Abstracts from the 15th annual meeting of the Belgian Society for Medical Oncology (BSMO)

5th Annual BSMO meeting, March 1-2 2013, Diegem, Belgium

On March 1st and 2nd, the Belgian Society for Medical Oncology (BSMO) organised its 15th annual meeting. Day one of the symposium focussed on the different aspects of melanoma, discussing the use of therapeutic vaccines, the different aspects of immunotherapy, the efficacy and side-effects of targeted therapy for metastatic melanoma and the management of melanoma patients with brain metastatses. However, the main event of the first symposium day was Prof. Dr. J. Vermorken receiving the title of commander in the order of Leopold by Mr. K. Peeters, Minister-President of the Flemish Government (*Figure 1*). The second day of the symposium featured the presentation of selected abstracts from junior BSMO members followed by an interesting lecture on chronobiology and chronotherapy of cancer and a view on personalised medicine using the lung cancer example.
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Figure 1. Prof. Dr. J. Vermorken receives the title of commander in the order of Leopold by Mr. K. Peeters, Minister-President of the flemish government.

Rab27B, an ex(o)citing machinery for 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 metastasis. We investigated the biological role and expression status of Rab27B, a regulator of exosome release, in breast cancer. Rab-27B-upregulation in oestrogen receptor (ER)-positive breast cancer cells promoted G1/S phase cell cycle transition and increased proliferation, F-actin reorganisation and invasion in cell culture and invasive growth and haemorrhagic ascites in a xenograft model. Proteomic analysis of purified Rab27B vesicles identified heat shock protein (HSP90) as key pro-invasive growth regulator. HSP90 secretion occurred in a Rab27B-dependent manner and was required for matrix metalloprotease (MMP)-2 activation. Endogenous Rab27B mRNA and protein expression significantly 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.

PTEN loss paradoxically reduces the anti-tumour efficacy of MTOR inhibitors in bladder cancer

E. Seront - UCL

The Phosphatidylinostitol 3-phosphate kinase (PI3K)/Akt/mammalian Target of Rapamycin (mTOR) cascade is a pathway that plays a key role in cell growth and

survival. PTEN acts as a negative regulator of this pathway. The PI3K/Akt/mTOR cascade is thus logically frequently activated in bladder cancer due to loss of functional PTEN. Preclinical studies have also shown that reduced expression of PTEN could enhance the toxicity of mTOR inhibitors in many cancer types.

Early this year, we reported the results of a phase II clinical study evaluating the efficacy of everolimus in patients with advanced bladder cancer. More recently, we performed immunohistochemical analysis on the archival tumours' samples and found that PTEN loss was paradoxically observed only in tumours of patients insensitive to everolimus. To understand the reasons of this paradox, we evaluated the efficacy of the mTOR inhibitor rapamycin on three bladder cancer cell lines with different PTEN status: UM-UC-3 with homozygous deletion of PTEN gene, UM-UC-9 with a decreased PTEN expression and UM-UC-14 with a wild-type PTEN expression. We first observed that similarly to our clinical observations, PTEN-deficient UM-UC-3 and UM-UC-9 were less sensitive to rapamycin than UM-UC-14. We next investigated whether a difference in Akt activation could account for this distinct behaviour. mTOR inhibition is indeed known to promote Akt activation via the relief of an inhibitory feed-back involving S6 kinase (a downstream effector of mTOR). We found that indeed, rapamycin induced Akt activation in PTEN-deficient UM-UC-3 cells but not in UM-UC-14. We also showed that inhibition of Akt activation by the PI3K inhibitor wortmannin dramatically enhanced the toxicity of rapamycin in UM-UC-3 (but not in UM-UC-14), further supporting the role of Akt in the resistance to rapamycin in PTEN-deficient tumour cells. Importantly, we confirmed these results in vivo by documenting that the association rapamycin-wortmannin was more efficient than rapamycin alone in UM-UC-3 (but not in UM-UC-14).

