OncoPoint

23-05-2012, Auditorium C, UZ-Gent; http://mellfire.ugent.be/public/oncopoint/

8.45-9.00	**Opening**
	Session I: Imaging in cancer research
09.00-09.30	Christian Van Hove (MEDISIP) INFINITY: The small animal imaging facility of Ghent University
9.30-10.00	Wim Ceelen (Department of Surgery) Kinetic modelling of dynamic contrast enhanced MR data: a tool for noninvasive assessment of tumour vascular physiology
10.00-10.30	**Short oral abstract presentations (part I)**
10.30-10.50	**Coffee break & poster viewing**
10.50-11. 50	**Selected abstract presentations (4)**
11.50-12.20	**Short oral abstract presentations (part II)**
12.20-13.30	**Lunch & poster viewing**
	Session II: Tumor environment
13.30-14.00	Olivier De Wever (Laboratory of Experimental Cancer Research) The tumor ecosystem : a source for therapeutic targets and biomarker discovery
14.00-14.30	Karim Vermaelen (Department of Pulmonary Medicine) Phenotypic, functional and epigenetic modulation of dendritic cells by the murine lung tumor environment
14.30-15.45	**Selected abstract presentations (5)**
15.45-16.15	**Coffee break**
	Session III: Emerging technologies in cancer research
16.15-16.45	Marleen Van Troys (Department of Biochemistry) Quantitative analysis of cell migration dynamics in 3D matrices at higher throughput
16.45-17.15	Pieter Mestdagh (Center for Medical Genetics) Exploratory tools to study non-coding RNA in cancer
	Session IV: Support in cancer research
17.15-17.45	Sofie Bekaert (BIMETRA) BIMETRA: facilitation of translational research @ campus
17.45-18.00	**Final conclusions, announcements of awards**

Accreditering wordt aangevraagd

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11.20 Abstract 37
Candy Kumps: Transcriptome analysis following ALK abrogation provides insights in the downstream signaling pathways in human and murine neuroblastoma cells

11.35 Abstract 26
An Hendrix: Rab27B, an ex(o)citing driver of breast cancer growth, invasion and metastasis

14.30-15.45 **Selected abstract presentations (5)**

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Gwen Sys: Tumour grafts derived from sarcoma patients retain tumour morphology, viability, invasion potential, and indicate disease outcome in the chick chorioallantoic membrane model

15.15 Abstract 29
Piet Ost: Salvage stereotactic body radiotherapy for patients with limited prostate cancer metastases: deferring androgen deprivation therapy

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Abstract 3: Evelyn Pauwels
Design and process evaluation of the OncoWijzer, an informative website tailored to breast cancer survivors' and intimate partners' post-treatment care needs

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Determining a role for germline mutations in intermediate risk breast and ovarian cancer susceptibility genes in the Belgian population

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Which Care Do Patients With Breast Cancer Want?

supported by

Karien Smet

ADOLESCENTS AND YOUNG ADULTS (AYAS) WITH CANCER: THE PERSPECTIVE OF AYAS AND THEIR PARENTS.

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Introduction There is growing recognition that the perception of AYAs diagnosed with cancer is more distinctive than this of children or adults. The main objective of this research is to understand how AYAs and their care giving parents experience cancer, diagnosis, hospitalisation, treatment and survivorship to open a debate on how their unique needs and experiences can be implemented in a Patient Centred Care pathway for primary and secondary care. Methods This qualitative study was based on the principles of grounded theory. 24 semi-structured interviews were held with AYAs between 15 and 25 years. Additionally 19 care giving parents were interviewed (data collection still in process). Sampling was based on situational diversity (e.g. gender, age, social context, education, time since diagnosis). The interviews were transcribed and coded (NVivo7) and constant comparison was used to analyse the data. Results Analysis revealed that AYAs see cancer as something temporarily passing their life-path. Their coping-strategies are focused on preserving identity and a normal life, not only during treatment, but also in follow-up and survivorship. Findings suggest that AYAs prefer care, tailored to their needs. When confronted with the cancer of AYAs, care giving parents face various fears and insecurities. They are constantly challenged in finding the right balance between care for the AYA, care for the other family members and care for themselves. Findings also demonstrates poor communication about emotions between the AYAs and the care giving parents. Conclusions This study revealed that cancer seems to have a different meaning for AYAs then for their care giving parents. The results can inspire caregivers to develop psychosocial pathways in accordance to the specific preferences and wishes of both AYA and the care giving parent. To guarantee quality of cancer care collaboration between primary and secondary caregivers should be orientated on these preferences.

Astrid De Boeck

Bone marrow-derived mesenchymal stem cells drive colorectal cancer progression: implication of a paracrine neuregulin 1/HER3 activation loop

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Emerging evidence suggest that bone marrow-derived mesenchymal stem cells (BM-MSC) play an undeniable role in the progression of several cancers, including colorectal cancer (CRC). This study aimed to identify the molecular mechanisms associated with these heterotypic cellular interactions and analyse their relevance in CRC. Paracrine interactions of BM-MSC with CRC cells were studied using collagen invasion assays, cell counts, flow cytometric cell-cycle analysis and tumor xenografts. The role of neuregulin 1 (NRG1) and the human epidermal growth factor receptor (HER) family pathways were investigated using tyrosine kinase assays, mass spectrometry, pharmacological inhibition, antibody-mediated neutralisation and RNA interference. To support the clinical relevance of our findings, transmembrane NRG1 (tNRG1), HER2 and HER3 expression were analysed in primary CRCs (n = 54) and liver metastasis (n = 3) by immunohistochemistry and the potential for NRG1 as a prognostic stromal marker for CRC was evaluated. We found that BM-MSC stimulate invasion, survival and tumorigenesis of CRCs through release of soluble factors. Neuregulin (NRG)1 secreted by BM-MSC was found responsible for the observed effects, and signalled through HER2/HER3-dependent activation of the PI3K/AKT/Bad pathway in CRC cells. In clinical specimens, high tNRG1 expression was observed in tumor-associated mesenchymal cells (T-MC) in the immediate vicinity of the HER2/HER3 expressing cancer cells and high tNRG1 expression was significantly associated with advanced tumor stage (P = .005) and invasion depth (P = .04) and decreased 5-year progression-free-survival (PFS) (P = .01). In conclusion, paracrine HER2/HER3 signals initiated by BM-MSC-derived NRG1 promote CRC progression, and high tNRG1 expression is associated with poor prognosis in CRC, suggesting that stromal NRG1 may serve as a novel prognostic marker for CRC.

Evelyn Pauwels

Design and process evaluation of the OncoWijzer, an informative website tailored to breast cancer survivors' and intimate partners' post-treatment care needs

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Introduction. As on-line provision of information during the transition phase after treatment carries great promise in meeting shortcomings in post-treatment care for breast cancer survivors and their partners, a tailored informative website was developed. Methods. The development process included quantitative and qualitative assessments of survivors' and partners' care needs and preferences. Participants' use and evaluation of the website were explored preliminary by conducting baseline and post-measurements. During the intervening 10-12 weeks 57 survivors and 28 partners were granted access to the website. Moreover, the pilot-study assessed whether survivors' and partners' characteristics were related to visiting the website. Results. The website distinguished itself for its limited number of buttons per page, the minimization of navigation required and visitors' ability to select information tailored to their needs. Fifty-seven percent (n=21) of survivors who took part in the post-measurement indicated that they had visited the website. Compared to non-visitors (n=16), they were more likely to have a partner and a higher income, reported higher levels of self-esteem and had completed treatment for a longer period of time. Partners who consulted the on-line information (42%, n=8) were younger and reported lower levels of social support compared to partners who did not visit the website (n=11). Visitors generally evaluated the website's content and lay-out positively, yet some believed the information was incomplete and impersonal. Conclusions. The website reached only about half of survivors and partners, yet was mostly well-received. A website containing clear-cut and tailored information could be a useful tool in a stepped approach to post-treatment care provision.

