NKI-AVL
The Netherlands Cancer Institute –
Antoni van Leeuwenhoek Hospital

Master Education at NKI-AVL
Master education at the NKI-AVL

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I. General information, how to apply for a placement (stage)

Why the NKI?
The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AvL) is an integrated cancer institute, combining hospital and research laboratories. The hospital has 180 beds, a large radiotherapy department and outpatient clinics. The laboratory covers all major areas of cancer research, with special emphasis on genetics, cell biology, immunology and translational research, i.e. using basic knowledge to improve diagnostics and therapy. Scientists from all over the world are attracted by the reputation of the NKI-AvL to come and work in our research departments. The result is a dynamic and inspiring international atmosphere, which prepares students optimally for a productive career in science. Students get the opportunity to be involved in cutting edge research with all the excitement and challenges connected to it. The NKI-AvL is very happy to receive Master students from universities and HLO schools for a placement. In the first place, students can make important contributions to our ongoing research projects. Secondly, investment in students is an investment in the future of (our) research. Graduate students and technicians are frequently recruited from placement students.

Teaching at the NKI
The NKI-AvL is an independent institute, not a university. Its non-clinical staff members generally are the heads of research groups (called project leaders). The groups (about 50) vary in size from 5-20 people (master students, PhD students, post-docs and technicians). The prime objective in the non-clinical departments is to do research. In clinical departments, staff members combine patient care and research. Education in fundamental and clinical cancer research is a regular activity of the Institute and includes master, PhD and post-academic training. Several staff members are professors at national universities and have additional teaching responsibilities. The NKI/Avl has a formal connection with the University of Amsterdam, Faculty of Science. The Institute is also a partner in the Oncology Graduate School Amsterdam, collaborating with the Free University and the University of Amsterdam. The NKI-AvL has no formal teaching obligation and no dedicated teaching staff, nor funding. Therefore, the institute sets limits to the amount of students that can be placed and requires that students stay for a certain minimal duration. Generally, 4 months is the minimum length of a placement. In principle, we only accept Master students.

Admission requirements for the placement
University students are accepted in the institute for a placement, provided that they have obtained a Bachelor’s degree with a major in Biology, Medical Biology, Medicine, Pharmacy, Chemistry or a closely related subject. In addition, within the Division of Psychosocial Research and Epidemiology, students with a theoretical background pertaining to these areas can be placed. Within the research facilities and Radiotherapy students with a background in informatics and or physics can be placed. The English language is the teaching medium in daily practice. Every Master student who does a placement in the institute in the area of Biology/Medicine/Chemistry for more than 4 months duration, is obliged to attend the course on Experimental Oncology and to do the exam connected with it (see Theoretical training). The student must make the arrangements with his/her university to obtain formal permission for the placement.

Placement of HLO students is usually arranged in a direct contact between the student’s supervisor at the school and a supervisor at the NKI. HLO students are not obliged to do the course and exam in Experimental Oncology. Coordinators for HLO students is Dr. J. Collard, e-mail j.collard@nki.nl, tel.: 020-5121932 and Dr. J. Hilkens, e-mail j.hilkens@nki.nl, tel.: 020-5122018.

For medical students, there are additional opportunities for training in the NKI/Avl. In particular, the University of Amsterdam offers students the possibility to do a short-term placement of a few months in our institute, in either hospital or laboratory. In the “Wetenschappelijke stagegids” published by the university, the subjects that are suitable for a placement are described. Students are also specifically referred to the Clinical Divisions in this guide. Information about clinical training is supplied by the heads of the clinical sections or by the coordinator for medical students Prof. Dr. A.J.M. Balm, E-mail a.balm@nki.nl, tel.: 020-5122550 (secretariat).
How to apply for a placement?
Study this guide for Master students [http://www.nki.nl/Research/Career/Masters+students/](http://www.nki.nl/Research/Career/Masters+students/) to get an impression of the work that is done in the various research groups. Select a few research groups of interest and express your interest to do a placement directly to the research group leader, preferably by e-mail. Indicate what your background is, when you want to start the placement and how long you want to stay. Make an appointment with one or a few group leaders of interest and discuss the options. After you decided for a research group, confirm that you want to come and when. If you contacted other research groups, make sure to inform them that you are not coming! This is not only polite, but also important, because these groups can then accept other candidates. To get more background on the research of the groups of your interest, you can look up publications in Entrez-PubMed on the web: [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). In addition, you can look in the annual report, which is available on [www.nki.nl](http://www.nki.nl) or on websites of the groups to which reference is made on the website as well. In case you have a certain interest, but cannot find the right contact with the aid of this guide or otherwise, you can ask the Dean for Master Studies for advice. The Dean is also the official connection between the NKI and your university.

Information:
Exclusively by e-mail: Dean for Undergraduate Studies, Prof. Dr. J. Borst, e-mail address: j.borst@nki.nl; for questions concerning the course on Experimental Oncology or general information, please contact the secretary, Ms. R. de Jong at e-mail address rm.d.jong@nki.nl, telephone 020-5122055.

Theoretical training in Experimental Oncology
Apart from training in experimental work, students have the opportunity to enhance their theoretical knowledge. The Netherlands Cancer Institute offers a course on Experimental Oncology for this purpose. For every student, who does a placement in the institute in the area of Biology/Medicine/Chemistry for more than 4 months duration, it is compulsory (verplicht) to follow this course and to do the exam connected with it. (Unless you attended and passed an equivalent course in your curriculum). Other learning opportunities are the institute’s Staff Evenings, regular work discussions and literature discussions within the division and within the research group you joined. Students are expected to participate in these meetings.

The course on Experimental Oncology is held twice a year, in March/April and October/November. It consists of lectures, discussions and guided tours, which generally take place in the morning and early afternoon. The rest of the day is meant for preparation of the discussions and further study. There is opportunity to continue laboratory experiments during the course, if you study in the evening and in weekends. The course itself lasts for 4 weeks and the exam takes place 1 week after the last lecture. The study material for the exam consists of hand-outs, reviews and notes made by the students themselves. The book Cancer Biology by Robert A. Weinberg is excellent for background reading, but you are not required to buy it. The teachers are institute staff members, both researchers and clinicians. The subject matter is state of the art cancer research in laboratory and at the bedside. To successfully complete the course, students must have a background in (medical) biology, medicine, pharmacy, (bio)chemistry or a closely related subject, with understanding of basic cell biology, immunology and molecular biology/genetics. The course is intended for students at the Master level. The mark for this course can be part of the final mark for the placement, or a separate mark for a theoretical subject, with consent of the university (6 ECTS). Announcements for the course are made on the institute website [www.nki.nl](http://www.nki.nl). The “onderwijsbalies” of the various universities are also informed. Registration and further information: Ms. R. de Jong, Secretary to the Dean, e-mail: rm.d.jong@nki.nl, telephone 020-5122055.

Scripties (minor thesis)
It is possible to write a “scriptie” under supervision of a staff member of the NKI. The student needs to obtain permission from the university to do so and should find an NKI staff member willing to coach him/her. It should be noted that NKI staff members have no obligation to assist students in this way. The supervisor must be a project leader and permanent staff member of the NKI to ensure sufficient expertise. The Dean can advise about subjects and supervisors.
Useful information

Name institute
Dutch: Het Nederlands Kanker Instituut-Antoni van Leeuwenhoek Ziekenhuis
English: The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital
Abbreviation: NKI-AVL

Address: Plesmanlaan 121
1066 CX Amsterdam
Tel.: 31-20-5129111
Fax: 31-20-6172625

The Institute can be reached by tram, line 2, and bus lines 18, 23 and 64, stop Antoni van Leeuwenhoek Hospital. The nearest train station is Amsterdam Lelylaan (10 min. walking distance from Institute).

Teaching committee (Onderwijscommissie)
Installation and maintenance of policies concerning students has been delegated by the scientific director to the so-called teaching committee:
Chairperson and Dean University Master students: Prof. Dr. J. Borst
Deans HLO students: Dr. J. Collard, Dr. Ir. J. Hilkens
General affairs: Prof. Dr. H. te Riele
Publicity, tours: Dr. Ir. J. Hilkens, Dr. J. Collard
Experimental Oncology course: Prof. Dr. J. Borst, Dr. R. Beijersbergen, Prof. Dr. H. te Riele
Clinical teaching: Prof. Dr. A.J.M. Balm.
Dean Oncology Graduate School Amsterdam (OOA): Prof. Dr. T. Sixma.

HOOGLERAREN EN LEEROPDRACHTEN

N.K. Aaronson, bijzonder hoogleraar Kwaliteit van leven bij chronische en/of levensbedreigende ziekten, Universiteit van Amsterdam

R. Agami, bijzonder hoogleraar Basic and Translational Oncology, Erasmus Universiteit Rotterdam

P. Baas, bijzonder hoogleraar Pulmonale Oncologie, Academisch Medisch Centrum Amsterdam

H. Bartelink, bijzonder hoogleraar Experimentele Radiotherapie, Universiteit van Amsterdam

R. Bernards, bijzonder hoogleraar Fysiologische Chemie in het bijzonder de Moleculaire Carcinogenese, Universiteit Utrecht

F. Balm, bijzonder hoogleraar Hoofd/Halsoncologie en -Chirurgie, Universiteit van Amsterdam

A. Begg, bijzonder hoogleraar Moleculaire Radiobiologie, Universiteit Nijmegen

A. Berns, bijzonder hoogleraar Experimentele Moleculaire Genetica van Erfelijke Aandoeningen, Universiteit van Amsterdam

J.H. Beijnen, hoogleraar Analytische Geneesmiddelenxicologie, Universiteit Utrecht

J. Borst, bijzonder hoogleraar Experimentele Oncologie, Universiteit van Amsterdam

P. Borst, buitengewoon hoogleraar Klinische Biochemie, Universiteit van Amsterdam

F. van Dam, bijzonder hoogleraar Kwaliteit van leven bij chronische en/of levensbedreigende ziekten, Universiteit van Amsterdam
J. Haanen, bijzonder hoogleraar Translationele Immunotherapie van Kanker, Universiteit Leiden

M. van Herk, bijzonder hoogleraar Oncologie, Universiteit van Amsterdam

F. Hilgers, hoogleraar Oncologisch Gerelateerde Stem- en Spraakstoornissen, Universiteit van Amsterdam

S. Horenblas, bijzonder hoogleraar Oncologische Urologie, Vrije Universiteit Amsterdam

F.E. van Leeuwen, bijzonder hoogleraar Epidemiologie van Kanker, Vrije Universiteit Amsterdam

M. van Lohuizen, bijzonder hoogleraar Biologie en epigenetische regulatie van normale en kanker stamcellen, Universiteit van Amsterdam

W.H. Moolenaar, bijzonder hoogleraar Moleculaire Cellbiologie, Universiteit Leiden

J. Neefjes, bijzonder hoogleraar Antigeen Processing en Presentatie, Universiteit Leiden

D. Peeper, bijzonder hoogleraar Functionele Oncogenomica, Vrije Universiteit Amsterdam

P.J. Peters, hoogleraar Ultrastructurele Cellbiologie, Vrije Universiteit Amsterdam

H. te Riele, bijzonder hoogleraar Genetische instabiliteit en carcinogenese, Vrije Universiteit Amsterdam

S. Rodenhuis, bijzonder hoogleraar Klinische Oncologie, Universiteit van Amsterdam

J.H.M. Schellens, hoogleraar Klinische Geneesmiddelen Toxicologie, Universiteit Utrecht

T. Schumacher, bijzonder hoogleraar Immuuntechnologie, Universiteit Leiden

T.K. Sixma, bijzonder hoogleraar Structuur en functie van eiwitten, Erasmus Universiteit Rotterdam

B. van Steensel, bijzonder hoogleraar Chromosoombiologie, Erasmus Universiteit Rotterdam

M. Verheij, hoogleraar Translationele Radiotherapie, Vrije Universiteit Amsterdam
II Student policies

1. Student registration

Prior to the placement, Master students have to get formal permission from their university/school for the placement. As part of this procedure, an agreement is made on the content as well as the duration of the placement. The procedure required depends on the university or HLO School. All formal communication with the University or HLO School has to be done either by the responsible project leader (NKI staff member) or by the relevant dean. In certain cases, an examiner is required at the NKI side who has a (professorial) appointment with the University in question. Various NKI staff members are in this way connected to universities. Check the guide for Master students at http://www.nki.nl/Research/Career/Masters+students for this information. J. Borst can act as examiner for UvA students.

University and HLO Master students who do a placement have to register with dean J. Borst via a form that can be downloaded from the intranetsite. This registration serves multiple purposes: 1. A record is kept for the NKI-AVL and the schools. 2. The student receives information about the course from the Dean’s office and is informed of the Dean’s role as consultant and mediator. 3. The Dean ensures uniformity in evaluation of the student’s performance and award of credit points (EC). 4. The placement is monitored by the Dean as impartial NKI representative, who can act as mediator in case a dispute arises about supervision and/or evaluation of the student’s performance. 5. The school has one central contact point for communication.

Finally, the student also has to register with the P&O department, via a form that can be downloaded from http://antonet/​layouts/FormServer.aspx?XsnLocation=/FormServerTemplates/stageformulier.xsn&OpenIn=​browser

This serves the purpose of having the student insured for accidents and registered. This registration is the responsibility of the project leader. Failure to register may have important consequences for award of credit points (ECTS).

2. Procedure supervision, performance and evaluation

This information is also available at: http://antonet/Research/Pages/MasterStudents.aspx

Although post-docs or PhD students often perform the daily supervision of the student, the formal supervisor must be a staff member of the NKI. This is without exception the project leader of the group in which the placement is done. An official examiner and/or the dean (see above) can take part in final evaluation of the student’s performance, if required. Many universities appoint a supervisor within the university who has a shared responsibility for the student and usually partakes in the evaluation.

