients’ T cells so that they bind to tumor antigens at high affinity.

SELECTED PUBLICATIONS


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Titia Sixma enjoys the beauty and solidity of protein structures. She is a crystallographer who combines biochemical analysis with structural studies in order to understand how proteins work.

Protein structures are more than beautiful. They show us how one protein activates another and how two different regions of a protein interact. The structure helps explain biochemical data and the role of the protein in cellular pathways leading to cancer. Structural and biochemical data together can help us design new drugs.

Much of what we study is basic biology, understanding how proteins collaborate in complexes, and to do so we are tackling a technically challenging goal — crystallizing complexes of different proteins rather than a single type of protein from pure solution. We have three main projects. The first is aimed at understanding how mistakes in DNA replication are detected and corrected. Such mistakes would lead to frequent genetic mutations were it not for an active mismatch repair system. If mutations occur in the repair system itself, however, then other mutations can go unchecked, and lead to cancer. We are especially interested in the protein MutS, which checks for the insertion of the wrong bases. A second project focuses on the process of modification by two proteins — ubiquitin and SUMO, especially in DNA repair and chromatin regulation.

Thirdly, in collaboration with the Free University in Amsterdam, we are working on the structure of a relative of the nicotinic acid receptor. It’s fun to study because it’s the only high-resolution crystal structure currently available of an entire class of pharmaceutically interesting proteins. Forty years of biochemistry have been made visible through our structural work, which in the long term may help in the design of compounds that combat nicotine addiction and smoking.

Much of our effort goes into studying the properties of our proteins and their complexes. This tells us a lot about the proteins and their interactions, and gives insight in their mechanisms.

SELECTED PUBLICATIONS


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As well as carrying out his own research and heading a group, Arnoud Sonnenberg is an editor for the Journal of Cell Science. He believes that to understand what goes wrong in cancer, you have to start by finding out how healthy cells work.

In our group we focus on finding out how cells know where they are in the body and know how they should develop. Interactions between the cell-surface proteins called integrins and the extracellular matrix that surrounds cells are crucial to this. Depending on the particular integrins present and the composition of the matrix, cells can behave in very different ways, with implications for tumor development.

We are currently excited about our work on an integrin called α6β4, which helps attach the self-renewing stem cells of the epidermis to a layer of matrix that keeps them from invading underlying tissues. Because they are naturally programmed to be capable of continued cell division, stem cells are prime candidates for cells that could relatively easily be transformed into cancer cells, and stem cells are thought to be the source of many cancers. Integrin α6β4 seems to be particularly important in normally restricting the epidermal stem cells to their correct niche and limiting their ability to grow.

We think that a mutation that causes the loss of α6β4 could allow a stem cell to leave its normal niche and start proliferating rapidly; this would encourage the accumulation of more mutations that could eventually transform these cells into full-blown cancer cells. Complete transformation probably entails the production of other integrins. When wounds heal, for example, skin cells make a new set of integrins that lets them migrate and settle in a different place. If our ideas on integrins turn out to be true, one could view skin cancer as an uncontrolled version of wound healing — a normal process gone wrong.

So far, most research on integrins uses cell cultures. The next big challenge is to establish good animal models in which we can apply our knowledge and discover the full role of integrins in cancer.
Vascular damage after radiotherapy

Fiona Stewart

After working at the Gray Cancer Institute in London, UK, radiation biologist Fiona Stewart came to the NKI in 1984. Planning to stay for just a short time, she found the Institute so inspiring that she is still here.

I mainly work on the damage that occurs to normal tissues during radiotherapy. More than half of the patients who are cured of their cancer have received this therapy, and we are becoming more and more concerned about its long-term effects. In the early years of radiotherapy, people were just relieved to have their cancer treated, but with survivors now living 30 years or more, we need to prevent serious long-term side effects.

We now know that people who have had radiation therapy are at increased risk of cardiovascular disease and stroke. Using mice that have been genetically engineered so that, like humans, they are susceptible to atherosclerosis, my group at the NKI has found that radiation accelerates its onset. It also makes blood vessels more likely to make thrombotic plaques, which are more likely to rupture and block an artery than are plaques formed in unirradiated vessels.

Radiation seems to trigger plaque formation by damaging the cells that line blood vessels. I’m now trying to work out why the atherosclerosis pathway keeps progressing rather than dying down once therapy stops. We are comparing biopsies from irradiated and unirradiated tissues in patients undergoing surgery many years after cancer treatment. I’m also using mice to test drugs that could improve the situation.

Damage to small blood vessels is also a serious issue. After radiotherapy, people can be left with reddened skin in which capillaries have become fragile. The skin breaks down easily, leading to bleeding and infection. Capillaries in internal organs also become damaged. In prostate cancer, bleeding from the rectum after radiotherapy can be so severe that the patient requires surgery. We urgently need to understand why the body doesn’t restore this tissue damage, and then try to block these unwanted reactions to aid recovery, or provide supportive therapies.

SELECTED PUBLICATIONS


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Gene modification: subtle is the oligo
Hein te Riele

Over the 18 years that Hein te Riele has worked at the NKI his original simple desire to beat cancer has matured into a determined effort to understand how most cells avoid becoming cancerous by repairing the mutations that can lead to uncontrolled cell proliferation.

My team works at the molecular end of cancer research, on the systems that cells use to prevent mutations. One way in which human cells protect the integrity of their 3 billion bases of DNA is an error-checking system that spots and repairs mismatched bases. This repair system also stops a cell with faulty DNA from dividing, and so prevents mutated genes from being copied into new cells.

In around 15% of tumors this safeguard has failed, and this is probably a major cause of the tumor. Making sense of what’s going on is complicated by the fact that we still do not fully understand how mismatch repair works in healthy cells.

One approach to finding out is to subtly alter the genes that build the mismatch repair system. To make these mutations we use a short single strand of DNA with a base sequence that plugs on to the gene we want to alter, but has a few differences from it. Paradoxically, we have to temporarily disable the mismatch repair system to do this, to stop it repairing our changes. We stop the cell making repair proteins for a couple of days using a technique known as RNA interference, which gives some cells time to accept the new mutation.

It’s a powerful technique, but not easy. Currently we’re the only lab in the world that can pull it off. We make mutations in cultured embryonic stem cells, which we use to generate mice with the desired mutations. We can then see if the mutation we’ve made predisposes the mice to cancer. In this way we are teasing apart the individual roles of the mismatch repair proteins.

SELECTED PUBLICATIONS


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Membrane rafts as gateways for drug
Wim van Blitterswijk

Coming to the NKI 36 years ago, Wim van Blitterswijk has spent most of his career here. He now concentrates on mechanisms of signal transduction, with a special interest in the role of lipid rafts in the signaling pathways that lead to cell death.

Twenty years ago I first learnt that certain lipids congregate in cell membranes as structures called ‘lipid rafts’. I also knew that an anticancer agent, the artificial lipid alkyl-lysophospholipid (ALP), kills cancer cells by acting on the cell membrane, but at that time we lacked the tools to investigate further.

We now have those tools, and ALP and lipid rafts have become hot research topics because of their connection with apoptosis—the induced cell suicide that appears to play an important role in the body’s natural defenses against cancer, and which is being explored as a target for new treatments. Progress has been fast, and ALP is now being tested in clinical trials at the NKI. My group’s research aims to provide an understanding of all its actions so that treatment can be tailored to the individual patient.