Kathleen Claes

Determining a role for germline mutations in intermediate risk breast and ovarian cancer susceptibility genes in the Belgian population

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Germline mutations in the coding and splice regions of BRCA1&2 are identified in approximately 15% of patients selected for genetic testing in the Belgian population. It is estimated that germline mutations in intermediate risk genes like PALB2, ATM, CHEK2, BRIP1 and BARD1 account for an elevated breast and ovarian cancer risk in approximately 5% of familial breast cancer. In this study we evaluated the mutation prevalence of PALB2 mutations in 285 Belgian patients from 256 independent BRCA1/2 mutation negative families with a young age at onset and/or family history of breast and/or ovarian cancer. The complete UTR, coding and splice site regions of PALB2 were analyzed with High resolution melting followed by Sanger sequencing of the aberrant melting curves. In silico predictions of variants with an unclear clinical significance was performed with the Alamut software. In total we identified 20 unique sequence variants in PALB2 of which 6 are previously unreported. Three novel unequivocal mono allelic mutations (PALB2 c.2834+1G>T c.2888delC and c.3423del4) were detected and shown to segregate with the disease in the families. PALB2 c.2834+1G>T is a splice site mutation which undoubtedly will lead to aberrant splicing; c.2888delC and c.3423del4 are both frame shift mutations in the WD40 protein domain, responsible for the interaction with BRCA2. Furthermore, we detected in nine patients 4 novel sequence variants (PALB2 c.-158G>C, c.498T>C, c.995T>A and c.1520C>G) of which the clinical significance is currently unknown. Several in silico analyses predict that PALB2 c.995T>A (p.Leu332His) could affect protein function. However, segregation analysis revealed that the mother of the patient, diagnosed with breast cancer at the age of 45 years did not carry the sequence variant. Deleterious PALB2 mutations were only identified in familial breast cancer patients (3/208=1.4%) and not in 48 sporadic patients with early onset or bilateral breast cancer. Interestingly, the average age at diagnosis for the mutation carriers (62y; range 48-71) was significantly higher compared to the average age at diagnosis (39y, range: 25-78) of the non carriers. This limited role for PALB2 germline mutations in the Belgian population, suggests locus heterogeneity and further investigations in other intermediate risk breast and ovarian cancer susceptibility genes (ATM, CHEK2, BRIP1 and BARD1) will be undertaken.

Elsie Decoene

Development of evidence-based guidelines for a nurse consultation in a breast unit:

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Background The purpose of this project is to develop guidelines for the organisation and content of a nurse consultation in a breast unit. Material and methods The project exists of five major phases: (1) a literature study, (2) a qualitative study with breast care nurses (BCN) and patients about their experiences of the care, (3) development of the guidelines, (4) multicentric implementation, and (5) evaluation of phase four. A phenomenological approach using ten focus groups to collect data was adopted. Information about the perspectives of breast cancer patients with the care of a BCN was collected. All patients completed their treatment (n= 36) and received care from a BCN. The interviews were transcribed and coded (Nvivo9) and constant comparison was used to analyse the data. Results Analyses revealed two major themes: how patients with breast cancer experience their illness and treatment and how BCN can support them. The BCN has to support the patient at the right moments by giving individualised information and psychosocial support. She represents humanity in a complex organisation and the clinical pathway the patient has to endure. The supporting activities of the BCN must be clear for the patient and not only problem based. Taking spontaneous contact with the patient is a comforting thought, as patients often wait to call for help from the BCN. Conclusions This project shows differences and similarities with the perspective of the BCN. Beside her role toward the patient, the BCN has an important task in taking action when care fails. Education, coaching en recognition of the BCN is essential for a patient centered and qualitative care. The guidelines must provide information and support for the BCN and her team how to organize a nurse consultation at each important phase in the total clinical pathway.

Elsie Decoene

Development of evidence-based guidelines for a nurse consultation in a breast unit: part 1: the perspectives of breast care nurses

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Background The objective of this project is to develop evidence-based guidelines for the organisation and content of a nurse consultation in a breast unit. Material and methods The project exists of five major phases: (1) a literature study of the information- and psychosocial needs of breast cancer patients, (2) a qualitative study with breast care nurses (BCN) and patients about their experiences of the care, (3) development of guidelines based on the first two phases and evaluation by an expertgroup, (4) a monocentric implementation of the guidelines in a breast unit and (5) an evaluation of the outcomes of phase four. A phenomenological approach using five focus groups to collect data was adopted. Participants of this part of the study were 30 BCN working 1 to 6 years in a breast unit. Three topics were explored: the role, the position and the competence of a BCN. Results Two main themes were identified: patient-centered roles en organisation-centered roles. In the first role themes such as assessing physical and psychosocial status of the patient, providing information, providing psychosocial support and being there for the family are explored. Having an important role as BCN in the total organisation of a breast unit was translated in themes as coordinating the entire care and acting as an important player in the multidisciplinary team. Conclusions In Belgium, the role of a BCN is not informed or implemented by evidence-based guidelines and there is no national education program for these specific (rather new) nurse roles. The guidelines will provide information and support for the BCN how to organize a specialised nurse consultation at each important phase in the total clinical pathway of a patient with breast cancer. The guidelines will be flexible and acceptable for implementation in all breast units in Belgium.

Annelies Fieuw

Enrichment analysis for MYCN pathway genes in focal genomic gains and losses identifies new components of the MYCN-miRNA regulatory network in neuroblastoma

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Background Particular patterns of large chromosomal deletions, gains and amplifications in neuroblastoma have been described in detail and delineate three major genomic subgroups. However, comprehensive analyses of focal aberrations have thus far received much less attention. The finding of a 5 kb gain containing exclusively the MYCN activated miR-17-92 cluster in a neuroblastoma cell line lead us to hypothesize that clinically relevant focal genomic gains and losses may be implicated in neuroblastoma and that such DNA copy number changes may specifically target MYCN regulated genes. Methods High resolution DNA copy number data of 190 neuroblastoma tumor samples and 33 neuroblastoma cell lines were analyzed for focal DNA copy number aberrations. Accompanying mRNA and miRNA data, available for most of these samples, were used for additional data mining purposes. Results We detected significant enrichment for up regulated MYCN target genes in focally gained and amplified regions, suggesting that DNA copy number variants can further reinforce particular MYCN downstream effects in tumor cells. In the deleted regions, enrichment for predicted target genes of MYCN up regulated miRNAs was observed. Using an integrated data mining approach and subsequent experimental validation, RGS5, homozygously deleted in one neuroblastoma cell line, was identified as a target of MYCN up regulated miRNAs. RGS5 encodes a regulator of G protein signaling implicated in vascular normalization and has not previously been reported in neuroblastoma oncogenesis. Conclusions With this unique approach, we show for the first time that focal genomic gains in neuroblastoma are enriched for direct MYCN target genes, while focal genomic deletions are enriched for genes down regulated through MYCN regulated miRNAs. Using an integrated genomic approach we confirmed the latter observation and expanded the MYCN-miRNA controlled regulatory network. Given the emerging role of RGS5 in tumor angiogenesis, this gene may represent an important target for anti-angiogenic therapy.

Abstract 8

Isabel Van Audenhove

Fascin loss-of-function by intracellular delocalisation with nanobodies

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Fascin is an actin bundling protein which is considered as a metastatic marker and therefore an important therapeutic target. We have generated a unique set of tools, nanobodies, enabling us to study endogenous fascin in cancer cells. Nanobodies, present in species of the Camelidae, correspond with the smallest antigen binding fragments which fully retain their binding affinity. We identified a fascin nanobody, FASNb5, which potently inhibits the actin bundling activity of fascin in vitro. When expressed in PC-3 prostate cancer cells, this nanobody prevents formation of filopodia, fingerlike protrusions important for adhesion and migration. FASNb5 also inhibits matrix degradation, an invasive process during which an underlying extracellular matrix is degraded. Another fascin nanobody, FASNb2, has no effect on actin bundling because it binds another epitope in fascin. This nanobody has no influence on filopodia formation and matrix degradation. However, as an alternative strategy we tagged this nanobody, causing delocalisation of the nanobody and fascin towards the mitochondrial outer membrane. Under these conditions, FASNb2 significantly reduces both filopodia formation and matrix degradation, thereby establishing a clear correlation between the subcellular localisation of fascin and its role in filopodia formation and matrix degradation. This reveals a new way to promote a functional protein knock-out using high affinity nanobodies, at the level of the endogenous protein.