A Master placement in the NKI has the following compulsory elements:

a) Doing practical work on basis of a work plan designed by the supervisor.
b) Successfully completing the exams connected to the course on Experimental Oncology (not for HLO students).
c) Attending work discussions, seminars and staff evenings.
d) Writing a report on the research performed.
e) Giving at least one work discussion, as a final oral report on the research project.

The written report is usually presented in the form of a research paper, but can have more details. Use of the English language is encouraged, but not compulsory. The student and the supervisor need to take care that the placement is completed within the period that has been agreed upon with the university/school. The written report must be handed in and the final oral report must be delivered within two months after the practical work has ended. The placement can only be extended after consultation of the dean and does not alter the requirement to accomplish the goals originally agreed upon. Notifying the university or school of an extension or interruption of the placement is at all times the student’s responsibility.
Evaluation (beoordeling) – Evaluation is based on three parts of the placement: practical work, written report and oral presentation. The mark and credits for the Experimental Oncology course can be counted separately, or be incorporated in the total mark and credits for the placement. The final mark is the weighted mean (gewogen gemiddelde) of these parts, in which the weighing factors correspond to the amount of time spent on each part. For the placement, the usual ratio for practical work, written report and oral presentation is 60%, 30% and 10% respectively. At all times, the combination of practical work and written report must determine the final mark for at least 80%. Credit points are based on the ECTS system, which is the European standard. In this system, 1 week of study (36 h) equals 1.43 EC points (one point forty-three). An example: total duration of the placement is 24 weeks. The student has done the course in Experimental Oncology (4 weeks) within this time frame and wants to incorporate the credit points and mark for the course in the final evaluation of the placement. Mark for placement was 8, mark for course 7. Mark for the placement \((20 \times 8) + (4 \times 7) : 24 = 7.8\). Total credit points: \(24 \times 1.43 = 34\) EC. You may enter the mark in decimals, or rounded off at 0.5 point. EC points are not given in decimals. The credits for the course when counted separately are rounded off at 6 EC points.

The procedure for evaluation of the placement is as follows:

a) At least 1 week prior to the oral report, the written report is handed in with the direct supervisor, the project leader and a chosen third member of the evaluation committee, who must be a staff member of the NKI-AVL. This extra member serves to ensure objectivity. If the approval form mentions a third person apart from supervisor and project leader as examiner or co-assessor, this person should be the third member of the committee.

b) The final oral presentation should be attended by the three members of the committee outlined under a) and ideally also the head of the Division. Directly after this presentation, the direct supervisor proposes a final mark for the placement to the committee. The following elements determine the mark: quality of the research and reports, difficulty, former experience of the student and the quality of the supervision and help. The mark has to be approved by the entire committee. When consensus cannot be reached, the head of the Division makes the final decision after having read the written report and having consulted the direct supervisor.

c) The direct supervisor or project leader notifies the dean in writing (email j.borst@nki.nl or rm.d.jong@nki.nl) of the exact number of weeks spent de facto on the placement (specifying whether this includes or excludes time spent on the course in Experimental Oncology), the mark for the placement and the desire of the student to incorporate the mark and credits for the course or to count these separately. The dean enters the final mark and credit points on the official form destined for the university or school. This can be a form supplied by the university or a form made by the dean’s office. On this form, de “vakgroep” is indicated as Experimental Oncology, specialisation can be indicated with the name of the Division. (Note: please do not drop in on the spur of the moment to get the final mark from the dean, but adhere to the protocol outlined here; we need information and time to prepare the form). Certain Universities also accept a signature from the project leader in stead of the dean. In all cases, however, please consult the dean by email for the marking and allotment of credits points.

Note on insufficient marks -
According to the criteria of the UvA, a 5.5 and higher is a sufficient mark. One may give an insufficient mark for a placement, in principle. However, at the NKI-AVL we will apply the following policy: Students should be made aware in an introductory discussion with the project leader that they may be sent away at an early stage if their performance is below the acceptable level. If you notice that your student is performing way below average, have a discussion with him/her as soon as possible (within 2 months after starting the placement) and let them know that they get a trial period (suggestion 1 more month). If the performance does not improve, you must have another consultation with the student, now in presence of the dean. At this point, it will be decided whether the student is better off leaving the institute and doing a placement elsewhere. If the placement is continued, it should be made clear to the student that there is the possibility that he/she will obtain an insufficient mark and thereby no credits. For the remainder of the placement period, the dean will act as co-supervisor. Final evaluation and grading will be done in consultation with the dean. For application of this policy, the project leader (not the supervising OIO or post-doc) is responsible.
3. Foreign students

Foreign students are welcome to do their placement at the NKI-AVL, provided that they have sufficient training (they should be at the level of a Dutch Master student). The project leader should verify that the student has appropriate background knowledge and experience before accepting the student. In case of doubt, please consult the dean.

Several issues need to be sorted out before a foreign student can start. These include among others visa, housing and financing. It is the responsibility of the student to arrange for this, but the institute can give some support. The project leader/supervisor should contact the personnel department for such support.

Students should evidently have permission to enter The Netherlands and to stay here. Students from Austria, Australia, Belgium, Canada, Cyprus, Denmark, Finland, France, Germany, Greece, Great Britain, Iceland, Ireland, Italy, Japan, Liechtenstein, Luxembourg, Malta, New Zealand, Norway, Portugal, Spain, Sweden and Switzerland do not require a visa to come to The Netherlands. Students from all other countries who will be staying for more than 3 months need a visa. They need to apply for an entry visa (Machtiging Voorlopig Verblijf) in their own country. As soon as they arrive in The Netherlands, they must go to the Foreign Police (Immigratie en Naturalisatie Dienst). They will be registered there, which starts their application for a visa (Verblijfsvergunning).

Housing and living costs in Amsterdam are high. A student should be aware of this, because the institute will as a general rule not give financial compensation during the placement. The Personnel department can provide help with housing.

4. Financial compensation

The Institute will reimburse daily travel expenses for commuting to and from the Institute according to the Collective Labor Agreement which applies for everyone working at the Institute. The Institute is not allowed to give a living allowance to University Master students. Students of HLO Schools receive a small monthly allowance according to the Collective Labor Agreement.
III Overview activities research groups arranged by Division

Division of Immunology

Head: Prof. Dr. J. Borst
Project leaders: Prof. Dr. J. Borst, Dr. C. Blank, Dr. J. Haanen, Dr. H. Jacobs, Prof. Dr. T. Schumacher, Dr. F. Vyth-Dreese,

Group Borst: Life/death decisions in lymphocytes

T- and B-lymphocytes have unique membrane receptors, which can recognize foreign antigens. Upon binding of antigen to the receptor, the cell is activated, divides and matures into a helper- or cytolytic effector T cell or into an antibody producing B cell. After the immune response, the majority of effector cells die by apoptosis, a regulated form of cell death. A small population is retained as memory cells, which allows a more efficient and rapid response upon renewed exposure to the same antigen. We are interested in the signals that keep lymphocytes alive upon their initial activation and the signals that kill them or protect them when they have performed their function. Apart from the antigen receptors, members of the Tumor Necrosis Factor (TNF) receptor family play an important role in the life/death decisions that take place in lymphocytes. Some of these receptors can induce apoptosis, while others promote survival. Knowledge about the mechanism of action of these receptors is not only relevant for understanding and manipulating the immune response to cancer. Our work has other connections with cancer therapy as well: The apoptosis signaling routes that are activated by TNF receptor family members also play a role in the response to anti-cancer drugs and radiation. Blocking of these routes may result in resistance to conventional or novel therapies, while their deliberate engagement may promote tumor regression.

We use advanced immunological, biochemical, molecular biological and microscopic techniques to study the regulation of cell survival and death in vitro as well as in vivo. These include gene expression profiling by micro-array, generation of DNA constructs, cell transduction, in vitro biochemical and cell biological assays and in vivo analysis of genetically engineered mouse strains.

Information: Jannie Borst, tel.: 020-5122055, E-mail: j.borst@nki.nl

Group Blank: Tumor Immune Escape and Homeostatic Proliferation

The aim of our research is to identify mechanisms that tumors use to escape from anti-tumor immune responses. The characterization of inhibitory molecules and pathways that are involved in these escape mechanisms, may help in designing novel approaches to improve anticancer immunotherapy. One of these immunotherapy approaches is adoptive transfer of tumor-reactive T cells. A prerequisite effective tumor growth control is sufficient expansion and survival of tumor-reactive T cells without exhaustion in vivo. This may best be achieved by transferring T cells into lymphopenic hosts. Upon the transfer of these peripheral T cells into lymphopenic recipients, acute homeostatic proliferation (HP) is observed. In the second line of our research we try characterize these HP T cells further aiming at the selective use of these T cells in immunotherapy.

Information: Christian Blank, tel.: 020-5122570, E-mail: c.blank@nki.nl

Group Haanen: Immunotherapy

The primary objective of this group is the development of novel immunotherapeutic strategies. In the past years we invested in the development of high through-put technologies to detect and follow tumor-specific T cell within tumors and in peripheral blood samples of patients treated with different forms of immunotherapy. This can be achieved by application of MHC tetramers, developed in Group Schumacher, that specifically stain tumor-specific T cells in peripheral blood and tissues from tumor-bearing mice and cancer patients. For patients with metastatic melanoma, we have developed several new immunotherapeutic strategies, amongst others DNA-based vaccinations, infusion of tumor-infiltrating lymphocytes and infusion of T cells transduced with melanomaspecific T cell receptors.

Information: John Haanen, tel.: 020-5126979, E-mail: j.haanen@nki.nl
**Group Jacobs: DNA damage tolerance in health and disease**

Our research is focused on two topics:

*The Role of Translesion DNA Synthesis in Immunity, Cancer Development and Cancer Treatment*

Secondary diversification of Immunoglobulin (Ig) genes comprises somatic hypermutation of variable regions to generate high affinity variants and class switch recombination between constant regions to alter the effector function of an Ig. Both processes are transcription-controlled and require the catalytic activity of the Activation Induced Cytidine Deaminase (AID). AID appears to induce DNA lesions. To tolerate such lesions, cells have two major pathways of DNA damage tolerance: error-free template switching (TS) and error-prone translesion DNA synthesis (TLS). Intriguingly, B cells of the germinal center take advantage of error prone TLS polymerases to diversify the primary antibody repertoire by somatic hypermutation and simultaneously risk oncogenic mutations. We aim to understand how these highly mutagenic processes are activated, controlled, and targeted to Ig genes and what are the consequences of their 'aspecific' targeting for genome integrity and the pathogenesis of lymphoma.

*Exploring DNA-Damage Tolerance as a Drug-Target for Chemosensitization*

DNA damage tolerance (DDT) enables DNA replication to continue in the presence of stalling lesions, caused for example by DNA damaging chemotherapeutics. DDT strongly depends on damage-inducible, site-specific ubiquitination of the DNA sliding clamp proliferating cell nuclear antigen (PCNA), implicating PCNA ubiquitination as an effective target for chemosensitization, the sensitization of cancer cells to DNA-damage based chemotherapy. To determine the role of DDT in mammals, we generated mice expressing a non-modifiable PCNA-K164R mutation. PCNA-K164R mutant mice develop normally and are not prone to develop tumors. Interestingly, the failure to ubiquitinate PCNA-K164R blocks DDT and renders PCNA-K164R mutant cells highly chemosensitive. The site-specificity of PCNA ubiquitination, implies PCNA-K164 ubiquitination as an ideal drug target for chemosensitization. By blocking site-specific PCNA-ubiquitination *in vitro* as well as *in vivo* we are revealing the therapeutic potential of a DDT blockade in cancer treatment. In addition, we determine the contribution of DDT in the development of chemoresistance and the impact of a DDT blockade in prohibiting the formation of chemoresistant mutations.

*Information: Heinz Jacobs, tel.: 020-5122065, E-mail: h.jacobs@nki.nl*

**Group Schumacher: Tracing and manipulating cytotoxic T cells**

Research in our group aims to analyse and improve virus- and tumor-specific T cell immunity, using innovative (and often home-grown) technologies. Our three major research lines concern 1) The development of novel technologies to detect tumor-specific T cells in patients samples, and to trace T cells in vivo at the single cell level. 2) The analysis of T cell fate in vivo by genetic tagging and by intravital imaging in mouse model systems 3) the clinical translation of our work by the development of clinical protocols for the induction of tumor-specific T cell immunity through gene therapy. For more detailed information on these projects, see: [http://www.nki.nl/Research/Faculty+and+Research/Divisions/Immunology/Schumacher.htm](http://www.nki.nl/Research/Faculty+and+Research/Divisions/Immunology/Schumacher.htm)

The research in this group involves a mixture of immunological and biochemical/ biotechnological techniques.

