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Antibody-independent enrichment of live circulating tumor cells (CTCs) from a variety of cancer types

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Background: Circulating tumor cells (CTCs) are used clinically as biomarkers for monitoring metastatic disease progression. However, CTC capture has been hampered in various cancer types mainly due to the limitation of antibody capture methods, primarily based on EpCAM. We developed a new CTC enrichment device, ApoStream™ that utilizes differences in cell morphology between normal cells and cancer cells. The device is based on dielectrophoretic field-flow fractionation in a continuous flow microfluidic chamber to separate CTCs from other healthy blood cells.
Materials and Methods: To demonstrate the performance of the ApoStream™ device, cancer cells were spiked into peripheral blood mononuclear cells (PBMCs) from normal donor blood and isolated based on their morphological differences. Cancer cell recovery, viability, immunofluorescence detection and FISH were demonstrated on cells isolated from the ApoStream™ device from lung, prostate and melanoma cancers. Lung cancer blood samples were compared in a paired sample study with CellSearch®. Cells isolated from ApoStream™ were stained for cytokeratin (CK+)/CD45-/DAPI+ and melanoma CTCs using S100+, and imaged using laser scanning cytometry (LSC). CTC morphology was also confirmed with H&E staining.
Results: ApoStream™ yielded a recovery of 67±15% with more than 1000 times enrichment from samples spiked with ovarian cancer cells (SKOV3). The viability of cells after ApoStream™ separation was more than 90% and isolated cells were grown successfully in culture. High CTC recovery from lung (n = 27), prostate (n = 10) and melanoma (n = 7) cancer patient blood samples was achieved with counts ranging up to 3500 CTCs per 7.5 ml blood. Further, there were no false-positive CTCs from normal donor blood controls demonstrating ApoStream™'s specificity. When compared to the CellSearch® system, ApoStream™ isolated a significantly higher number of CTCs from lung cancer patient blood samples (range:0–1213, mean:259 versus range:0–340, mean:14) showing the effectiveness of ApoStream™ in isolating EpCAM-negative CTCs. In addition, FISH analysis was successfully performed on ApoStream™ enriched cells.
Conclusion: ApoStream™ technology permits antibody-independent enrichment of CTCs from various types of cancers with high recovery for further downstream characterization including protein, RNA and DNA analysis. Viable CTCs enriched by 'ApoStream™' allow for culturing of these rare cells for drug screening experiments and ultimately implementation for personalized cancer therapy.

PP 26

IGF-1R signaling in ER+ and triple negative breast cancer − a potential target for therapy?

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Background: IGF1 and its signaling network control growth, development and regulate proliferation and survival. IGF-1R is overexpressed in many types of solid tumors including ER+ and triple negative breast tumors. There appears to be a crosstalk between the IGF1R system and ER signaling in ER+ breast cancer. Indeed, there is in vitro evidence showing that increased signaling through IGF1R confers resistance to ER targeting agents. Consequently, inhibitors of the IGF1R are in active development. Since IGF-1R is involved in the progression of breast cancer and resistance to systemic (including endocrine) treatment; suppression of this pathway by administration of IGF-1R mAB may therefore be efficacious in ER+ and TNBC models.
Materials and Methods: To examine this possibility, BIIB022 (a recombinant human AB directed against IGF-1R) was treated in ER+ (MCF7 & T47D) and TNBC cells (HCC1937 & MDA-MB-231).
Results: Treatment of ER+ and TNBC cell lines with BIIB022 in vitro showed: (1) 15−25% cell killing and 50−60% anti-proliferative activities by MTT and 3D-ON TOP colony formation assay respectively, (2) dose- and time-dependently blocked p-AKT (both at Ser473 and Thr308), (3) blocked activation of P70S6K in ER+ cells, but not in TNBC cell lines, (4) both IGF1 and estrogen-induced p-AKT and pP70S6K were abrogated by prior treatment of BIIB022 in ER+ cells, (5) IGF1-induced AKT phosphorylation was inhibited, but phosphorylation of downstream effectors of mTOR (p70S6K or S6K Ribosomal Protein) was not inhibited in TNBC cells by prior treatment of BIIB022, (6) inhibition of IGF-1R by BIIB022 lead to activated ERK both in ER+ and TNBC cells, (7) BIIB022 significantly reduced HIF1α expression following IGF1 stimulation in ER+ cells, and (8) integrin-dependent breast tumor cell migration was significantly abrogated with BIIB022 in association with RAC1-GTP inhibition.
Conclusion: Our preclinical in vitro data demonstrate (1) IGF-1R signaling cascade is not completely inhibited by prior treatment of BIIB022, (2) Blocking IGF-1R signaling inhibits estrogen-mediated AKT-mTOR pathway activation (non-genomic function of ER), (3) From this study, we can suggest that the RAS-MAPK or mTOR kinase inhibitor must be used in conjunction with IGF-1R inhibition for maximum anti-tumor effect in both ER+ and TNBC models.