Joline Goossens

Fertility and –preservation in cancer patients: needs and experiences

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BACKGROUND The main focus after the diagnosis of cancer is establishing a treatment plan and surviving cancer. Therefore, long term complications such as fertility have a lower priority. However, fertility and future parenting is important for many patients, and a possible loss can result in psychosocial problems. Research shows that cancer patients have a high need for information on fertility and –preservation options. Although patients express their need for fertility related information, it is often bad timed, inadequate and difficult to access. METHODS In October 2011, a two-year research project was initiated at the Nursing and Midwifery Science unit of Ghent University, funded by the Flemish League against Cancer. The project aims (1) to assess the fertility related needs and experiences of cancer patients/survivors and (2) to develop a guide to support caregivers in counseling cancer patients confronted with potential loss of fertility. First, two systematic reviews will be conducted based on the principles of The Cochrane Collaboration (1) to explore the fertility related information– and communication needs of cancer patients and (2) to investigate the attitude and knowledge of patients and caregivers towards fertility/–preservation. Second, a qualitative approach, using semi-structured interviews will be applied to identify the fertility related needs and experiences of both cancer patients (n =40) and caregivers (n =20). RESULTS Based on the results of the systematic reviews and the interviews with patients/caregivers, a guide will be developed and tailored to the fertility related needs of cancer patients/survivors. Preliminary results of this study will be available by May 2012. DISCUSSION Fertility problems have an impact on the quality of life of cancer patients. These patients have the right to be informed in a sufficiently way. Therefore, we strive to develop a guide that will improve the quality of counseling services related to fertility issues.

Abstract 10

Kaat Durinck

Functional interplay of NOTCH1 and PHF6: balancing between normal T-cell development and T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy arising from uncontrolled proliferation and arrested differentiation of immature precursor T-cells. Oncogenic NOTCH1 activating mutations are of particular interest as they are found in more than 50% of T-ALL cases, marking NOTCH1 as an important therapeutic target. In 2010, we discovered loss-of-function mutations in PHF6 in up to 40% of T-ALL patients marking this gene as one of the major tumor suppressor genes implicated in T-ALL development. PHF6 is structurally characterized by the presence of two 'plant homeodomain zinc finger' (PHD) domains. These PHD-domains have been described to serve as binding modules to specific histone modifications, suggesting a role for PHF6 as an epigenetic regulator of gene expression. In order to unravel the role of PHF6 in normal T-cell development and T-ALL oncogenesis, we established PHF6 perturbation cell model systems by means of lentiviral transductions of PHF6 wildtype cell lines with doxycyclin-inducible short hairpin RNA (shRNA) encoding constructs. The transcriptional response of all protein coding genes and 8000 lncRNAs was assessed following induced PHF6 down regulation (Agilent). In addition, we explored the regulatory network governed by PHF6 by means of ChIP-sequencing (Illumina Genome Analyser IIx). Integrating our own ChIP-seq data with publically available NOTCH1 ChIP-seq data (Wang et al., PNAS, 2011) showed a remarkable overlap of NOTCH1 and PHF6 binding peaks on a genome-wide scale. We present the first landscaping of the transcriptional program regulated by PHF6 and provide a lead towards unraveling the functional relationship between mutant NOTCH1 and PHF6 loss-of-function in T-ALL in the context of global gene regulation.

Anneleen Beckers

Genomic profiling of MYCN and ALKF1174L transgenic neuroblastoma mouse models provides insights into the dynamic process of tumor formation

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INTRODUCTION: Establishing mouse neuroblastoma (NB) models that faithfully recapitulate human disease is of utmost importance in order to understand the complex biology of the disease in more detail and to offer modalities for pre-clinical in vivo testing of new therapeutic compounds. AIMS AND METHODS: Two new transgenic NB mice models, driven by the MYCN and ALKF1174L oncogene, respectively, were characterized using genome, m(i)RNA-ome profiling to assess how well they recapitulate human NB and can be used for cross genomics analyses. RESULTS: ALKF1174L driven tumors occur with 20% penetrance and appear after 130 days or later. Genomic profiles of four tumors were remarkable in that they represented the diverse spectrum of aberrations observed in human NB tumors, as exemplified by an endogenous MYCN amplification. The TH-MYCN mouse with accelerated tumor formation showed silent array CGH profiles or few genomic imbalances. Interestingly, the number of genomic aberrations was correlated with the time of tumor appearance. Of further interest, the most frequently occurring imbalance in all mouse tumors is gain of chr3, which is often the only imbalance in TH-MYCN mice. Since chr3 contains no syntenic regions implicated in human NB, the significance of this intriguing finding remains to be established. In relation to transcriptome analysis, we analyzed two expression signatures that were established on human NB tumors. High MYC/MYCN miRNA activity score was observed in the TH-MYCN tumors whereas high ALK mRNA activity scores was measured in ALK tumors. Inversely, a mRNA and miRNA signature established on the mice data, did show to correlate with survival in human NB tumors. CONCLUSIONS: Our data shed new light onto the dynamics of genomic alterations in TH-MYCN and ALKF1174L driven NB formation. Furthermore, we show that transcriptional perturbations mimic those observed in human NB thus validating these mouse models for cross species integrated genomics.

Evelien Mets

High-throughput 3'UTR screen identifies a cooperative miRNA network targeting important T-ALL tumor suppressor genes and oncogenes.

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy arising through cooperation between genetic defects affecting proliferation, survival, cell cycle and T-cell differentiation. Multiple protein coding genes with tumor suppressor or oncogenic functions are either linked to specific genomic subgroups or alternatively implicated in all T-ALL genomic entities. Recently, we have identified several miRNAs as part of a cooperative microRNA – tumor suppressor gene network in T-ALL (Mavrakis et al., 2011). In order to further explore the regulatory miRNA network, we performed a 3'UTR screen for five important tumor suppressor and oncogenes that are implicated in T-ALL using a library of 470 miRNA mimic molecules. Repeated screening underscored the reproducibility of the assay and top hits included previously reported bona fide miRNAs for T-ALL. Various predicted as well as non-predicted miRNAs were validated. For each of these miRNAs, correlation analysis of the corresponding target gene and miRNA expression levels was performed in a large data set of 64 primary T-ALL samples, 20 T-ALL cell lines and 9 normal T-cell subsets of 3 human donors representing various stages of normal T-cell development. Integrative mRNA – miRNA expression analyses (http://www.mirnabodymap.org)) revealed putative miRNA functions (Mestdagh et al., 2011). Currently, the role of these newly identified miRNAs, is being assessed both in vitro and in vivo in normal T-cell development and T-ALL oncogenesis. Mavrakis et al., Nature Genetics (2011). Mestdagh et al., Nucleic Acids Research (2011).

Stijn Vanhee

HUMAN PRIMITIVE TYPE BLOOD CELLS ARE GENERATED INDEPENDENT OF NOTCH SIGNALLING IN VITRO.

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Abstract 14

Reinhart Speeckaert

Indoleamine 2,3-dioxygenase, a new prognostic marker in sentinel lymph nodes of melanoma patients

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Background: Indoleamine 2,3-dioxygenase (IDO), an enzyme with immunosuppressive properties is considered as a factor that impairs the antitumour immune response in melanoma. In this study, we investigated the expression of IDO in sentinel nodes of melanoma patients to determine its prognostic relevance. Patients and methods: One hundred and sixteen melanoma patients were enrolled in this study with a median follow-up time after diagnosis of 71 months. The expression of IDO and forkhead box P3 (Foxp3) in the sentinel lymph nodes was determined by immunohistochemistry and correlated with progression-free survival and overall survival. In 42 patients, regulatory T cells were investigated by flow cytometry. Results: Cox regression survival analysis showed a significant negative effect of IDO expression on progression-free survival ($p = 0.015$) and overall survival ($p = 0.010$). High IDO expression was correlated with a significant higher frequency of Foxp3-positive cells in uninvaded lymph nodes ($p = 0.016$). The presence of IDO expression in the sentinel nodes was not associated with an increased frequency of circulating regulatory T cells (Tregs) but was significantly correlated with an increased mean fluorescence intensity of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) in Tregs ($p = 0.019$). After CD3CD28 stimulation, peripheral blood mononuclear cells of patients with high IDO expression showed a lower production of interferon-gamma (IFN-c) ($p = 0.025$). Conclusions: This study points to an independent predictive role of IDO on survival, especially in melanoma patients with uninvolved sentinel nodes. Investigating IDO expression in the sentinel nodes of melanoma patients may be a useful marker to pre-identify patients with a less favourable prognosis in stage I and II disease.