*Information: Ton Schumacher, tel.: 020-5122072, E-mail: t.schumacher@nki.nl*
Division of Gene Regulation

Head: Dr. R. Agami
Project leaders: Dr. R. Agami, Dr. J-H Dannenberg, Dr. F. van Leeuwen, Dr. B. van Steensel

**Group Agami: Unraveling cancerous genes and microRNAs**
Most human tumors harbor multiple genetic alterations that activate oncogenes, inhibit tumor suppressors and induce genomic instability. As each tumor contains many genetic alterations, the study of the contribution of each variation to carcinogenesis and to the maintenance the cancerous phenotype is obscured. We developed a vector-based system that mediates suppression of gene expression through RNA interference (pSUPER). We used this gene inactivation system to identify and characterize novel genes involved in oncogenesis, tumor-suppression and DNA-damage checkpoints. Recently, we also developed a novel microRNA-expression library and a barcode array. microRNAs are endogenous small RNAs that function as master regulators of gene expression capable of defining and altering cell identity. We use this tool to identify cancer-causing and cancer suppressing microRNAs. Our efforts have resulted in the identification of several novel cancerous protein-coding and miRNA genes. For more recent and detailed information please take a look at our website: [http://research.nki.nl/agamilab/](http://research.nki.nl/agamilab/)

Information: Reuven Agami, tel.: 020-5122079, E-mail: r.agami@nki.nl

**Group Dannenberg: Lysine deacetylation and demethylation in development and treatment of cancer**
Proteins are targeted by a vast number of modifying enzymes that balance post-translational modifications such as phosphorylation, ubiquitination, sumoylation, methylation and acetylation. Acetylation and methylation of lysine residues in proteins is as widespread as protein phosphorylation and controls the activity and function of numerous proteins. Acetylation of lysine residues is controlled by histone acetyl transferases (HATs) and histone deacetylases (HDACs). Although these enzymes were identified as histone-modifying enzymes, hence their names, it has become evident that also non-histone proteins are substrates for these enzymes. Both classes of proteins have been linked to tumorigenesis as cancer-specific mutations are found in HATs (e.g. CBP) while many tumor types display elevated levels of HDAC1, HDAC2 and HDAC3. Owing to their anti-tumor activity, HDAC-inhibitors (HDACi) have entered the clinic in the treatment of cutaneous T-cell lymphoma and are tested in various clinical trials for combination therapy. Despite their clinical efficacy little is known about the actual mode of action of HDACi and which HDACs they are targeting to elicit anti-tumor activity.

Methylation as well as demethylation has been linked to tumorigenesis by the identification of lysine methylases and lysine demethylases as oncogenes and tumor suppressor genes. As a consequence several small molecule inhibitors of lysine demethylases are being developed and tested in clinical trials. In order to understand the biological consequences of inhibiting these enzymes, genetic approaches are required to understand the role of lysine demethylases in the context of an organism.

Our group is focusing on dissecting the function of HDACs in development and tumorigenesis by using mice and cells carrying histone deacetylase and demethylase conditional knockout alleles. Our goal is to obtain in-depth knowledge of histone deacetylases and methylases that are relevant for tumorigenesis and can serve as promising drug targets in anti-cancer therapy. To this end we have several projects available in which students can participate who are interested in developing and/or applying state-of-the-art approaches including but not limited to genetics, biochemistry, cell biology and bio-informatics.

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Tumor-suppressor gene inactivation or oncogene activation in cancer cells can be caused by genetic changes as well as stable and heritable changes in gene expression. These stable changes in gene-expression are dictated by epigenetic mechanisms, which affect the structure of chromatin, i.e. DNA and histone proteins packaged into nucleosomes. Methylation of DNA and post-translational modification of histone proteins have been identified as key events in gene silencing or activation in cancer. However, the mechanisms by which epigenetic imprints are established or prevented are still poorly understood.

Many chromatin modifications are conserved from yeast to humans. We are using budding yeast as a powerful model system to study the mechanisms of epigenetic regulation. By using a combination of molecular biology, biochemistry, and high-throughput genetics, we are trying to unravel the function of the histone methyltransferase Dot1, which is involved in human leukemia. In addition we are developing new tools and assays to investigate how histone proteins and their post-translational modifications are inherited when cells undergo DNA replication and cell division. One of the main goals of our lab is to unravel how histones and their post-translational modifications can lead to stable and heritable changes in gene expression.

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Group van Steensel: Chromatin Genomics

In the living cell, gene expression is controlled by an extremely complex network of protein-DNA interactions. Hundreds of regulatory proteins, such as transcription factors and other chromatin proteins, control the transcription of tens of thousands of genes by binding to regulatory elements nearby these genes. Our understanding of this complex network is limited, mostly because we know only a tiny fraction of the molecular interactions that occur at the DNA-protein interface. Our group studies the role of chromatin proteins in the regulation of gene expression. For this, we use whole-genome approaches. We develop novel genomics technologies to study protein-DNA interactions and chromatin structure in the living cell, and apply these technologies to gain understanding of the complex networks of gene regulation. Among others, we use DamID, a technique that we developed in our laboratory for large-scale mapping of in vivo target DNA sequences of chromatin proteins. With this technique, we construct high resolution full-genome maps of protein binding in Drosophila, mouse and human cells.

For the analysis of these maps (up to a few million data points per experiment) we develop new bioinformatics tools for data visualization and functional interpretation. The results give us extensive insights into the functions and molecular interactions of chromatin proteins. Projects are available for students who are interested in developing and/or applying new genomics technologies. In addition, we have bioinformatics projects available that involve programming in R or Perl. Other techniques that are used in the lab include state-of-the-art molecular biology techniques, cell culture and transfections, microarray technologies, immunofluorescence, confocal microscopy, and bioinformatics.

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Division of Cell Biology I

Head: Dr. A. Sonnenberg
Project leaders: Dr. A. Sonnenberg, Dr. J. Collard, Dr. M. Innocenti, Dr. K. Jalink, Prof. Dr. W.H. Moolenaar, Dr. E. Roos

**Group Sonnenberg: Cell-matrix receptors**

Hemidesmosomes are multiprotein complexes that mediate adhesion of epithelial cells to the underlying basement membrane. The transmembrane components of hemidesmosomes include the integrin 6 4and BP180. These proteins are connected, via the hemidesmosomal plaque proteins HD1/plectin and BP230, to the keratin intermediate filament system. The integrin 6 4 has a central role in the assembly and maintenance of hemidesmosomes since mice, carrying a targeted deletion of the genes encoding 6 or 4 are unable to assemble hemidesmosomes, which results in extensive blistering of the skin. Also in human, the absence of 6 4 due to mutations in the genes encoding 6 or 4, is associated with loss or hypo-plasticity of hemidesmosomes. By providing binding sites for the different hemidesmosomal components at the basal aspects of keratinocytes, the integrin 6 4 initiates the formation of hemidesmosomes. We have identified and characterized, on the 4 cytoplasmic domain, binding sites for each of the other three hemidesmosomal components and shown that binding of 4 to plectin and BP180 is crucial for their localization into hemidesmosomes.

Current studies focus on the signaling pathways by which growth factor receptors regulate the assembly/disassembly of hemidesmosomes. It has been shown that during wound healing when keratinocytes have to migrate over a matrix to close the wound, growth factor receptors are activated and hemidesmosomes disassembled. Furthermore, in many tumors hemidesmosomes are not formed and their loss has been associated with the ability of tumor cells to migrate and invade into surrounding tissues. One approach we are pursuing to assess the role of 6 4 and hemidesmosomes in tumor progression is to generate a conditional knock-out mouse strain for 4 that allows us to inactivate 4 in tumor cells and study the properties of 4-positive and -negative tumor cells in vitro and in vivo.

A second direction of our research involves the cross-talk between different adhesion junctions. Specifically, we are studying the effects of 1 integrins that are concentrated in focal contacts, on the stability of E-cadherin based cell-cell junctions, and study the role of focal contacts in the formation of hemidesmosomes.

A third direction is to unravel the signal transduction pathways that are involved in the translation of integrin-mediated adhesion information into a growth response. It has long been recognized that the interaction of cells with the extracellular matrix is a prerequisite for the progression of the cell cycle. Furthermore, many tumors have become independent of these signals and can grow in suspension, a phenomenon called ‘anchorage independent cell growth’. What the exact signals are that integrins transmit and that control cell proliferation is not known. These research projects are designed to further our understanding how signals mediated by growth factor receptors and integrins regulate important cellular processes such as migration and proliferation.

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**Group Collard: Tumor progression and metastasis**

The research within this group is focussed on the genetic control of tumor progression and metastasis. We have developed function-based screening methods to identify genes involved in processes such as invasion and metastasis. These studies have led to the identification of various invasion-inducing genes including the *Tiam1* gene, which encodes an activator (guanine exchange factor) of the Rho-like GTPase Rac. Currently, we are studying the role of Tiam1 and Rho-like GTPases in processes such as cell adhesion, migration and transformation, employing in vitro and in vivo experiments. Moreover, we have generated Tiam1-deficient mice to study the role of Tiam1 and Rac in the formation and progression of tumors in vivo.
Research lines currently ongoing in this group are: Studies on the function of various Rac GTPases with emphasis on Rac1 and Rac3; Studies on the function of the Rac activator Tiam1 in various aspects of tumor formation and progression, and studies on the function of Tiam1 in cell polarization. With respect to the latter, we found that Tiam1 in conjunction with the Par polarity complex regulates apical-basal cell polarization as well as front-rear cell polarization and directional migration in epithelial cells. In addition the Tiam1-Par complex controls T-cell polarization in processes of homing and egress. Which molecular mechanisms control cell polarization and how other polarity complexes contribute to the establishment of cell polarization is currently a major topic in our group. For background information on the research of the group see: Malliri et al., Nature 417: 867-871, 2002. Mertens et al., Trends in Cell Biology 16: 308-316, 2006; Gerard et al., J.Cell Biol., 176:863-875, 2007; Iden & Collard, Nature Rev Mol cell Biol. 2008.

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**Group Innocenti: Acting Dynamics in cancer cells**  
Actin dynamics control the remodeling of the plasma membrane and are essential to support cell migration. Not surprisingly, sophisticated mechanisms have evolved to harness the activity of actin nucleators, enzymes required for actin to efficiently form filaments. To date, the Arp2/3 complex and Formins are the best characterized actin nucleators in mammalian cells.

The WASP/WAVE family of Nucleation-Promoting Factors (NPFs) stimulates the weak basal activity of the Arp2/3 complex. WAVE proteins are involved in the formation of lamellipodia, veil-like protrusion of the plasma membrane. They confine Arp2/3-complex activity to the lamellipodium tip, which faces the plasma membrane. In this way, localized actin polymerization allows the plasma membrane enclosing lamellipodia/ruffles to advance and cells to move. WASP proteins are instead implicated in endocytosis and trafficking: they activate the Arp2/3 complex on clathrin-coated pits and cytosolic vesicles.

Actin polymerization by the Diaphanous-related Formin mDia2 controls the formation of filopodia, finger-like extensions of the plasma membrane. mDia2 also regulates vesicle trafficking, which provides supplies to the leading edge of crawling cells.

**Regulation of WAVE2- and N-WASP-based complexes**  
WAVE- and N-WASP-based core complexes have been shown to spatially and temporally restrict Arp2/3-dependent actin polymerization. However, these core complexes fail to provide a mechanistic explanation for the high versatility of the WASP/WAVE proteins. We have found that dedicated subunits are required to confer functional specificity to both WAVE and N-WASP core complexes. Currently, we are characterizing some of them in great detail.

**Regulation of mDia2**  
How mDia2 regulates actin dynamics is still matter of debated. In order to fully understand the biological function(s) of mDia2, we have isolated its interactome. This information has revealed new roles for mDia2 that will be the focus of future investigation. Biochemically, we are taking advantage of recombinant full-length mDia2 to dissect its mechanism(s) of action.

**Mechanisms of formation and roles of filopodia in cell migration**  
We have recently generated a genetically modified cell line that allows us to induce filopodium formation in vitro and to perform systematic studies. This tool enables us to undertake pioneering loss-of-function genetic and proteomic screens that will inventory filopodium-regulatory proteins and reveal the filopodium protein signature. This knowledge will allow us to understand the role of filopodia in cell migration.

**Publications**  
Galovic M, et al., Interplay between N-WASP and CK2 optimizes clathrin-mediated endocytosis of EGFR. JCS, accepted 2011  
Beli P at el., WAVE and Arp2/3 jointly inhibit filopodium formation by entering into a complex with mDia2. Nat Cell Biol. 2008

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**Group Jalink: Biophysics of cell signaling**  
Intracellular signals that regulate the (patho-) physiological behavior of living cells are in many cases restricted in time and space, i.e. they are transient (short-lived), and limited to a subcellular region. A detailed understanding of these signals requires techniques to study them with high spatiotemporal resolution in single cells. (Bio-)physical techniques, such as electrophysiology, spectrometry, and imaging techniques, offer this resolution and are increasingly complementing the traditional biochemical analysis methods. Our group aims to 1) contribute to the development and validation of these techniques, and 2) apply them in biological research. Our lab is equipped with the necessary advanced equipment to perform a variety of determinations. We are engaged in the basic development of measurement schemes (e.g., sensing of membrane PIP₂ content by Fluorescence Resonance Energy Transfer, or FRET) and in the application of these schemes in ongoing research projects.

Students can participate in both development and application of measurement technology.
An exceptionally broad range of techniques can be learned, including molecular biology, transfection, protein biochemistry, confocal- and wide-field life-cell imaging, FRET, FRAP (Fluorescence Recovery After Photobleaching), flash-photolysis of caged compounds, microinjection, and the major electrophysiological techniques. Please see our website http://research.nki.nl/jalinklab

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Group Moolenaar: The autotaxin-LPA receptor axis: a new signaling network

Autotaxin (ATX) is a secreted phosphodiesterase that generates the lipid growth factor LPA (lysophosphatidic acid) from more complex phospholipids. LPA signals through specific G protein-coupled receptors (GPCRs) present in almost every cell type. ATX is overexpressed in several human cancers and promotes tumor metastasis and angiogenesis in mice. Targeted disruption of the ATX gene results in severe vascular defects. Furthermore, in collaboration with the group of A. Perrakis, the crystal structure of ATX was recently determined. Yet many questions remain, particularly how the ATX-LPA receptor regulates tumor progression.

