Poster Presentations

PP 65

Are GSK2118436 and GSK1120212 effective in melanoma cell lines harboring V600BRAF mutations different from the common V600EBRAF variant?

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Background: Melanoma is the most aggressive form of skin cancer and its prognosis depends by staging at diagnosis. Recent data from trials investigating targeted agents or immune modulators suggest new promising strategies for the treatment of patients with advanced melanoma. Mutations at V600 codon of BRAF gene are present in 40−60% of patients with primary cutaneous melanomas. The most common mutation is V600E, but others like V600K, V600D, V600R and V600L have been identified. To date, activity of BRAF inhibitors has been demonstrated for V600E and V600K mutations, but the data has yet to be demonstrated in all other mutations. The aim of this study was to determine the in vitro efficacy of a combination of GSK2118436 (a BRAF inhibitor) with GSK1120212 (a potent MEK inhibitor) on inhibiting proliferation of the human V600R/DBRAF mutated melanoma cell lines, and to evaluate the putative mechanisms of resistance induced in melanoma cell clones.
Materials and Methods: To assess selectivity of the drugs, 15 melanoma cell lines were tested. To determinate viability, the cells were plated 2×104 in 96-well plates and 100 ul of appropriate complete medium, containing increasing concentrations (0.5 to 100 nM) of the GSK2118436 and GSK1120212 molecules for 72 hrs. The MTT assays were performed using standardized approaches. To evaluate the inhibition of MAPK pathway and the consequent inhibition of cellular proliferation, the phosphorylation of ERK was examined by Western Blot analysis performed on total protein extracts from cell lines after treatment with above-mentioned inhibitors.
Results: Considering cell viability after treatment, the IC50 values were <100 nM; the Western Blot analysis showed a reduced phosphorylation of ERK following treatment, highlighting the effectiveness of the drugs in inhibiting cell proliferation. For both compounds, this activity was detected at the same concentrations independently on types of V600 mutations (V600E, V600D, or V600R) carried by melanoma cell lines. The association of these two inhibitors were also tested using the same drug concentrations; preliminary data show that the combination of GSK2118436 and GSK1120212 may enhances the inhibition of cell proliferation.
Conclusion: The availability of drugs, such as GSK2118436 and GSK1120212, which may exert their activity against a wider range of mutation types, could be helpful to increase the subsets of melanoma patients to be addressed to a more effective targeted treatment.

PP 99

Effect of temozolomide on the U-118 glioma cell line

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Background: Glioblastoma (GBM) are brain tumors that account for more than 50% of the tumors that arise within the central nervous system. They are highly proliferative, angiogenic, and develop resistance to the alkylating agents used in chemotherapy. The median survival time for GBM patients remains approximately 12−14 months in patients treated with temozolomide (TMZ) which is considered the main chemotherapeutic agent. The mechanisms of TMZ action and the pathways by which GBM cells escape from death are not fully elucidated and until now it is not explained the reduced efficacy of TMZ in GBM treatment. The reduced efficacy of TMZ was initially attributed to the activity of MGMT that removes the DNA adducts. However, it was demonstrated that even when the MGMT promoter was methylated the median survival was 21.7 months. These results suggest that the mechanism of TMZ action could be overlapped by the survival signaling pathways such as ERK1/2, Pi3K/Akt and autophagy. The activation status of cell survival pathways Pi3K/Akt, ERK1/2 and of autophagy in GBM cells treated with TMZ is poorly understood. Therefore, the main purpose of this work was to evaluate the activation status of Pi3K/Akt, ERK1/2 and autophagy in GBM cells treated with TMZ.
Materials and Methods: For that, U-118 glioma cells were incubated with different concentrations of TMZ for different periods of time. Proliferation was evaluated using a BrdUrd kit. Activation of autophagy and of PI3K/Akt and MAP kinase was evaluated by western blot. Apoptosis was addressed by confocal microscopy and by flow cytometry.
Results: The results indicated that in glioma cells treated with TMZ there was an increased expression of LC3 indicated that TMZ activated autophagy. The results also indicated that PI3K/Akt and ERK1/ERK2 were constitutively active in the U-118 cells and also that the active state was maintained in glioma cells treated with TMZ. Our study also demonstrated that TMZ induced a low level of apoptosis which was not accompanied by cell cycle arrest.
Conclusion: Our results seem to indicate that the resistance of GBM cells to TMZ could be associated to the activity of Pi3K/Akt and/or ERK1/2 MAP kinase and to the activation of autophagy. Further studies using inhibitors of these signaling pathways are needed to clarify their role in U-118 survival and proliferation.

PP 7

The non-small cell lung cancers exhibit distinct response phenotypes to telomerase inhibitor imetelstat

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Background: Telomerase is active in more than 80% of non-small cell lung cancers (NSCLC) but is not active in most somatic cells, making it an attractive target for cancer therapy. Furthermore, telomerase is required for cancer stem cell maintenance, so targeting telomerase could target this chemo-resistant subpopulation. Imetelstat, a 13-mer N3′ P5′-thio-phosphoramidate, inhibits telomerase by binding to the RNA template component leading to shortened telomeres and associated cell death. Because imetelstat is currently in clinical trials for NSCLC, it is important to determine the spectrum of activity of imetelstat in NSCLC.
Materials and Methods: We examined the in vitro effect of short-term imetelstat treatment in colony formation on a panel of NSCLC cell lines. For the colony formation assay, cells were plated at clonal density, drugged with 3 uM imetelstat, and allowed to grow for 2−4 weeks. The end point was determined by control (untreated) cells in colony formation and was dependent on inherent population doubling time.
Results: We found an array of responses ranging up to 96% inhibition of colony formation. The panel can be divided into 3 groups: responders (>70% inhibition), intermediate (20−70%), and non-responders (<20% inhibition). Interestingly, response to imetelstat in colony formation assay was independent of baseline telomere length. A biomarker signature is being generated based on gene expression levels in the three response groups.
Conclusion: Our studies indicate a wide range of response of NSCLC to imetelstat emphasizing the importance of predicting which patients are most likely to respond to imetelstat therapy.

