may play a role in rapamycin-induced apoptosis. Identification of critical molecular markers in tumor cells will help to identify patients who shall benefit from mTOR inhibitors.

Wednesday 29 September

Poster Sessions

New drug targets

36 POSTER

Transcriptional signature associated with sensitivity to ET-743 (Yondelis) in low passage sarcoma cell lines

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ET-743 (trabectedin, Yondelis) is a marine anticancer agent that has shown to induce long lasting objective remissions and tumor control in a subset of patients with pretreated/resistant soft tissue sarcoma. Drug induced tumor control is achievable in a 22% of such patients, but there is not clear indication of the molecular features correlated with clinical sensitivity/resistance to ET-743.

Nine low passage soft tissue sarcoma cell lines explanted from chemo naïve patients with different patterns of sensitivity (IC50 range, 0.4 to 100nM), have been profiled with a cDNA microarray containing 6700 genes relevant in cancer development and drug resistance. The molecular signature of these cell lines was analyzed at baseline and at 4 different time points after ET-743 exposure at the clinically relevant concentration of 10 nM. Additionally, association of p53 mutation and p73 expression levels with ET-743 sensitivity and cell cycle kinetics after treatment were also analyzed.

Gene expression profile analysis revealed upregulation of 86 genes and downregulation of 244 genes in response to ET-743, showing a strong inhibition of gene transcription by the drug. ET-743 gene expression signature reveals a group of genes related with cell cycle control, stress and DNA damage response, such as JunB, ATF3, CS-1. SAT, GADD45B, and ID2 that are upregulated in all the cell lines studied independently of its sensitivity and of the histological subtype.

Transcriptional signature 72 hrs after ET-743 administration, associated with ET-743 sensitivity, showed a more efficient induction of genes implicated in DNA damage response and apoptosis, such as Rad17, BRCA1, PAR-4, p21 and p53DINP1 in the sensitive cell lines group.

Flow cytometry studies showed cell cycle arrest and/or apoptosis in the sensitive cell lines. The presence of p53 mutations correlate with sensitivity. Data produced in this translational program provides with a rational to explore at the clinical level whether this signature can contribute to the identification of the subset of patients that can benefit from ET-743 therapy.

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Sensitivity and resistance of human leukemic blasts to aplidin; molecular signature by gene expression profiling (GEP)

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Aplidin (APLD) is a marine anticancer drug discovered in the Mediterranean tunicate *A. albicans*. The antitumoral activity of APLD has been related to a cascade of events including cell cycle G1 arrest and G2 blockage, an acute apoptotis induction with JNK/p38 sustained activation; and inhibition of VEGF autocrine loop, reducing VEGF secretion and down regulating of the VEGFR-1. The phase I clinical program with APLD has been completed with evidence of a positive therapeutic index and lack of bone marrow toxicity. Phase II clinical studies are currently underway in hematological tumors with special focus on leukemia and multiple myeloma. In vitro and in vivo studies in leukemia models have demonstrated cytotoxicity at concentrations reachable in patients well below the recommended dose, and lack of cross-resistance with conventional agents. A translational program in pediatric acute lymphoblastic (ALL) and acute myeloid (AML) leukemia has produced evidence of variable in vitro sensitivity to APLD in blast from patients (Leukemia 2003, 17: 1338) at concentrations that do no affect normal bone marrow and peripheral blood samples.

Blast cells from 17 ALL and 12 AML patients with differential sensitivity to APLD have been analyzed by Gene Expression Profiling using a cDNA microarray that contains 6700 genes relevant in cancer development, apoptosis and drug resistance. The in vitro sensitivity to APLD of the patient blasts, measured as IC_{75}, ranged from 0.012 to 0.096 mM and 0.011 to 0.153 mM for primary and relapsed ALL, and 0.012 to 0.088 mM for AML, respectively. The IC_{75} median values, used as cut off for classifying the samples as sensitive or resistant were 0.028, 0.014 and 0.045 mM for ALL, ALL-r and AML respectively. Gene expression profiles reveal a specific signature in AML and ALL samples that correlate with the extent of sensitivity to APLD.

AML samples sensitive to APLD presented high expression of genes related to signal transduction, metalloproteases and drug metabolism. Genes in APLD-resistant AML samples are involved mainly in NF-kB activation. In contrast, ALL samples sensitive to APLD presented higher expression of DNA damage response genes.

The GEP model generated in this study will be incorporated in the translational research studies within the phase II program with APLD in resistant leukemia.

38 POSTER

The role of sample preparation on gene expression profiling: impact on clinical use of microarray technology

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Background: Gene expression profiling is quickly entering the clinical arena in areas such as development of new diagnostic markers and pharmacoprediction. Although seminal findings have been reported using transcriptom analysis, numerous challenges remain for its routine usage. One of the foremost is that gene expression patterns in tissues, e.g. tumors or peripheral blood greatly depend on temporal and interindividual variations. In addition, technical aspects of sample processing, isolation of cellular components, RNA preparation methods and other facets such as time from biopsy or blood withdrawal to RNA-isolation and different experimental conditions have been suggested to affect gene expression patterns. However, these issues are poorly investigated in gene expression analysis using microarrays.

Materials and Methods: Peripheral blood from healthy individuals and cancer patients were used as a model to assess the influence of pre-analytical factors on gene expression profiles. Several methods to isolate different cell types and RNA (PAXgene, QIAamp, Ficoll, BD-CPT) and two different blood processing techniques (Buffy Coat vs venipuncture blood) were compared using Affymetrix HG-U133A microarrays. A total of 68 individual array experiments were included in this analysis. Furthermore, the influence of physical factors such as temperature (room temperature, 8ºC), cryopreservation and time delay in sample preparation were also analyzed.

Results: Overall, the pre-analytical conditions have a strong and significant impact on gene signatures outweighing e.g. interindividual differences. Particularly delayed sample handling revealed a striking impact on gene signatures. We observed an induction of genes related to hypoxia, concomitant with down regulation of genes associated with cell cycle, metabolism and apoptosis. Similarly, gene expression was strongly influenced by the choice of cell and RNA preparation technique: e.g. the use of the PAXgene system, solely providing stabilization of the gene expression profile of blood samples, revealed overall decrease of present calls, highest variability and decreased sensitivity for changes in expression patterns of lymphocytes and monocytes. Cryopreservation, different temperatures during cell isolation or the source of the blood sample introduced minor changes, nevertheless, they were biologically relevant as exemplified by regulation of the IL-8 gene by different temperatures during cell isolation.

Conclusions: Clinical utilization of microarray technology will require improved standardization. Careful annotation of sample collection, transportation or storage and of RNA isolation techniques needs to become a prerequisite during clinical use of this technology. Based on our results, we suggest immediate preparation of RNA prior prolonged sample transportation or storage.

39 POSTER

Discovery and development of multiplex angiogenesis inhibitors that target EphB4: validation with a novel chemical-genetics based in vivo model

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EphB4 is a receptor tyrosine kinase (RTK) that plays a critical role in blood vessel development. EphB4 knockout mice die *in utero* from multiple