A major line of research concerns the mechanisms of signal transduction by individual LPA receptors. In particular, we are focusing on a newly discovered signaling protein that is activated by the LPA-induced RhoA pathway, a master regulator of the actin cytoskeleton, cell shape and cell motility. In addition to various molecular approaches (RNA interference, protein biochemistry, mutational analysis, etc.), we make use of advanced cell biological and live-cell imaging techniques (collaboration group Jalink) to elucidate the function of this new signaling molecule and how it influences tumor cell behavior.

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Division of Cell Biology II

Head: Prof. Dr. J. Neefjes
Project leaders: Prof. Dr. J. Neefjes, Dr. R. Michalides, Dr. H. Ovaa, Prof. Dr. P. Peters

**Group Neefjes: Antigen presentation by MHC molecules in living cells**
The immune system is continuously searching for cells that are infected by pathogens. The immune response then ensures the control of these infections. The identical system is used to detect tumor cells, which can also be destroyed by the immune system. One goal is to transfer the knowledge of the immune system into an active and specific vaccination protocol for tumors.

We study how antigens can be presented to the immune system. Critical are so-called MHC molecules that present small fragments of intracellular antigens at the cell surface for consideration by cytotoxic T cells. This implies that a number of processes have to occur before the immune system can be notified on an intracellular infection. These are: protein degradation to make fragments; fragment transport; loading of fragments onto MHC molecules and transport of MHC molecules to the plasma membrane.

We have established various techniques allowing the visualization of these processes in living cells. This means that we combine molecular biology with protein chemistry with a variety of microscopical techniques to visualize these processes at any level of resolution. In addition, we apply laser technology to (in)activate fluorescence in order to follow biochemical processes in living cells. These techniques have resulted in the identification of a new protease as an essential intermediate in fragment preparation, a new source of antigens, the half-life of fragments in cells and the specificity of this process, the identification of the transport process (ie the molecular motor proteins) and the visualization of an MHC molecule and its chaperone in living cells.

We have established techniques to follow protein half-life in yeast (as a model system). We are currently using these to identify factors controlling the degradation of large stable protein and protein-RNA complexes such as the proteasome and ribosomes. We have recently established tools to identify novel players in the MHC class II antigen presentation pathway. Using siRNA screening and high throughput FACS analyses, we have identified many new proteins clustering in various signalling pathways controlling this immune response. How these novel components act in controlling immune responses has to be determined using various types of cell biological and immunological tools. We already identified various proteins potentially involved in controlling transport. Transport of MHC class II molecules and late endosomes is governed by the motor proteins dynein and kinesin and further controlled by the small GTPase Rab7. Using a series of unique technologies, we aim at understanding these processes at the molecular level in living cells. All our projects apply a host of techniques to show how biochemical processes work in living cells.

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**Group Ovaa: chemistry of protein degradation and antigen presentation.**
The chemical biology laboratory at the Netherlands Cancer Institute specializes in the design and development and importantly the use of diagnostic and proteomics tools using a variety of approaches with the ultimate goal of early diagnosis and treatment of cancer.

The group develops techniques to profile cellular enzymatic activities associated primarily with ubiquitin-mediated proteasomal degradation and antigen presentation and uses various techniques including classical biochemical approaches but also organic synthesis and mass spectrometry driven approaches combined in a single multidisciplinary lab in order to gain further understanding of the biochemical processes under investigation. The research comprises the identification of small molecule activity modulators and small molecule reporters on enzymatic activities by both rational design and high throughput screening of small molecule libraries with robotics and development and use of novel research tools. The current goal is grouped around three sub-themes:

**Theme 1: ubiquitin and ubiquitin-like systems**
**Theme 2: proteasome inhibition**
**Theme 3: immune chemistry**
More information can be found at our website: http://research.nki.nl/Ovaalab
 Currently we are actively looking for several trainees on several exciting projects in various scientific disciplines.

Selected reading


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**Group Peters: Visualizing protein networks**
It is becoming increasingly important to visualize, identify, and co-localize multiple molecular protein complexes within cells. Thanks to proteomics, our knowledge of the various molecular machines within cells is expanding rapidly. Determining the organization and movement of these components at the cellular and molecular level is a critical step in understanding cell function. Technical advances have turned three-dimensional electron microscopy into an ideal tool to elucidate structural mechanisms by examining tertiary and secondary structure. Using freezing techniques and cryoelectron microscopy it is possible to trap macromolecules in distinct states that can be directly correlated as functional intermediates. Electron tomography is now a mature technique, and is currently experiencing a period of rapid growth.

We recently finished our first attempt to do three-dimensional imaging of receptor networks in cells. Electron cryo-tomography of unfixed high pressure frozen ultra thin cryosections were used for determining the three-dimensional structures of large macromolecular assemblies in cell organelles. Tomograms constructed from cryo-sectioned cells reveal a remarkable internal membrane network composed of stacks. X-ray crystallographic studies provide constraints on the possible modes of receptor packing in the membrane. By placing receptor molecules into the density map derived from tomography, we are beginning to construct a three-dimensional molecular model for the membrane protein network.

The current excitement in this field arises from the expectation that the ongoing advances in microscope instrumentation and computational methods may ultimately make it possible to routinely obtain resolutions in the range of 20 Å, i.e. potentially good enough to locate individual proteins in a cell. We very much welcome students that would like to participate in this endeavor. An ultrastructural project on prions or mycobacteria is an alternative.

Recent Papers

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Division of Molecular Carcinogenesis

Head: Prof. Dr. R. Bernards
Project leaders: Prof. Dr. R. Bernards, Dr. R. Beijersbergen, Dr. R.M.F. Wolthuis

Group Bernards:
Functional genetic approaches to identify cancer-relevant genes
The focus of this group is on the application of innovative functional genomics tools to identify novel genes that have a role in the biology of cancer. We use both high-throughput gain-of-function genetic screens with retroviral cDNA expression libraries and loss-of-function RNA interference genetic screens to identify novel components of cancer-relevant pathways.

Gain-of-function genetic screens
We use retroviral cDNA expression libraries to identify novel genes that act in pathways, which are frequently deregulated in human cancer. In short, these genetic screens involve the infection of a cell population with a high-complexity retroviral cDNA expression library, selection of cells with altered phenotype, followed by identification of the cDNA responsible for the phenotype. In the past years, we have successfully used this approach to identify novel genes that act in several cancer-relevant pathways and to identify biomarkers of drug resistance.

Loss-of-function genetic screens
Mammalian genetic approaches to study gene function have been hampered by the lack of tools to efficiently generate stable loss-of-function phenotypes. To overcome this limitation, we designed a mammalian expression vector (pSUPER) that directs the synthesis of short hairpin transcripts that get processed intracellularly into siRNA-like molecules. Using this technology, we have generated a number of "gene family" knockdown libraries, in which all members of a gene family are individually targeted for suppression by shRNA vectors. One particular focus has been the family of de-ubiquitinating enzymes (DUBs), which act as antagonists of ubiquitin ligases to remove ubiquitin moieties from proteins. Using this DUB knockdown library, we have identified the cylindromatosis tumor suppressor gene (CYLD) as a regulator of the anti apoptotic transcription factor NF-kB and USP1 as a regulator of the Fanconi anemia D2 protein (FANCD2). More recently, we have completed the construction of a collection of some 55,000 shRNA vectors, targeting 23,000 mouse and human genes.

One of our recent innovation in the screening of such complex shRNA libraries is the development of so called "bar code screening" technology, in which tens of thousands of shRNA vectors can be screened in parallel for their role in a specific process. We have used large-scale loss of function genetic screens to identify novel components of the p53 pathway and to identify biomarkers of resistance to specific cancer therapeutics.

Key publications


Reviews

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**Group Beijersbergen: Construction and application of tools to perform large scale RNAi screens in mammalian cells.**

RNA interference (RNAi) is a powerful tool to perform loss-of-function genetic screens in lower organisms, which greatly facilitates the identification of novel components of cellular signalling pathways.

In mammalian cells, such screens have become possible by the generation of expression vectors that direct the synthesis of short hairpin RNAs (shRNAs) that act as short interfering RNA (siRNA)-like molecules to stably suppress gene expression. We have constructed a set of retroviral vectors encoding 23,742 distinct shRNAs, which target 7,914 different human genes for suppression. Using this RNA interference library, we have identified novel modulators of the p53 tumor suppressor pathway. To facilitate large-scale screening with shRNA libraries we have developed a novel strategy: siRNA bar code screens that can be used for the analysis of large pools of shRNA vectors in parallel. The abundance of each shRNA vector is quantitatively assessed by hybridization to high-density oligonucleotide arrays. This approach facilitates the rapid identification of individual shRNA vectors associated with specific phenotype and can also be used to identify shRNA vectors that are associated with cellular lethality or synthetic lethal interactions. The lab is performing different types of cell-based assays with the tools described above. These cell-based screens are supported by the high throughput and robotics facility that we have set up for the use of shRNA library collections. This facility involves automated tissue culture, robotic liquid handling and automated high throughput imaging.

The research in my laboratory evolves around the identification of novel drug targets in cancer, modulators of drug response (enhancers and resistance) and the elucidation of mechanisms of cancer drug action in tumors using large-scale cell-based screening technologies. We use genome wide chemical siRNA and vector based shRNA collections in cell based assays with the goal of identifying essential components in disease- or treatment related pathways that can be explored as drug targets in cancer therapy. In addition to single parameter read-outs for proliferation, survival and apoptosis, we use high throughput/ high content imaging technologies for specific (sub) cellular phenotypes. A major research line in the lab is the search for synthetic lethal interactions with specific tumor-associated genetic alterations including tumor-suppressor genes p53, pRB and PTEN and activated oncogenes for RAS, MYC and PI3K.

A second line of research is the development of *in silico* models of therapy response in breast cancer. We use RNAi and chemical inhibitors for perturbation experiments of pathway components in breast cancer cell lines. In this way, we map network structures, network interactions and the connection of network states to cellular phenotypes. The effects of pathway perturbation are determined by quantification of the activation status of individual pathway components using our luminex platform. These measurements are then linked to the phenotypical outcome of pathway stimulation using cell based assays for proliferation, survival and metabolism. The data obtained from these experiments are used to create and validate a dynamic and quantitative computational model of pathway behavior and the biological phenotypes in relation to drug response. We will extrapolate the *in vitro* responses to mouse models for breast cancer and subsequently link *in vitro* drug responses to clinical outcome in patients. With this approach we anticipate to maximize the success rate of available targeted therapies against MAPK and PI3K pathway components in the treatment of breast cancer. Techniques we use are tissue culture, transfection and retroviral infection, DNA and protein analysis, RNAi, micro-array analysis, deep sequencing and proteome profiling.

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**Group Wolthuis: Control of Mitosis in Normal and Cancer Cells**
At the end of the cell cycle, the chromosomes which have been duplicated in S-phase, are distributed over two new daughter cells during mitosis. Our group aims to understand how mitosis works, both in normal and in cancer cells. The main research interests are to reveal the roles of Cyclin-Cdk complexes and other kinases in mitotic progression and to decipher the regulation of protein destruction in mitosis, critical to coordinate cell division and sister chromatid separation. Using mammalian cells as model systems, we continue to develop and apply a combinatorial approach of biochemistry, molecular cell biology, RNAi-genetics and live-cell fluorescence imaging.

An important object of study is the control of mitotic protein destruction by a multi-subunit Ubiquitin ligase, the Anaphase-Promoting Complex or Cyclosome (APC/C) and the unknown role of its activator Cdc20 in this process. Our recent work revealed unexpected roles for the potentially oncogenic Cks protein family in directing Cdc20-dependent destruction of Cyclin A, and in coordinating the concurrent destruction of Cyclin B1 and Securin. We are using our new findings to learn how Cdc20 activates the APC/C but also to understand how Cdc20 could be inhibited by the mitotic spindle checkpoint. Furthermore, we think that depending on the way the APC/C is inhibited, distinct molecular programs may be activated which affect the cellular responses to a prolonged arrest in mitosis.

The next research goal will be to understand how oncogenic mutations may impinge on critical steps in mitotic entry, mitotic progression and mitotic exit, but also how they determine the way cells respond to errors in mitosis, or to anti-mitotic drugs. It is anticipated that answers to these questions could create exciting opportunities to develop novel anti-cancer drugs.

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Division of Biochemistry

Head: Prof. Dr. T. Sixma
Project leaders: Prof. Dr. T. Sixma, Dr. A. Perrakis

Group Sixma: Protein structure and function, X-ray crystallography
In cancer, deregulation of normal cellular pathways results in uncontrolled growth. In the protein structural group we study these pathways at the atomic level, creating three-dimensional structures of the proteins involved and characterizing them by a variety of \textit{in vitro} biochemical and biophysical techniques. This enables interpretation of the effects that oncogenic mutations will have at the level of the individual proteins. We can interpret these results in the light of the organism in collaboration with cell-biologists, geneticists as well as pathologists to give a better understanding of the fundamental processes that underlie cancer.

Meanwhile these crystal structures serve a valuable role in structure-based drug design. Close analysis of ligand-binding is used for the design of new drugs that target specific proteins in the cell. Our studies are focused on the details of ubiquitin conjugation processes in the cell cycle and on DNA repair mechanisms.

