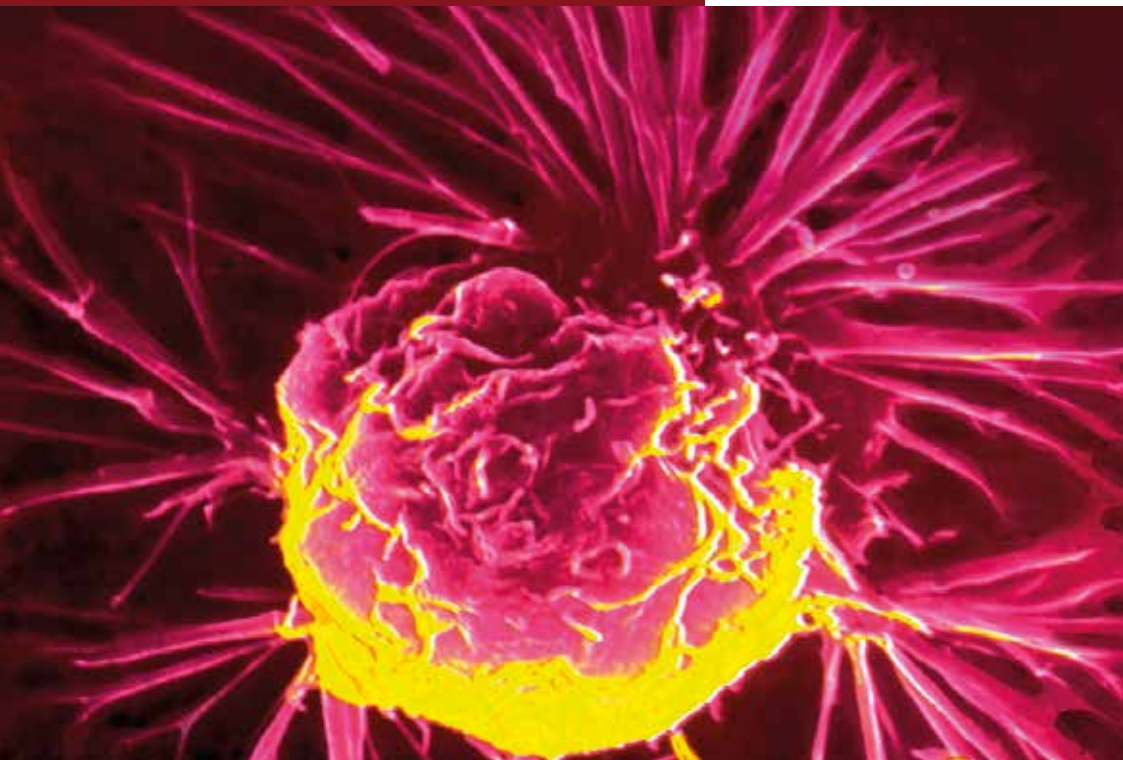# 18th ANNUAL BSMO MEETING 2016

BSMO

Programme and abstracts

Friday 26 February and Saturday 27 February 2016

Golden Tulip Brussels Airport Diegem

www.BSMO.be

INTRODUCTION

Dear colleague,

Welcome to the 18th Annual Meeting of the BSMO, a unique annual opportunity to gather as a community and exchange ideas and engage in cooperation. These are exciting but also challenging times for the medical oncologist with quickly evolving treatments and getting treatments in a timely fashion to the right patients.

The Friday morning meeting is dedicated to the BSMO Breast Cancer Task Force and the afternoon to the BSMO Gynaecological Cancer Task Force. The general meeting is on Saturday morning. Everybody is welcome at these task force meetings.

On Saturday morning the symposium relates to several aspects of our profession with a balanced clinical and scientific mix. It is these scientific insights combined with compassionate patient care that make the medical oncologist.

On behalf of the board, we would like to wish you a fruitful meeting!

Jacques De Grève, BSMO President

08.30   **Registration**

09.30 - 13.00 BSMO Breast Cancer Task Force Meeting
Chairs: Ahmad Awada, Institute Jules Bordet, Brussel & Hans Wildiers, UZ Leuven

- Neoadjuvant dual HER2 blockade
- Platinum in triple negative breast cancer
- Endocrine therapy and biological therapy in ER+ metastatic breast cancer

11:00   **Break**

Discussion on guideline adaptations and new projects

13:00   **Registration other delegates & lunch**

13:30   **Welcome**

14:00 - 17:00 BSMO Gyneacological cancer task force
Chairs: Peter Vuylsteke, CHU UCL Namur & Wim Demey, AZ Klina, Antwerpen

14:00   **Systemic treatment of ovarian cancer**
Hannelore Denys, UZ Gent

14:30   **Chemotherapy and pregnancy**
Kristel van Calsteren, UZ Leuven

15:00   **Hereditary aspects**
Jacques De Grève, UZ Brussel

15:30   **Break**

16:00   **Rare ovarian cancers**
Luc Dirix, GasthuisZusters, Iridium kankernetwerk Antwerpen

16:30   **Cervix Cancer: Locally advanced and recurrent/metastatic disease**
Christine Gennigens, CHU Liège

17.00   **Meeting closure**

17.30   **BSMO General Assembly 2016 (members only)**

19:00   **Reception and dinner**

08.30 - 12.30 BSMO 18th Annual Meeting

08.30   **Registration and coffee**

08.55   **Introduction**
Jacques De Grève, BSMO President

09:00   **Oncology Paper of the year 2016 presentation & award**
Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA)
Chair: Jolanda Verheezen, Sint-Trudo ziekenhuis, Sint-Truiden
Speaker: Hans Wildiers, UZ Leuven