Christian Vanhove

INFINITY: The small animal imaging facility of Ghent University

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Introduction. INFINITY (INnovative Flemish IN-vivo Imaging TechnologY) is the small animal imaging facility of Ghent University. The laboratory houses a collection of state-of-the-art instrumentation for in vivo imaging and therapy in pre-clinical research. Methods. Until the beginning of the twenty-first century, clinical imaging was primarily focused on anatomical imaging, with computed tomography (CT) and magnetic resonance imaging (MRI) as the major imaging technologies. These structural imaging modalities can offer images with exquisite spatial resolution, but they share the limitation of not being able to detect lesions until structural changes are large enough to be seen by these imaging technologies (e.g., tumour growth). Because a lot of diseases initiate as a fundamental change on the cellular or molecular level, rather than a structural change, very sensitive imaging technologies are required that can show cellular or molecular abnormalities present at the very early stage of the disease. Functional imaging modalities using radioactively labelled imaging probes, such as positron emission tomography (PET) and single-photon emission computer tomography (SPECT), offer the potential to detect cellular or molecular changes before lesions are large enough to cause structural changes. During the last decade, all these clinical imaging technologies are translated into small animal imaging systems for in vivo pre-clinical research. Results. A lot of these small animal imaging systems are currently available at INFINITY (μCT, μMRI, μUS, μPET and μSPECT). Next to these in vivo imaging technologies, INFINITY also contains a small animal radiation research platform (SARRP) to deliver image guided conformal radiotherapy to small animals. An important advantage of the laboratory is that all these equipment is available in adjacent rooms. Conclusion. Both fundamental and applied research can benefit from in vivo small animal imaging and therapy. INFINITY offers a wide variety of state-of-the-art pre-clinical equipment for in vivo imaging and therapy of small animals.

wim ceelen

Kinetic modelling of dynamic contrast enhanced MR data: a tool for nonivasive assessment of tumour vascular physiology

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Quantitative magnetic resonance imaging holds considerable promise in functional tumour imaging. Compartmental modelling of dynamic contrast enhanced (DCE)MRI data allows calculation of physiological parameters such as microvascular permeability, flow, and blood volume. In xenograft models, DCE-MRI was used to quantify early response to radiotherapy, anti-angiogenic therapy, and combination therapy. Also, novel macromolecular and targeted MR contrast agents were used to further improve the specificity of the calculated parameters. An overview will be given of previous, current, and future work in this field.

Anneleen Beckers

MiR-137 is epigenetically silenced in MYCN amplified neuroblastomas and targets the polycomb repressive complex 2 (PRC2) component EZH2

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INTRODUCTION: Aberrant epigenetic modifications are important events in the process of tumor formation. Recent studies have shown that epigenetic silencing of tumor suppressor miRNA's can directly add to tumor formation. AIMS AND METHODS: Given the established role of miRNA's in neuroblastoma (NB), we hypothesized that epigenetic silencing of miRNA's would contribute to NB. To investigate this hypothesis, we performed an integrated analysis of miRNA profiles from 14 murine neuroblastomas (TH-MYCN and ALK-driven) and 200 human NB, and MBD-sequencing data marking genome-wide DNA methylation at high resolution in 8 human NB cell lines and 45 primary tumors. RESULTS: Among 523 profiled murine miRNA's, 21 miRNA's were differentially expressed in MYCN-driven versus ALK-driven tumors. For miR-137, we confirmed significant lower expression levels in MYCN-driven human tumors and low expression levels were associated with more aggressive phenotype ($p<0.001$). Interestingly, as observed in other cancer entities, its transcription start site (TSS) was more frequently hypermerhylated in more aggressive human NB tumors and MYCN amplified (MNA) NB cell lines, corresponding with reduced expression of miR-137. This DNA hypermethylation coincided with loss of the activating histon mark H3K4me3 at the level of the predicted TSS in MNA NB cell lines as opposed to non-MNA NB cell lines. Through integration of m(i)RNA expression data and survival data, targets of miR-137 were subsequently prioritized. EZH2, a known target of miR-137 and a core component of the Polycomb Repressor Complex 2, displayed increased expression in more aggressive tumors, matching its recent reported status of oncogene in NB. A similar expression pattern was apparent for AKT2, a major downstream effector of the PI3K pathway, one of the most potent pro-survival pathways in cancer. CONCLUSION: Our results indicate that miR-137 is methylated in MNA NB and that this miRNA may act as a tumorsuppressor through down regulation of EZH2 and AKT2.

Elly De Vlieghere

Myofibroblast-encapsulated microparticles capture free cancer cells from the peritoneal fluid

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Stéphanie Blockhuys

Myosin IIA-dependent collagen type I strap formation and matrix contraction by irradiated breast cancer cells

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Background and purpose: Tumor progression and treatment response are accompanied by extracellular matrix reorganizations. Radiation-induced fibrosis and collagen type I accumulation are adverse effects of radiotherapy. We investigated radiation-induced collagen type I matrix contraction by breast cancer cells. Methods and results: Collagen type 1 matrices allow the visualization and quantification of the dose-dependent increase of collagen strap formation by 6MV X ray exposed breast cancer cells. Western blot analysis indicated an increased myosin IIA expression (decreased myosin IIB expression) and an increased myosin II activity. The role of the actomyosin contractility was further confirmed by (1) genetic inhibition of myosin IIA (MCF-7/6 shMYH9) and (2) pharmacological inhibition of myosin II, F-actin polymerization and cell-matrix interaction by blebbistatin, cytochalasin D and bèta1-integrin antibody respectively. Furthermore, the collagen strap-forming cells are characterized by increased cell plasma membrane ruffling (video-microscopy) and metabolic activity (MTS assay), but not increased invasion capacities (transwell collagen invasion assay). Clinical relevance of our in vitro results is confirmed by an ex vivo experiment, whereby single cells isolated from a breast tumor mammectomy are seeded and irradiated on top of collagen matrix. Conclusions: Irradiated breast cancer cells induce a myosin IIA-dependent collagen type I strap formation and contraction. Our observations suggest that the ECM reorganization after radiotherapy is partly due to RI molecular and functional changes of the cancer cells. Perspectives: We propose the actomyosin system as a possible target for prevention of tumor-associated ECM stiffness induced by the irradiated cancer cells.

Abstract 20

Fjoralba Zeka

Outcome prediction of neuroblastoma patients using microRNA gene expression profiling in both fresh frozen and archived tumor samples

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Introduction: Current risk classification criteria for neuroblastoma patients result in suboptimal classification, contributing to less effective cancer therapy. By developing a miRNA prognostic signature we aim at achieving higher risk stratification accuracy and thus better neuroblastoma survival rates. Methods: 430 mature human microRNAs were profiled in 51 fresh frozen tumor samples upon which a set of 25 prognostic miRNAs was identified. The signature was tested in 179 fresh frozen tumor samples and validated in an independent set of 304 fresh frozen samples and 75 formalin-fixed paraffin-embedded (FFPE) samples. Results: The 25-gene miRNA signature could accurately predict progression-free survival (PFS) and overall survival (OS) ($p<0.0001$) in the test cohort, independently from currently used risk predictors. Patients with increased risk for shorter PFS and OS could also be identified within the high-risk subgroups from the test cohort and the validation cohort. Remarkably, the signature could also predict OS and PFS in the FFPE sample set ($p<0.01$). Conclusions: In this study we present the largest neuroblastoma miRNA expression study so far, including more than 500 neuroblastoma samples originating from fresh frozen primary tumor biopsies and 75 FFPE samples. We established and validated a robust miRNA classifier, able to identify patients with higher risk for adverse outcome within the current high-risk group. Given the successful classification using FFPE material, we are currently collecting large FFPE sample cohorts to allow prognostic classification within current treatment groups. Given the low survival rates and low response to treatment, special attention will be dedicated to identification of ultra high-risk patients in the current high-risk, allowing more accurate patient assignment to new upcoming treatment strategies.