References:

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Group Perrakis: Structural biology
The genome projects delivered one-dimensional information (the nucleotide sequence) of the genetic configuration of a variety of organisms from \textit{archaea} to man. The function of gene products - proteins - remains to be understood as we are cruising in the post-genomic era towards systems biology. Understanding the function of proteins and macromolecular complexes is invaluable in understanding the emerging properties of biological systems such as the cell and the organism; in turn understanding function requires structural knowledge of protein complexes with ligands, DNA, RNA, or other proteins. That is usually sufficient detail to understand the malfunctions of proteins and system pathways that happen to be the cause of cancer and other diseases of genetic origin. We study a variety of systems relevant to cancer and basic biological pathways by X-ray crystallography and other biophysical techniques; our current focus is on the mechanisms that cells use to ensure correct DNA inheritance: chromosome separation and the subsequent DNA replication. We specifically study components of the spindle assembly checkpoint (SAC), the Polo kinase and FoxM1 proteins that regulate the function of many of the SAC components and also the DNA replication license. We aim to understand the function of such complex systems at detail sufficient to provide the basic knowledge that can allow rational interference with these systems and will lead to the basis for the development of new drugs.

In parallel we work on two proteins that are important drug targets: Autotaxin, a protein involved in various pathologies including cancer, inflammation, pain and fibrosis; and JBP1, a protein restricted to parasites as Leishmania and Trypanosoma species, and thus a drug target for parasitic diseases.

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Division of Molecular Biology

Head: Prof. Dr. H. te Riele
Project leaders: Prof. Dr. H. te Riele, Prof. Dr. P. Borst, Dr. J. Jonkers, Dr. S. Linn, Dr. A.H. Schinkel, Dr. K. de Visser (VIDI fellow), Dr. L. Wessels

Group Te Riele:

Genetic instability and cancer
Genetic instability is a hallmark of human cancer. One type is called microsatellite instability (MIN or MSI) and has been identified in tumors associated with the non-polyposis form of hereditary colon cancer (HNPCC). MIN is indicative of defects in the cells’ DNA mismatch repair (MMR) machinery which accelerates the accumulation of mutations in proto-oncogenes and tumor suppressor genes and hence the development of cancer. Another form of genetic instability, chromosome instability (CIN), is found in the majority of human tumors. One of the causes of CIN are defects in Fanconi anemia (FA) genes. Defects in MMR and FA genes also alter the DNA damage response of cells, albeit in an entirely different way. We are studying how these types of genetic instability contribute to oncogenic transformation and cellular and mouse models and affect the response of tumors to chemotherapeutic interventions.

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Gene modification by small single-stranded DNA oligonucleotides
We have recently found that small single-stranded DNA oligonucleotides can be used to introduce subtle modifications in the mouse genome as small as the deletion, insertion or substitution of a single nucleotide. However, ‘oligo targeting’ is strongly suppressed by DNA mismatch repair (MMR) activity. We have therefore developed methods to transiently down regulate MMR protein levels allowing oligonucleotide-directed gene modification to occur effectively without the accumulation of spontaneous mutations. We are exploiting this revolutionary site-specific mutagenesis method to assess the phenotypic consequences of unclassified allelic variants of MMR genes in the human population. Furthermore, we are trying to improve the efficiency of oligo targeting in order to extent its application to in vivo gene therapy.

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Cell cycle control and tumor suppression in the absence of retinoblastoma proteins
The retinoblastoma suppressor protein pRB and its homologs p107 and p130 act as key regulators of the G1/S transition of the cell cycle. Deregulated cell cycle control is found in most if not all human tumors and in many cases is caused by loss of the retinoblastoma gene family. Indeed, we found that tumorigenesis in mice often requires inactivation of pRB plus one of its homologues. However, loss of function of the RB gene family alone is not sufficient for oncogenic transformation. We are studying how the cell cycle is controlled in the absence of pRB proteins and which additional events are required for oncogenic transformation of pRB defective cells.

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A novel procedure for loss-of-function screens
Genome-wide genetic screening using synthetic RNAi libraries is a powerful tool to identify tumor suppressor genes. However, current libraries are complex and often not readily available. We have developed a novel procedure to enzymatically produce RNAi-expressing vector libraries that are already enriched for putative tumor suppressor genes. We are exploiting this method to identify tumor suppressor pathways that prohibit transformation of pocket-protein defective cells.

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Group P. Borst:

DNA base J

Base J β-glucosyl-hydroxymethyluracil (base J), which we discovered in African trypanosomes in 1993 (Gommers-Ampt et al., Cell 1993; 75:1129-1136), is a base present in kinetoplastid flagellates and in Euglena. It replaces 1% of thymine in nuclear DNA and is predominantly located in repetitive sequences, such as telomeric repeats. We have characterized a J-binding protein (JBP1) that binds with high specificity to J-containing duplex DNA (Cross et al. EMBO J 1999; 18: 6573-6581). We have shown that JBP1 is a thymidine hydroxylase that catalyses the first step of J biosynthesis, the conversion of T in DNA into hydroxymethyluracil. JBP1 appears to belong to the family of Fe²⁺ and 2-oxoglutarate-requiring dioxygenases, as does a second putative hydroxylase, JBP2. In the kinetoplastid Leishmania, a JBP1 KO is lethal. In contrast, JBP2 is dispensable in Leishmania under normal growth conditions, but JBP2 KO strains are hypersensitive to bromodeoxyuridine (BrdU). During growth in BrdU, Leishmania loses its J, which is located for > 98% in telomeric repeats in this organism. How J loss leads to cell death is unclear. We do not find alterations in DNA integrity or cell cycle blocks. A recent breakthrough came from the discovery by R. Sabatini (University of Georgia, US) that trypanosomes have J at transcriptional start and stop sites. Using immuno-precipitation of J-DNA and deep sequencing, we have also found the 1% of non-telomeric J in Leishmania at specific chromosome-internal positions, partly at transcriptional stops (collaboration with NKI-AVL deep sequencing unit and Peter Myler, Seattle). We have shown that loss of this internal J leads to massive readthrough of RNA Polymerase II transcriptional stops, as shown in Fig. 1, suggesting that transcriptional termination is a major function of J. We have also found an interesting interaction between J and histone H3/H3V (H3 variant) and the tools are on board to determine the role of the chromatin environment in J function. With Anastassis Perrakis (NIKI-AVL) we are trying to determine the structure of JBP1-J-DNA complexes by crystallography. In 2010 the structure of the DNA-binding domain of JBP1 was solved. Interestingly, this domain has a unique structure not seen before in DNA-binding proteins and the specific binding of JBP1 to J-DNA was shown to be dependent on a single aspartate residue interacting with the glucose-moiety of base J. Information: Henri van Luenen, tel.: 020-5122097, E-mail: h.v.luenen@nki.nl or Piet Borst, tel.: 020-5122880, E-mail: p.borst@nki.nl

Multidrug resistance of cancer cells and MRPs

A common mechanism for drug resistance in cancer cells is due to glycoproteins in the plasma membrane able to pump out drugs from the cell. We are interested in a sub-class of these pumps, the Multidrug Resistance-associated Proteins (MRPs). We study the substrate specificity of MRPs in transfected cells, in polarized kidney cell monolayers and in insect cell vesicles (Baculo virus system). We try to find inhibitors that block these pumps, and we study the physiological function of these pumps by disrupting MRP genes in mice. By a systematic investigation of compounds missing in the blood of Mrp3 KO mice using LC/MS we have found that this transporter is indispensable for transporting the glucuronidederivatives of phyto-estrogens from gut wall into the blood. This has opened new ways to study the potential beneficial effects of these compounds on the health of mammals. We are extending this approach to a range of other transporters. Information: Koen van de Wetering, tel.: 020-5122082, E-mail: k.vd.wetering@nki.nl or Piet Borst, tel.: 020-5122880, E-mail: p.borst@nki.nl

Drug resistance studies in a realistic animal model for mammary tumors

In collaboration with the group of Jos Jonkers, we are analyzing drug resistance in mouse mammary tumors with mammary gland-specific deletion of Brca1 and p53. Whereas all attempts to get drug resistance in xenografts of human tumors in immunocompromised mice have failed thus far, the spontaneous Brca1- and p53-deficient tumors become readily resistant to the maximal tolerable dose of chemotherapeutics also used in the treatment of human breast cancer, such as doxorubicin, docetaxel and topotecan. Using gene expression profiling, quantitative RNA analyses, specific antibodies against drug transporters, and specific inhibitors of drug transporters, we have shown that resistance to drugs transported by P-glycoprotein is often associated with upregulation of Pglycoprotein- encoding genes, whereas resistance to topotecan is associated with upregulation of Bcrp1, a drug transporter known to be able to transport topotecan out of cells. We have introduced null-alleles for Bcrp1 and for P-glycoprotein into the tumor model, to analyze other resistance mechanisms that will protect the tumor when the relevant drug transporters are not available anymore.

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This tumor model is highly suitable for testing new experimental drugs. One example is a new inhibitor for an enzyme involved in DNA repair, poly-ADP-ribose polymerase. This inhibitor is very effective in this tumor model, but unfortunately also upregulation of P-glycoprotein abrogates effectiveness. A most unusual result is obtained in this tumor model with cisplatin. The tumor readily responds to treatment, but does not become resistant to the drug. Nevertheless, the tumor cannot be eradicated and invariably relapses from a small fraction of tumor cells not killed by the cisplatin treatment. We have shown that these remnant cells are also damaged by the cisplatin, but somehow manage to survive. Our analysis of this remnant fraction was helped by the isolation of tumor cell lines that closely resemble the properties of the original tumors in this model system. We are testing a variety of experimental drugs that block DNA damage-sensing to see whether it is possible to eliminate the remnant cells and eradicate the tumor. We have also started an investigation of predictive markers, i.e. alterations in gene expression that can be used to predict the response of tumors to current chemotherapy. Information: Sven Rottenberg, tel.: 020-5122082, E-mail: s.rottenberg@nki.nl or Piet Borst, tel.: 020-5122880, E-mail: p.borst@nki.nl

Group Jonkers: Conditional mouse models of breast cancer
Approximately five percent of the women who develop breast cancer carry an inherited mutation in one of the breast cancer susceptibility genes BRCA1 or BRCA2. The proteins produced by these genes play an important role in DNA repair. To study the role of BRCA1 and BRCA2 in breast tumor development, we have produced Brca1 and Brca2 mouse mammary tumors by generating mouse strains with mammary gland-specific deletion of Brca1 and p53 (or Brca2 and p53). Indeed, the females from these strains of mice develop mammary tumors with high incidence, demonstrating that combined loss BRCA and p53 is needed for mammary tumorigenesis. We are currently performing gene expression profiling of the Brca1 and Brca2 mouse mammary tumors to look for differences in gene expression between these groups of tumors. In addition, we have developed a method for high-resolution, genome-wide analysis of DNA amplifications and deletions in mouse tumors. Using this approach, we hope to identify candidate cancer genes that are specifically amplified or deleted in the Brca1 or Brca2 mammary tumors, and, therefore, could represent mutations that cooperate with loss of BRCA function in breast oncogenesis. Information: Jos Jonkers, tel.: 020-5122000, E-mail: j.jonkers@nki.nl

Group Linn: Mechanism of resistance to endocrine treatment of breast cancer
The majority of breast cancers in patients is hormone-dependent and need estradiol for their growth. Disruption of the functioning of the receptor for estradiol, ER, is commonly used in the clinic for treatment. However, resistance to this form of treatment is a major problem. We found that resistance to a commonly used anti-estrogen, tamoxifen, is due to phosphorylation of specific sites in the ER, and furthermore, that combinations of these specific phosphorylations can predict resistance to other anti-estrogens in in vitro studies as well. Since patients, who developed resistance to one particular anti-estrogen, can still be treated with other anti-estrogens, this rational prediction of resistance opens a way of “personalized” medicine in breast cancer treatment. We want to study the molecular details of resistance to anti-estrogens, which genes are turned on and how, and to apply our findings in the clinic. Information: Sabine Linn, tel.: 020-5122449, E-mail: s.linn@nki.nl

Group Schinkel: Basic insights for optimizing anticancer chemotherapy - a molecular-genetic approach
In order for anticancer drugs to work properly, many conditions need to be met. The drug must be readily taken up in the body, it must easily distribute to the tissues where tumors and their metastases reside, and it must be able to penetrate the tumor cells themselves. Moreover, the drug should not be rapidly degraded in the body, and it should not be excreted too quickly. Our group aims to obtain insight into the molecular systems that are responsible for these processes, as this will greatly improve the development of optimally functioning anticancer drugs and optimal drug administration regimens. We focus on three important systems, each of which can handle a wide variety of different drugs: 1. Active multidrug efflux pumps of the ATP binding cassette (ABC) protein family. These membrane proteins are important limiting factors for the uptake of drugs from the intestine, and their penetration into various tissues and tumor cells. Moreover, they actively excrete drugs into bile, feces and urine.
2. Drug uptake systems of the OATP and OCT family, which are responsible for the uptake of drugs across the cell membrane. These proteins generally facilitate the uptake and distribution of drugs from the intestine and into various tissues and tumor cells.

3. Drug metabolizing systems of the Cytochrome P450 3A subfamily. These highly multispecific enzymes break down more than 50% of the currently used drugs. Due to their highly variable activity between patients, they form a major factor in unpredictable toxicity of drugs.

The activity of these systems can vary widely, due to genetic polymorphisms, or due to intentional or coincidental inhibition or induction by co-administered drugs or food compounds. This can lead to unpredictable toxicities, but also to undertreatment of cancer. Both can have fatal consequences. In order to understand the basic functioning of these systems, we have developed a number of knockout and transgenic mouse models. These mouse models are analyzed with respect to physiological, pharmacological and toxicological alterations. This yields insight into the normal physiological roles of these proteins and into their impact on the pharmacological and toxicological behavior of drugs. For example, we have discovered that BCRP, one of the ABC transporters, is responsible for pumping vitamin B2 into milk, but also that it plays a major role in restricting the uptake of anticancer drugs from the intestine, and in restricting the drug penetration into fetuses and brain.