ALP is taken up by lipid rafts and moves into the cell as small packets of membrane pinch off inwards. We’ve shown that, once inside, it blocks production of a major membrane lipid — phosphatidylcholine. The cell becomes metabolically stressed and goes into apoptosis. But if you disrupt the rafts, ALP cannot enter the cell and has no effect.

We also discovered that some cells can develop resistance to ALP by no longer making sphingomyelin, one of the lipids needed for raft formation. We found that these cells are also resistant to other agents that normally trigger apoptosis, such as gamma irradiation and compounds that stimulate the so-called ‘death receptors’. These were thought to act by different pathways, but our findings show that the rafts must be a common feature. The next step will be to see if resistance against ALP develops in clinical trials.

SELECTED PUBLICATIONS


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Genetic alterations in breast cancer
Marc van de Vijver

Marc van de Vijver interrupted his clinical training in the 1980s to spend more than four years doing basic research at the NKI. This led to an important contribution to breast cancer research and inspired his present career, in which he combines research with clinical pathology. He encourages other MDs to follow suit.

During my postgraduate studies I found that some breast cancer patients carry extra copies of a gene called HER2. This turned out to encode a receptor (HER2) for a growth factor, which helped us understand why these breast cancers grow more rapidly. Subsequent research, mainly by scientists in the United States, led to the development of the anticancer drug Herceptin, which blocks the action of the HER2 protein.

My group now has two goals. First, we want to elucidate the biology of breast cancer development by identifying the initial genetic changes that occur and determining how these lead to the abnormal growth of breast tissue.

Second, we are trying to identify genetic profiles in breast cancers that tell us how long a patient is likely to survive (their prognosis), and the likely effects of different drug treatments. This will help guide clinical decision-making for each patient, enabling them not only to live longer but also to avoid unnecessary treatment and its unpleasant side effects.

Targeted drugs like Herceptin are expensive, and there are many more in the pipeline, making the ability to predict a patient’s response an extremely important future goal.

In collaboration with Laura Van ’t Veer (see page 63) and René Bernards (see page 30), we have already identified a profile composed of 70 genes whose pattern of expression seems to be linked to longer survival in breast cancer patients. Before applying this routinely in the clinic we need to test whether the association holds true for large numbers of patients. Together with 15 other hospitals in the Netherlands, we are following up to 600 patients to see if their progress matches with the presence or absence of this 70-gene signature. The next step will be an international trial involving thousands of patients.
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. She is also applying epidemiology to identify those at risk from the late side effects of cancer treatment.

Epidemiological studies help identify factors affecting health and disease that may not be immediately obvious. Once these links are known, they can help people avoid disease and may also help us improve treatment.

I’m currently interested in hormone-related cancers in women — breast and ovarian cancers. My group recently carried out a systematic review of the literature that strongly suggests that women who take more exercise are less likely to get breast cancer. We believe this could be because exercise influences hormone levels. If our theory is correct, this is very exciting. Many risk factors that influence a woman’s chance of breast cancer are either out of her control or are an intrinsic part of her lifestyle. But if exercise protects, then women could help themselves by deciding to take more. Other hormone-related choices that women make are their use of oral contraceptives and hormone-replacement therapy, and we are looking to see if these change the risk of cancer. Part of this study involves following a group of women who had hormone stimulation as part of fertility treatment.

As more people survive cancer, we are realizing that 20 or 30 years after receiving treatment, they are at increased risk of new tumors and cardiovascular disease. One study we are doing in this field is following 3,500 patients treated for Hodgkin’s lymphoma in the 1960s through to the 1990s — it’s already found that female survivors are at a high risk of developing breast cancer.

To get the most out of epidemiology I collaborate with experts in molecular biology. At the NKI this is easy because we know each other, and everyone’s offices and labs are close by. My group is working with Laura Van ’t Veer to put together her genetic profiles of patients with epidemiological data on cancer survivors. Combining the two should help us predict which patients are most at risk of this late-onset cancer.

SELECTED PUBLICATIONS


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Epigenetics in yeast
Fred van Leeuwen

Beginning his scientific career at the NKI by working on African trypanosomes, Fred van Leeuwen became fascinated by epigenetics – the process by which genes are permanently switched on or off. This took him to Seattle, USA, and back to the NKI in 2003.

We’re interested in how cells maintain their identity and pass a memory of which genes are switched on or off to daughter cells through cell division. Understanding the mechanisms will give us insights into how some cancers may arise.

Every cell in the body has the same genes, but varies according to which ones are switched on or off. And this pattern of gene activity is passed on to future cell generations. The process by which this happens – known as epigenetics – involves ‘packaging’ of DNA, in the nucleus by wrapping it around proteins called histones. Small changes in this packaging can affect the ease with which the cellular machinery can ‘read’ its sequence.

As well as being caused by mutations, certain cancers can arise from epigenetic changes. For example, patients with an inherited form of colon cancer often have a mutation in MLH1, a protein involved in DNA repair. In contrast, people who develop the disease spontaneously, with no family history, usually have a normal version of the gene. But it is often switched off, rather than inactivated by mutation.

We would like to be able to revert the epigenetic state of a tumor cell to a normal cell. But before we can do that we need to understand the epigenetics of normal cells.

As an experimental system we are studying budding yeast. We use reporter genes that give the cells a color to reveal which parts of the genome are turned on or off as the yeast colonies grow. These colors let us watch the pattern of gene activity as it propagates through the generations. We have two broad aims: first, to find new players – enzymes that introduce modifications on the histone proteins that cause genes to become ‘silent’, and second, to understand how such modifications are passed on to new cells.

SELECTED PUBLICATIONS


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Cell fate control by Polycomb silencers
Maarten van Lohuizen

Maarten van Lohuizen has come full circle since gaining a PhD at the NKI in 1992 studying oncogenes with Anton Berns. After a postdoc on cell-cycle genetics at the University of California, San Francisco, he returned to the NKI in 1995 and is now head of the Division of Molecular Genetics.

I’m fascinated by the master switches that control cell and tissue development, and how these go wrong in cancer. Our group is working on one set of these switches, the Polycomb-group proteins, which control cell fate and identity both during embryonic development and throughout the rest of your life. We know these proteins are involved in tumor formation. Understanding this in detail could lead to entirely new types of drugs that act in more precise ways than simply killing rapidly dividing cells.

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. 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 gut lining. We suspect that many cancers are the result of stem cells losing their normal response to their immediate environment and continuing to divide when they should be starting to differentiate. 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.

In collaboration with Anton Berns (see page 31) and the Sanger Institute in the UK, we are using large-scale screens for mutations caused by retrovirus insertion to systematically find genes and pathways involved in cancer. With these screens we have already identified one Polycomb gene, BMI1, with a 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.

SELECTED PUBLICATIONS


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Chromatin genomics
Bas van Steensel

Bas van Steensel envisions the thousands of genes that make up a genome as musicians in an orchestra. Each gene has to act in perfect coordination with all others, and correct timing and levels of expression are essential. Unlike an orchestra, however, the genome is not controlled by a single conductor, but by several hundreds. Chromatin proteins and transcription factors are these ‘conductors’ of the genome.

Our lab wants to know how proteins work together to coordinate the expression of thousands of genes. We have developed a genome-wide technique named DamID that can identify genes which are directly controlled by a chromatin protein or transcription factor. The technique works by linking the protein of interest to an enzyme from E. coli bacteria. When the protein binds to a gene the enzyme adds a unique chemical tag to the region. This tag can be subsequently mapped in the genome using special microarrays, and the genes by the protein can thus be identified.