09:30   **MSD Oncology Award**
Vibeke Kruse, UZ Gent

09:35   **Oral abstracts**
Chairs: Hans Prenen, University Hospitals Gasthuisberg, Leuven & Joelle Collignon, CHU Liège

O.1 - The immune infiltrate in colorectal liver metastases depends on the histopathological growth pattern
Laure-Anne Teuwen, Antwerp University

O.2 - Interrogating the impact of pregnancy on breast cancer (BC) genomics using DNA copy number profiling
Bastien Nguyen, BCTL, Brussels

O.3 - Deciphering the genetic background of high-risk BRCA 1/2 mutation-negative breast cancer patients
Cedric Van Marcke, Cliniques universitaires St. Luc, Brussels

O.4 - Efficacy of an enzyme-activated doxorubicin prodrug in patient-derived dedifferentiated liposarcoma and synovial sarcoma xenograft models
Jasmien Cornillie, UZ Leuven

O.5 - PLX9486 Shows anti-tumor efficacy in a patient-derived gastrointestinal stromal tumor (GIST) xenograft model resistant to standard tyrosine kinase inhibitors (TKI)
Jasmien Cornillie, UZ Leuven

O.6 - Evaluation of subclonality in the CTC and DTC compartment of patients with metastatic breast cancer using DEPArray sorting and AmpliSeq panel sequencing
Anja Brouwer, Antwerp University

O.7 – Presence of tumor-specific cytolytic T cells in human primary breast carcinoma
David Schröder, de Duve Institute, Brussels

O.8 - The function of the Hippo pathway effector YAP in and around liver cancer
Laura van den Mooter, UZ Leuven

O.9 - Reproductive behaviors and risk of developing breast cancer according to subtype. A systematic review and meta-analysis
Matteo Lambertini, Institut Jules Bordet, Brussels

O.10 - Inflammatory breast cancer cells show a particular pattern of canonical and non-canonical TGFß signaling, possibly affecting cancer cell motility
Charlotte Rypens, Antwerp University
O.11 - Patient-derived xenograft (PDX) models of soft tissue sarcoma (STS) - a preclinical platform for early drug testing
Agnieszka Wozniak, KU Leuven
O.12 - The impact of an interventional counseling procedure in families with a BRCA1/2 gene mutation: efficacy and safety
Erica Sermijn, University Hospital Brussels

11:05	**Coffee break**
11:30	**What you should not have missed the last year** *Chair: Evandro de Azambuja, Institute Jules Bordet, Brussels* *Speaker: Luc Dirix, GasthuisZusters, Iridium kankernetwerk Antwerpen*
12:30	**Meeting closure & lunch**

ABSTRACTS INVITED SPEAKERS

BSMO Gyneacological cancer task force

14:30 Chemotherapy and pregnancy
*Kristel Van Calsteren, UZ Leuven*

Department of Development and Regeneration, KULeuven
Division feto-maternal medicine, University Hospital Gasthuisberg Leuven

Cancer is diagnosed in 1 in 1000 to 2000 pregnancies. Most frequently diagnosed are breast cancer, hematological malignancies and cervical cancer. The complex medical, ethical, psychological and religious issues arising in pregnant women with cancer demand care from a multidisciplinary team.

Based on current knowledge, the administration of certain chemotherapeutic agents is possible from 14 weeks gestational age onwards. The placental barrier function partly protects the fetus. Chemotherapy after 35 weeks is not recommended as an interval of at least 3 weeks is preferred between chemotherapy administration and delivery. Recent data show that the long-term outcome of children antenatally exposed to chemotherapy is comparable to non-exposed children of the same age. Nevertheless, a higher rate of neurodevelopmental problems was encountered after preterm birth, also in this selected patient population. A subgroup of patients receiving chemotherapy seems to be at increased risk for IUGR and preterm contractions.

The physiologic gestational changes reduce the maternal serum levels of chemotherapy, although the pharmacodynamic impact remains unknown.
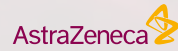
16:30 Cervix Cancer: Locally advanced and recurrent/metastatic disease
Christine Gennigens, CHU Liège

Cervical cancer is the fourth most common cancer worldwide and the fourth cause of cancer death, while it remains the second most common cancer in developing regions. In 2011, an estimated 530,000 cases of cervical cancer were diagnosed worldwide, with 275,100 deaths.

This global burden is attributable to the disproportionately high incidence of cervical cancer in developing, low-income countries lacking adequate healthcare infrastructure and screening programs.

Despite advances in screening, vaccination and treatment of early stage disease, a proportion of patients will be diagnosed with advanced stage, recurrent or persistent cervical cancer.

The treatment of cervix cancer can be divided in 3 parts (early stage/locally-advanced/recurrent or metastatic disease) ; my presentation will focus on locally advanced (LACC) and recurrent/metastatic disease.

The gold standard treatment for LACC is chemoradiotherapy followed by brachytherapy. How can we increase survival ? Strategies include: modified concomittant CT-RT ? ; para-aortic pretherapeutic staging ?; neo- / adjuvant CT ? ; hysterectomy after CT-RT ? ; ...

Between 15-61% of patients will present persitent or will develop recurrent disease within the first two years following completion of primary treatment. These patients are currently treated with platinum-based chemotherapy, but their prognosis is poor. This has prompted researchers to look for new drugs, such as anti-angiogenic agents. Significant improvement in OS was found with the addition of bevacizumab (GOG-240) to a chemotherapy backbone (17,0 vs 13.3 months).