Mieke Van Bockstal

Pathologic features, HER2 protein expression, and HER2 and CEP17 copy numbers in ductal carcinoma in situ.

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Background and methods The reported prevalence of HER2 overexpression and amplification in ductal carcinoma in situ (DCIS) varies considerably, which might partly be due to different assessment methods. To further investigate this issue, we performed both HER2 immunohistochemistry (IHC) and HER2 dual probe FISH analysis, evaluated HER2 and CEP17 copy numbers and correlated these data with histopathological characteristics. In this study, 78 DCIS cases were included and whole mount slides were used for all analyses. Results Using HER2 IHC, 23% of DCIS were scored negative, 18% equivocal and 59% positive. According to HER2/CEP17 ratio, 57% showed HER2 amplification. The amplification status correlated with the IHC score ($p<0.001$). Of all amplified cases, 89% were assigned a positive IHC score, and remarkably, all these cases but one showed HER2 clusters on FISH analysis. Amplified lesions more frequently showed nuclear atypia grade 3 ($p<0.001$), extensive comedonecrosis ($p<0.001$), stromal inflammation ($p<0.001$) and myxoid stromal architecture ($p=0.037$). All lesions with micro-invasion were amplified ($p=0.034$). There was no correlation with patient age, hormone receptor status or other pathological variables. In the amplified group, high-grade nuclear atypia and moderate to extensive stromal inflammation were both associated with a higher mean HER2 copy number ($p<0.001$ and $p=0.007$, respectively) and HER2/CEP17 ratio ($p=0.004$ and $p=0.008$, respectively), while this was not the case in the non-amplified group. CEP17 copy numbers did not correlate with nuclear atypia, nor with stromal inflammation. Conclusions The correlation between HER2 amplification and adverse pathological features, including micro-invasion and high-grade nuclear atypia, underscore that HER2 is a driver of DCIS aggressiveness and possibly of recurrence as non-invasive cancer as well. The prevalence of HER2 overexpression, amplification and cluster formation was much higher than in invasive carcinoma, suggesting that HER2 might play a less important role in transition from DCIS to frankly invasive cancer.

Abstract 22

Karim Vermaelen

Phenotypic, functional and epigenetic modulation of dendritic cells by the murine lung tumor environment

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Although previously considered as a “poorly immunogenic” tumor, investment into immunotherapeutic approaches for lung cancer have surged over the past years. Dendritic cells control the balance between immunity vs tolerance, and we propose that the lung tumoral microenvironment influences the response to immunotherapy by modulating DC biology. We developed an orthotopic mouse model of lung cancer in which we examined the impact of the lung tumor environment on DC’s. We found an enrichment of CD11b+ (inflammatory type) DCs within lung tumors. In sharp contrast to DCs in immediately adjacent peritumoral lung tissue, intratumoral DCs displayed a strong upregulation of the T-cell co-inhibitory molecule PD-L1, are defective in antigen presentation to T-helper cells, and to a lesser extent, cross-priming to cytotoxic T-cells. DCs exposed to lung carcinoma-conditioned medium produced less of the immunostimulatory cytokine IL-12 and more of the immunosuppressive IL-10. In addition, transcriptome profiling of purified tumor-infiltrating DCs unveiled upregulation of genes typically associated with hypoxic response and immunosuppressive tumor-associated macrophages. These results suggest that entire biological networks rather than individual factors are altering tumor-exposed DC’s in this system. miRNAs are short nucleotides that can individually repress whole molecular pathways. Hence, we aimed to explore the hypothesis that unbridled tumor growh relies on a subversion of the anti-tumoral immune response by reprogramming of DCs at the miRNA level. We succeeded at performing large-scale profiling of the miRNA repertoire in DCs extracted from thoracic lymph nodes of tumor-bearing vs healthy lungs. This revealed differential expression of a specific set of miRNAs, depending on whether the DCs have been exposed to the tumor micro-environment or not. Strikingly, lung tumors in CD11c-Cre x Dicer fl/fl animals showed a delayed growth and improved host survival. Our findings unveil an extensive reprogramming of DCs exposed to the lung tumor micro-environment, with acquisition of immune suppressive as well as tumor-supporting features. This is an important message towards efforts to target antigens to endogenous DCs for cancer immunotherapy.

Abstract 23

Sofie De Langhe

Pre-treatment nocturia, radical prostatectomy and TGFβ1 SNPs are associated with radiation-induced nocturia

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Purpose. After radiotherapy for prostate cancer, approximately 50% of the patients experience acute genitourinary symptoms, mostly nocturia. This may be highly bothersome with a major impact on the patient's quality of life. In the past, nocturia is seldom reported as a physiologically-distinct endpoint but as part of a single score consisting of multiple symptoms. It is assumed that in addition to dose-volume parameters and patient- and therapy-related factors, a genetic component contributes to the development of radiation-induced damage. In this study, we investigated the association between dosimetric and clinical factors, TGFβ1 polymorphisms and the development of acute radiation-induced nocturia in prostate cancer patients. Methods and Materials. Data were available for 322 prostate cancer patients treated with primary or post-operative intensity-modulated radiation therapy (IMRT). Five genetic markers in the TGFβ1 gene (-800 G>A, -509 C>T, codon 10 T>C, codon 25 G>C, g.10780 T>G), and a high number of clinical and dosimetric parameters were considered. Toxicity was scored using an in-house developed symptom scale. Results. Radical prostatectomy ($p<0.001$) and the presence of pre-treatment nocturia ($p<0.001$) are significantly associated with the occurrence of radiation-induced acute toxicity. The -509 CT ($p=0.030$) and codon 10 TC ($p=0.001$) genotypes are significantly associated with an increased risk for radiation-induced acute nocturia. Conclusions. Radical prostatectomy, the presence of pre-treatment nocturia symptoms and the heterozygous genotypes of TGFβ1 -509 C>T and codon 10 T>C are identified as factors involved in the development of acute radiation-induced nocturia. These findings may contribute to the research on prediction of late nocturia after IMRT for prostate cancer.

Mieke Van Bockstal

Prognostic significance of morphological and immunohistochemical characteristics in ductal carcinoma in situ of the breast.

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Background The incidence of the pre-invasive lesion DCIS has increased since the introduction of screening mammography. Nowadays, the Van Nuys Prognostic Index (VNPI) is often used as a risk score to predict disease recurrence after breast-conserving surgery. Although the VNPI is a valuable tool, recurrence prediction is still not completely accurate and hence, it could be improved by the identification of additional prognostic markers. Methods In this study, 78 DCIS cases were included. The following histopathological features were analyzed: architecture, nuclear atypia, necrosis, calcifications, stromal inflammation, stromal morphology, lesion size and margin width. Immunohistochemistry (IHC) for the following stromal proteins was performed: alpha-smooth-muscle-actin (SMA), aquaporin-1, caveolin-1, caveolin-2, CD34, CD10, decorin, laminin-beta-2 and necdin. Hormone receptor status was determined, and Her2 IHC and HER2 dual probe FISH analysis were performed and scored according to ASCO/CAP guidelines. Because of lack of follow-up data, nuclear grade was used as a surrogate prognostic marker. Results In univariate analysis, high grade DCIS lesions were significantly associated with the presence of extensive comedo necrosis, myxoid stroma and extensive stromal inflammation. Moreover, high grade lesions more often showed reduced stromal decorin expression. Stromal expression of aquaporin-1, caveolin-1, caveolin-2, SMA, CD34, CD10, laminin-beta-2 and necdin did not correlate with nuclear atypia. Surprisingly, we also noted a remarkable correlation between reduced stromal decorin expression and both HER2 gene amplification and Her2 protein overexpression. In multivariate logistic regression analysis, both stromal inflammation and decorin expression were able to predict nuclear grade. Conclusions Through thorough analysis of several histopathological and immunohistochemical features in DCIS, we demonstrated that reduced stromal decorin expression and extensive stromal inflammation are able to predict high-grade nuclear atypia in DCIS lesions. Both of these pathologic characteristics seem very promising as a prognostic marker in DCIS and warrant further investigation.