So far we have created genome-wide maps of DNA binding of around 30 proteins. These maps offer us a more general view of how things work compared to traditional methods examining single genes individually. For example, analysis of these maps using advanced bioinformatics techniques tells us how each protein may contribute to the coordination of gene expression, and by which mechanism this is done. By comparing the binding maps of the different proteins, we are also starting to see relationships between proteins that no one has seen before. This lets us see which proteins form complexes together, and which complexes control which genes. We think of this as a kind of combinatorial code that we hope to decipher in the future. We work with cells from fruit flies and humans. Fruit flies have a much smaller and simpler genome than humans and are therefore an easier model system for the development of new techniques and concepts. The skills and insights that we obtain from working with this organism make it much easier to do genome-wide mapping studies in human cells.
Molecular profiles of breast cancer
Laura van ‘t Veer

Trained in molecular biology, Laura van ‘t Veer applies her expertise to cover the whole range from cancer diagnostics and genetic counseling in the NKI’s family cancer clinic to research into understanding the roles that genes play in cancer.

Most of our research focuses on breast cancer – identifying the genetic factors that make women susceptible to the disease and also fine-tuning diagnostic tests to predict outcome more accurately, and make better decisions about therapy.

We are part of large international collaborations, the Breast Cancer Association Consortium, which involves around 50,000 patients and an equal number of controls, and the International BRCA-1/2 Carrier Cohort Study. Our focus is to look at the DNA that is passed to future generations in sperm and egg to identify new gene mutations that confer a high or moderate risk of developing breast cancer. And we look at how different genes interact with each other and with environmental factors to promote disease.

Predicting the clinical course of breast cancer based on improved molecular assessment of the tumor biology and the presence of circulating tumor cells is the other main focus of our research. Especially large-scale gene expression analysis has helped us to dissect breast cancer into therapeutically meaningful subgroups.

I am one of the co-founder’s of the NKI’s spin-off biotech company Agendia, which develops the research findings into diagnostic tests for breast cancer and other diseases. The first commercial test, the 70-gene ‘signature’ or pattern of gene activity, can indicate the chance of future metastasis. Physicians can use this to decide if a patient should embark upon chemotherapy, and weigh-up the likely benefits over side effects.

SELECTED PUBLICATIONS


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Marcel Verheij spends half his time as a radiotherapy specialist at the Antoni van Leeuwenhoek Hospital and the rest in three different labs. This helps him keep his laboratory work focused on clinical need, and his clinical work scientifically up to date.

After gaining my medical degree, I started a residency in radiation oncology at the NKI, and also spent two years at the Memorial Sloan-Kettering Cancer Center in New York. There I got interested in apoptosis, or cell suicide, and started to investigate the signaling mechanisms by which radiation induces it. I returned to the Netherlands with enough data for my PhD and to establish a new line of research at the NKI.

I have three main areas of research, all of which have the aim of making apoptosis a more powerful weapon against cancer. Working with Wim van Blitterswijk (see page 57), we have brought a new group of anticancer lipids into potential clinical use as radiosensitizers. We are also investigating how lipid rafts in cell membranes sense apoptosis-related signals and how they pass this information into the cell. In collaboration with Jannie Borst (page 32), we are developing a promising therapeutic combination of radiation and a drug that binds to the ‘death receptors’ to trigger apoptosis.

In collaboration with Renato Valdés Olmos we have introduced an imaging method that can detect radioisotope-labeled proteins that specifically bind to the surface of cells about to undergo apoptosis. This will enable us to judge whether therapies are working. We already have indications that tumors with a high proportion of apoptotic cells are more susceptible to therapy than those with fewer suicidal cells.

As a third line of assault, I’m focussing on combinations of chemotherapy and radiotherapy, because we know that these increase the chance of survival for patients with some certain tumors. With scientific and clinical facilities close by, and keen to collaborate, the NKI is a great place to make advances in this type of therapy.

SELECTED PUBLICATIONS


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Lodewyk Wessels brings an unusual perspective to the NKI. He trained first as an electrical engineer and worked in machine learning (ML) at the Technical University of Delft before joining the NKI in 2006 as a full-time bioinformatician.

My first project at the NKI was to adapt computer algorithms I’ld helped develop for ML, and use them to predict the mutation status of breast cancers from comparative genomic hybridization data. The aim was to produce an alternative means of identifying carriers of germline mutations in the BRCA1 gene from a high-risk population.

We compiled a ‘predictive classifier’ — a set of chromosomal regions where the number of copies present indicates the mutation status of known cancer-causing genes.

Our ultimate goal is to build classifiers that make reliable predictions on data from new cases, not just cases on which the predictors were trained. We are now concentrating on incorporating data on whole pathways, rather than single genes, to make prediction more accurate. This ties in with our group’s other research, which is on systems biology — the study of how all the components of a cell work together.

As well as our own research on systems biology we provide statistical and bioinformatics expertise to other groups at the NKI. Often, such projects require tailor-made bioinformatics solutions, which open up new topics for bioinformatics research. For example, we’re collaborating with the teams of Jos Jonkers’s, Anton Berns and Maarten van Lohuizen (page 61) to interpret data from insertional mutagenesis studies in mice.

Our task is to develop algorithms for deciding which insertions lead to cancer and which have no effect. In the meantime, the approaches we developed for insertional mutagenesis proved to be applicable to a wide range of other genomic datasets.

Another challenging, but exciting collaboration is with Jos Beijnen — using proteomics to detect cancer in its early stages by the proteins present in easily sampled tissues such as blood. We’re calibrating his measurement techniques and our algorithms, using known proteins, to ensure reliability and reproducibility of results.


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Steeds minder kankerpatiënten naar kwakzalver

Onderstaand interview met prof. dr. F.S.A.M. van Dals, vicevoorzitter van de Vereniging tegen de Kankerzonde, werd onlangs gepubliceerd in het Tijdschrift Ontmoeting.

Het geeft een goed beeld van de motieven van kankerpatiënten om hulp te zoeken in het alternatieve geneesmiddelengebruik en de risico’s die daarmee gepaard gaan. Nauwkeurige wetenschappelijke informatie in dit verband is nog erg zeldzaam.

Voor het leggen van de kansen van de kwakzalver bij kanker is het van groot belang om zowel de gedachten van de patiënt als de ervaringen van de behandelaar te overwegen. Het is belangrijk om een goede communicatie tussen patiënt en arts te garanderen. Des te meer omdat de kwaakzalveren soms hun behandelmethoden niet tijdig delen met de patiënt.

Er zijn in Nederland nog vele kwaakzalveren die met allerlei medicatie en behandelingen proberen kanker te bestrijden. Dit blijkt uit onderzoeken die werden gedaan door de Nederlandse Kankerfondsen (NKI) en de Amsterdamse Universiteit. Zij hebben gevonden dat er sprake is van veel variatie in de kwaliteit en effectiviteit van deze behandelingen. Er is dus ruimte voor verbetering.

Lokale interleukine 2 is effectief tegen kanker, maar hier wordt weinig in geïnvesteerd, omdat het commercieel niet interessant is.

Moet de overheid de ontwikkeling van goedkope middelen tegen kanker stimuleren?

Nee, want dat vergt veel tijd en geld, wat een probleem is.

Ja, want goedkope middelen zijn belangrijk, hoe hij inzetten voor 11.000.

Is er sprake van effectiviteit? Ja (78%)

Totaal eenstemmig: 279.
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 treatment protocols, for example, or find new disease markers and drugs. The success of the division’s research is due to extensive collaborations both within the NKI, with the departments of pathology, molecular pathology, radiotherapy and surgical oncology, and elsewhere.

