



Division head, group leader Flora van Leeuwen



Group leader Matti Rookus

Flora Van Leeuwen PhD Group leader

Matti Rookus PhD Group leader

Marjanka Schmidt PhD Group leader

Berthe Aleman MD PhD Academic staff

Frans Hogervorst PhD Academic staff

Nicola Russell PhD Academic staff

Emiel Rutgers MD PhD Academic staff

Michael Schaapveld PhD Academic staff

Laura Van 't Veer PhD Academic staff

Senno Verhoef PhD Academic staff

Anouk Pijpe PhD Post-doc

Suzanne Rebers PhD Post-doc

Sandra Van den Belt-Dusebout PhD Post-doc

Martijn Verheus PhD Post-doc

Eric Vermeulen PhD Post-doc

Naomi Boekel MSc PhD student

Richard Brohet MSc PhD student

Anja van Eggermond MSc PhD student

Lieske Schrijver MSc PhD student

Mandy Spaan MSc PhD student

Rianne van Nimwegen MSc PhD student

Sandra van den Broek MSc PhD student

Janneke Verloop MSc PhD student

Cherita Sombroek MSc Junior scientific researcher

Thea Mooij MSc Statistical analyst

Marie-José Blom MSc Data manager

Juliet Boessenkool-Pape MSc Data manager

Ellen Engelhardt MSc Data manager

Eduard Ivanov MSc Data manager

Cora Knol MSc Data manager

Judith de Lange MSc Data manager

Harmke Groot MSc Research assistant

Esther Janssen Research assistant

Kiki Jeanson MSc Research assistant

Kim Kersten MSc Research assistant

Marianne Kuenen Research assistant

Gabey Ouwens Research assistant

Saskia Pelders MSc Research assistant

Frederiek Diemer Research assistant

DIVISION OF PSYCHOSOCIAL RESEARCH AND EPIDEMIOLOGY

EPIDEMIOLOGY

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

Risk factors for hormone-related cancer In our nationwide cohort study in families with a *BRCA1/2* mutation (GEO-HEBON study), we are studying whether 1) hormonal/life-style factors modify cancer risk in *BRCA1/2* families, 2) common genetic alterations are associated with the risk of breast cancer among *BRCA1/2* carriers.

We continued the coordination of the data collection of the International *BRCA1/2* Carrier Cohort Study (IBCCS), a follow-up study on 3823 *BRCA1/2* mutation carriers, and the harmonization of the epidemiologic risk factor data for the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). The CIMBA risk factor database now includes 10,031 *BRCA1/2* carriers. We coordinate the data cleaning process that is being conducted by eight international groups for specific risk factors and by us for exogenous hormones. The CIMBA DNA database contains more than 20,000 *BRCA1/2* carriers. Three approaches were taken to find new genetic modifiers of *BRCA1*- and *BRCA2*- related cancer risk: 1. targeted single nucleotide polymorphisms (SNPs), found to be related with cancer risk in the general population or among *BRCA1/2* carriers in earlier studies, 2. SNPs with highest significance in GWA studies in the general population, and 3. new GWA studies within CIMBA. The first approach, though generally based on plausible biological pathways, was not very successful in general. One of the exceptions was the finding that the minor allele of *CASP8* D302H, a key enzyme involved in the initiation of apoptosis, was associated with a reduced risk of breast cancer and ovarian cancer for *BRCA1*, but not for *BRCA2* mutation carriers. As an example of the second approach, the top 350 SNP list of two GWAS studies in the general population were examined as genetic risk modifiers for *BRCA1* and *BRCA2*. A total of 8 SNPs in *BRCA1* mutation carriers and 12 SNPs in *BRCA2* carriers were associated with breast cancer risk. SNPs in *SNRNPB* and *CAMK1D* were strongest in *BRCA1*, whereas SNPs in *LOC134997* and *FBXL7* were most significant in *BRCA2*. Furthermore, the SNP at 9p22.2 (*rs3814113*) that was identified in a GWAS on ovarian cancer in the general population showed a reduced risk of ovarian cancer in *BRCA1* as well as in *BRCA2* carriers. Thirdly, two new GWAS studies, with several replication phases, were conducted in *BRCA1* and *BRCA2*, respectively. In *BRCA1* carriers five SNPs on 19p13 were associated with breast cancer risk, two of which showed independent associations (*rs8170* and *rs2363956*). Genotyping these SNPs in population-based cases and controls identified a similar association with estrogen receptor–negative breast cancer and an association with estrogen receptor–positive disease in the opposite direction. The five SNPs were also associated with triple-negative breast cancer. The GWA study in *BRCA2* indicated that SNPs that modify *BRCA2* penetrance are similar to variants that modify risk of sporadic breast cancer. Functional studies are ongoing to identify the biological pathway related with the newly discovered SNPs in *BRCA1*, while more in-depth genetic analyses are being conducted for *BRCA2*.