In conclusion, we showed that PTEN-negative bladder cancer cells are more resistant to mTOR inhibitors due to their incapacity to abrogate feedback Akt activation. Combination of mTOR and PI3K/Akt inhibitors (or the use of dual inhibitor such as the recently launched BEZ235 compound) could thus provide a direct benefit in patients with PTEN-negative bladder tumours.

Prognostic value of disseminated tumour cells in bone marrow and peripheral blood of patients with breast cancer

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Despite optimal treatment, 30-40% of patients with initially localised breast cancer (BC) will develop metastatic disease over time. These patients are believed to have microscopically disseminated disease at the time of diagnosis. Here we evaluate the prognostic significance of the detection of disseminated tumour cells (DTC) in bone marrow (BM) and circulating tumour cells (CTC) in peripheral blood (PB) in a large cohort of 148 patients with localised or metastatic BC. The initial analysis of this study was published after a median follow up of 2.5 years (Benoy et al. Br J Cancer, 2006). We now report on these data after a follow-up period of almost 10 years.

PB and BM samples were collected from 116 patients with localised (M0 subgroup) and 32 patients with metastatic (M^+ subgroup) BC before the initiation of local or systemic treatment. PB of healthy volunteers and BM of patients with a nonmalignant breast laesions or a haematological malignancy served as control group. DTC were detected by measuring a normalised relative gene expression (RGE) value for cytokeratin-19 (CK-19) and mammaglobin (MAM), using a quantitative RT-PCR detection method. Evaluated end points were overall (OS) and distant disease-free (DDFS) survival in the M0 group and OS and progression-free (PFS) survival in M^+ patients.

Elevated expression of CK-19, MAM or both in BM was strongly associated with shorter OS in both M0 patients ($p=0.069$) and M^+ patients ($p=0.010$). Elevated CK19 and/or MAM expression was furthermore highly predictive for earlier distant relapse (DDFS) in M0 patients ($p=0.034$) and shorter PFS in M^+ patients ($p=0.003$). For PB, no statistically significant difference in any of the studied endpoints was observed between patients with or without elevated CK-19 and/or MAM expression.

The presence of DTC, measured as an elevated CK-19 and/ or MAM mRNA expression, in BM was highly predictive for OS, DDFS and PFS in patients with localised and metastatic BC. Furthermore, using an identical RT-qPCR method, at the time of diagnosis, DTC detection had superior prognostic significance in comparison to CTC detection in peripheral blood in this patient cohort.

The composition and organisation of lymphocytes infitrating human breast cancer

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Breast cancer (BC) is a heterogeneous disease classified

into at least four distinct molecular subtypes: Luminal A, Luminal B, HER2+ and triple negative (TN). Recent studies show that strong links exist between host immunity and clinical outcome. The presence of CD3+ tumour- infiltrating lymphocytes (TILs) is thought to reflect host anti-tumour immune responses, and associated with favourable prognosis. The clinical relevance of TILs in BC is currently controversial due to variation in the pattern of immune cells detected in tumours.

To more fully delineate the immune composition of BC, we prospectively evaluated TIL's in tumours compared to non-adjacent normal breast tissue (n=66) in fresh GentleMACS™ homogenates. We further examined residual tumours from patients treated with neoadjuvant chemotherapy (n=13).