The head of the Division of Medical Oncology, Sjoerd Rodenhuis, is responsible for the diagnosis and management of cancer, and for devising treatment protocols for the division’s clinical trials. His particular interest in breast cancer has helped turn the AVL hospital into a major referral center for this disease. Consequently, there is a large population of patients with breast cancer to which the NKI has access for research. The NKI also specializes in research into and treatment of head and neck tumors, as well as cancer of the stomach, colon, kidney and skin.

The right diagnosis
Having an accurate diagnosis is crucial to making the best treatment decisions. Cancer diagnosis involves identifying not just the tissue of origin, such as breast or lung, but also the tumor subtype. And even within a single subtype there can be many variants — and which one the patient has will affect their response to treatment.

Even in cancers affecting the same tissue there is often a diversity in genetic and biological properties that is only beginning to be understood. In breast cancer, for example, traditional histology provides a useful indication of whether the tumor has originated from the basal cells of the milk duct epithelium, or the cells lining the lumen of the duct. Immunocytochemistry can then reveal whether or not the tumor is carrying high levels of the estrogen receptor or of another receptor, Her-2. Patients can be accordingly assigned treatment with either hormones or the drug Herceptin, but these techniques do not actually predict whether individual patients will or will not respond to their treatment. Only one in four patients with advanced breast cancer and who test positive for Her-2 will respond to treatment with Herceptin alone, and there is currently no way of predicting who they are at diagnosis. The drug is often combined with another for better results. But it would be preferable to know in advance who is most likely to benefit, so as to avoid treating patients unnecessarily.

The Division of Medical Oncology is working closely with the Division of Diagnostic Oncology (page 82) to make cancer diagnosis more precise than is currently possible. More precise diagnoses would help clinicians choose drug combinations that better suit
individual patients, thus avoiding unnecessary treatments and side effects. In the race against time before a cancer spirals out of control, this could make the difference between life and death.

A major collaboration between NKI scientists, pathologists, surgeons and medical oncologists has led to pioneering work on the use of microarray technology for defining patterns of gene expression in breast cancer that can be used to predict the chance that a patient’s disease is likely to metastasize. The success of the collaboration has led to the setting up of a commercial company within the NKI, Agendia, to provide molecular diagnostic tests and services for other clinicians and researchers. Medical oncology specialists are now closely involved in taking the use of microarrays further to predict responses to drug treatment.

The thoracic oncology group within the division is investigating whether microarrays can help predict the course of non-small-cell lung cancer, while another genetic study is looking for the presence or absence of mutations in a growth factor receptor, EGFR. This information indicates which patients may benefit in future from a new drug aimed at this receptor. Meanwhile, the group is also investigating other methods for improving the diagnosis of various types of lung cancer, such as the use of fluorescently activated markers that are preferentially taken up by tumor tissue, and immunocytochemistry for detecting the presence of a particular maker called HTERT in the lung tissue of smokers, which might indicate those most at risk of developing smoking-related cancer and who could receive chemotherapy as a means of prevention.

**Tailoring treatment**

A crucial decision in medical oncology is when to switch a patient to another drug combination if the first appears not to be working. The use of high doses of drugs known as alkylating agents, for example, was once considered effective, until it was found that only a minority of patients benefited, while the majority could suffer severe side effects. In collaboration with the Department of Pathology, the Medical Oncology Division is testing to see which patients have tumors that are most susceptible to these drugs, and whether lower and safer doses could be used. To do so, they are using various molecular techniques and magnetic resonance imaging (MRI).

**Quality drugs**

Another important area of research in medical oncology is clinical trials to test different treatment combinations, using both existing and new pharmaceutical products. Currently, the NKI is involved in around 120 different clinical trials with collaborators in the Netherlands and abroad.

Underpinning this, the Department of Pharmacy and Pharmacology led by Jos Beijnen (page 29) provides...
an extensive facility for designing, manufacturing and evaluating new pharmaceutical formulations. Besides its routine work for the AVL, the department supplies top-quality preparations of new investigational drugs to hospitals and biotech companies around the world. The profits from this are used to fund the department’s research, which involves around 25 PhD students working on improving drug design and formulation, and bioanalysis of drug effects in patients.

The Department of Pharmacy has also recently established a Biotherapeutics Unit for the manufacture of DNA-based products, including DNA vaccines for the treatment of cervical cancer and melanoma, in collaboration with medical oncologist and immunologist John Haanen (page 37). In a separate collaboration with the NKI’s bioinformatics expert Lodewyk Wessels (page 65), the department is exploring the potential of proteomics — the analysis of proteins in biological samples — for the diagnosis and monitoring of breast cancer, colorectal cancer, renal cancer, and other solid tumors.

In addition the Division of Immunology is investigating the effects of using cytokines to stimulate the immune system before surgery for renal carcinoma, to see whether this boosts the action of the body’s T cells against tumors.

**Monitoring new treatments**

A vital part of assessing a new treatment strategy, with either existing or new drugs, is monitoring their effects in patients. The Department of Pharmacy and Pharmacology monitors patients during clinical trials to determine, for example, which drugs can be taken orally, which doses are most effective and when, and how to minimize side effects. The department also conducts its own research into improving cancer treatment. One project involves screening patients for genetic differences that could indicate how long a drug persists in the body. Ultimately, this information could help guide different dose regimes for each individual.

Another project tackles a major problem that results when particular transporter proteins in a patient’s gut wall block the uptake of drugs taken orally, and so prevent them entering the bloodstream. To receive an effective dose, these patients must be given the drugs intravenously, over a prolonged time in a hospital bed, rather than in more convenient pill form. To address this problem, researchers led by medical pharmacologist Jan Schellens (page 50), in collaboration with the Division of Experimental Therapy, are investigating the use of new agents that block these drug transporters, thus allowing anticancer drugs taken orally to enter the body.

Basic research by Alfred Schinkel (page 51) and others is contributing to our understanding of these drug transporters and our potential ability to manipulate them.

The Gastroenterology Department is investigating new treatments aimed at controlling incurable forms of cancer in the gut. These include cancer of the stomach and the mid-gut, as well as tumors arising from the neuroendocrine system — the nervous tissue that lies alongside the gut. Different combinations of chemotherapy and radiotherapy are being evaluated. The department is also using genetic studies to identify which patients are likely to experience treatment side effects.

The Division of Medical Oncology is at the forefront of research in a wide range of clinical specialties, aimed at improving both diagnosis and treatment of many types of cancer. The research involves the development and application of many different technologies from the laboratory to the clinic, confirming the NKI’s position as a leading European research institute.
SELECTED PUBLICATIONS


Surgical Oncology

The Division of Surgical Oncology is a large and diverse department, with over 50 academic staff and more than 50 medical residents and research students. The division’s research interests reflect clinical reality and extend far beyond surgery itself into areas such as diagnostics and therapies for inoperable tumors.

One of the largest projects the division has undertaken is the development of lymphatic mapping, which can identify likely sites at which secondary tumors will arise. The fluid within tissues drains through lymphatic vessels to lymph nodes before being recirculated via the lymphatic system into the blood. This fluid can carry cells from a tumor into the lymph nodes, where they become trapped and set up a secondary tumor. A given tumor only drains into a few lymph nodes at most, and if these ‘sentinel nodes’ can be identified, the surgeon can remove them to determine whether the cancer has spread. Effective treatment can then be given at an early stage, reducing the chance of the cancer recurring.