Van Troys Marleen

QUANTITATIVE ANALYSIS OF CELL MIGRATION DYNAMICS IN 3D-MATRICES AT HIGHER THROUGHPUT

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Tumor cell dissemination and metastasis involve cell migration within the extracellular matrix, a complex 3D-environment of varying density in which cells move individually or collectively. The search for anti-invasion drugs for improving patient outcome, creates a demand for in vitro assays that allow detailed quantitative analysis of migration dynamics in 3D-matrices and that can be performed at higher throughput. Based hereupon identification of candidate agents with higher physiological relevance are anticipated - more than by using 2D-assays or end point assays. We present an in vitro workflow that covers videomicroscopie, automated image analysis and quantitative analysis of cancer cell migration dynamics in 3D-matrices. Newly, it combines high complexity and content (i.e. migration kinetics of fully matrix-embedded cells) with throughput (96 samples in parallel). 3D-matrix properties (matrix composition/density), treatment regimes and gene expression modulations can be easily applied. Quantitative analysis of the image-based data relies on CELLMIA: newly developed, fully automated software that is highly performant on both unlabelled and fluorescently labeled cells in different 3D-matrices. Within one sample, velocities and/or directionality of cells from large populations of individually and of collectively moving cancer cells are simultaneously extracted. Data quality control and statistical guidelines for in-depth comparison of the multiple tested conditions, have been developed. Validation of the approach (experimental set ups, image analysis, data processing) was done by analyzing the effects of motility-related drugs on the migration of cancer cell lines under different conditions. Output quality in relation to sensitivity, reproducibility and statistical robustness is demonstrated, proving the advantage of the invasion platform for both targeted studies and screening approaches addressing (cancer) cell invasion.

Abstract 26

An Hendrix

Rab27B, an ex(o)citing driver of breast cancer growth, invasion and metastasis

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Cancer cells implement various exocytic routes, modulated by small Rab GTPases, to relay crucial information for fostering growth, invasion and matrix degradation. We investigated the biological role and expression status of Rab27B, a regulator of exosome release, in breast cancer. Rab27B-upregulation in estrogen receptor (ER)-positive breast cancer cells promoted G1/S phase cell cycle transition and increased proliferation, F-actin reorganization and invasion in cell culture, and invasive tumor growth and haemorrhagic ascites in a xenograft mouse model. Proteomics of purified Rab27B vesicles and the secretome of Rab27B-expressing breast cancer cells identified HSP90α as key pro-invasive factor. HSP90α secretion occurred in a Rab27B-dependent manner and was required for MMP-2 activation. Endogenous Rab27B mRNA and protein, but not Rab3D and Rab27A mRNA, associated with lymph node metastasis and differentiation grade in ER-positive breast cancer samples. In conclusion, Rab27B regulates invasive growth and metastasis in ER-positive breast cancer cell lines, and increased expression is associated with poor prognosis in humans. Because of the relationship between Rab27B and cancer progression, elucidating the role of exosomes in metastatic niche formation will be the next step forward in cancer research.

Joke Tommelein

Radiotherapy-induced changes in tumor-associated myofibroblasts: consequences for colorectal cancer progression

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An Van Hecke

Research protocol: Adherence in patients using oral tyrosine kinase inhibitors: development of an intervention to improve adherence

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Background The use of oral chemotherapeutic drugs in cancer management is increasing. Oral tyrosine kinase inhibitors (TKI) are often prescribed for patients with e.g. Chronic Myelogenous Leukemia (CML), breast cancer and gastrointestinal stromal tumors. Adherence to this medication is crucial for treatment success. Previous research by Noens et al. (2009) revealed however that only 14.2% of the CML patients taking TKI were perfectly adherent. No systematic review of determinants and interventions for enhancement of medication adherence is available. Understanding of underlying factors influencing medication adherence and persistence to oral TKI are critical to allow adequate counseling of these patients. Methods/design/results In January 2012 a two-year research project was initiated at the Nursing Science department of Ghent University, funded by the Flemish League against Cancer. The project aims (1) to perform a systematic review of determinants and interventions to enhance TKI adherence / persistence, (2) to provide insight into the factors influencing adherence and persistence to oral TKI in patients with cancer and (3) to develop an intervention to enhance adherence and persistence with this medication. The literature review is based on the Cochrane approach. A qualitative approach, using in-depth interviews will be applied to identify underlying processes leading to (non-)adherence. Current good practices to improve medication adherence/persistence will be observed, focusing on nursing interventions in different settings (hospitals, out-patient settings,…). Based on the results of both interviews and observations, an intervention will be developed based on a model for the development of complex nursing interventions (MRC framework 2008, Van Meijel et al. 2004). Preliminary results of this study will be available by May 2012. Discussion The outcomes of this study will enable us to detect patients at risk and to formulate recommendations to implement the intervention, in order to improve patient adherence.

Piet Ost

Salvage stereotactic body radiotherapy for patients with limited prostate cancer metastases: deferring androgen deprivation therapy

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Aims The management of patients with asymptomatic low-volume metastatic prostate cancer (PCa) remains controversial. We investigated whether repeated Stereotactic body radiotherapy (SBRT) of oligometastatic disease is able to defer the initiation of palliative androgen deprivation therapy (ADT) in patients with low-volume bone and lymph node metastases. Materials and Methods Patients with up to 3 synchronous metastases (bone and/or lymph nodes) diagnosed on positron emitting tomography, following biochemical recurrence after local curative treatment, were treated with (repeated) SBRT to a dose of 50 Gy in 10 fractions. Androgen deprivation therapy-free survival (ADT-FS) defined as the time interval between the first day of SBRT and the initiation of ADT was the primary endpoint. ADT was initiated if more than 3 metastases were detected during follow-up even when patients were still asymptomatic or in case of a PSA rise above 50ng/ml in the absence of metastases. Secondary endpoints were local control, biochemical and clinical progression free survival. Toxicity was scored using the Common Terminology Criteria for Adverse Events. Results: We treated 24 patients with a median follow-up of 24 months. Ten patients started with ADT resulting in a median ADT-FS of 38 months. The 2-year local control, biochemical and clinical progression free survival was 100%, 26% and 42% respectively. Eleven and 3 patients respectively required a second and third salvage treatment for metachronous low-volume metastatic disease. No grade 3 toxicity was observed. Conclusions: Repeated salvage SBRT is feasible, well tolerated and defers palliative ADT with a median of 38 months in patients with limited bone or lymph node PCa metastases.

Katrien Van Impe

Simultaneous control of cancer cell invasion & cell cycle regulation

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The cytoskeleton of eukaryotic cells contains three different types of filaments. Cross-talk between microtubules and actin filaments must occur but the proteins that fulfil these functions have largely remained elusive. CapG is commonly known as an actin binding protein that is upregulated in different types of cancer. CapG is essential for cell motility, as it regulates the growth of actin filaments to generate propulsive force. Using single chain antibodies (nanobodies) as protein antagonists, we discovered a nanobody that inhibits the actin binding and capping activities of CapG. This inhibitory nanobody restrains the migratory and invasive capacities of MDA MB-231 breast cancer cells stably expressing the GFP-tagged inhibitory nanobody. Flow cytometry of these breast cancer cells uniquely revealed a pattern of multinucleated cells, in contrast to MDA MB-231 cells transfected with siRNA targeting CapG. Mass spectrometry analysis of CapG interaction partners in MDA MB-231 cells using the inhibitory nanobody show a specific pattern of tubulin binding in contrast to a control nanobody, suggesting that inhibition of actin binding promotes association with tubulin which possibly explains the cell division defects. In contrast to siRNA, this study with nanobodies has uncovered a new function of the actin binding protein CapG in the regulation of the cell cycle through a fine tuned control of actin and tubulin binding.