Flow cytometric analysis detected CD45+ leukocytes in both tissue types but revealed they are present at higher densities in the tumour. Extensive infiltrates are found in all BC subtypes; however, their frequency is elevated in HER2+ and TN. The composition of the immune infiltrate, similar between Luminal A, Luminal B and HER2+, was dominated by CD3+ T-cells (65-86% of CD45+). The majority of CD3+ TILs (CD4+ and CD8+) have an activated, effector phenotype (CD25+ CD45RO+). Among the other leukocytes, CD14+ monocytes represent 6-12% and CD19+ B cells 5-11% of the CD45+ cells. Interestingly, in TN BC the percentage of CD19+ B cells was considerably elevated (27%) and paralleled a decrease in CD3+CD8+ T-cells. Conversely, residual tumours after neoadjuvant chemotherapy show a lower density of immune cells with lower level of CD19+ B cells and CD4+ T-cells in parallel with an increase in CD8+ T-cells compared to untreated tumours. Confocal microscopy was used to analyse immunofluorescent antibody labeled FFPE sections to examine TIL organisation in BC. These experiments revealed that in extensively infiltrated tumours, T-cells are present in both peri- and intra- tumoural locations while B cells are principally clustered with T-cells in the B cell follicles of tertiary lymphoid structures.

We found that TIL recruitment varies between BC subtypes and between tumours within a given subtype, with extensively-infiltrated tumours detected at higher frequencies in the HER2+ and TN subtypes. These data have important implications for understanding how effective anti-tumour immune responses are generated.

Early effect of PI3K-Akt-mTOR blockade on in vitro 18F-FDG uptake in HER-2 NEU positive (HER2+) breast cancer cells

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Aberrant activation of the PI3K-Akt-mTOR pathway is an important driver of resistance to HER2 targeted therapies and is involved in glucose homeostasis. 18F-FDG-PET has been proposed for early response assessment for targeted therapies, but knowledge on the effects of blocking this pathway on FDG uptake dynamics is limited.

The aim of this study was to investigate the effect of pharmacological PI3K-Akt-mTOR blockade on in vitro FDG uptake in HER2+ breast cancer cells resistant and sensitive to trastuzumab. The JIMT-1 cell line is trastuzumab-resistant and sensitive to everolimus therapy. The SKBR3 cell line is sensitive to both therapies. The IC50 at 72h of trastuzumab (HER-2 receptor antagonist), everolimus (mTOR inhibitor) and PIK90 (PI3K inhibitor) were determined by xCelligence and used as reference dose. Cells were treated for 24, 72 and 96h followed by incubation with FDG. Uptake was measured in the cell suspensions and all supernatant media and corrected for radioactive decay. Cellular FDG uptake is expressed as the mean percentage of counts per minute per 10E4 cells relative to untreated control samples. The number of viable cells was counted using an automated cell analyser (Muse, Millipore).

IC50 concentrations of everolimus and PIK90 for JIMT1 at 72h were 9.5 nM and 40 μM, respectively. As expected, the IC50 of trastuzumab was not reached. IC50 concentrations of everolimus, trastuzumab and PIK90 for SKBR3 at 72h were 4,5nM, 80nM and 16 μM, respectively.

At 24h and 72h, everolimus increased FDG uptake for JIMT1 by 157% and 120% respectively, while at 96h everolimus decreased FDG uptake by 76%. In contrast, PIK90 treatment resulted in lower FDG uptake at 72h and 96h. With trastuzumab, FDG uptake was consistently higher compared to controls. At 24h and 72h, everolimus decreased FDG uptake for SKBR3 by 94% and 52% respectively, while at 96h everolimus increased FDG uptake by 180%. The same is true for trastuzumab. With PIK90, FDG uptake was higher compared to controls (*Table 1*).

Blockade of PI3K-Akt-mTOR in trastuzumab resistant and sensitive HER-2+ breast cancer cells affects *in vitro* ^{18}F-FDG uptake in transient and opposite ways, depending on the pharmacological target and duration of treatment. Therefore, further validation is necessary to

Table 1. Mean % of FDG uptake as compared to untreated control.

	Everolimus	PIK90	Trastuzumab			
	JIMT1	SKBR3	JIMT1	SKBR3	JIMT	SKBR3
24h	157*	94	107	104	246*	99
72h	120	52*	33*	154*	103	66
96h	76	180	32	195	211*	122

* p<0.05

elucidate the cellular mechanisms involved in tracer uptake prior to routine clinical use for early response assessment.