To carry out lymphatic mapping, the doctor injects small volumes of a low-level radioactive tracer at the site of the primary tumor — most commonly a breast tumor, skin tumor (melanoma) or penile tumor. The tracer’s movement is followed with a gamma-ray camera to see which nodes it collects in; these nodes are then prime candidates for surgical removal. Lymphatic mapping can highlight those nodes at greatest risk of developing a tumor long before any lump can be felt, and the NKI has moved the procedure from research into clinical use. A recently completed study of patients with penile cancer showed that lymphatic mapping improves long-term survival rates.

Evaluating diagnosis and screening

The division is continually evaluating current diagnostic and screening techniques for their reliability and validity. For example, the presence of the protein S100B in the blood is used as a diagnostic marker for melanoma. However, researchers in the division have shown that this marker is of limited use, and fails to predict the presence of non-palpable secondary tumors in lymph nodes. If patients have S100B in their blood then their chance of recurrent melanoma is high, but a lack of S100B does not, unfortunately, rule out melanoma.

Women at known high risk of developing ovarian cancer are screened twice a year, using vaginal ultrasound and measurement of blood levels of the ovarian protein CA125. Research at the NKI has suggested, however, that this screening has only limited success at detecting early-stage cancer, and is collaborating with the Amsterdam Medical Center to develop a more reliable test that will be based on a spectrum of blood proteins, not just a single one. A nationwide study coordinated from the NKI is also looking at the psychosocial impact of removing a woman’s ovaries to reduce her risk of cancer, to determine whether the reduced fear of cancer balances out the hormonal disturbances caused by the loss of the ovaries.

Heat treatments for metastatic cancer

A novel therapy being pioneered at the NKI is hyperthermic intraoperative chemotherapy (HIPEC) to treat tumors that have arisen in, or tend to
spread to, the abdominal cavity. All of the visible tumor is first removed, and the entire abdominal cavity is then perfused with a solution of chemotherapeutic drugs warmed to 40–41°C. The combination of chemotherapy and heat is thought to work better than drugs alone, because heat increases uptake of the drug into the tumor cells and may even reverse the drug resistance that can arise during chemotherapy. In addition, heat itself can kill tumor cells. The world’s first prospective randomized trial of this treatment was carried out recently at the NKI, and showed significantly increased survival when compared with conventional treatment. So far, HIPEC has been used to help patients with certain colorectal tumors and there are plans to test it in women with ovarian cancer, which has a tendency to reappear within the abdomen.

Another approach to treating metastatic cancer is to either surgically remove the metastatic cells or to destroy them using radiofrequency thermal ablation (RFA). This involves inserting a miniature transmitter that has been placed inside a long needle into the tumor. The transmitter acts like a microwave oven and destroys cells around it. If the growths are not large, and can be completely removed by RFA, the treatment can improve long-term survival. RFA also leaves the patient with less pain and discomfort than surgery, and can sometimes be used when conventional surgery is impossible.

Which treatment?
Deciding the best treatment for each patient is vital. To determine the best treatment for some types of breast cancer, the NKI is coordinating the AMAROS trial (after-mapping of the axilla: radiotherapy or surgery), an international, multicenter phase III clinical trial in patients with operable invasive breast cancer whose tumor has spread to the lymph nodes. Patients will be randomly assigned to receive either radiotherapy or surgical removal of the nodes.

In thoracic surgery, such as that for lung cancer, the division’s research focuses on the use of radiotherapy or chemotherapy before surgery, while also looking for ways to reduce postoperative pain. One project is investigating whether CT-guided RFA could replace surgery as a treatment for some secondary or metastatic lung tumors.

The division has pioneered the technique of isolated limb perfusion to treat melanoma and sarcoma with chemotherapeutic drugs. Tubes lead into the major artery and vein supplying the affected limb, and are connected by a pump, creating an isolated circulation through which chemotherapeutics can be given at a higher concentration than usual, as they are kept within the limb.

In 2004, the Antoni van Leeuwenhoek hospital started day-care surgery for breast cancer. Careful additional local anesthesia can give thorough pain relief while avoiding many usual
postoperative problems such as the nausea and vomiting associated with strong painkillers such as opioids. In addition, the Division has developed the technique of ‘awake fibercapnic intubation’ to make intubation easier in patients with difficult airways, typically patients with a tumor in the upper airway.

**Maintaining function**
Cancer of the head or neck is particularly problematic to treat because it is so difficult to remove tumors surgically without damaging vital tissues and organs. The division is engaged in many projects that aim to spare healthy tissue or restore function to areas damaged by surgery. The head and neck department has a leading role in rehabilitating the voice and the airway after a total laryngectomy. The head and neck department is also working closely with the Division of Radiotherapy to develop combinations of radiotherapy and cisplatin and other radiosensitizing agents (page 78) with the aim of helping people with large tumors while leaving as much function as possible.

Sparing healthy tissue is also important in the case of bladder cancer, where removal of the bladder is sometimes the only chance of survival. The department of urology has been in the forefront of developing reconstructive surgery that aims to restore normal anatomy and sexual and urinary function, with substitute bladders and the reinstatement of means of urine storage and evacuation.

To help rebuild disease-damaged areas, the NKI’s plastic surgeons recently investigated the risk of surgical complications of a method that involves saving the breast’s skin during a mastectomy and immediate inserting implants to maintain the breast’s overall shape. They are now able to offer guidance for patients with breast cancer or for those who have inherited a specific risk of developing breast cancer. Working with the Department of Psychology, members of the surgical staff are also developing methods that spare a woman’s nipple during surgery. Other methods that aim to enhance the cosmetic result of breast reconstruction include refining lipofilling techniques, which involve making use of the patient’s own fatty tissue. So far this looks promising.

In reconstructive surgery that follows surgical removal of a tumor and surrounding tissues ‘free flaps’ are frequently used. These flaps of muscle, subcutaneous tissue and skin are transplanted from one site of the body to the part of the body that needs restoring and provides an optimal means of covering the defect. Getting the flap to graft in the new location requires that it contains a good set of blood vessels, and that these vessels can be microsurgically fixed into an adequate blood supply. Unfortunately, radiotherapy used to treat the initial tumor often damages blood vessels in the area, and the Department of Plastic and Reconstructive Surgery is collaborating with the Division of Radiotherapy to investigate the severity of damage to these vessels or tissues and their clinical implications.

The NKI has played a pivotal role in successfully developing the clinical application of photodynamic therapy. The patient is given an intravenous photosensitizing chemical and then red light from a laser is shone onto the tumor. Only the combination of the chemical and light kills cells, and thus destroys the tumor while causing minimal damage to surrounding healthy tissue, leaving excellent
function and good cosmetic results. Another recent development is a device that delivers light to tumors that have infiltrated deep into tissue. These techniques are being tested in nasopharyngeal cancer in collaboration with universities in Jakarta and Yogyakarta in Indonesia.

Robotic surgery
Reducing scarring is just one advantage of the NKI’s latest piece of surgical technology — a remotely controlled robotic surgeon. With this da Vinci® Surgical System, the patient lies on a surgical table and laparoscopy style instruments are inserted so that they reach the tumor. The surgeon moves to the other side of the operating theatre and maneuvers the instruments using joysticks, watching what is happening on a 3D computer display. This filters out any tremor in the surgeon’s hands, and allows hand movements to be scaled up to allow greater precision than is possible manually. The patient benefits from an operation performed with greater precision, minimizing the chances of damaging surrounding tissue. The NKI first used robot surgery for prostate cancer, where removing the tumor often damages nerves, resulting in incontinence or impotence. In the United States, surgeons use robots to perform 30% per cent of prostatectomies. Europe is likely to catch up soon, and the NKI will be in the forefront.