To increase power for the assessment of the risk of hormone-related cancers after fertility treatment, such as ovarian stimulation for in vitro fertilization (IVF), we expanded our nation-wide OMEGA-cohort of 25,152 women treated for subfertility (collaboration with CW Burger, Erasmus MC Rotterdam). The original cohort comprises 19,145 women who received ovarian stimulation for IVF between 1983 and 1995 and 6,007 subfertile women not treated with IVF. For the expansion of the IVF cohort ($n = 8,800$), we collected detailed data regarding IVF treatment (number of cycles, drug doses, number of oocytes retrieved) from the 12 collaborating IVF-clinics through computerized databases and medical charts. We also identified 4,100 subfertile women to enlarge the comparison group of women not treated

with IVF, comprising women who underwent tubal surgery or intra-uterine insemination, or who withdrew from the IVF waiting list. The original cohort has recently been linked with the Netherlands Cancer Registry to update cancer risk and analyses of breast cancer risk ($n = 609$ cases) are ongoing. For the expanded cohort 50% of the addresses have been updated. Three pilot studies including 643 IVF-treated women have been performed; the average response rate of 51% was not influenced by the use of an internet questionnaire, the collection of DNA (toenails), or the study region. More responders filled in the internet questionnaire (55%) compared to the paper questionnaire (45%). To increase the response rates, the lay-out of the questionnaire, the study leaflet and the website have been adapted, and media attention has been sought. In total, approximately 20,000 women will be invited, starting November 2010. Data will be collected regarding life-style, reproductive factors, and health outcomes through (internet and paper) questionnaires. A new prospective cohort study (the Nightingale Study) among nurses in the Netherlands was initiated in collaboration with the Institute for Risk Assessment Sciences (IRAS, Utrecht University) and the BIG-register (of the Ministry of Health, Welfare and Sport). Exposure to light-at-night has been suggested as a contributing cause of breast cancer (IARC classification 'probable human carcinogen, 2A'). Since shift- and night-time work is prevalent and increasing in modern societies, this exposure may contribute to the continuing increase of breast cancer incidence and may be of public health concern. This study will provide insight into, amongst others, the potential association between occupational exposures (e.g. shift work, electromagnetic fields) and the risk of cancer and other diseases, and on potential biological mechanisms. Women will be asked to complete a (web-based or paper) questionnaire, sign an informed consent, and donate toenails for DNA analyses (e.g. clock genes). Specific attention will be directed to potential shift-work related selection bias.

Late effects of cancer treatment Now that curative treatment is available for a substantial group of cancer patients, it is increasingly important to evaluate how the occurrence of late complications of treatment affects their long-term survival. We aim to evaluate the risk of second cancers and cardiovascular disease (CVD) after radio- and chemotherapy (CT) for Hodgkin's lymphoma ($n=3,400$), testicular cancer ($n=3,745$) and breast cancer ($n=80,000$) over a period of up to 30 years after primary treatment.