The obtained insights will help in making the administration of drugs more effective, safe, reliable and comfortable for the patient, as well as more affordable. Due to the wide spectrum of affected drugs, insights from this work will also impact the use of many other drugs next to anticancer drugs. Possible student projects within the group cover a wide variety of approaches and techniques, including in vivo animal experiments: they can involve molecular- and cell-biological techniques (cloning, RT-PCR, Western blot analysis, micro-array expression analysis, cDNA transfection and transduction, tissue culture, cellular drug resistance and drug transport assays, FACS sorting); animal experiments include e.g. clinical-chemical, hematological and pathological characterization of mouse strains, pharmacokinetic studies looking at tissue and plasma distribution and excretion of drugs, microsurgical cannulation of gall- and urinary bladder. In vitro assays for Cytochrome P450 enzyme activity and subsequent HPLC or other bio-analytical techniques are also applied.

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Group De Visser: Inflammation and breast cancer

Cancer is a progressive disease, arising as a consequence of activated oncogenes and/or deactivated tumor suppressor genes. Besides these genetic alterations, tumor-host interactions are essential for cancer development. One of the key stromal players involved in cancer development is the immune system. A deeper understanding of the temporal and spatial mechanisms regulating the inflammatory response and downstream pathways by which the immune system modulates cancer growth is critical for development of novel combinatorial therapies that target both cancer cells and tumor-supportive host responses. The overall goal of our research is to address the role and underlying pathways of the inflammatory tumor microenvironment during breast cancer progression, metastasis formation and cancer therapy. We utilize a mouse tumor model that faithfully recapitulates human invasive and metastatic lobular carcinoma. Mammary carcinomas arising in this mouse model are characterized by abundant presence of innate immune cells, regulatory T cells, antibody depositions and increased levels of pro-inflammatory mediators. By genetic elimination and pharmacological inhibition of specific subsets of the adaptive and innate immune system, we are currently investigating the functional significance of components of the immune system in a temporal manner.

We use a broad range of techniques, including in vivo analysis of experimental animals, immunohistochemistry, microscopy/confocal microscopy, flow cytometry, PCR, cell culturing, Western blotting, ELISA, etc. Please contact me if you are interested in performing a project in the field of “Inflammation and Breast Cancer”

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Group Wessels: Bioinformatics and Statistics
The Bioinformatics and Statistics group provides leadership on the collection and analysis of data for the research programs of the institute, by conducting research in bioinformatics and statistics and by performing state of the art analyses of a wide array of data types. Research topics include stratifying tumors into groups with distinct and homogeneous outcome and therapy response; the characterization of genes and pathways involved in tumorigenesis and understanding molecular regulatory mechanisms. A number of exemplary projects are presented below in more detail.

**Systems Biology**

In collaboration with the Beijersbergen, Bernards and Jonkers groups we have established the Cancer Systems Biology Center. The aim of this center is to develop a strategy to tackle the complexity of molecular networks that govern breast tumorigenesis. This strategy is rooted in a modeling and experimental validation cycle spanning multiple levels of complexity including cell lines, mouse models and patients. As a start we are focusing on breast cancers for which no effective targeted therapies exist: ‘triple negative’ and invasive lobular carcinomas. Since there are strong indications for the involvement of PI3K and MAPK signaling pathways in these subtypes, we are generating in silico models of therapy response by employing:

1) normal and tumor cell lines as in vitro model systems;
2) quantification of functional activation of pathway components and associated cellular phenotypes; 3) computational modeling to create quantitative models of pathway behavior and resistance mechanisms; and
4) mouse tumor models as in vivo model systems. The identified models will be directly validated in proof-of-concept pre-operative trials. We hope that this strategy will yield improved diagnostic tools and tumor-specific treatments resulting in more tailored cancer therapy.

**Extracting oncogenic pathways from insertional mutagenesis screens**

We have developed combinatorial association logic networks (CALs): an approach to extract simple Boolean logic circuits which employ combinations of insertion loci to predict the expression pattern of downstream targets. In classical one-dimensional analyses, direct interactions between the insertion patterns and transcription levels across tumors are detected. However, when the insertion loci themselves interact, direct associations between the individual loci and transcript levels may become undetectable. Therefore, our method detects associations between transcript levels and the outputs of small Boolean logic networks that combine multiple genetic loci. The detection of logic networks requires solving a demanding optimization problem. By reformulating the objective function and applying a customized branch and bound algorithm, we obtain runtimes of up to four orders of magnitude faster than exhaustive search. We demonstrated our method on an insertional mutagenesis dataset, combining insertion data with transcriptional information from the same sample, finding known and novel associations between genes involved in Notch signaling.

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Head: Prof. Dr. M. van Lohuizen
Project leaders: Prof. Dr. A. Berns (Scientific Director), Dr. E. Citterio (VIDI fellow), Dr. A. Haramis, Dr. J. Hilkens, Dr. J. Jacobs, Prof. Dr. M. van Lohuizen, Dr. D. Peeper

Group Berns: Identification and mechanisms of action of oncogenes and tumor suppressor genes

A number of genetic alterations, usually in proto-oncogenes and tumor suppressor genes, are required for the development of cancer. Once a tumor is established many different mutations have occurred. Our group focuses on two different aspects: 1) finding new genes involved in cancer and 2) determining their specific role in the tumorigenic process by studying tumor predisposition of mice in which one or more of these genes have been mutated by gene targeting strategies.

To identify new oncogenes and tumor suppressor genes we utilize high throughput transposon tagging. In brief, tumors are induced in mice by infecting them with replication competent murine retroviruses. During the life cycle of these viruses the viral genome is more or less randomly inserted in the mouse cellular DNA. This is a mutagenic event and since multiple insertions per cell can take place in many millions of independent cells, some of these insertions will activate or disrupt genes resulting in a selective growth advantage to that specific cell which then gives rise to a clonal tumor with only a small number of insertions. Cloning the flanking DNA of these insertions enables the direct identification of genes involved in the tumorigenic process. Students can participate in this "genomics" project by cloning and identifying genes flanking proviral insertions. Students will not be involved in animal experiments, but utilize stored tumor material for these analyses.

To study the specific role of defined oncogenes and tumor suppressor genes in tumorigenesis we perform in vivo studies. These studies are not suitable for short-term projects. However, cells isolated from these mice represent a powerful tool for studying growth and transformation properties of these mutant cells in vitro. This can provide ample information about the genes involved. Students can participate in these latter studies. Students are introduced in a range of techniques ranging from typical genomics strategies to cell culture, transformation assays, microscopy, and biochemical experiments depending on the specific project.

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Group Citterio: Maintenance of genome integrity by histone (de)ubiquitinating enzymes

The maintenance of genome stability and its organization into chromatin is crucial for preventing aberrant gene expression that could drive cancer development. Post-translational modification of proteins by ubiquitination is emerging as a key regulatory mechanism in genome maintenance. However, how protein ubiquitination regulates DNA damage signaling/ DNA repair pathways is still poorly understood. Recent work by us and other groups revealed that (mono)-ubiquitination of histones is part of the cellular response to DNA damage. Our research focuses on the molecular mechanisms and the enzymes involved in (de)ubiquitination of histone proteins and how deregulation of this process influences genome stability and tumorigenesis.

By a biochemical approach, we have recently identified USP3 (ubiquitin-specific protease 3), as a major deubiquitinating enzyme (DUB) for histone H2A and H2B. Our results implicate USP3 in normal S-phase progression and in the response to ionizing radiation (IR). Ubiquitinated H2A is deposited by the Polycomb-group (PcG) proteins Ring1b/Bmi1, which are frequently deregulated in human cancer.
Together, these findings involving uH2A in Polycomb-mediated gene silencing as well as in DNA damage signaling strongly connect this modification to human cancer.

We are focusing on two main lines of research. First, we are using biochemical approaches combined with in vitro studies in mammalian cells (employing RNAi-based techniques) and with the generation of in vivo mouse models to explore the mechanism of USP3-mediated DNA damage response. The second line of investigation is a proteomic approach aimed at the identification of novel players in chromatin regulation and in the damage response. Protein chromatography techniques are combined with in vitro ubiquitination assays and MALDI mass spectrometric analysis.

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Group Haramis:

Analysis of energy metabolism during development and implications for cancer

During development of an organism, mechanisms that sense nutrient availability are intimately linked with growth control pathways in order to coordinate energy conditions with organ growth and tissue homeostasis. Tumor cells employ several of the same mechanisms that coordinate nutrient availability with growth during development to ensure tumor cell proliferation. To improve cancer therapy, we need a better understanding of the molecular pathways that link energy metabolism with growth control during development and how are those affected in cancer. Our research focuses on understanding how vertebrate organisms sense and respond to energetic stress, using zebrafish as our central experimental model.

We have generated and characterized zebrafish mutant in the tumor suppressor LKB1. LKB1 is a serine-threonine kinase that is mutated in a cancer-predisposition syndrome in humans as well as in over 30% of lung adenocarcinomas. Biochemically, LKB1 activates AMPK a critical energy checkpoint at the cell and organism level. This cascade leads to “shut-down” of energy-consuming processes such as transcription and translation and engages pathways that serve to produce energy, enabling an organism to survive.

We found that lkb1 zebrafish mutants are unable to sense and respond to energetic stress, exhibit a fast metabolic rate, deplete their energy reserves prematurely and die of premature starvation. We are currently characterizing the molecular mechanisms affected by LKB1 that lead to this dramatic deregulation of whole-organism energy homeostasis and perform chemical genetic screens using the lkb1 mutants with the aim to identify synthetic lethal interactions.

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Group Hilkens:

Genes and Genetic Pathways Involved in Breast Cancer

Background: Breast cancer is the most common cancer among women in the western world. In the Netherlands 1 out of 9 women will contract this disease and almost 50% of them will die of it. Nearly all human breast cancers arise from a glandular epithelial cell in the breast. Several sequential genetic changes are needed for such a cell to become fully malignant; i.e. has lost normal growth regulation and acquired invasive and metastatic properties. This process of gradual increase in malignancy is called tumor progression. The objective of our research is to identify and characterize (biochemically and functionally) novel genes involved in breast cancer and to unravel the collaborating genetic pathways leading to breast cancer progression.

Identification and characterization of novel mammary cancer genes using insertional mutagenesis

Novel oncogenes involved in breast cancer will be identified by mouse mammary tumor virus (MMTV) induced insertional mutagenesis. MMTV is a retrovirus that replicates in mammary gland epithelium. The viral DNA randomly integrates in the cellular genome as part of its replication cycle. The powerful promoter/enhancer of the inserted provirus can activate an adjacent oncogene, and thus render the cell into a tumor cell. MMTV remains replicating and subsequent integrations can activate additional genes leading to progression of the tumor and eventually metastasis. The integrated virus also acts as a genomic marker and therefore relatively easily allows PCR mediated amplification and sequencing of the flanking genomic DNA.
Since the sequence of the mouse genome is almost completely known, identification of nearby oncogenes can be relatively easily accomplished. As a result of the enormous sequence information that recently became available, this "retroviral insertion mutagenesis" technology is one of the most efficient methods to identify novel oncogenes, but is yet relatively unexplored in mammary cancer. We have recently identified over 17 novel putative oncogenes using this method. This knowledge will aid us in understanding the genetic pathways that lead to a fully malignant cancer and is essential if we are to identify novel targets for cancer therapy.

Students will be involved in the ongoing search for novel oncogenes and will take part in the validation of the newly identified genes as true oncogenes using *in vitro* and *in vivo* assays. Subsequently, the trainee will take part in the biochemical and functional characterization of one or more of the novel oncogene products, or he/she will be involved in identifying the human ortholog of the novel oncogenes and the involvement of these genes in human breast cancer using microarrays and RT-PCR methods.

Methods: Until recently, identification of retroviral target genes was very laborious, and no efforts have been reported to exhaustively identify oncogenes by insertional mutagenesis. However, newly developed PCR technologies and the availability of the complete mouse genome sequence allow efficient high-throughput screening for retrovirally tagged genes. This project involves various modern molecular biological techniques including: gene cloning, various PCR techniques, northern and Southern blotting, sequencing, transfection and/or retroviral transduction, immunoprecipitation, gel electrophoresis, western blotting, various tissue culture techniques, etc.

Further reading:

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Group J. Jacobs:
Chromosome end protection by telomeres
Our main research interest is in telomeres, the protective caps at the end of our chromosomes. Telomeres consist of tandem TTAGGG DNA repeats and multiple proteins that bind directly or indirectly to these repeats. Telomeres shorten with every cell division. This happens in vitro in cultured cells, but also in vivo in the cells of our body as we get older. At some point telomeres become so short that they can no longer protect chromosome ends from being recognized as damaged DNA. Such damaged or dysfunctional telomeres trigger a DNA damage response that causes cells to die (apoptosis) or to stop dividing permanently (senescence). This response represents an important anticancer barrier as it eliminates would-be cancer cells. However, it is also responsible for the loss of replicative capacity of our body cells that underlies many of the diseases and discomforts arising when we get old. If this response is not efficiently executed, telomere dysfunction can actually promote tumorigenesis because uncapped chromosome ends are subject to DNA repair activities, which result in telomere fusions and dicentric chromosomes that can lead to chromosome instability.