SELECTED PUBLICATIONS


Radiotherapy

The NKI enjoys the privilege of being linked with one of the top radiotherapy departments in the world. Not only does this have the latest commercially available equipment, but there is an integrated team of technology experts, scientific researchers and healthcare providers who are constantly developing new ways of making radiation treatments more effective. This means giving the right treatment to the right patient, and then controlling the way that treatment is given.

Staff in the NKI’s radiotherapy department take a four-pronged approach to making radiation treatment more effective at killing cancer cells while leaving healthy ones unscathed. First, is the search for chemical agents that, when combined with radiation, massively increase its efficacy. This involves trying to understand the underlying cellular biology. Secondly, they are developing new equipment that can deliver radiation to a tumor with greater precision. Third they are looking to increase the dose of radiation given to patients, and finally they are analyzing the genes in tumors to find profiles that will provide finely tuned, personalized therapy for future patients.

The department’s overall strategy is to look for ways of improving the outcome of therapy for patients with the most common tumors — those of the breast, lung and prostate and, to a lesser extent, the rectum. The rationale is that even a modest improvement in these will have a dramatic effect on tens of thousands of people worldwide. NKI has already made a more than modest contribution.

This work builds on just over a century of innovation using radiation. Only a couple of years after German physicist Wilhelm Conrad Röntgen discovered X-rays in 1895 in Würzburg, Germany, doctors started experimenting with radiation to combat cancer, and by the 1920s X-rays had become a major weapon in their armory. Although other types of treatment are now available, radiotherapy is still the major therapy for more than half of cancer patients.

The underlying principle is simple. As radiation penetrates cells it damages DNA — a cell’s store of genetic information. While the damage is not so severe that the cell dies outright, its presence activates biochemical machinery that either halts cell growth and allows the DNA to be repaired, or, if the damage is great enough, causes the cell to commit suicide — to enter apoptosis.

The problem with radiotherapy is that this DNA damage will occur in any cell the radiation passes through, whether cancerous or healthy. There is, however, some bias towards damaging tumor cells as these tend to be faster growing than normal cells, are often less equipped to deal with DNA damage, and consequently are more vulnerable to radiation injury.

Combination therapies

Department head Harry Bartelink’s involvement with combination therapy goes back to 1992, when he and his co-workers published a pioneering paper showing that adding the cytotoxic drug cisplatin to radiotherapy dramatically improved the ability to control cancer. At the time the side-effects were so great that it did not become a standard therapy, but since then advances in supportive therapies have turned cisplatin and radiotherapy into one of the most frequently used therapeutic options for an increasing number of solid tumors.
The difference it makes is huge. In anal cancer, for instance, giving chemotherapy alone cures no one, while radiotherapy alone has the possibility of curing around half of the patients. Combining the two leads to a cure in around 8 out of 10 patients.

Currently, clinician Marcel Verheij (page 64) and scientist Wim van Blitterswijk (page 57) are collaborating to take a close look at the synthetic alkyl-lysophospholipids (ALPs) as a combination therapy. These lipids are cytotoxic, that is they can kill cells. Unlike many other cytotoxic drugs, however, ALPs do not attack a cell’s DNA, but instead are taken up by rafts of lipids that float in the cell membrane. Laboratory experiments have revealed much about how ALP then interferes with lipid metabolism in the cell, throwing it into a state of metabolic stress. The cell’s response to stress is to stop growing or to consider killing itself — add radiation-induced DNA damage to this and the cell is very likely to undergo apoptosis. In studies with mice, Verheij and van Blitterswijk have found that while treatment with ALP or radiation alone has some effect, combining them results in complete and long-lasting tumor regression. With the hospital right next to the lab it is only a short step to move ALP into clinical trials.

Immunologist Jannie Borst (see page 32) is investigating the possibility of combining a protein called TRAIL with radiation therapy. TRAIL latches onto and stimulates the so-called ‘death receptors’ that trigger apoptosis, and once these receptors are stimulated the cell enters apoptosis and dies. Laboratory studies showed that TRAIL predominantly affects tumor cells, and triggers apoptosis by a different pathway from that used by DNA-damaging agents, making it particularly exciting as a new therapeutic approach.

Right on target
While combining radiation with chemicals increases its punch, the radiation will still damage healthy tissue. A team of almost 50 medical physicists within the division is working on creating better ways of determining exactly where a tumor is within the patient, and then directing the radiation just to that region. This would be a simple matter if the patient and their internal organs never moved, because you could measure where the tumor was and then hit it perfectly — but in reality the patient is very much alive and moving. Each breath, heartbeat or movement of gas through the intestines will alter the position of internal organs, and thus shift the tumor.

The conventional approach has been to irradiate not only the zone where the tumor is predicted to be, but also a sizeable margin around it. So even if the tumor moves, it will still be irradiated. That approach causes considerable damage to surrounding organs, and, depending on the position of the tumor, can lead to heart, lung, or rectal damage.
To avoid such damage, the NKI’s medical physicists and computer technologists have developed equipment that can follow the tumor at the same time as delivering the radiation. At the center of this advance is a technique called computer tomography-cone beam (CTCB) guided radiotherapy. This involves making a PET scan before starting therapy to determine the location of the actively growing mass of cells, and carrying out CT scans that show where this tumor is within the body. A computer then merges the two. When the patient lies on the platform to receive radiation, a third imaging modality, also developed at the NKI, comes into play. This generates a fairly low-resolution image, but one that can run while the radiation is given.

A computer constantly matches this image with the pre-treatment high-resolution image, and drives the platform on which the patient lies so that the tumor is held in the same place even if the patient moves a little. In 2004, the NKI was the first institute in the world to use this technique in clinical practice. By 2006 there were about 20 machines using this technology around the world, with a 10-fold increase expected each year.

**Boosting the dose**

One consequence of this technology is that you can increase the dose of radiation, knowing that you are only hitting diseased cells. In the case of prostate cancer, this has significantly reduced the number of patients who experience rectal bleeding, as previously radiation frequently damaged the colon.

The arrival of more careful targeting has triggered a global review of radiation doses, and many clinical trials are looking at the effect of giving increasingly large doses. Until recently, a dose of 60 gray was considered to be the maximum acceptable for patients with lung cancer, but NKI clinicians are now considering doses of up to 100 gray. Initial results from trials at the NKI show that high doses enable the cancer to be treated without having to resort to removing the whole organ, which can make a huge difference to the patient’s life.

Harry Bartelink has coordinated a trial across Europe to determine the difference in outcome if patients with
early breast cancer are given either the standard 50-gray dose or a 65-gray dose. Early indications are that the higher dose gives a 50% reduction in local recurrence with only a minimal increase in side effects. Similarly, fellow researcher Joos Lebesque has coordinated a Dutch trial in prostate cancer showing that an increase in the radiation dose to the tumor from 68 to 78 gray resulted in a 30% reduction of signs of recurrence.

**Prognosis profiling**

Other trials are being carried out to determine whether there is a way of predicting who is most likely to benefit from a particular intervention before exposing them to the inconvenience and risk of receiving it. By using microarrays to search for specific gene mutations and patterns of gene activity, the NKI’s researchers are beginning to build a picture of the genetic profiles of those who respond well. The genes most likely to serve as useful markers are those involved in controlling cell growth and DNA repair. Currently, the Radiotherapy department is conducting a trial that already includes more than 400 patients, comparing how people with different genetic profiles respond to two different levels of radiotherapy.