In 2010, we evaluated the long-term risk of colorectal cancer following Hodgkin's lymphoma treatment. Results show that Hodgkin's lymphoma patients have a 3.7-fold (95% confidence interval (CI) 2.6-5.0) increased standardized incidence ratio (SIR) of developing colorectal cancer compared with the general population, with an absolute excess risk of 7.8 colorectal cancer cases per 10,000 patients/year. After a median follow-up of 20 years, 40 colorectal cancer patients were identified (23 colon, 17 rectum). The highest SIR (7.5, 95% CI 3.4-14.3) was seen for patients treated before age 25. Cumulative incidence was 1.9% (95% CI 1.3-2.7) at 30 years of follow-up. Especially for colon cancer, the SIR increased with longer follow-up duration (9.3, 95% CI 3.4-20.2 in 30-year survivors). No increased SIR was found for colorectal cancer in patients treated with RT alone. However, a significantly increased SIR was found for patients treated with RT and CT; the SIR for patients treated with CT and RT below the diaphragm was (6.9 95% CI 3.9-11.2). We also found that a 40-year old HL-survivor treated before age 25 has the same CRC risk as a 55-60-year old person from the general population.

After we published our results on treatment-related risk factors for stomach cancer after Hodgkin's lymphoma we contributed our data to an international multicenter case-control study of stomach cancer after Hodgkin's lymphoma, coordinated by the U.S. National Cancer Institute. The study includes 89 cases with stomach cancer and 190 matched controls. Preliminary results show that stomach cancer risk increases with larger radiation doses to the mean stomach, with 5.3-fold increased risk for radiation doses of 30-39 Gy compared with less than 0.5 Gy. The study confirms our finding that procarbazine increases the risk of stomach cancer. By the end of 2009 we established the nationwide Dutch **BETTER** (Better care after Hodgkin lymphoma: Evaluation of long-Term Treatment Effects and screening

Key publications

Antoniou AC, Sinilnikova OM, Rookus MA, Chenevix-Trench G and 133 other authors. *Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers. Hum Mol Genet* 2009;18:4442-4456

Antoniou AC, Wang X, Rookus MA, Couch FJ and 168 other authors. *A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet* 2010; 42:885-892

Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Huntsman D and 38 other authors. *Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med.* 2010;7:e1000279

Braat DDM, Schutte JM, Bernardus RE, Mooij TM, Van Leeuwen FE. *Maternal death related to IVF in the Netherlands 1984-2008. Hum Reprod* 2010;25:1782-86

Broeks A, Braaf LM, Wessels LF, van de Vijver M, De Bruin ML, Stovall M, Russell NS, Van Leeuwen FE, Van 't Veer. *Radiation-associated breast tumors display a distinct gene expression profile. Int J Radiat Oncol Biol Phys* 2010;76:540-7

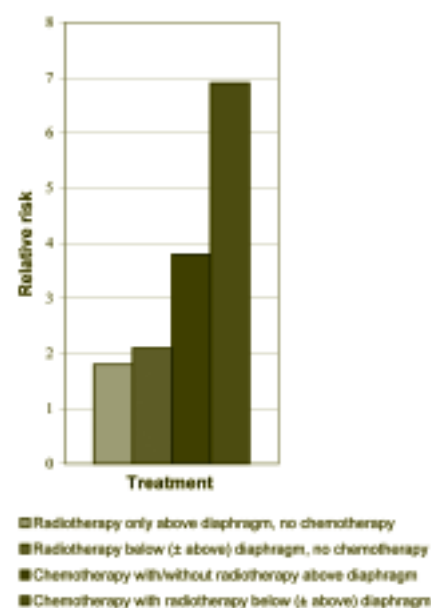


Figure 1: Risk of colorectal cancer after treatment of Hodgkin's lymphoma in 2667 patients (Relative risk is compared to the general population)

Publications (continued)

Cardous-Ubbink MC, Geenen MM, Schade KJ, Heinen RC, Caron HN, Kremer LCM, Van Leeuwen FE. Hypertension in *Long-Term Survivors of Childhood Cancer: A Nested Case-Control Study*. *Eur J Cancer* 2010;46:782-90

Devilee P, Rookus M. A Tiny Step Closer to *Personalized Risk Prediction for Breast Cancer*. *New England Journal of Medicine* 2010;362:1043-1045

Enciso-Mora V, Broderick P, van Leeuwen FE, Houlston RS, and 41 other authors. *A genome-wide association study of Hodgkin's lymphoma identifies new susceptibility loci at 2p16.1 (REL), 8q24.21 and 10p14 (GATA3)*. *Nat Genet* 2010;42:1126-1130