Even with the results shown in GOG-240, the prognosis for patients with recurrent or metastatic cervical cancer remains poor and represents an urgent unmet need worldwide. Therefore, better alternatives to conventional therapy must be explored, including novel approaches such as immunotherapy. The rationale for immunotherapy is based on the causative role of HPV infection in this disease.

BSMO 18th Annual Meeting

09:00 Oncology Paper of the year 2016 presentation & award
Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA)
Wildiers H., Krop I., Kim S., González-Martín A., Lorusso P., Ferrero J., Smitt M., Yu R., Leung A., on behalf of the TH3RESA study collaborators; (2014);

Methods This randomised, open-label, phase 3 trial included patients (≥18 years, left ventricular ejection fraction ≥50%, Eastern Cooperative Oncology Group performance status 0–2) with progressive HER2-positive advanced breast cancer who had received two or more HER2-directed regimens in the advanced setting, including trastuzumab and lapatinib, and previous taxane therapy in any setting. Patients were randomly assigned (in a 2:1 ratio) to trastuzumab emtansine or physician's choice. Coprimary endpoints were investigator-assessed progression-free survival (PFS) and overall survival in the intention-to-treat population. We report the final PFS analysis and the first interim overall survival analysis. This study is registered with ClinicalTrials.gov, number NCT01419197.

Findings From Sept 14, 2011, to Nov 19, 2012, 602 patients were randomly assigned (404 to trastuzumab emtansine and 198 to physician's choice). At data cutoff (Feb 11, 2013), 44 patients assigned to physician's choice had crossed over to trastuzumab emtansine. After a median follow-up of 7,2 months in the trastuzumab emtansine group and 6,5 months in the physician's choice group, 219 (54%) patients in the trastuzumab emtansine group and 129 (65%) of patients in the physician's choice group had PFS events. PFS was significantly improved with trastuzumab emtansine compared with physician's choice (median 6,2 months vs 3,3 months; stratified hazard ratio [HR] 0,528; p<0 0001). Interim overall survival analysis showed a trend favouring trastuzumab emtansine (stratified HR 0,552; p=0 0034), but the stopping boundary was not crossed. A lower incidence of grade 3 or worse adverse events was reported with trastuzumab emtansine than with physician's choice (32% vs 43%).

Interpretation Trastuzumab emtansine should be considered as a new standard for patients with HER2-positive advanced breast cancer who have previously received trastuzumab and lapatinib.

O1 - The immune infiltrate in colorectal liver metastases depends on the histopathological growth pattern

M.N. Teuwen[1], K. Marien[1], K. Schats[1], P.J. Van Dam[1], H. Nystrom[1], A. Reynolds[2], S. Van Laere[1], L. Dirix[1], M. Kockx[3], P. Vermeulen[1]

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Objective: To characterize the immune infiltrate by immunohistochemistry in colorectal liver metastases with different histopathological growth patterns (HGP)
Background: Liver metastases grow in distinct histopathological patterns. In the desmoplastic HGP, tumor cells are separated from liver parenchyma by desmoplastic stroma that contains new blood vessels that result from sprouting angiogenesis. In the replacement HGP, tumor cells replace hepatocytes, thereby recruiting sinusoidal blood vessels as a means of blood supply. In order to successfully metastasize, disseminated cancer cells must adapt to and interact with foreign tissue micro-environments and find a way to avoid the antitumor host immune response. This can be achieved by escaping or suppressing the immune response. We hypothesize that the mechanism of immune avoidance could be related to the histological growth pattern of liver metastases.

Methods: Ten tissue microarrays with 20 cores each, originating from 67 unique patients with primary colorectal or breast cancer were stained for CD8 (cytotoxic T cells), CD20 (B cells), CD163 (macrophages), programmed cell death ligand 1 (PD-L1) and vascular endothelial growth factor-A (VEGF-A). Protein expression signals were quantified in four regions of interest (tumor center, Invasive margin at ?tumor side, invasive margin at ?normal liver side, adjacent normal liver).

Results: An average of 34% of the included cores showed a desmoplastic, 25% a replacement and another 25% a mixed HGP. The remaining cores included only normal liver tissue. We found significantly higher staining of CD8 in samples that showed a desmoplastic HGP compared to these with a replacement HGP (p < 0,05). Similarly, CD20 staining was increased in desmoplastic metastasis, especially at the invasive margin (p < 0,05). For CD163, staining was higher throughout the complete tumor region (p < 0,05). There was no significant difference between the two growth patterns in VEGF-A expression, nor for PD-L1 positivity in immune cells.

Conclusion: We found significantly higher amounts of cytotoxic T cells, B cells and macrophages in liver metastases that show a desmoplastic HGP compared to those with a replacement HGP. These results imply that the histological growth pattern of liver metastasis influences the tumor's immune invasion strategies.

O2 - Interrogating the impact of pregnancy on breast cancer (BC) genomics using DNA copy number profiling

B. Nguyen[1], D. Brown[2], G. Pruneri[3], S. Majjaj[2], F. Peccatori[4], C. Sotiriou[2], A. Azim Jr[2]

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[3]European Institute of Oncology, Milan, Italy
[4]Division of Gynecologic Oncology, European Institute of Oncology, Milan, Italy

Background: Epidemiological evidence indicates a clear relationship between pregnancy and BC risk, yet little is known regarding the impact of pregnancy on BC biology. DNA copy number aberrations (CNA) play an important role in breast carcinogenesis. BC during pregnancy is a rare disease, yet it could serve as a good model to study the impact of pregnancy on BC biology.