**The bottom line**

Because of the headline-grabbing cost of new equipment, it is easy to make the false assumption that radiotherapy is expensive. But once installed, a machine runs for years, so that less than 5% of the total expenditure on an individual patient’s care is down to the radiotherapy. While the chemotherapy elements may cost €100,000, outlay on radiotherapy can be less than €20,000.

This means that radiotherapy is not only clinically effective, it is also cost efficient, and therefore a valuable technology when well used. The work at the NKI is increasing its impact.

**SELECTED PUBLICATIONS**


Diagnostic Oncology

The Division of Diagnostic Oncology comprises the Departments of Radiology, Nuclear Medicine, Pathology, Clinical Chemistry and the Family Cancer Clinic. Both clinical work and research in this division are carried out by several independent groups with wide-ranging expertise but with a common focus on cancer diagnosis. Each group has close ties to other departments at the NKI and is developing or applying different techniques to the goal of improving a physician’s ability to determine exactly the type and extent of growth of each tumor and to plan the most effective treatment.

Pathology goes molecular
The Pathology Department performs both routine diagnosis and translational research on human tissues removed at surgery with the patient’s consent. Over the past 20 to 30 years, the department has amassed a considerable bank of frozen tumors and normal tissues, which supports research across all departments of the NKI. Using the latest genetic techniques, DNA and RNA samples isolated from the tumors are being investigated to expand our understanding of each type of cancer.

The output of the division has grown as new techniques have been introduced. In 1983, the molecular pathology group was founded, and began to use the then novel technique of PCR to detect mutations in cancer-associated genes and chromosome rearrangements in lymphoma. The group continues to use a wide range of molecular techniques, particularly in hematology for the diagnosis of leukaemia and lymphoma, as well as to diagnose tumors of the breast, head and neck, and lungs.

The rapid expansion of genetic tests for cancer-related markers has led to an increasing demand for testing and counseling for affected families, particularly in relation to cancer of the breast, ovary, and lower gut. If a person is diagnosed with a form of cancer linked to a gene mutation, then other family members can be screened to see if they too carry the mutation. Its presence may indicate that they or their children are at high risk of developing the disease. In the case of breast cancer, for example, women carrying high-risk mutations in the BRCA1 or BRCA2 genes might wish to consider preventive mastectomy.

To meet the growing demand for these tests, the NKI began the Family Cancer Clinic in 1995. This administers both tests and genetic counseling and has served more than 2000 families. The clinic also serves as a conduit for recruiting volunteers for research, and together with the Department of Epidemiology contributes data to several national and international research projects, such as GEO-HEBON (gene–environment interactions in hereditary breast and ovarian cancer). This nation-wide study is looking at how genetic and other factors, such as lifestyle, diet, and hormonal changes such as during breast feeding, may alter the risk of breast or ovarian cancer in people with a family history of these diseases.

The Pathology Department has pioneered the use of DNA microarray technology for detecting patterns of gene activity in a patient’s tumor that can be used to predict the course of the disease. Areas of prediction not only include the likelihood of disease spreading to other parts of the body, but also the response to different kinds of treatment. Analyzing the results and making accurate and reliable predictions is a complex and specialized task that requires close collaboration with members of the
Bioinformatics Unit. So far, most progress has been made in applying this technology to breast cancer, with the identification of a 70-gene ‘signature’ that predicts the likelihood of metastasis. The department has initiated a collaborative study with other hospitals in the Netherlands (the RASTER study), to determine the feasibility of introducing the test into routine clinical practice. Similar research is ongoing for the diagnosis and prognosis of lymphoma, although this has yet to be translated into a diagnostic test.

The Bioinformatics Unit also analyses the data from animal experiments aimed at identifying new genes involved in cancer and understanding how they interact. These in turn could serve as targets for new drugs in future.

**Diagnostic imaging**

Two departments within the Division of Diagnostic Oncology are at the forefront of cancer diagnosis and monitoring using radiological imaging, which provides important information without invasive surgery. A special unit within the Department of Radiology, the Diagnostic Imaging Laboratory, is devoted to the pursuit of new imaging techniques, image processing and analysis. In particular, the unit is researching the use of magnetic resonance imaging (MRI) for different applications, such as measuring the local spread of breast tumors before surgery, and monitoring the effect of chemotherapy on tumor size. The unit is also assessing whether computer-aided enhancement of MRI images can improve the accuracy of breast cancer diagnosis in women who are at a greater risk of developing the disease.

The Department of Nuclear Medicine focuses on the use of imaging techniques that involve the detection of radioactive substances injected in or around tumors. This research aims to improve diagnosis, detect local spread of disease, monitor treatment and guide surgery. Positron emission tomography (PET) scanning after injection of radioactively labeled compounds such as glucose or estrogen, for example, enables ‘functional imaging’, which shows which area of a tumor is most active. Radiological imaging using tracer compounds injected into breast tumors is also proving extremely useful to guide surgery for those tumors that have already invaded surrounding tissue.

Computer tomography (CT) scans, on the other hand, indicate the impact of radiotherapy or chemotherapy by showing whether or not the tumor is shrinking. Such information can spare months of treatment with the wrong drugs if a patient’s tumor is failing to respond and is instead continuing to grow. It enables a physician to switch the patient rapidly on to different drugs.

**Signs from biological fluids**

The Clinical Chemistry Department is involved in both pre-clinical research on animal models and clinical
investigations on patients. Using a wide variety of the most up-to-date techniques, the department is analyzing mostly fluids such as blood and cerebrospinal fluid for research aimed at improving diagnosis and treatment. For stomach cancer, for example, the Clinical Chemistry Department is assessing blood tests for marker proteins that are produced by the tumor cells and enter the blood. The concentration of these proteins in the blood appears to predict how quickly the tumor will grow and spread, and hence the patient’s chance of survival.

Cerebrospinal fluid from patients with breast cancer can yield information on whether the disease has spread early to the brain, a condition known as leptomeningeal metastasis. This occurs in around one in twenty patients, and can be devastating if it is not detected and treated immediately. The problem is that current diagnostic tests fail to detect the presence of the brain tumor in a high proportion of cases. The department is exploring the possibility that proteomics — the search for patterns of multiple proteins in the cerebrospinal fluid using mass spectroscopic analysis — will give a more accurate early diagnosis.

As with the lining of the gut drug transporters in the lining of the brain’s blood vessels pump therapeutic drugs out of the brain as fast as they enter. A project with the Department of Pharmacology is focused on improving the access of anticancer drugs to tumors in the brain. The Department of Clinical Chemistry is carrying out tests in animals of new inhibitor drugs that block the drug transporters and allow anticancer drugs such as topotecan to enter the brain, for the treatment of brain tumors and brain metastases.

The Division of Diagnostic Oncology is at the hub of interactions between many clinical departments of the Antoni van Leeuwenhoek Hospital and research departments at the NKI. Its activities aim to improve the diagnosis and treatment of a number of types of cancer, using the latest technologies and setting new standards for what can be achieved in future.


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, however, 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.

Animal facility
Much of the 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 strains of mice 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 animals’ living conditions are carefully controlled and the welfare of every mouse is monitored daily. 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. 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. They also help create the transgenic and knockout mice that are now powerful tools in genetic research. The animal facility has two pathologists and five technicians who sample 25,000 to 30,000 tissues per year, a workload equivalent to that of a pathology laboratory in a medium-sized hospital.

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 will be equipped with radiotherapy equipment and advanced imaging systems, enabling scientists to treat and study the mice in ways comparable to treating humans in a clinic.