Engel C, Versmold B, van Leeuwen FE, Schmutzler RK and 46 other authors. *Association of the variants CASP8 D302H and CASP10 V410I with breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers*. *Cancer Epidemiol Biomarkers Prev* 2010;19:2859-2868

Fles R, Hoogendoorn WE, Platteel I, Scheerman CE, Leeuw-Mantel G, Mourits MJ, Hollema H, van Leeuwen FE, van Boven HH, Nederlof PM. *Genomic profile of endometrial tumors depends on morphological subtype, not on tamoxifen exposure*. *Genes Chromosomes Cancer* 2010;49:699-710

Gaudet MM, Kirchoff T, Rookus MA, van Leeuwen FE, Chenevix-Trench G and 170 other authors. *Common genetic variants and modification of penetrance of BRCA2-associated breast cancer*. *PLoS Genet* 2010;6:e1001183

Kaas R, Verhoef S, Wesseling J, Rookus MA, Oldenburg HS, Peeters MJ, Rutgers EJ. *Prophylactic mastectomy in BRCA1 and BRCA2 mutation carriers: very low risk for subsequent breast cancer*. *Ann Surg* 2010;251:488-492

Marees T, Van Leeuwen FE, Schaapveld M, Imhof SM, De Boer MR, Kors WA, Ringens PJ, Moll AC. *Risk of third malignancies and death after a second malignancy in retinoblastoma survivors*. *Eur. J. Cancer* 2010;46:2052-58

Recommendations) consortium, consisting of hemato-oncologists and radiation oncologists of the NKI, all 8 University Medical Centers and 3 large peripheral hospitals. The consortium aims to increase the life expectancy and enhance the quality of life of HL survivors in the Netherlands by reducing morbidity and mortality from late adverse effects of HL treatment. In 2010, two meetings have been organised, in which draft national screening guidelines were developed. Furthermore we are also developing a website for HL survivors with information about late effects of treatment and possibilities to reduce these risks. An evaluation of the website focussing on knowledge, risk perception and worries about late effects, is in progress. In UMCN, UMCU and Medisch Spectrum Twente we also started identifying HL survivors, who are eligible to be recalled for medical surveillance, based on the screening guidelines. **Figuur 2 Flora van Leeuwen** To further study the effects of radiotherapy, chemotherapy, reproductive and genetic factors on the risk of breast cancer after Hodgkin lymphoma, we are performing a nationwide case-control study (collaboration with Division Experimental Therapy, Annegien Broeks, Laura van 't Veer). So far we have identified 170 case patients, who were individually matched to 462 controls. All women who are still alive receive a questionnaire on lifestyle factors and hormone use and are asked to provide a blood sample for genetic analyses.

Within our nationwide cohort, comprising 3,745 testicular cancer patients treated in the Netherlands during 1965-1995, we recently examined the risk of late relapse after successful treatment of a testicular germ cell tumor. Late relapses, defined as a relapse occurring 2 years or more after treatment of a testicular germ cell tumor which resulted in a complete response, are rare events. Incidence, characteristics and tumor-specific survival for late relapses have not been well defined in previous studies. Our study cohort had long-term and very complete follow-up. After a median follow-up of 19 years 79 patients experienced a late relapse. Almost 80% of these relapses occurred within 10 years after completion of treatment, giving a 10-year cumulative incidence of 2.7%. Beyond the 10th year follow-up the annual rate of late relapse was very low, i.e. 0.06% per year. Only initial stage predicted late relapse risk, while age, histology, period of diagnosis and initial treatment did not. The 10-

year cumulative incidence reached 11.3% among stage III non-seminoma patients. The 10-year testis cancer-specific survival following a late relapse was 70%. Older age (≥ 50 years) at late relapse, a symptomatic presentation or a late relapse detected by increasing serum markers and no histological confirmation of the relapse were all associated with significantly worse testis cancer-specific survival. Surgery for late relapse was associated with better survival.