Methods: We used a dataset of 54 pregnant and 113 non-pregnant BC patients matched for age and stage with complete clinicopathological, gene expression and 5-year follow-up data (Azim et al; 2014). The samples were profiled using Affymetrix OncoScan FFPE arrays to evaluate DNA CNA. We used a logistic regression model to identify CNAs associated with pregnancy. We then identified genes whose expression were associated with CNAs and evaluated their impact on disease free survival (DFS) through Cox proportional-hazards models. P-values were adjusted for multiple testing.

Results: After quality controls, CNA profiles were obtained for 38 pregnant and 87 non-pregnant BC patients. We found 61 CNAs associated with pregnancy ($p<0.05$), 27 of which, were further associated with the expression of their corresponding gene (FDR<0.05). Of particular interest, we found a significantly higher prevalence of 16q23.3-16q24.3 region gain/amplification in the pregnant cohort compared to the control group (18,4% vs. 3,4%, p=0.02). The gene dosage effect was associated with the expression of 61 genes across the chromosome 16q arm (FDR<0.05). Of those, we found two oncogenes, CDT1 and GINS2, whose high expression was associated with poorer prognosis in pregnant (HR=4.57, p<0.05, and HR=4.10, p<0.01 respectively) but not in the control group (HR=1.26, p=0.67 and HR=0.71, p=0.39).

Conclusions: By combining gene expression and CNA profiles, we found relevant genomic alterations associated with pregnancy. More data will be presented during the conference on other chromosomal regions, the related gene expression changes and their associations with prognosis. This could further elucidate the impact of pregnancy on BC risk.

O3 - Deciphering the genetic background of high-risk BRCA 1/2 mutation-negative breast cancer patients

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Background: Breast cancer is the most frequent malignant disease and the second cause of cancer death among women. Approximately 10% of the cases are considered hereditary, following an autosomal dominant inheritance pattern. Among these, about 30% are attributed to germline defects in the tumor suppressor genes BRCA1 and BRCA2. Different technologies enabled to identify several other breast cancer predisposition genes of high, moderate or low penetrance. It is however considered that these variants only explain 40% of the inherited risk of breast cancer.
We used whole-exome sequencing to investigate BRCA 1/2-negative high-risk familial breast cancer patients, aiming to identify their patterns of genetic variation within the breast cancer predisposition genes.

Methods: Germline exomes of 48 breast cancer patients with strong familial history were sequenced on a 5500 SOLiD(™) System. Variants were called within a panel of 236 genes already associated to cancer or to DNA repair. Synonymous variants and variants with minor allele frequency in a default global population above 1,5% were discarded.
Candidate variants were validated with Sanger sequencing. Familial validation through co-segregation with the disease is ongoing.

Results: Quality and gene-panel filtering could narrow the list of variants to 308 from the 3.628.494 identified. Of these, 123 were kept after alignment validation and literature study. 116 were validated by Sanger sequencing. Out of them, 11 variants affected a splice site region and 105 resulted in an amino acid substitution. Nineteen variants were known in the COSMIC database.
Most patients (46 out of 48) had at least one validated variant; mean number of variants per patient was 2.1 (range: 0 to 5). Each variant was found in 1 to 5 patients (mean 1.4). Variants were detected in the known high-to-moderate penetrance breast cancer susceptibility genes ATM, PALB2, MSH2, MSH6, PMS2, RAD51C and MRE11A for 17 patients. Co-segregation has so far been revealed for altogether 25 variants.

Conclusion: In these high-risk BRCA 1/2 mutation-negative breast cancer patients, massively parallel whole exome sequencing enabled us to detect several germline variants in genes linked to breast cancer or related to DNA repair. Further segregation analysis is needed to corroborate their clinical significance.

O4 - Efficacy of an enzyme-activated doxorubicin prodrug in patient-derived dedifferentiated liposarcoma and synovial sarcoma xenograft models

J. Cornillie[1], A. Wozniak[1], L. Vreys[1], Y. Gebreyohannes[1], J. Wellens[1], U. Vanleeuw[1], P. Pokreisz[2], A. Cassazza[2], D. Hompes[3], M. Debiec-Rychter[4], R. Sciot[5], P. Schöffski[1]

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[5]Department of Pathology, KU and UZ Leuven, Leuven, Belgium

Background: Dedifferentiated liposarcoma (DDLPS) and synovial sarcoma (SynSa) are aggressive mesenchymal tumors with limited sensitivity to the standard-of-care treatment doxorubicin (doxo). We evaluated the efficacy of PhAc-ALGP-doxorubicin (ALGP-doxo), a prodrug that is metabolized to doxo by peptidases in the tumor microenvironment and/or tumor cells, in two DDLPS and one SynSa patient-derived xenograft.

Methods: NMRI mice (n = 24 for each model) were transplanted subcutaneously with human DDLPS (models UZLX-STS3 and -STS5) or SynSa (UZLX-STS7) and randomized as follows: control (vehicle), doxo (total dose of 0.03 mmol/kg for UZLX-STS3; 0.04 mmol/kg for -STS5 and -STS7) or ALGP-doxo (1.2 mmol/kg). Treatments were administered via intraperitoneal pumps, continuously releasing drugs for 7 days. Half of the mice were sacrificed after 7 days of treatment; the remaining animals were monitored for another 14 days. Treatment efficacy was assessed by tumor volume, histology, immunohistochemistry for markers of proliferation (phospho-histone H3, Ki-67) and apoptosis (cleaved caspase 3), and Western blotting (WB). Statistical analysis was performed with Wilcoxon and Mann-Withney U-test.