The animal facility also houses zebrafish, which are increasingly being used in genetic studies, and small colonies of the frog Xenopus, which has long-been used in developmental research.
Microarray facility
High-throughput technologies for studying genes and gene expression are essential in today’s biomedical research, but present huge challenges to the individual researcher. One of these technologies, DNA microarray, involves spotting many thousands of gene fragments onto a microscope slide which are then used to screen samples for genes that are being expressed.

The NKI’s microarray facility was set up in 1998, right at the start of this particular technological revolution, with substantial financial support from the Dutch Cancer Society. Rather than using a commercial platform, which is expensive and tends to be inflexible, staff at the NKI’s facility have developed their own highly versatile system for printing microarrays. Printing arrays in-house saves money, allowing NKI researchers to carry out projects that are usually only feasible for a large pharmaceutical company.

The facility has automated many of the stages required to carry out a microarray experiment and analyze the results. These include automation of the ‘hybridizing’ stage, when the sample to be tested is mixed with the microarray, and computer-aided scanners that read the results. The scanners are linked directly to a web-based database, so researchers can call up their data any time they need, even if they are away from the lab.

Any scientist wanting to use the facility is given basic training in all the stages of the work, ensuring that they fully understand the process. If an experiment requires a large number of microarrays, the staff will train the researchers to carry out the experiment themselves, but if it needs only a few, the microarrays are processed by facility staff. This means that everyone gets high-quality data.

Microscopy facility
The hospital attached to 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 six digital microscopes, most 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 six 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. A simpler digital microscope is used for monitoring changes in cells growing in multi-well plates. Aided by a robot, it can take images of cells at set time intervals over a period of a few days.

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. The latest development in electron microscopy, 3D reconstruction of protein complexes, will be introduced soon at the NKI.

Robotics and Screening Center
As researchers build ever-larger collections of agents that perturb the expression of particular genes, they need technology that can rapidly perform the thousands of individual experiments required to test the effects of disrupting gene expression in cells. To do this efficiently, the NKI has built a highly flexible, high-throughput automated facility that includes an automated liquid-handling system together with a robotic arm that can also access an automated tissue culture system, plate-washing station and plate readers. This robotic set-up runs cell-based screens at a throughput of more than 10,000 samples per day. The facility is designed to accommodate several different experimental set-ups to support the many different research lines pursued in the institute, ranging from the construction of large collections of genomic tools, to small-molecule drug screens and yeast genetics.

The robotic system includes an automated fluorescence confocal microscope that can analyze thousands of samples a day for complex cellular phenotypes. In combination with robotics and followed by computer-assisted automated image analysis, large-scale screens that test thousands of genes can be performed to look for genes that affect protein localization, chromosome separation, receptor activation, or even the movement and migration of living cells.

Flow Cytometry Facility
Flow cytometry plays an important role in many of the research lines of the NKI. A flow cytometer is able to measure the size and molecular characteristics of individual cells; thousands of cells can be examined 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 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.

The Flow Cytometry Facility accommodates four bench-top analyzers and two multicolor high-speed sorters, each of which can detect (depending on the number of detectors) three to nine different fluorescent labels.

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

All in all, this facility enables the researchers to find the cellular equivalent of a needle in a haystack, such as a single stem cell hidden among 100,000 other cells.

Tissue and Serum Bank
Over the past 20 years the Antoni van Leeuwenhoek Hospital has collected and stored tumor samples from patients. While identities are kept confidential, these samples can be linked to medical records. This means that researchers can study the precise genetic nature of the tumor that a person had a decade or more ago, link it to the treatment they received, and compare this information with the treatment outcome. This tissue bank is a phenomenally valuable asset in the fight against cancer.
**Technology Transfer**

The Technology Transfer Office (TTO) supports the NKI-AVL 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 organized to identify and protect the institute’s intellectual property without impeding scientific research, and is responsible for patenting, licensing, consultancy, and scientific collaborations and research agreements. The TTO is the first point of contact for licensing requests and research collaborations. When commercial partners are required in EU or other research consortia, the TTO assists with consortium agreements and project management.

**Biometrics Department**

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 120,000 registrations.

**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, configuration of network software on PCs and Apple Macintosh computers of end users and, if necessary, the development and maintenance of custom server and/or client-side software. The Central 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.

Synthetic peptides find wide use within the NKI. Peptides have been synthesized on-site since 1988, making it possible to obtain peptides with specific modifications in any quantity. The facility makes use of two synthesizers. One monitors the complete reaction process while the other machine can synthesize up to 60 peptides simultaneously. Peptides up to 40 amino acids in length can be synthesized. The quality and identity of all peptides is monitored by HPLC or LCMS and peptides can be purified by preparative HPLC to meet the highest-quality standards.

In addition to departmental laboratories licensed for radioactive work, which are present on nearly each floor of the research building, there is a central radiochemistry facility available for specific and general experimental use. The staff of the Radionuclide Laboratory offer help and advice on all aspects of radioactive work. The department is equipped with up-to-date gamma and scintillation counters, gamma analyzers and HPLC apparatus. There are also separate facilities for animal experiments with radioactive tracers. Radiation safely courses are regularly organized for students and scientists.

The Sequence Facility offers a service for DNA sequence and fragment analyses to the research departments and the clinical DNA-diagnostics laboratory.

The institute also has a central cryogenic storage facility, a glassware kitchen, an electro- and technical workshop and an audiovisual department.
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 scientist have professorships at Dutch universities.
On a regular base we seek assistant professors. These AvL-fellows 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 an AvL-fellowship are invited to contact the scientific director.

Postdoctoral fellows
About 70 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 the annual NKI-AVL postdoc retreat. Topics typically discussed during the retreat are: how to succeed as a scientist in academia or outside; communication skills; setting personal expectations; obtaining and negotiating a promotion; responsibilities beyond the laboratory; laboratory management; mentoring and being mentored; project management; understanding the (inter)national funding process and getting funded; getting published; technology transfer; and setting up collaborations.

Any postdoctoral fellows interested at training at the NKI are invited to contact one of the group leaders or look at our website.

Graduate students
Graduate students do research in a challenging environment while following a PhD program offered by the Oncology Graduate School Amsterdam (OOA). This joint program
between the oncology researchers from the NKI, the Free University Amsterdam and the University of Amsterdam offers detailed graduate courses. OOA graduate students enjoy frequent opportunities to interact, including a three-day annual retreat, where students present their work to fellow students. Each graduate student has a PhD committee consisting of NKI faculty members who oversee the quality and progress of the project, and which discusses the performance of both the supervisor and the student.

Graduate students are welcome to check for positions that we advertise regularly on our website, and to contact directly either our group leaders or the dean of the graduate students Prof. Dr. Titia Sixma (email: t.sixma@nki.nl).

Masters students also have the opportunity to take part in cutting-edge research and to enjoy its associated excitement and challenges. The NKI-AVL is happy to receive Masters students from universities and HLO schools for rotation projects. University students may undertake projects in the institute provided they have obtained a Bachelor’s degree with a major in 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 Masters student who does a project in the institute in the area of Biology/Medicine/Chemistry for more than 4 months is obliged to pursue a course and examination in experimental oncology.

Masters 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 (email j.borst@nki.nl, university students) or Dr. John Collard (email: j.collard@nki.nl, HLO students). Information about clinical training is supplied by the heads of the clinical divisions or by the coordinator for medical students, Prof. Dr. Fons Balm (email: a.balm@nki.nl).
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