PP 82

Pleiotropic influences of radio- and chemotherapy on auto-antibodies warrant caution for their use as biomarkers of tumor response: the anti-GRP78 paradigmatic example

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Background: Radio- and chemotherapy are common anticancer strategies applied nowadays to treat cancer patients. Although biomarkers are developed to predict and track tumor response, pleiotropic effects of anticancer treatments on global patient biology, and thus on the relevance of observed changes in biomarkers levels, are largely underestimated. In particular, biomarkers based on the immune response could be misleading considering the myelotoxicity of chemotherapy and the local pro-inflammatory effects of ionizing radiations. Here, we examined how an emerging class of seric cancer biomarkers, namely auto-antibodies (AAbs) directed against tumor-associated antigens (TAAs), may be influenced by conventional anticancer modalities.
Materials and Methods: We first performed serological proteome analysis (SERPA) as an integrated proteomic workflow to identify TAAs in mice bearing syngeneic lung carcinoma. We then applied radiotherapy (16 Gy) and chemotherapy (100 mg/kg cyclophosphamide) on tumor-bearing mice and studied the consequences on AAbs production.
Results: We first identified different protein candidates showing a specific immune response against the serum of tumor-bearing mice and selected GRP78 (Glucose-Regulated Protein 78) for further evaluation. Although we used irradiation and cyclophosphamide regimens that inhibited tumor growth to the same extent, we found that chemotherapy was associated with a reduction in the serum titer of anti-GRP78 AAbs ($p < 0.02$, $n = 10$), whereas radiotherapy increased it ($p < 0.001$, $n = 15$). We next documented that the decrease in anti-GRP78 AAbs (and total circulating IgG) after cyclophosphamide administration was related to a net reduction in the population of antibody-producing B-cells. Local tumor irradiation did not influence the number of B cells even though it led to an overall decrease in circulating antibodies due to their massive trapping in irradiated tumors. This phenomenon however did not preclude the post-radiation increase in circulating GRP78 AAbs concentration that we could attribute to a local increase in GRP78 protein in the irradiated tumors.
Conclusion: In this study, using anti-GRP78 antibodies a paradigmatic immune-based marker of tumor growth, we showed that conventional anticancer therapies may dramatically influence the titer of AAbs for other reasons than a direct tumor response. This should draw the attention of investigators towards the therapeutic status of cancer patients recruited in studies aiming to identify new AAbs as cancer biomarkers.

PP 92

Clinical and genetic characterization of dihydropyrimidine dehydrogenase deficiency in fluoropyrimidine-treated patients carrying the DPYD*2A allele

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Background: The therapeutic efficacy and toxicity of fluoropyrimidines are, at least in part, related to the balance between anabolism of the drug to its nucleotides, which inhibit thymidylate synthase and are incorporated into RNA and DNA, and the catabolic pathway dependent on dihydropyrimidine dehydrogenase (DPD), which is the initial and rate-limiting step in pyrimidine degradation. Over the last decade, it has become clear that DPD regulates the amount of 5-FU available for anabolism thereby affecting its pharmacokinetics, toxicity, and efficacy. Moreover, an uncommon variant of the DPD gene, consisting of a G to A mutation in the splicing recognition sequence of intron 14 (IVS14+1G>A) of the DPD-encoding gene (DPYD*2A), produces a non-functional enzyme due to skipping of exon 14 and is potentially associated with life-threatening toxicity.
Materials and Methods: Six patients given FOLFOX, capecitabine or 5-FU test dose (425 mg/mq) were genotyped. They suffered from the following toxicities (WHO criteria): diarrhea and febrile neutropenia grade 3−4, nausea-vomiting, stomatitis, piastrinopenia, alopecia, hand-foot syndrome grade 3 and anemia grade 2. Blood samples for DNA analysis were collected and used to screen patients for DPD polymorphisms by PCR and automatic sequencing of the entire coding region.