In a previous cohort study we showed increased risks of cardiac morbidity and mortality among breast cancer patients treated between 1970 and 1986. To evaluate the long-term cardiovascular morbidity and mortality in survivors of breast cancer treated with more contemporary regimens we are conducting two new large cohort studies.

The first is a population-based cohort of patients with invasive breast cancer ($n=97,747$) and ductal carcinoma in situ ($n=13,545$) of the breast diagnosed between 1989 and 2004 in the Netherlands. Patients have been identified through the Netherlands Cancer Registry. The cohort has been linked with the heart intervention registry maintained by the Steering Committee Heart Interventions Netherlands (BHN) and the Dutch Hospital Discharge registry (LMR) for cardiovascular disease and with the Dutch general population registry (GBA), the Central Bureau for Genealogy (CBG) and Statistics Netherlands (CBS) for date and cause of death, respectively. Additional treatment information is currently being supplied by hospital registries, nine RT institutes, Pharmo, clinical trials, and regional documentation projects.

The second cohort is hospital-based and consists of 24,000 patients treated between 1970 and 2004 in the Netherlands Cancer Institute or the Erasmus MC, Daniel den Hoed Cancer Center. For this cohort detailed treatment information and cardiovascular risk factors are being collected from medical files and through general practitioners. Our first analyses were restricted to a sub cohort of 10-year survivors treated for breast cancer between 1970 and 1989 before the age of 46 years ($n=854$). We observed an increased risk of cardiovascular disease for patients irradiated to the internal mammary chain nodes compared to patients not irradiated to these nodes ($HR=3.81$; $CI=1.80-8.05$). Risk was also elevated for patients treated after 1980 with more recent radiotherapy techniques.

Publications (continued)

Marees T, Dommering CJ, Imhof SM, Kors WA, Ringens PJ, Van Leeuwen FE, Moll AC. *Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. Human Reprod* 2009;24:3220-24

Pijpe A, Manders P, Brohet RM, Collee JM, Verhoef S, Vasen HF, Hoogerbrugge N, van Asperen CJ, Dommering C, Ausems MG, Aalfs CM, Gomez-Garcia EB, Van't Veer LJ, van Leeuwen FE, Rookus MA. *Physical activity and the risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat* 2010;120:235-44

Pijpe A, Manders P, Hooning MJ, Kluij I, Vasen HF, Hoogerbrugge N, van Asperen CJ, Meijers-Heijboer H, Ausems MG, van Os TA, Gomez-Garcia EB, Brohet RM, HEBON, van Leeuwen FE, Rookus MA. *Body weight and risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat.* 2010

Pijpe A, Manders P, Mulder RL, van Leeuwen FE, Rookus MA. *Reliability of self-reported diagnostic radiation history in BRCA1/2 mutation carriers. Eur J Epidemiol* 2010;25:103-113

Schmidt MK, Tommiska J, Broeks A, Van Leeuwen FE, Van 't Veer LJ, Pharaoh PDP, Easton DF, Shah M, Humphreys M, Dörk T, Reincke SA, Fagerholm R, Blomqvist C, Nevanlinna H. *Combined effects of single nucleotide polymorphisms TP53 R72P and MDM2 SNP309, and p53 expression in survival of breast cancer patients. Breast Cancer Res* 2009;11:R89

van den Belt-Dusebout A, Aleman B, Gietema J, de Wit R, Van 't Veer M.B., Lugtenburg A.D.G., Krol S., van Leeuwen F.E. *Langetermijncomplicaties na behandeling voor zaadbalkanker en Hodgkinlymfoorm. Ned Tijdschr Geneesk* 2010: 154

Van der Pal HJ, Van dalen EC, Hauptmann M, Kok WE, Caron HN, Van den Bos C, Oldenburger F, Koning CC, Van Leeuwen FE, Kremer LC. *Cardiac Function in 5-years survivors of childhood cancer; a long-term follow-up study. Arch Int Med* 2010;170: 1247-55

Voskuil DW, Van Nes JGH, Junggebur JMC, Van de Velde CJH, Van Leeuwen FE, De Haes JCM. *Maintenance of physical activity and body weight in relation to subsequent quality of life in postmenopausal breast cancer patients. Ann. Oncol* 2010;10:2094-101