We are specifically interested in how mammalian cells precisely perceive and respond to loss of telomere function, how telomeres precisely protect chromosome ends and how they are maintained. These are very important issues because, as explained above, telomere function and the response of cells to telomere problems have large impacts on aging and cancer.
To identify novel factors involved in telomere function or telomere damage signaling we are taking two kinds of approaches: 1) candidate-driven approaches in which we investigate specific proteins, 2) unbiased functional genetic screening in which we aim to identify and subsequently functionally characterize novel (potentially unsuspected) factors using cDNA and RNA-interference libraries. A better understanding of telomere function and of how cells monitor and respond to telomere dysfunction might help us find targets for treatment or prevention of cancer and aging-related diseases.

Students will work on one or a few subprojects corresponding to one of these research lines and will be introduced to a variety of techniques, ranging from functional genome-wide screening and cell culture to molecular biology, cell biology and biochemical experiments.

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Group Van Lohuizen:

Role of Polycomb-group genes in stem cell fate control, cell cycle regulation and cancer

Polycomb-group (Pc-G) proteins form large conserved repressive protein complexes, which induce stable silencing of specific target genes, such as Hox gene clusters and the INK4a/ARF tumor-suppressor locus, which forms an important protection mechanism against immortalization and oncogenic transformation. We are studying the molecular mechanism of Pc-G silencing, which acts at the level of chromatin structure. In addition, we are studying the role of Pc-G silencing in controlling stem cell fate and cancer formation. Techniques used: Cell culture of ES cells, neural stem cells, mammary epithelial progenitor cells, mammary fat pad transplantations to investigate stem cells and cancer stem cells in vivo and primary mouse embryo fibroblasts; advanced imaging techniques using live cells; biochemical techniques; genome-wide expression profiling with microarrays, chromatin immunoprecipitations (CHIPs) and DAMId (a new technique to find binding sites for chromatin proteins in a genome-wide fashion).

Our work encompasses a number of new mouse models for solid cancer, to investigate the role of Pc-G (cancer) stem cell fate. These include models for breast cancer, brain cancer, colon cancer, prostate cancer and pancreatic cancer as well as conditional regulatable short-hairpin RNAi transgenic models for the Polycomb genes Bmi1 and EZH2.

We also use Embryonal stem cells and neural stem cells derived from these conditional models to investigate the role of Pc-G regulation on neural differentiation in defined cell culture models. These cells are also used with large scale proteomic and biochemical methods to study changes in Pc-G protein complexes during defined lineage differentiation. In addition, these models are being used how Pc-G protein complexes are being regulated by signaling pathways and post-translational modifications (phosphorylation, ubiquitination and SUMOylation).

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Group Peeper: Dissecting cancer cell signaling networks & identifying therapeutic cancer targets

The objective of the Peeper laboratory at NKI Amsterdam is to dissect essential cancer cell signaling networks and identify novel targets for therapeutic intervention. To achieve this, we are asking several questions: Are there intrinsic cellular mechanisms protecting us against cancer? Can we exploit those clinically? How can we best dissect tumor-suppressing genetic networks? How can we effectively identify essential cancer genes, which may serve as novel drug targets? Can we use our laboratory results to make a difference in the clinic, for example by finding ways to augment the therapeutic window of cancer therapeutics?

In a nutshell, these are the fundamental as well as clinically relevant questions that we are taking up in our laboratory. To achieve this, we are combine advanced techniques, including screens with 100.000-vector shRNA libraries and next-generation sequencing, with classical biochemical and genetic approaches.
Highlights include our discoveries that a genomic screen for anoikis resistance can be used to identify metastasis genes (Nature 2004); that Oncogene-Induced cellular Senescence (OIS) serves as a potent tumor suppressor mechanism limiting cancer progression (Nature 2005; New Engl J Med 2006); the identification of several OIS-associated oncogenes (Nat Cell Biol. 2005; Nat Rev Cancer 2006); and that OIS is associated with the activation of an inflammatory transcriptome (Cell 2008; Nat Rev Cancer 2009; Genes Dev 2010). Recently, we identified a prognostic breast cancer genetic signature and a factor essential to drive metastasis.

Currently, we are shifting towards more translational research, in that we are setting out to identify novel therapeutic targets for melanoma and breast cancer, in the context of cell-based and in-vivo genomic screens. For more information about ongoing research, click here.

Daniel Peeper is a group leader in the Division of Molecular Genetics at the Netherlands Cancer Institute. He is also a professor (part time) in Functional Oncogenomics at the VU University medical center (VUmc) Amsterdam. He received the 2007 Junior Researcher award from the Society for Melanoma Research, a Queen Wilhelmina Award (2 million €) from the Dutch Cancer Society in 2009 (see below), he was elected as EMBO YIP member in 2005 and as EMBO member three years later. For more info, visit the Peeper lab website: http://research.nki.nl/peeperlab/

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Molecular Radiobiology

**Group Stewart: Mechanisms of radiation-induced late vascular damage and fibrosis formation**

Vascular damage in normal tissues is a serious late complication of cancer patients after radiotherapy. Vascular injury develops from months to years after irradiation and manifests in small vessels as telangiectasia, which are characterized as dilated, tortuous, and thin walled blood vessels. Telangiectasia are prone to rupture causing vascular bleeding which might require surgical intervention. In addition to vascular injury, irradiation causes excessive connective tissue formation (fibrosis) that leads to hardening and scarring of the underlying tissues and organs thereby creating obstructions which impede lymph drainage. Both fibrosis and telangiectasia formation are probably caused by an erroneous and unbalanced repair response in the irradiated tissue.

We study irradiated mouse kidneys as model for a micro vascular rich tissue. We have identified the transforming growth factor-beta (TGF-β) signalling pathway as being critically involved in the development of late normal tissue damage. Mice expressing reduced amounts of the TGF-β coreceptor endoglin (Eng+/- mice) display less radiation-induced vascular damage and fibrosis. We are currently investigating the molecular and cellular mechanisms of this difference and are applying this knowledge to the development of new intervention/treatment strategies. In addition, we analyse skin biopsies from irradiated breast cancer patients with telangiectasia in order to be able to translate our findings into the clinic. Reducing the risk of normal tissue injury is an essential part of improving the therapeutic ratio of cancer treatment and to decrease the common and distressing side effects of radiotherapy in cancer survivors. Possible projects in our group cover a broad range of techniques, including qPCR, Western Blot analysis, immunohistochemistry/immunofluorescence, microscopy/confocal microscopy, quantitative image analysis and others.

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**Group Verheij: Mechanisms, modulation and prediction of radiation-induced cell death**

Our group focuses on improvement of clinical results by the incorporation of novel biological response modifiers into radiation treatment protocols. The rationale to use these biologicals is that they target specific pathways that are deregulated in cancer cells, thereby increasing tumor response while limiting normal tissue damage. Apoptosis has emerged as an attractive target for intervention and several strategies to enhance radiation-induced tumor cell death are pursued at the NKI-AVL. These include: (1) synthetic alkylphospholipids (Perifosine and ErPC); (2) death receptor ligands (TRAIL and CD95L); (3) small molecular inhibitors of anti-apoptotic Bcl-2 family members (Gossypol/AT-101 and ABT-737); (4) DNA repair inhibitors (NAD depletor APO866 and PARP inhibitor olaparib). The ability of these agents to enhance radiation-induced cell death is evaluated *in vitro* and upon proven synergism *in vivo* using a variety of (xenograft/spontaneous) mouse tumor models. *In vivo* induction of apoptosis can be studied non-invasively by 99mTc-annexin V scintigraphy. In patients this imaging method has been shown to be useful as a predictor for treatment response. We collaborate with the Divisions of Immunology (J Borst), Experimental Therapy (C Vens) and Cell Biology (W Moolenaar).

*Information: Marcel Verheij, tel.: 020-5122153, E-mail: m.verheij@nki.nl*
Molecular Pathology

Group Broeks, Nederlof, Schmidt

The Molecular Pathology and Epidemiology Group is involved in research aimed at elucidating the molecular basis of cancer development and prognosis. There is strong emphasis on translational research and the actual implementation of developments from the laboratory in clinical practice. The research focuses on both hereditary genetic variants and genetic tumor profiles in trying to answer the following questions:

A. Which germline variants contribute to increased breast cancer risk?
B. Which germline variants impact prognosis of breast cancer?
C. Which breast carcinomas will develop distant metastases?
D. Which breast carcinomas will develop local recurrence, especially after breast conserving therapy?
E. Can factors be identified that predict response to hormonal therapy and chemotherapy?

For this research there is intensive collaboration between researchers in (Molecular) Pathology, Clinical Genetics, and Epidemiology.

Information: Annegien Broeks tel.: 020-5122037, E-mail: a.broeks@nki.nl, Petra Nederlof tel.: 020-5122750, E-mail: p.nederlof@nki.nl, Marjanka Schmidt tel: 020-5122767, E-mail: mk.schmidt@nki.nl

Molecular Cancer Therapy

Group Schellens: Mechanism of action of cytostatic drugs

Pharmacological support of phase I studies

We investigate mechanisms involving oral uptake of important anticancer agents to guide execution of the clinical development of oral taxanes, platinum drugs and capecitabine. Enhanced absorption is achieved by inhibition of drug transporters such as ABCB1 (P-glycoprotein) and ABCG2 (BCRP) and/or CYP3A. Boosting agents such as ritonavir and elacridar are being used. In addition, we monitor circulating tumor cells and pharmacodynamic endpoints in these cells to support drug development. We focus on tyrosine and non-tyrosine kinases and cell cycle proteins suchs as CDC2. We also determine other new endpoints in early clinical trials. As the mechanism of these agents is totally different from classical cytotoxic drugs implementation of new PD endpoints may help to interpret dose- and concentration-effect relationships. We determine pharmacogenetic variability of DPYD coding for the detoxifying protein of 5fluoropyrimidines and implement individualization of 5FU/capecitabine in poor metabolizers of DPD. We also develop methods for structured health technology assessment in early and advanced breast cancer.

Information: Jan H.M. Schellens, tel.: 020-5122446, E-mail: j.schellens@nki.nl
Sectie Psychosociaal Onderzoek en Epidemiologie

Hoofd: Prof. Dr. F.E. van Leeuwen
Leden: Prof. Dr. F.E. van Leeuwen, Prof. Dr. N.K. Aaronson, Dr. M.A. Rookus, Dr. E.M.A. Bleiker, Prof. Dr. W.H. van Harten, Dr. S.B. Schagen, Dr. M.K. Schmidt

Binnen het psychosociaal onderzoek (hoofd: N. Aaronson) staan 3 onderzoekslijnen centraal: studies gericht op het in kaart brengen van en, waar mogelijk, het verbeteren van de kwaliteit van leven en de gezondheidstoestand van kankerpatiënten (N. Aaronson), cognitief functioneren na behandeling voor kanker (S. Schagen), en de psychosociale aspecten van screening en counseling bij erfelijke en familiale vormen van kanker (E. Bleiker, en onderzoek dat zich richt op procesverbetering en technologyassessment (W. van Harten).

De epidemiologie (hoofd: F. van Leeuwen) richt zich op vier belangrijke onderzoekslijnen nl.:
1. de etiologie van hormoongerelateerde kanker bij vrouwen (M. Rookus en F. van Leeuwen);
2. de langetermijn neveneffecten van kankerbehandeling, in het bijzonder m.b.t. het risico op het ontwikkelen van tweede tumoren en hart- en vaatziekten (F. van Leeuwen);
3. moleculaire en genetische markers van borstkankerrisico, behandeling, prognose en mortaliteit (M. Schmidt en M. Rookus); patiënten informatie en informed consent voor gebruik van lichaamsmateriaal in wetenschappelijk onderzoek en biobanking (M. Schmidt)

Psychosociaal onderzoek
1. Het ontwikkelen, testen en toepassen van kwaliteit-van-leven vragenlijsten en andere ‘patient-reported outcomes’ in klinisch-oncologische studies en in de dagelijkse klinische praktijk.

2. Het ontwikkelen, testen en toepassen van interventies ten behoeve van het verminderen van klachten en symptomen, en het verbeteren van de kwaliteit van leven.
   Informatie: Neil Aaronson, tel.: 020-5122481, E-mail: n.aaronson@nki.nl

3. Psychosociale aspecten van erfelijkheidsonderzoek bij mensen met een verhoogde kans op kanker.
   Informatie: Eveline Bleiker, tel.: 020-5126072, E-mail: e.bleiker@nki.nl

4. Cognitief functioneren na kanker en kanker behandeling:
   - Het onderzoeken van de incidentie, de aard, de ernst en de oorzaken van cognitieve problemen t.g.v kanker en kankerbehandeling.
   - Het onderzoeken van strategieën om dergelijke problemen te verminderen of te voorkomen.
   Er wordt gebruikt gemaakt van neuropsychologisch onderzoek, imaging technieken, sociaal psychologische studies en dier experimenten.
   Informatie: Sanne Schagen, tel.: 020-5122328, E-mail: s.schagen@nki.nl

5. Procesverbetering in de oncologische (ziekenhuis) zorg met gebruikmaking van methoden uit de bedrijfskunde en toegepaste wiskunde.