Results: Five patients were found heterozygous IVS14+1GA (DPYD*1/*2A) and one patient was homozygous mutant IVS14+1AA (DPYD*2A/*2A). The homozygous patient was initially tested with a reduced 5-FU test dose and showed diarrhea grade 2, mucositis grade 3, anemia grade 1, pistrinopenia grade 3, febrile neutropenia grade 4, complete alopecia and Staphylococcus aureus sepsis. This patient required 20 days of hospitalization and was managed with antibiotics, platelet transfusion, port removal, G-CSF administration and parenteral nutrition.
Conclusion: Although the frequence of DPYD*2A allele is low, the screening for DPD mutation is clinically relevant to avoid the severe toxicities or death in patients trated with fluoropyrimidine-containing regimens.

PP 25

Preclinical efficacy of a dual PI3K-mTOR inhibitor, BEZ235 in triple negative breast cancer

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Background: Pathway-targeted therapy has not been established for the treatment of Triple Negative (TN) subset of breast cancer (BC). Studies demonstrated that the frequent activation of the PI3K pathway is part of the natural history of ER-negative BC, and PTEN protein/function is down regulated in ~40% of breast tumors (BT) including TNBT (Saal et al., 2007, 2008). We hypothesize that the inhibition of PI3K/mTOR pathway by BEZ235 will have anti-proliferative, anti-angiogenic, and anti-migratory effects on TNBT cells.
Materials and Methods: The effects of BEZ235 were studied on: (a) the cell survival/proliferation (MTT, SRB, & cell titer-GLO assay), (b) IGF-induced upregulation of HIF-1α, (c) the cellular signals for proliferation and apoptosis, (d) fibronectin-directed migration (scratch-assay), and (e) the organization of polymerized-actin (confocal microscopy) in TNBT cell lines.
Results: The results show that, (1) the effect of BEZ235 was pronounced only after 96 hrs of the treatment in TNBT cell lines (HCC70, HCC1937, MDA-MB231, SUM149), in contrast to HER2+ cell lines wherein the EC50s can be determined as early as 48 hrs, (2) the range of EC50s in TNBT cells varied from 1−5 uM as compared to 10−70 nM in HER2+ cells, (3) PTEN-null and ATM kinase mutated MDA-MB468 cell line exhibited 200 uM EC50 (72 hrs), (4) BEZ235 treatment decreased cellular-ATP levels within 48 hrs, (5) IGF-induced HIF-1α expression was abrogated by BEZ235 in MDA-MB468 cells, (6) BEZ235 treatment (50nM) decreased pAKT-S473 and pP70S6K after 1 and 3 hrs, (7) the decrease in pAKT-S473 was reversed after 48 hrs while the decrease in pP70S6K was reversed partially after 48 hrs, (8) treatment with BEZ235 time dependently increased cleaved-caspase9 and cleaved-PARP, and (9) BEZ235 treatment dose dependently inhibited fibronectin-directed migration and altered organization of actin-cytoskeleton in TNBT cells.
Conclusion: BEZ235 has anti-proliferative/pro-apoptotic, anti-angiogenic and anti-migratory effects on TNBT cells. We are currently pursuing studies to, (a) delineate the relationship between the anti-proliferative effects (3D-ON-TOP clonogenic assay) of BEZ235 and the status of the PI3K-PTEN-mTOR pathway using PIK3CA-mutated and PTEN-null cell lines, (b) demonstrate the effect of BEZ235 on integrin-directed real-time migration of live TNBT cells, and (c) find out the effect of BEZ235 on vascular-mimicry in TNBT cells; the results of which will be presented in the meeting.

PP 80

Identification of translocations involving the PRDM16 locus in hematological malignancies with 1p36 alterations