Abstract 32

Evi Mampaey

Study of correlation between miRNA-expression and SNPs in genes involved in miRNA biogenesis in primary colorectal tumors

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AIMS: It is hypothesized that the binding between miRNA and target can be affected by single nucleotide polymorphisms (SNPs) residing in the miRNA sequence. The increase or decrease in miRNA binding caused by the SNP would probably lead to a corresponding decrease or increase in the target mRNA translation. Thus, SNPs in miRNA genes could potentially alter various biological processes by influencing the processing and/or targeting selection of miRNAs. In this study we are investigating if there is also a correlation between the variating miRNA-expression in tumor cells and the SNPs which are present. We will examine approximately 800 miRNAs and fourty SNPs divided over eight genes that are contributed to the miRNA biogenesis. A database of 120 patients was made, which provide clinical data (i.e. relapse or not, disease-free survival, KRAS mutation status, tumor stage, differentiation grade…). METHODS: A cohort of 120 patients with primary colorectal tumors is available. We have acces to all the clinical data of these patients, including the KRAS mutation status. miRNA-expression: -Total RNA isolation of tumor and normal tissue: miRNeasy mini kit (Qiagen) -Quantification: NanoDrop -Qualification: Experion -Measure the expression: Stem loop Reverse Transcriptase RT-PCR SNP detection: -DNA-isolation of normal tissue: DNeasy kit (Qiagen) -Quantification & qualification: NanoDrop -Identifying SNPs: RFLP / TaqMan assays / qPCR / HRM RESULTS: We presenting preliminary results of the SNPs in the different samples. CONCLUSION: he clinical data will be correlated with the findings from miRNA en SNPs.

Abstract 33

Dr. Nicolaas Lumen

Surgical outcome of robot-assisted radical prostatectomy after a training program in a high-volume robotic centre.

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Objectives To assess the value of a training program at a high-volume robotic centre for a surgeon starting with robot-assisted radical prostatectomy (RARP). Methods Before starting RARP, two young urologists followed a 6-month training program at a high-volume robotic centre. Surgical outcome of the first 50 RARPs are evaluated and compared with a cohort of the last 50 open radical prostatectomies (ORP) performed by an experienced urologist at the same institution. Tumour stage and grade were similar in both groups. Nerve-sparing (uni-or bilateral) was significantly more performed with RARP. Results Operation time was longer with RARP (231 vs. 179 min; p=0.0004). Hospital stay was similar in both groups (6.2 vs. 6.9 days for respectively RARP and ORP). Catheter stay was significantly shorter (8.6 vs. 15.2 days; p<0.0001) and blood loss was significantly less in RARP (decline of haemoglobin on postoperative day 1: 2.1 vs. 4.05g/dl; p<0.0001). Complication rate was not significantly different among groups but tended to be more severe in ORP. Positive surgical margin rate was 6% and 24% for respectively RARP and ORP (p=0.022). At 12 months follow-up, urinary continence was 81% and 82.9% for respectively RARP and ORP (p=1). Conclusions Training in a high-volume robotic centre can reduce the learning curve. RARP was associated with significantly less positive surgical margins, shorter catheterisation duration and less blood loss compared to ORP.

Eveline Crevits

THE CONFRONTATION WITH CANCER: EXPERIENCES of FRIENDS of ONCOLOGICAL PATIENTS

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Introduction Research has shown that the social network of cancer patients reduces within a few months after diagnosis. In contrast to the patient's perspective, the perspective of friends is barely studied. Methods This qualitative study was based on the principles of grounded theory. Twenty eight semi-structured interviews were held with relatives and friends of oncological patients. The interviews were transcribed and constant comparison was used to analyse the data. Data-collection and –analyses took place in a cyclic process. The study was approved by the Ethics Committee of the participating hospital. Results The (open) attitude of the patient regarding his friends turns out to be crucial in maintaining contact with the patient. The diagnosis of cancer confronts friends of patients with 'fear of death', 'uncertainty about the future perspectives' and 'sadness about the suffering'. The first contact with the patient seems to be easier when the friend is informed about the reaction of the patient on the diagnosis. Friends want to support and comfort the patient. Therefore they feel they can't always talk openly about the cancer or the future. Cancer changes the roles and relations between friends and patients. The relationship becomes more intimate and intense, but at the same time less reciprocal. The patient is mostly in the 'spotlight'. Patients mostly appeal to friends for distraction and entertainment. Activities are always chosen attuned to the possibilities of the patient. Friends often use an informal network to inform each other on the condition of the patient. Distance and the daily hustle and bustle make it difficult to plan visits and activities with the patient. Conclusion Friends are led by the patient in their contact. Interventions could focus on this to remain or create a social network that meets with the needs of the patient.

Abstract 35

Aurélie Van Lancker

The prevalence and control of symptoms in elderly with incurable cancer: instrument development and validation.

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1) Introduction Insight in symptom prevalence and symptom control in the elderly with incurable cancer is lacking. 2) Aim Development and validation of an instrument to collect data on symptom prevalence and symptom control in the elderly with incurable cancer. 3) Method This study consisted of two phases. In the first phase domains and items were identified based on an extensive literature review. The initial set of items was validated by a Delphi procedure including a panel of 11 experts in oncology, palliative care and/or geriatric care. Face- and content validity of the preliminary instrument was assessed. The experts were invited to evaluate all domains and items on clarity of wording and relevance. Relevance was quantified according to the Item Content Validity Index (I-CVI, target value > 0.8). 4) Results The instrument to collect data on symptoms contained 58 items and enables to measure frequency and intensity of (1) physical, (2) psychological, (3) social, and (4) existential symptoms. The instrument to collect data on interventions consisted of 86 items and has a focus on eight symptoms: (1) pain, (2) nausea, (3) vomiting, (4) dyspnoea, (5) obstipation, (6) cachexia, (7) fatigue, and (8) depression. The I-CVI of the included items was > 0.8 and resulted in a final inclusion of 40 items in each instrument. To assess clarity of wording for the aimed population, the validated instrument on symptoms was pilot tested in a sample of ten geriatric patients. 5) Conclusion This study resulted in two validated instruments, which will be used to assess symptom prevalence and symptom control in the elderly with incurable cancer. These instruments will be used to acquire knowledge about frequency and intensity of symptoms and interventions aimed at controlling these symptoms.

Mireille Van Gele

The three-dimensional human reconstructed skin model: a tool to study RNAi-induced depigmentation and melanoma invasion

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Because current skin whitening products often have insufficient efficacy and side effects, effective and safe therapeutics are warranted. We established a human pigmented full-thickness skin model as a first step in the development of novel siRNA-based depigmenting agents. Histological characterization revealed that our model had a similar morphology as normal human skin, expressed keratinocyte differentiation as well as basement membrane markers, and showed a high degree of pigmentation. A correct deposition of melanocytes into the basal layer was confirmed by performing immunohistochemical stainings with the melanocyte-specific marker Melan-A. The utility of the model to study RNAi-induced depigmentation was validated by incorporation of melanocytes transfected with siRNA against tyrosinase, a key enzyme in skin pigmentation. This resulted in a strong reduction in pigmentation and inhibition of melanin transfer proving that siRNA-mediated gene silencing in melanocytes worked successfully in our model. Therefore, our established 3D skin model will be a useful and easy tool to validate the whitening potential of candidate genes with a presumed function in melanin synthesis or transfer. In addition, our results stress the importance of self-made human skin models as research tools in order to obtain RNAi-based 'proof-of-principles', which is a necessary step before evaluating the biological effect of topical applied siRNA-carriers onto skin in vitro or in vivo. Interestingly, replacing normal melanocytes with melanoma cells results in a melanoma skin reconstruct. Such a model allows to investigate cell-matrix and cell-cell interactions between different cell types, changes which also occur during melanoma progression. We will illustrate that skin reconstructs are efficient tools to study not only transformation mechanisms of melanocytes, but also invasion of melanoma cells. We conclude that 3D reconstructed skin models are valuable models bridging the gap between in vitro and in vivo studies.