Results: Tumor volume in the control and doxo group increased steadily, while ALGP-doxo caused tumor volume stabilization in UZLX-STS3 and -STS5. The average relative tumor volume under ALGP-doxo in UZLX-STS7 decreased to 60.1% on day 7 (p < 0.001) and 4.6% on day 21 (p = 0.016) as compared to baseline. The prodrug induced a significant decrease in proliferation and increase in apoptosis on day 7 in all models. This was also observed on day 21 in UZLX-STS3 and -STS5, while in -STS7 the analysis was impossible as tumor tissue was replaced by fibroblasts. pHH3 decrease in ALGP-doxo treated UZLX-STS5 and -STS7 tumors was confirmed by WB.

Conclusions: ALGP-doxo shows considerably higher antitumor activity than doxo in patient-derived DDLPS and SynSa xenografts. The delivery of a 30-40 fold higher dose of ALGP-doxo than doxo is tolerated, while a comparable dose of doxo is lethal. These results warrant further testing in anthracycline-sensitive and -resistant preclinical models of human malignancies.

O5 - PLX9486 Shows anti-tumor efficacy in a patient-derived gastrointestinal stromal tumor (GIST) xenograft model resistant to standard tyrosine kinase inhibitors (TKI)

Y. Gebreyohannes[1], A. Wozniak[1], T. Van Looy[1], J. Wellens[1], J. Cornillie[1], G. Habets[2], J. Lin[2], A. Marimuthu[2], M. Debiec-Rychter[3], R. Sciot[4], P. Schöffski[1]

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Objective: GIST is mainly driven by activating mutations in the KIT gene, leading to the constitutional activation of KIT kinase and its downstream signaling pathways. Imatinib (IMA) or sunitinib are TKI, which are highly effective in GIST patients but with time resistance develops, mainly through secondary mutations affecting the activation loop of the KIT receptor, encoded by exon 17. In the present study we tested the in vivo efficacy of PLX9486, a TKI targeting primary KIT mutations as well as secondary changes affecting exon 17 of the KIT gene in a TKI-resistant GIST xenograft model.

Methods: NMRI nu/nu mice (n=23) were transplanted bilaterally with human UZLX-GIST9 (KIT p.P577del;W557LfsX5;D820G, passage 10) xenografts. Mice were divided into three treatment groups: vehicle [NMP: diluent (1:10 v/v); n=7], IMA (100 mg/kg/qd; n=7), PLX9486 (100 mg/kg/qd; n=9), and dosed orally for four weeks. Efficacy was assessed by tumor volume measurement (3x/week), histopathology [hematoxylin & eosin staining (H&E)], immunohistochemistry [phospho-Histone H3 (pHH3), cleaved PARP, phospho-MAPK (pMAPK)] and Western blotting for KIT signaling pathways. Mann Whitney U test was used for statistical analysis, p values <0.05 were considered significant.

Results: In this PDX model, PLX9486 resulted in tumor volume stabilization, while IMA treated xenografts continued to grow. PLX9486 treatment significantly reduced the mitotic index on H&E (>50 fold decrease in comparison to control and IMA, p<0.0001), which was confirmed by pHH3 immunostaining. PLX9486 led to a pronounced decrease in MAPK activation, and other downstream proteins of the KIT signaling pathway though there were no effects on KIT phosphorylation. All these effects were much more prominent than in IMA treated tumors. Mice appeared to tolerate all treatments well.

Conclusions: PLX9486 showed anti-tumor efficacy in a TKI-resistant GIST xenograft model, mainly through inhibition of proliferation. These and other preclinical efficacy and safety data warrant further testing of PLX9486 in the clinical setting.

O6 - Evaluation of subclonality in the CTC and DTC compartment of patients with metastatic breast cancer using DEPArray sorting and AmpliSeq panel sequencing

A.J. Brouwer[1], D.J.E. Peeters[2], M. Peeters[2], S. Van Laere[1], L.Y. Dirix[3]

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A growing understanding of the molecular biology of cancer and the identification of specific aberrations driving cancer evolution have led to the development of various targeted agents. It is well known that tumors can exhibit significant heterogeneity and change over time as a result of selective pressure. Circulating tumor cells (CTCs), shed from multiple tumor sites, have demonstrated to represent a patient's overall tumor burden. We report the first results of an ongoing comparative study on mutation profiles of CTCs and synchronously isolated DTCs from metastatic effusions of patients with clinically progressive MBC.

CTCs are enriched from 7.5 ml blood samples using the CellSearch system and further purified and sorted into several batches of 1-100 CTCs using the DEPArray system. DNA is isolated and amplified using the Ampli1-kit and subjected to Ion Torrent AmpliSeq panel sequencing. DTCs, fresh frozen tissue from solid metastases or the primary tumor, and bulk CTC (CellSearch Profile) were sequenced as comparators for mutation profiles. DNA of leukocytes was sequenced to enable somatic mutation analysis. Only somatic variants with good quality metrics, coverage >20x, variant allele frequencies >10%, and being non-synonymous or splice site variants, were taken into account.

Sequencing was performed on 67 samples of three patients with a mean coverage depth of 1000x. Coverage width of the 57 genes was near 100% for gDNA samples and 89% in single CTC/DTC samples. In patient 1, a PIK3CA hotspot and a FLT-3 variant were detected (sub)clonally in all tumor samples. Furthermore, various private mutations, including several TP53 hotspot mutations, were found in both CTCs and DTCs, but in WBC. In patients 2, another PIK3CA hotspot was present in all CTCs at heterozygous frequencies. In patient 3, a FLT-3 variant was detected at subclonal level. Furthermore, heterogeneity was observed between all CTC and DTC samples.

These data demonstrate the feasibility to become comprehensive SNV profiles of single and pools of CTCs and DTCs. Beside detection of targetable aberrations, evaluation of heterogeneity is of clinical importance, as the effect of targeting subclones is currently being explored in clinical trials like TracerX and Darwin.