Efficacy of a phosphoinositol 3 kinase (PI3K) inhibitor in gastrointestinal stromal tumour (GIST) models

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PI3K signaling is crucial for GIST proliferation and survival. We assessed the efficacy of the PI3K inhibitor NVP-BEZ235 (BEZ), alone or in combination with imatinib (IM), in GIST xenografts.

Nude mice were grafted bilaterally with human GIST, carrying either KIT exon 9 (UZLX-GIST2, dose-dependent IM resistant) or exon 11 (UZLX-GIST3, IM sensitive) mutations. Animals, randomised into four groups (n=8/group) were dosed orally for two weeks with either vehicle, IM (50mg/kg/bid), BEZ (10mg/kg/qd), or IM+BEZ. Treatment efficacy was assessed by tumour volume, histopathology and Western immunoblotting. Moreover tumour regrowth was evaluated for three weeks after treatment cessation.

As a single agent IM and BEZ stabilised tumour growth of both UZLX-GIST2 and -GIST3. Moderate to significant tumour regression was observed in UZLX-GIST2 under BEZ (by 27%), and IM+BEZ (66%), and also in UZLX-GIST3 under IM (75%) and IM+BEZ (75%). In UZLX-GIST2 significant reduction in mitotic index was observed under BEZ (8.5-fold) and IM+BEZ (8.5-fold) as compared to control. In UZLX-GIST3 mitotic activity was virtually absent under all regimens. Apoptotic activity increased significantly after treatment with IM (5.5-fold) and IM+BEZ (14.0-fold) in UZLX-GIST3, whereas it was almost unaffected by BEZ as single agent, as well as in all treatment groups in UZLX-GIST2. By Western, PI3K signaling was incompletely inhibited in all groups in UZLX-GIST3, and after BEZ in UZLX -GIST2. Complete inhibition of PI3K signaling was observed only after combination treatment. After treatment cessation long-lasting growth-inhibition was observed in IM+BEZ treated UZLX-GIST3. Moreover, mitotic index after BEZ and BEZ+IM in UZLX-GIST3 was lower than in control even after treatment withdrawal.

BEZ shows significant efficacy in GIST xenografts. Furthermore, combination with IM shows synergistic and long-lasting effects even after treatment withdrawal, which is not the case with drugs routinely used for GIST treatment.

A functional driver mutation in exon 21 of the HER3/ERB3 receptor in non-small cell lung cancer

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Somatic mutations found within the tyrosine kinase domain of the human epidermal growth factor (HER) family of transmembrane receptors have been implicated in non-small cell lung cancer (NSCLC). These mutations have been identified in the EGFR (epidermal growth factor receptor), HER2 and HER4 genes, but not in HER3, which is considered to lack kinase activity. Here, we report the case of an adolescent patient with advanced NSCLC in whom we identified a novel V855A (Valine → Alanine) somatic mutation situated in exon 21 of the HER3 tyrosine kinase domain. The mutation maps at a position homologous to the frequently described EGFR tyrosine kinase inhibitor (TKI)-sensitive L858R (Leucine → Arginine) activating mutation situated in exon 21 of the EGFR tyrosine kinase domain.

In vitro functional analysis in a null Ba/F3 background reveals that HER3-V855A when combined with its HER2 dimerisation partner leads to neuregulin 1β-induced HER3 and HER2 receptor activation and transforms interleukin-3 (IL-3) dependent Ba/F3 cells to neuregulin 1β-dependent growth. Afatinib, a pan-HER small molecule inhibitor, has anti-proliferative and pro-apoptotic effects on the mutant HER3: wild-type HER2 Ba/F3 derivative that are logarithmically higher than the effect obtained in the wild-type HER3: wild-type HER2 combination. These findings demonstrate that the HER3

gene possesses a kinase domain that can be mutated and that the HER3-V855A mutation confers a gain-of-function phenotype that is associated with sensitivity to afatinib. Although the mutation was rare (1/210 lung cancers examined), this finding could be relevant for the treatment of NSCLC or other cancers carrying such mutations.

