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