O7 - Presence of tumor-specific cytolytic T cells in human primary breast carcinoma

S. Schröder[1], J. Carrasco[2], O. Bricard[1], K. Missault[1], N. Remy[1], G. Hames[1], M. Berlière[3], C. Galant[3], J.L. Canon[2], P. Coulie[3]

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Background: Immunotherapy through stimulatory antibodies targeting the CTLA-4 or PD-1 pathways has a demonstrated clinical efficacy in a fraction of patients with various cancers. It is likely that the main immune effectors of these therapies are CD8+ cytolytic T lymphocytes (CTL) recognizing tumor-specific antigens. Highly antigenic tumors such as metastatic melanomas are often immunogenic, i.e. they stimulate spontaneous anti-tumor CTL responses. This immunogenicity, of which the presence of tumor-infiltrating T cells (TILs) is probably a surrogate marker, might be a predictive marker for clinical benefit to immunostimulatory antibodies. The immunogenicity of primary breast carcinomas for CD8+ T cells has not been studied, but the amounts of TILs have been positively correlated with patients' survival. Here we wished to obtain evidence for the presence of tumor-specific CD8+ T cells in TILs from primary breast carcinomas.

Methods: From 5 tumors (2 ER+/HER2-, 2 ER+/HER2+, 1 ER-/HER2-) we isolated TILs and derived a random set of ±100 CD8+ clones. We screened these clones for recognition of candidate tumor-specific antigenic peptides selected through tumor exome sequencing and gene expression profiling. These peptides were encoded either by mutated genes or by cancer-germline genes of the MAGE family.

Results: For one tumor, 6 out of 49 T cell clonotypes recognized 4 out of 119 candidate mutated peptides. All these clones displayed absolute specificity for the mutated vs wild-type peptide. No anti-MAGE CTL were found. For each of the 4 other tumors, we screened 53-123 T cell clonotypes for recognition of 25-70 candidate mutated peptides, without any positive result.

Conclusions: Some human primary breast carcinomas are clearly immunogenic, as one tumor contained at least 10% of tumor-specific cells among the CD8+ TILs. Interestingly this tumor expresses ER, not associated with a prognostic value of TILs. More work is required to understand the reasons for the negative results in the 4 other patients. Our results warrant more investigations on the activation or inhibition of tumor-specific T cells at early stages of human breast cancer development.

O8 - The function of the Hippo pathway effector YAP in and around liver cancer

L. Van den Mooter[1], I. Moya[2], G. Halder[2]

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The Hippo signaling pathway is heralded as a new target for cancer therapy. This is because hyperactivation of the Hippo pathway effector YAP is sufficient to drive cell proliferation and malignancy of tumor cells, and because YAP is commonly hyperactivated in a broad range of different human carcinomas, while it is dispensable for homeostasis of most adult organs in the mouse. Thus, targeting the activity of YAP may specifically affect cancer cells. However, the effects of systemically targeting YAP are not known. Therefore, my projects aim to understand the effects of systemic and targeted inhibition of YAP in tumor and peritumoral cells of livers bearing cholangiocarcinomas. I use cholangiocarcinoma as a model system because they are one of the deadliest and incurable cancers. I found that YAP is essential in hepatocytes for tumor formation and that YAP is crucial in tumor cells for tumor maintenance and survival. Surprisingly, however, systemic YAP loss-of-function resulted in tumors that were more aggressive and invasive. These results thus indicate that although tumor specific deletion of YAP eliminates the tumors, the systemic inhibition of YAP increases cholangiocarcinoma aggressiveness and metastatic potential.

O9 - Reproductive behaviors and risk of developing breast cancer according to subtype. A systematic review and meta-analysis

M. Lambertini[1], L. Santoro[2], L. Del Mastro[3], B. Nguyen[1], L. Livraghi[4], D. Ugolini[3], A. Peccatori[2], A. Azim Jr.[1]

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Background: Breast cancer is composed of distinct subtypes defined mainly based on the expression of hormone receptors (HR) and HER2. For years, reproductive behaviors were shown to impact breast cancer risk but it is unclear whether this differs according to subtype. In this meta-analysis we evaluated the association between parity, age at first birth, breastfeeding and the risk of developing breast cancer according to subtype.

Methods: PUBMED and EMBASE were searched with no date restriction up to October 31st, 2014 to identify eligible studies. Boolean operators were used to combine specific keywords for each database and free text terms. Epidemiological studies (cohort studies or case-control studies) that evaluated the impact of parity and/or age at first birth and/or breastfeeding on breast cancer risk with available information on HR and HER2 were included. Tumor subtypes were defined as: luminal (HR+, HER2 +/-), HER2 (HR-, and HER2+) and triple-negative (HR-, HER2-). The MOOSE guidelines were applied and summary risk estimates (pooled odds ratio [pOR]) and 95% confidence intervals (CI) were calculated using random effects models.

Results: This meta-analysis evaluated 15 studies, including 21,941 breast cancer patients and 864,177 controls. Parity was associated with a 25% reduced risk of developing luminal subtype (pOR = 0.75, 95% CI = 0.70 to 0.81; P < .0001). Old age at first birth was associated with an increased risk of developing luminal subtype (pOR = 1.15, 95% CI = 1.00 to 1.32; P = .05). Subgroup analysis suggested a high risk of developing HER2 subtype by increased age at first birth (P = .02). Ever breastfeeding was associated with a reduced risk of developing both luminal (pOR = 0.77, 95% CI = 0.66 to 0.88; P = .003) and triple-negative (pOR = 0.79, 95% CI = 0.66 to 0.94; P = .01) subtypes.