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Background: The PRDM16 gene on chromosome 1p36 is rearranged in acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) with t(1;3)(p36;q21). PRDM16 rearrangements are currently not explored in clinical practice.
Materials and Methods: We studied 120 cases of hematological malignancies (74 myeloid, 44 lymphoid and 2 undifferenciated malignancies) with karyotypic 1p36 rearrangements. We used a contig of bacterial artificial chromosomes (BAC) clones to study the 1p36 region by fluorescence in situ hybridization (FISH). Using TaqMan® gene expression assays, we studied the expression of PRDM16 in 8 cases with available RNA.
Results: The PRDM16 locus was the most frequently rearranged locus, as 39 out of the initial 120 cases harbored a translocation involving PRDM16. The various breakpoints were clustered within a region of less than 400 kb in or 5× of the PRDM16 locus. BAC probes RP11–181G12 and RP11−22L13 allowed the identification of all cases. PRDM16 rearrangements were more frequent in myeloid than in lymphoid cases (37/2), with an overrepresentation of therapy-related myeloid malignancies in this series. We found PRDM16 to be rearranged with the RPN1 locus (3q21) in 30 cases and with other loci in 9 cases. We describe novel translocation partners, including transcription factors ETV6 and IKZF1. There was an overexpression of PRDM16 in all studied cases (range of $2^{-\Delta\Delta C_t}$: 4.8 to 737). Survival data of the 32 patients with available data interestingly suggest that patients with AML/MDS and PRDM16 translocations have a poor prognosis whatever the partner gene, RPN1 versus others, as the median overall survival (OS) was 18 months [95% CI, 6 to 31 months] and 5-year OS was 25.7% [95% CI, 8.4–43.0%].
Conclusion: Our data support the proposal for the addition of a "PRDM16"-entity in the World Health Organization classification of acute myeloid leukemias, as is already the case for the "EVI-1"-entity. In our series, PRDM16 is constantly overexpressed in cases where PRDM16 is rearranged by FISH. Given the apparent bad prognosis associated with this finding, we propose to screen hematological malignancies with karyotypic 1p36 alterations by FISH, using BAC probes RP11–181G12 and RP11−22L13. As 95% of positive cases arose from the myeloid lineage, screening for PRDM16 alterations could be restricted to myeloid malignancies. Before implementing this screening into clinical practice, survival data should be confirmed prospectively in a clinical trial.

PP 8

The role of StarD13 in astrocytoma malignancy: tumor suppressor or oncogene

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Background: Astrocytomas are tumors occurring in young adulthood. Astrocytic tumors can be classified into four grades according to histologic features: grade I, grade II, grade III and grade IV. Malignant tumors, those of grade III and IV, are characterized by uncontrolled proliferation, which is known to be regulated by the family of Rho GTPases. StarD13, a GAP for Rho GTPases, has been described as a tumor suppressor in hepatocellular carcinoma.
Materials and Methods: In the present study, we used immunohistochemistry on tissues taken from human patients of different grade astrocytomas. We also used astrocytoma cell lines. We knocked down Stard13 by transfecting the cells with StarD13 siRNA and we overexpressed Stard13 by transfecting the cells with a GFP-Stard13 construct. We measured cell proliferation and cell death using the MTT and WST kits and doing cell cycle analysis by flow cytometry.
Results: In the present study, IHC analysis on Grade I-IV brain tissues from patients showed StarD13 to be overexpressed in grade III and IV astrocytoma tumors when compared to grade I and II. However, when we mined the REMBRANDT data, we found that the mRNA levels of StarD13 are indeed higher in the higher grades but much lower than the normal tissues. The overexpression of a GFP-StarD13 construct in astrocytoma cells led to the increase in cell death and a decrease of cell viability. Knocking down StarD13 using siRNA led to a decrease in cell death and an increase in cell viability. When looking at the mechanism, we found that the tumor suppressor effect of StarD13 is through the inhibition of the cell cycle and not through the activation of apoptosis. When knocking down StarD13, we also saw an increase in p-ERK, uncovering a potential link between Rho GTPases and ERK activation.
Conclusion: In conclusion, we found StarD13 to be a tumor suppressor in astrocytoma. It is underexpressed in comparison to normal brain and when knocked down in astrocytoma cells, this leads to a decrease in cell proliferation.

PP 56

Suitability of advanced non-small cell lung cancer biopsies for prospective, multiple molecular analyses in clinical trials

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Background: Accessing somatic molecular data from cancer tissues is a critical requirement underpinning the development of novel personalised therapy. Presently, there is a lack of clarity on the amount of tumour tissue that is sufficient to support prospective exploratory research in clinical trials. We describe the feasibility of multiple laboratory assessments including array-based analyses on routine archival specimens in a clinical trial setting.
Materials and Methods: An open-label, single-arm, phase II, multicentre study in the UK/Ireland was conducted (with appropriate approvals/informed consents) to correlate thymidylate synthetase (TS) expression and progression free survival. Enrolled patients (n = 70, ECOG PS 0−1) with stage IIIB/IV non-squamous non-small cell lung cancer (NSCLC) received pemetrexed (pem)/cisplatin induction followed by pem maintenance