Candy Kumps

Transcriptome analysis following ALK abrogation provides insights in the downstream signaling pathways in human and murine neuroblastoma cells

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Activating ALK mutations are present in almost 10% of neuroblastomas (NB) and serve as new therapeutic targets for treatment. We analyzed aberrant ALK downstream signaling to identify vulnerable nodes that might offer novel targets for combined pharmacological therapy. Differential gene expression analysis in a panel of NB cell lines (n=10) before and after small molecule ALK inhibition allowed delineation of a 150-gene signature representative for high ALK activity in NB. This signature, which was correlated with poor prognosis in NB patients, was significantly enriched for genes implicated in PI3K/AKT/mTOR and MAPK/ERK signaling. Additionally, we identified several robustly ALK deregulated genes implicated in neuronal differentiation, growth control or affecting MYCN transcriptional activity either through stabilization of the protein or by interfering with MYCN target binding. Using a cross-genomics analysis, this signature could be validated in an ALKF1174L transgenic murine model of NB. In this study, we obtained for the first time a detailed picture of the transcriptional consequences of sustained ALK signaling in human NB cells, which could be recapitulated in an ALK-driven NB mouse model.

Gwen Sys

Tumour grafts derived from sarcoma patients retain tumour morphology, viability, invasion potential, and indicate disease outcome in the chick chorioallantoic membrane model

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The chick chorioallantoic membrane (CAM) assay was used to evaluate whether xenotransplanted sarcomas retained biochemical and functional characteristics of the original tumours. Metabolically active tumour tissue from 28 patients with a bone or soft-tissue tumour was applied on the CAM. Angiogenesis, graft- (viability, necrosis, infiltration) and host (fibroblast infiltration, vascular ingrowth) behaviour were evaluated. We found that essential features and immunohistochemical characteristics of the original tumours were maintained, illustrating the diversity of sarcoma. We suggest using the xenograft CAM assay to generate a sarcoma study population that could be profiled for prognostic assessment and randomized for prospective treatment with targeted agents.

Kaat Durinck

Unraveling a NOTCH1-lncRNA-miRNA regulatory network in acute T-cell lymphoblastic leukemia and normal T-cell development

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Introduction: NOTCH1 acts as a central player in oncogenesis and is mutated in >50% of T-cell acute lymphoblastic leukemia (T-ALL) patients. In this study, we aim to expand our knowledge on the transcriptional network controlled by NOTCH1 towards long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). Methods: Pharmacological inhibition of NOTCH1 signaling was applied in four T-ALL cell lines and the transcriptional response of all protein coding genes and 8000 lncRNAs was assessed using gene expression microarrays (Agilent). In parallel, lncRNA expression profiles were established for two sorted normal T-cell progenitor populations derived from CD34+ thymus cells cultured either with or without NOTCH1 stimulation using OP9-DL1 or OP9-GFP feeder layers. Finally, a top-50 ranked list of highest expressed lncRNAs in eight different T-ALL cell lines was generated upon qRT-PCR profiling of 1250 lncRNAs (Biogazelle). In addition to lncRNAs, the miRNAome (756 miRNAs) for all experimental conditions as well as nine distinct sorted subsets of normal developing T-cell populations and 20 T-ALL cell lines were profiled using high-throughput stem-loop qRT-PCR (Applied Biosystems). Results: Using a unique integrated experimental approach, we were able for the first time to identify a subset of lncRNAs and miRNAs acting downstream of the NOTCH1 signaling cascade with a presumed function in both normal and malignant T-cell development. A subset of these candidate lncRNAs will be further evaluated for their physiological role in vitro by means of overexpression or siRNA-mediated knockdown in the appropriate model systems. Conclusion: We performed the first landscaping of an integrated network of lncRNAs and miRNAs acting downstream of the NOTCH1 signaling pathway in T-ALL and normal T-cells. These data pave the way towards development of novel therapeutic strategies impacting on hyperactive NOTCH1 signaling.

Janneke Ronse

Which Care Do Patients With Breast Cancer Want?

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Background : It is often assumed that cancer patients suffer from a variety of psychosocial problems. Evidence about the effect of psychosocial care is inconclusive. There is a lot of funding for different programs. The reasons why patients do or do not participate at a particular program are not yet understood. Methods: A literature review on the determinants of participation was conducted. Next, an observational study was done. Participation, demographic and disease variables were registered for 191 patients. Analysis were done with SPSS16. At the same time, 46 women with breast cancer were interviewed for a qualitative research, using grounded theory methods. Results: The literature review showed few obvious determinants of participation. Only a problem solving coping style, believe in the effect, believe in the positive attitude of important others, disease specific distress, referral from a central caregiver, have a positive relation with participation. The observational study showed that the determinants of participation like familial situation, type of surgery, matter for other psychological caregivers, but disappear for the breast care nurses. The qualitative research shows that the diagnosis of cancer means chaos. The option of therapy offers hope. Patients adopt a positive attitude that they try to maintain. They expect their caregivers to support this attitude. Because of this, patients appreciate their breast care nurse. The idea of a psychologist reminding them of their difficult emotions, makes them hesitate to consult. Conclusion: Since it difficult to identify clear determinants of participation, screening remains difficult. Therefore the central caregiver of the patient can make the most adequate judgement about the need for psychosocial care and the risk for non-participation. In order to do this, this caregiver should build a relation of trust with the patient. Because breast care nurses are very accessible they are appropriate central caregivers.

Abstract 41

Pieter Mestdach

Exploratory tools to study non-coding RNA in cancer

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Over the last years, non-coding RNAs (e.g. microRNAs and long non-coding RNAs) have emerged as an important layer of the transcriptome. In order to elucidate their role in cancer biology, multiple tools have been developed. In this presentation, I will discuss different approaches on how to explore the non-coding RNA landscape in cancer using high-throughput genomics and transcriptomics, integrative data-mining strategies and functional genomics.

The tumor ecosystem : a source for therapeutic targets and biomarker discovery

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Background: Invasion, the hallmark of malignancy, occurs within an ecosystem where a continuous communication exists between cancer cells and a wide network of tumor-associated stromal cells. Kindlin-1, an epithelial-specific regulator of integrin functions, is responsible of Kindler syndrome, a genetic disorder characterized by skin blistering, atrophy and photosensitivity. Despite expanding interest in understanding kindlin-1 functions, its biological implication in cancer remains unknown.
Methods: Kindlin-1 expression was quantified in several human cancer types using quantitative realtime polymerase chain reaction and published microarray datasets. Kindlin-1 levels were analyzed by immunohistochemistry in breast tumors and matching distant metastases. The correlation between kindlin-1 expression and patient outcome was assessed with Kaplan–Meier analyses. Breast tumor growth and lung metastasis were evaluated in a syngeneic mouse model. Effects of ectopic expression or silencing of kindlin-1 on cell signaling, cell proliferation, migration and invasion were assessed in human breast cancer cell lines. Statistical tests were two-sided.
Results: Kindlin-1 is a prognostic indicator in breast and lung adenocarcinomas (log-rank test, p =.001 in both cancers). Overexpression of kindlin-1 induced changes characteristic of epithelial-mesenchymal transition through TGFβ signaling, down-regulation of E-cadherin, up-regulation of slug and twist *CDH1* repressors, and constitutive activation of cell proliferation, motility, and invasion. Consistently, kindlin-1 depletion in an orthotopic mouse model significantly inhibited breast tumor growth and lung metastasis.
Conclusion: Our findings identify kindlin-1 as a key regulator of breast cancer lung metastasis and lung tumorigenesis. The role of kindlin-1 in cancer progression likely extend beyond breast cancer, since its overexpression was observed in many epithelial cancers. Our data advance our understanding of kindlin-1 as a regulator of TGFβ/Smad signaling. Further clinical validation may reveal the therapeutic potential and prognostic role of experimentally validated pro-invasive molecules.

Abstract 43

Sofie Bekaert

BIMETRA

Bimetra is the Clinical Research Center of Ghent University Hospital and Ghent University that wants to reinforce their leading position, acting as a catalyst for translational research from bench to bedside and from bedside to community and as a central point of contact to integrate:

1. better understanding of diseases related to immunology, oncology, neurosciences and cardiovascular/metabolic disorders besides others.

2. enhanced diagnostics, improved prevention methodology, targeted therapies

and hence leveraging personalized and individualized medicine.

Bimetra is organized around 5 facilitating platforms from research& valorization facilitation (funding application, contracting and IP) over biobanking and translational data management, to experimental clinical research.

During a short presentation an overview is given of the main facilitation activities of Bimetra.