Does single nucleotide polymorphism genotyping improve patient selection for prostate biopsy when combined with a prostate cancer risk calculator?

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Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) associated with higher risk of prostate cancer (PCa). The present study aimed to evaluate whether published SNPs improve the performance of a clinical PCa risk-calculator in predicting prostate biopsy result.

A total of 214 patients with a history of prostate biopsy (110 positive biopsies, 104 negative biopsies) was enrolled. After literature search, 11 SNPs in 8 chromosomal regions (7q32, 8q24 regions 1, 2 and 3, 10q11, 17q12, 17q24.3, 19q) were selected for their statistically significant association with increased PCa risk. Genotypic odds ratios were computed and a new logistic regression model was built that integrated the clinical risk score (i.e. prior biopsy results, PSA level, prostate volume, transrectal ultrasound, and digital rectal examination) and the genetic information. Areas under the receiver operating characteristic curves (AUC) of the clinical score alone vs. the integrated regression model were compared. The added value of SNP genotyping was assessed using the Integrated Discrimination Improvement (IDI) statistics.

Eight of the eleven SNPs were concordant with the literature in terms of PCa risk association, especially four of them with a previously reported large effect- size (OR≥1.4). When using this set of four SNPs to compute a multilocus genetic score, the performance of the clinico-genetic model (AUC=0.771) was not statistically significantly higher (IDI= 0.017, p-value = 0.147) than the clinical score alone (AUC = 0.765).
Despite the small sample size, SNPs with large effect-size were associated with increased PCa risk. However, the improvement of the predictive value was small which led to lack of significance in this small cohort of patients.

Prostate specific antigen glycosylation profile may serve as a diagnostic biomarker for prostate cancer

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Prostate cancer (PCa) is the most common malignancy in men. PCa is mostly a slowly growing tumour that is restricted to the prostatic gland. Prostate specific antigen (PSA) assays are widely used for early detection of PCa. However, those analyses are associated with considerable sensitivity and specificity problems (especially in the diagnostic gray zone with a serum PSA [sPSA] concentration of 4-10ng/mL), complicating the distinction between various forms of prostate disease. Moreover, there is a risk of overdiagnosing indolent PCa and missing potentially aggressive PCa's.

In this study we determined the N-glycan profile of prostatic proteins in the urine of healthy volunteers (HV; n=15), patients with benign prostate hyperplasia (BPH; n=39), PCa patients (n=29) and patients with prostatitis (n=14) by means of DNA-sequencer assisted fluorophore-assisted capillary electrophoresis. Statistical analysis was performed to examine whether differences in N-glycan profile were statistically significant between the four subject groups.

N-glycan profile analyses have pointed out differences in the N-glycan profile between patients with BPH and PCa patients. The changes were associated with a decrease in triantennary structures (NA3) and a decrease in fucosylation of bi- and triantennary structures. This isolated test was not statistically better than sPSA measurement (AUC after ROC curve analysis are 0.805 ± 0.056 and 0.737 ± 0.063 for sPSA screening and the glycosylation marker respectively). It gives however an added value to sPSA screening. ROC curve analysis shows that the combination of these assays reached an area under the curve of 0.854 ± 0.049 for all patients. In the diagnostic gray zone of sPSA between 4 and 10 ng/mL sPSA was not retained in the logistic regression model.
In this study, we have found a statistical significant difference in the glycosylation patterns of patients with BPH versus PCa patients. These changes in N-glycosylation could lead to the discovery of a new biomarker for PCa, particularly in the diagnostic gray zone. Also differences between PCa patients and patients with prostatitis were observed. A larger sample size can show us if this glycosylation marker can be used as a clinical usable assay in the future.