Conclusions: The effect of the different reproductive behaviors on breast cancer risk differs according to subtype. This information could guide counseling of women on their risk of breast cancer and could have relevant implications on risk reduction strategies.

O10 - Inflammatory breast cancer cells show a particular pattern of canonical and non-canonical TGF signaling, possibly affecting cancer cell motility

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Background: Inflammatory breast cancer (IBC) is rare and aggressive type of breast cancer, characterized by its rapid invasion and spread. Recent evidence suggests that TGF signaling may be an important driver of the disease. Here, we describe data from patient samples and preclinical models that corroborate this hypothesis.

Methods: The xCELLigence system was used to profile a series of subtype-matched IBC and non-IBC (nIBC) cell lines for the cell motility inducing capacity of 11 chemokines. Significant results were confirmed using wound healing assays (WHA). RNAseq was performed on the same cell lines after TGF 1 treatment at either 1 hr, 4 hrs or 14 hrs. A series of 79 IBC and 133 nIBC patient samples was evaluated for nuclear SMAD2 and -3 protein expression using immunohistochemistry (IHC). In a subseries of these samples, protein and gene expression data were integrated and significantly correlated gene sets were translated into signal transduction networks. Expression2Kinases was used to identify key components of TGF signaling in IBC.

Results: Whereas TGF induced cell motility in all nIBC cells, we noted an 18-fold reduction of cell motility in IBC cells. These results were confirmed using WHA. RNAseq revealed a SMAD3-dependent, transcriptional program in nIBC cells, while in IBC cells, target genes of MYC were found to be overexpressed. IHC demonstrated an attenuated SMAD3 nuclear staining, combined with an increased SMAD2 nuclear staining. When integrating protein and gene expression data, we revealed that absence of SMAD3 activity in samples from patients with IBC correlates with elevated transcriptional activity of MYC and that increased nuclear SMAD2 expression was linked to activation of the classical TGF signaling pathway.

Discussion: We show that IBC cells do not induce cell motility in response to TGF stimulation. This observation can be explained by impaired non-canonical TGF signaling in IBC, which is essential for SMAD3-driven epithelial to mesenchymal transition (EMT). SMAD2 on the other hand is a proven driver of EMT-independent modes of cell motility. Our results strengthen the vision that EMT is not required for IBC cell invasion.

O11 - Patient-derived xenograft (PDX) models of soft tissue sarcoma (STS) - a preclinical platform for early drug testing

A. Wozniak[1], J. Cornillie[1], K Gebreyohannes[1], B. Boeckx[2], H. Li[1], T. Van Looy1, G. Floris[3], J. Wellens[1], L. Vreys[1], D. Hompes[4], M. Stas[4], F. Sinnaeve[4], D. Lambrechts[2], M. Debiec-Rychter[5], R. Sciot[3], P. Schöffski[1]

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Background: STS constitutes a rare and very heterogeneous family of mesenchymal tumours. limited treatment options available for advanced STS underline the need for reliable preclinical models to test novel therapeutic strategies.

Methods: A panel of patient-derived xenografts (PDX) was established by subcutaneous implantation of fresh, surgically resected tumour specimens in immunodeficient athymic nude NMRI mice. Once tumour growth was observed, pieces of tumour were re-transplanted to next generations of mice. At each passage tumour fragments were collected for histopathological and molecular characterization. Model was considered established after observing stable histological and molecular features for at least two passages. To evaluate the stability of the genomic profile we performed low-coverage whole-genome sequencing on DNA isolated from tumours obtained from at least two passages. In addition RNA-seq was used to better characterize the mutational profile of the ex-mouse tumours.

Results: Until now 139 STS samples from consenting patients treated at the University Hospitals, Leuven, Belgium, have been transplanted. Twenty six well-characterized, stable STS PDX models have been established, maintaining the histopathological and molecular features of the original tumor. Seventeen models, analysed with low-coverage DNA sequencing, showed a stable genomic profile in at least two different tumour passages. At this point the panel includes models of gastrointestinal stromal tumour (6 models), myxofibrosarcoma (5), dedifferentiated liposarcoma (3), malignant peripheral nerve sheath tumour (3), synovial sarcoma (2), leiomyosarcoma (3) epithelioid haemangioendothelioma (1), mesenchymal chondrosarcoma (1) and undifferentiated high grade sarcoma (2). Some of these models have already been successfully used for in vivo testing of novel agents, including both targeted and cytotoxic (pro-)drugs, and results served as a rationale for at least four prospective clinical trials. In addition 22 other xenografts are still in early stages of engraftment, not yet fulfilling our criteria of an 'established model'.

Conclusion: Our panel of mesenchymal PDX models is characterized by stable histological and molecular features. These clinically well-annotated models can contribute to reliable preclinical studies for new anticancer treatments for STS. The availability of unique, rare STS xenografts also allows to study the biology of these diseases. The platform is made available to collaborators from academia and industry.

O12 - The impact of an interventional counseling procedure in families with a BRCA1/2 gene mutation: efficacy and safety

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Background: Predictive genetic testing has high impact on cancer prevention for BRCA carriers and passing this information in BRCA families is important. Mostly, this is proband-mediated but this path is defective and denies relatives lifesaving information.
Objective: To assess the efficacy/safety of an intervention, in which relatives are actively informed.

Design: Sequential prospective study in new BRCA families. The proband informed relatives about predictive testing (phase I). After 6 months, a letter was sent to adult relatives who had not been reached (phase II). Then a phone call was made to obtain a final notion of their wishes. All subjects received psychometric testing (State-Trait Anxiety Inventory, STAI), an interview and routine counseling.