   Informatie: Wim van Harten; tel.: 020-5122860, E-mail: w.v.harten@nki.nl

Epidemiologie
1. Risico op hormoongerelateerde tumoren bij vrouwen

* Hebon studie: Binnen families waar veel borst- en/of ovariumkanker voorkomt wordt het risico op deze tumoren onderzocht in relatie tot de BRCA1 en BRCA2 mutaties en andere genetische variaties. Daarnaast wordt de invloed van leefgewoonten en omgevingsfactoren op dit risico bestudeerd en de gen-omgeving interacties. Met dit landelijke onderzoek werken we actief samen in twee internationale studies.
Informatie: Matti Rookus tel.: 020-5122491, E-mail: m.rookus@nki.nl

* DESnet project: In een cohort van vrouwen die in utero blootgesteld zijn aan Dietylstilbestrol (DES dochters) wordt onderzocht of hun kankerrisico is verhoogd en hoe effectief de screening is; daarnaast wordt nagegaan of de 3e generatie een verhoogd risico heeft op aangeboren afwijkingen.
Informatie: Matti Rookus tel.: 020-5122491, E-mail: m.rookus@nki.nl

* Nightingale Studie: In een cohort van (voormalig) verpleegkundigen worden de associaties tussen blootstellingen in de werk- en leefomgeving en gezondheidsrisico's (kanker, hart- en vaatziekten en neurologische aandoeningen) onderzocht.
Informatie: Matti Rookus tel.: 020-5122491, E-mail: m.rookus@nki.nl

* Omega Studie: In een landelijk cohort van vrouwen die onvruchtbaarheidbehandelingen – zoals IVF – hebben ontvangen wordt het risico op hormoongerelateerde tumoren onderzocht. Ook wordt het risico op kanker bij kinderen geboren uit IVF onderzocht.
Informatie Floor van Leeuwen, tel.: 020-5122483, E-mail: f.v.leeuwen@nki.nl

2. Langetermijn neveneffecten van de behandeling van kanker.

* In een landelijk cohort van Hodgkin lymfoom patiënten gediagnosticeerd vanaf de jaren 60 van de vorige eeuw wordt onderzoek gedaan naar het risico op tweede tumoren, cardiovasculaire ziekten en premature menopauze na radiotherapie en chemotherapie. Gekeken wordt naar de bestralingsdosis, grootte van de bestralingsvelden en de doses van de verschillende cytostatica; ook de rol van genetische factoren en leefgewoonten wordt onderzocht.
* Vergelijkbare cohort onderzoeken vinden plaats bij patiënten met borstkanker en testiscarcinoom.
Informatie Floor van Leeuwen, tel.: 020-5122483, E-mail: f.v.leeuwen@nki.nl

3. Moleculaire en genetische markers van borstkankerrisico, behandeling, prognose en mortaliteit.

4. Patiënten informatie en informed consent voor gebruik van lichaams materiaal in wetenschappelijk onderzoek en biobanking.
Informatie: Marjanka Schmidt, tel.: 020-5122276, E-mail: mk.schmidt@nki.nl
Division of Radiotherapy

Head: Prof. Dr. M. Verheij
Project leaders: Prof. Dr. H. Bartelink, Prof. Dr. M. van Herk, Prof. Dr. M. Verheij

Group Van Herk: Advanced imaging for precision radiotherapy - image guided radiotherapy

Radiotherapy aims to deliver a high dose of radiation to a precisely specified location in the body with the aim to kill a malignant tumor, while minimizing damage to the surrounding tissues. A safety margin is necessary to ensure that the target receives adequate dose even when inevitable small geometrical errors occur. To limit side effects, smaller and smaller safety margins are being used around the target, which increases the risk of missing the target. Radiotherapy techniques generally consist of the following four steps:

1) External patient marking, usually under simulator image guidance.
2) CT scanning while aligning the patient with lasers to the external patient marks.
3) Treatment planning, delineation the tumor and planning beams relative to the patient marks as visible on the CT scan.
4) Treatment with a linear accelerator, before which the patient is aligning with lasers on the patient marks.

Several ongoing projects in our department deal with measurement and improvement of the accuracy of each of these steps to allow safe delivery of advanced techniques. For instance, movement of the tumor due to respiration is being measured using high-speed scanners. Target volume delineation is improved by multi-modality image registration including PET scanning gated to the breathing cycle.

We are also investigating and implemented tools to improve the accuracy the actual treatment: image-guided radiotherapy. The most advanced systems that we have available integrate so-called cone-beam CT scanner on the treatment machine, which allows visualization of the patient anatomy in three dimensions just prior to irradiation. This system has come into clinical use a few years ago and provides a wealth of clinical data. Several projects focus on the application of this system emphasising of tumors in difficult anatomical sites such as lung, prostate, bladder, breast and stomach. Finally, models are being developed to allow correct implementation of statistical knowledge of the geometrical errors in treatment planning.

There are many opportunities to define student projects around one of these topics. For instance, recent students worked on implementation of statistical decision criteria for correction of geometrical errors, automatic localization of the bladder in cone-beam data, and improvement of image quality of cone beam data in the presence of anatomical motion.

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Division of Medical Oncology

Head: Dr. J.B.A.G. Haanen
Project leaders: Dr. P. Baas, Dr. A.M. Bergman, Prof.dr. J.H. Beijnen, Dr. C.U. Blank, Dr. A. Cats, , Dr. S.C. Linn, Prof. Dr S. Rodenhuis, Prof.dr. J.H.M. Schellens

Clinical pharmacology
The application of new drugs and new treatment strategies against cancer is under investigation. This concerns phase I and phase II studies, pharmacokinetics and pharmacodynamics. Students will be introduced to the oncological patient care; treatment according to study protocols (following Good Clinical Practice guidelines); data management and computerized processing of the data; classical and population pharmacokinetics and -dynamics; sample handling for pharmacokinetics and –dynamics; determining levels of medication and metabolites in biological matrices (HLPC, mass spectroscopy) according to Good Laboratory Practice; and performing calculations based on pharmacokinetic and pharmacodynamic models. A major point of attention is the connection and integration of patient-oriented research and laboratory-oriented pharmacokinetics and pharmacodynamics. Current research is aimed at, amongst others, strategies to enable oral treatment with anticancer drugs, phase I and pharmacological studies with novel anticancer drugs, and pharmacogenetics.

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Longtumoren
De afdeling thoraxoncologie voert onderzoek uit naar de behandeling van longtumoren. Daarnaast wordt er in grotere series patiënten gezocht naar factoren, die predictief zouden kunnen zijn voor metastatisch recidief en respons op chemotherapie. Ook is er aandacht voor nieuwe geneesmiddelen, zoals nieuwe orale middelen en immunologische behandelingen. Het NKI-AVL is referentiecentrum voor patiënten met een maligne pleuraal mesotheliom. In deze patiëntencategorie worden combinatiebehandelingen en onderzoek met nieuwe middelen regelmatig uitgevoerd.

Informatie: P. Baas, longarts, tel.: 020-5122958, E-mail: p.baas@nki.nl.

Hemato-oncologie
Het NKI-AvL heeft een gemeenschappelijke afdeling hemato-oncologie met het AMC (hoofd Prof. Dr. M.H.J. van Oers). De groep participeert in de HOVON- en EORTC studies. De belangstelling ligt hierbij vooral op de lymfatische maligniteiten. Er wordt onderzoek gedaan naar de ontwikkeling van immunotherapie, chemo-immunotherapie, fase I en II studies, de late effecten van therapie bij patiënten met een Hodgkin of non-Hodgkin lymfoom, de rol van de ‘micro-environment’ en micro-array
diagnostiek bij lymfatische maligniteiten. Ook wordt in het kader van EORTC en HOVON studies onderzoek gedaan naar de waarde van PET-scans bij diagnostiek en behandelstrategie. AvL/NKI stafleden betrokken bij de hemato-oncologie zijn: Dr. J.W. Baars, Dr. J.P. de Boer, Dr. J.M. Kerst, internisten; Dr. R.L.M. Haas, Dr. B.M.P. Aleman, Dr. L. Dewit, radiotherapeuten; Dr. C. Hoefnagel, Dr. R. Valdes Olmos, Dr. M. Stokkel en Dr. W. Vogel, nucleair geneeskundigen; Dr. D. de Jong, Dr. O. Balague-Ponz, pathologen; Dr. A.D.R. Huitema, Prof. Dr. J.H.M. Schellens, klinisch farmacologen (bij fase I/II onderzoek).

Informatie: Dr. J.W. Baars, interniste, tel.: 020-5122568, E-mail: j.baars@nki.nl

The Medical Treatment of Patients with Breast Cancer
Progress in systemic treatment of breast cancer is mainly responsible for the recent decline in breast cancer mortality. In particular, endocrine and cytotoxic chemotherapy in the ‘adjuvant’ setting have contributed to this effect, while drug treatment in the advanced disease setting continues to be life-prolonging rather than curative. Many clinical trials, in all clinical stages of breast cancer, are in progress and many of these attempt to relate clinical findings (such as response) to tumor- and patient characteristics. All studies require intensive collaboration among medical oncologists, other clinicians, (molecular) pathologists and imaging experts. Students will observe maximally intensive chemotherapy with advanced supportive care, treatments with targeted agents and response evaluations. They will participate in multidisciplinary conferences, explore the roles of pathology, molecular biology and pharmacology in breast cancer research. Their research focus will mainly be on the development of predictive tests for systemic treatment benefit with the use of retrospective analyses of available patient data.

Information: S. Rodenhuis, medical oncologist, tel.: 020-5122870; E-mail: s.rodenhuis@nki.nl or S.C. Linn, medical oncologist, tel.: 020-5122951; E-mail: s.linn@nki.nl

Treatment of hormone refractory Prostate cancer
Prostate cancer, refractory after hormonal treatment, has a poor prognosis. Subsequent chemotherapy has only limited effect on the course of this disease. Therefore, studies into new drugs and treatment modalities are warranted for this highly prevalent cancer. Multiple clinical trials are currently worldwide in progress and also in our institute several studies into prostate cancer treatment are active. There are studies into new drugs, combined with current state of the art therapies, and studies into new drug combinations. The latter trials are combined with laboratory studies into the biology of prostate cancer and new techniques to evaluate the outcome of treatment. As a student you will be able to witness the course from diagnosis to treatment, attend multidisciplinary meetings and participate in data collection and evaluation.

Information: A.M. Bergman, medical oncologist, tel.: 020-5122569, E-mail: a.bergman@nki.nl or H.G. van der Poel, urologist, tel.: 020-5122553; E-mail: h.vd.poel@nki.nl

Specifieke bepalingen
1. De minimumduur van stages dient tenminste 4 maanden te bedragen (tenzij anders overeengeomen).
2. In principe lenen stages by sectie X zich vooral voor studenten geneeskunde met enige klinische ervaring.
3. De aard van het onderzoek vereist motivatie om ook op avonden en in weekends te werken.
Division of Surgical Oncology

Board: Prof Dr. T. Ruers (Head), Dr. M. van Beurden, Dr. M. Van den Brekel

The Division of Surgical Oncology consists of six specialties (project groups) i.e. general surgery, head and neck surgery, urology, gynecology, plastic and reconstructive surgery and anesthesiology. The common denominators of research activities are:

1. Technical innovations in surgical treatment and local tumour control
2. Genetic profiling and tailored therapy
3. Immunology and experimental therapy
4. Rehabilitation after mutilating surgery.
5. Early detection of regional tumor spread.
7. Regional perfusion (limb, abdomen).

Clinical-pathological evaluation of patients treated with modified radical mastectomy between 1988-2002

Ongoing debate exists on the indications for adjuvant radiotherapy after modified radical mastectomy. The NKI-AVL has treated a large series of patients with a modified radical mastectomy, predominantly performed after 1988. Before 1988, usually a Halsted radical mastectomy was the standard of care. In this retrospective analysis, local control, overall and disease specific survival will be evaluated in relation to the applied adjuvant radiotherapy. Results will also be compared with international series and the former NKI-AVL Halsted mastectomy series (from this latter series a database is available). The aim of this study is to improve indications for adjuvant radiotherapy after mastectomy.

Information: E.J.Th. Rutgers, H.S.A. Oldenburg, Surgeons; J.L. Peterse, Pathologist; N. Russell, N. Bijker, Radiotherapist, tel.: 020-5122551; E-mail: e.rutgers@nki.nl

The effects of the multidisciplinary Breast Tumour Board between 1993-2004

In 1993 a multidisciplinary Breast Cancer Tumour Board was installed, including a weekly meeting to discuss every patient with breast cancer preoperatively. This study will evaluate the accuracy of needle biopsy diagnosis and triple diagnosis (needle biopsy, imaging and physical examination). Further, the relation between the initial planned therapy, the actual performed therapy and the accordance with treatment guidelines. Samples of 100 files per year will be analysed with respect to essential treatment factors.

Information: E.J.Th. Rutgers, H.S.A. Oldenburg, Surgeons; J.L. Peterse, Pathologist, tel.: 020-5122551; E-mail: e.rutgers@nki.nl

Local control after neoadjuvant chemotherapy in locally advanced breast cancer

Since 1990, the multidisciplinary treatment protocol for locally advanced breast cancer at the NKI-AVL includes neoadjuvant or up-front chemotherapy. A retrospective series of about 150 patients (90 within a randomised trial) will be analysed for the prognostic factors with respect to local control. These patients will be compared with earlier series of patients who were treated with radiotherapy alone. The aim of this study is to evaluate the impact of neoadjuvant chemotherapy with respect to local control.

Information: E.J.Th. Rutgers, Surgeon; S. Rodenhuis, Internist; J.L. Peterse, Pathologist, tel.: 020-5122551; E-mail: e.rutgers@nki.nl

Reconstruction of surgical defects using pedicled pectoralis myocutaneous island flap

Reconstruction by using a pedicled pectoralis myocutaneous island flap is a well-established method for primary and secondary reconstruction of surgical defects. A retrospective analysis of >500 patients will give us insight in the value of this reconstructive surgery for daily clinical practice.

Information: A.J.M. Balm, M.W.M. v.d. Brekel, F.J.M. Hilgers, I.B. Tan, Otolaryngologists, tel.: 020-5122550; E-mail: kno@nki.nl
Oncology Related Voice and Speech Disorders
http://studiegids.uva.nl/web/uva/sqs/nl/c/8817.html
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