Results: Twenty families were included. Twenty-four of the relatives could not be reached, 59 were 'decliners', 47 participated by the proband and 42 by the letter. Predictive testing was performed in 98 % of the participants of which 30 were mutation carriers. The intervention is psychologically safe: the 95 % CI for the estimated mean difference in STAI DY1 between phase II/I subjects (mean difference -1.07, 95 % CI -4.4 to 2.35, p = 0.53) shows that the mean STAI DY1 score (measured at first consult) for phase II is no more than 2.35 units higher than for phase I, which is not relevant.

Conclusions: A protocol directly informing relatives nearly doubles the number of relatives tested and is psychologically safe. This should lead to a change in counseling guidelines in families with a strong germline predisposition for cancer.

LIST OF SELECTED POSTERS

P01 - Platinum chemotherapy in metastatic, castrate-resistant prostate cancer (CRPC)
E. Dewaele[1], B. De Laere[2], S. Van Laere[2], A. Rutten[2], L.A. Teuwen[3], A. Prové[3], J. Vandebroek[3], L. Dirix[3]
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P02 - Single-center experience with advanced liposarcoma (LPS): overall survival (OS), prognostic factors and chemotherapy outcome.
J. Cornillie[1], T. Van Cann[1], R. Vandeweyer[1], A. Wozniak[1], M. Debiec-Rychter[2], R. Sciot[3], D. Hompes[4], P. Schöffski[1]
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P03 - Phenotypic and genomic intra-patient heterogeneity of single circulating tumor cells in patients with metastatic and castration-resistant prostate cancer.
B. De Laere[1], U. Pyy[2], J. Rönkä[2], S. Oeyen[1], A. Brouwer[1], P.J. Van Dam[1], A. Rutten[3], L.A. Teuwen[1], G. Van den Eynden[3], P. Vermeulen[3], S. Van Laere[1], J. Vandebroek[3], L. Dirix[3]
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P04 - Detection of aberrant androgen receptor transcripts in circulating tumour cells as a predictor of resistance to AR directed treatment in patients with castration resistant prostate *cancer*.
B. De Laere[1], P. Van Oyen[2], C. Ghysel[2], J. Ampe[2], B. Brouwers[2], P.J. Van Kerkhove[2], J. Vandebroek[3], A. Rutten[3], P.J. Ost[4], W. Demey[5], W. Lybaert[6], D. De Maeseneer[7], D. Schrijvers[8], L. Hoekx[9], J. Van den Brande[9], N. Beije[10], S. Sleijfer[10], D. Peeters[9], S. Oeyen[1], G. Van den Eynden[3], S. Van Laere[1], P. Vermeulen[3], L. Dirix[3]
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P05 - Assessment of renal toxicity in oncology patients treated with targeted agents
T. Vermassen, F. Poelaert, N. Lumen, K. Geboes, J. Delanghe, S. Rottey,
Ghent University Hospital, Ghent, Belgium

P06 - Single-center experience with metastatic leiomyosarcoma: survival, prognostic factors and outcome of chemotherapy.
T. Van Cann[1], J. Cornillie[1], R. Vandeweyer[1], A. Wozniak[1], M. Debiec-Rychter[2], R. Sciot[3], D. Hompes[4], P. Schöffski[1]
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P07 - Pneumocystis jiroveci pneumonia (PJP) in non-HIV immunocompromised individuals.
K. Schoovaerts, A. Rutten, J. Van Schaeren, B. Van Herendael, S. Van Grieken, L. Heytens, L. Dirix
GZA Sint-Augustinus, Wilrijk, Belgium

P08 - Low level laser therapy in the treatment of chemo-induced grade III palmar-plantar erythrodysesthesia
S.B. Latifyan[1], M.T. Genot[2], E. Chevalier[2], D. Laenen[2], M.F. Scharll[2], M. Vandenhoucke[2]
[1]CHU Brugmann/ Institut Jules Bordet, Brussels, Belgium, [2]Institut Jules Bordet, ULB, Brussels, Belgium

P09 - The Leuven Connective Tissue Oncology Repository (LECTOR), a sarcoma database
R.O. Vandeweyer[1], T Van Cann[2], J Cornillie[2], D Hompes[3], M Stas[3], F Sinnaeve[4], R Sciot[5], A Wozniak[2], P Schöffski[2]
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P10 - A retrospective study of the combination adjuvant chemotherapy with weekly paclitaxel and cyclophosphamide in early breast cancer patients older than 65 years.
S. Joris, Fontaine, Decoster, Vanacker, Schallier, Vanhoeij, Verfaillie, Lamote, De Grève,
UZ Brussel, Jette, Belgium

P11 - Selective internal radiotherapy (SIRT): a single centre experience.
T.F.A. Van den Mooter, M. Peeters, S. Van Hecke, I. Huyghe,
Universitary Hospital Antwerp, Edegem, Belgium

P12 - COMBI-Rechallenge: A phase II clinical trial on dabrafenib plus trametinib in BRAFV600-mutant melanoma patients who previously experienced progression on BRAF(MEK)-inhibition
M. Schreuder, V. Kruse, J. Jansen, B. Neyns,
Universitair Ziekenhuis Brussel, Brussel, Belgium

P13 - Description of first-line treatments in patients with non-resectable colorectal cancers in Belgium
P. Chevalier, C. Van Gils, M. Lamotte,
IMS Health, Vilvoorde, Belgium

P14 - A pilot, phase Ib feasibility study of ARGX-110 in patients with nasopharyngeal carcinoma (NPC).
A. De Meulenaere, P. Deron, L. Ferdinande, S. Rottey,
Ghent University Hospital, Ghent, Belgium

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