PP 36

Highly-specific and sensitive hydrolysis probe-based real-time PCR detection of epidermal growth factor receptor variant III in oral squamous cell carcinoma

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Background: The tumor-specific EGFR mutant, EGFRvIII, is characterised by ligand-independent constitutive signalling. Tumors expressing EGFRvIII are resistant to current EGFR-targeted therapy. The prevalence of EGFRvIII in head and neck squamous cell carcinoma (HNSCC) is disputed and may vary by specific sub-site. The purpose of this study was to measure the occurrence of EGFRvIII mutations in a specific HNSCC subsite, oral squamous cell carcinoma (OSCC), using a novel real-time PCR assay.
Materials and Methods: We evaluated the presence of EGFRvIII in pre-treatment formalin-fixed paraffin embedded (FFPE) tumor specimens from 50 OSCC patients were evaluated for the presence of EGFRvIII using a novel hydrolysis probe-based real-time PCR assay. EGFR protein expression was measured in tissue microarrays using quantitative fluorescent immunohistochemistry (IHC) and AQUA® technology.
Results: We detected EGFRvIII in a single OSCC patient in our cohort (2%). We confirmed the validity of our detection technique in

an independent cohort of glioblastoma patients. We also compared the sensitivity and specificity of our novel real-time EGFRvIII detection assay to conventional RT-PCR and direct sequencing. We found that our assay can specifically detect EGFRvIII and can discriminate against wild-type EGFR in FFPE tumor samples. AQUA® analysis revealed that the presence of EGFRvIII transcript is associated with very high EGFR protein expression (98th percentile). Contrary to previous reports, only 44% of OSCC over-expressed EGFR in our study.
Conclusion: The EGFRvIII mutation is rare in OSCC. Our results corroborate previous reports of EGFRvIII expression only in tumors with extreme over-expression of EGFR. Our results suggest that EGFRvIII-specific therapies may not be ideally suited as first-line treatment in OSCC. However, EGFRvIII targeting might be a valuable addition to therapy in recurrent/metastatic OSCC where EGFRvIII may be over-represented due to the reduced responsiveness of EGFRvIII-positive tumors to conventional therapies. Since tumors expressing EGFRvIII are refractory to EGFR-targeted therapy, this could explain the poor success of EGFR targeting in clinical trials in recurrent/metastatic HNSCC patients. We conclude that highly specific and sensitive methods, such as the real-time RT-PCR assay and AQUA® analysis described here, are essential for the accurate assessment of EGFR mutation frequency and EGFR expression, and will facilitate the selection of optimal tailored therapies for OSCC patients.

PP 15

BRCA1 expression is required for efficacy of vinorelbine and is a predictive biomarker in malignant mesothelioma

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Background: Malignant mesothelioma is an aggressive tumor refractory to current therapies. Vinorelbine has been shown to exhibit useful clinical activity in mesothelioma. BRCA1 regulates sensitivity to microtubule poisons; however its involvement in regulating apoptosis in mesothelioma has not been investigated. The purpose of this study is to demonstrate that loss of BRCA1 confers resistance to vinorelbine induced apoptosis.
Materials and Methods: Dose-response curves were generated and BRCA1 expression was studied in a panel of 6 mesothelioma cell lines. Two resistant cell lines were also generated. The role of BRCA1 in regulating apoptotic response was shown by measurement of the percentage of apoptotic cell population and caspase 3/7 activity after transfection with siRNAs targeting BRCA1 or Caspase8. BRCA1 negativity percentage was also evaluated in 3 different cohorts of patients by immunostaining.
Results: Vinorelbine induced cytotoxicity correlated with BRCA1 expression level. The downregulation of BRCA1 expression by siRNA blocked caspase3 activation, PARP cleavage and the percentage of subG1 cell population. Moreover, when cells were selected for resistance to vinorelbine, this was associated with a reduction in BRCA1 expression compared to parental cells and re-expression of BRCA1 restored sensitivity. Data obtained after silencing of BAX and BAK showed that vinorelbine mediates toxicity irrespective of a functional mitochondrial apoptosis pathway; however the silencing of caspase8 decreased sensitivity. A high percentage of BRCA1 negativity was observed in primary mesotheliomas.
Conclusion: Our data highlight BRCA1 as a candidate predictive biomarker for vinorelbine induced apoptosis suggesting a potential utility in personalizing therapy with this agent.

PP 44

Myeloid zinc finger 1 regulates thymidylate synthase expression in patients with metastatic colorectal cancer showing the same promoter gene polymorphism

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Background: Background: Thymidylate Synthase (TS) is the target enzyme for fluoropyrimidine anticancer drugs. Its expression is regulated by the number of functional upstream stimulatory factor (USF) E box consensus elements present on its 5′ untranslated region. To date are known different polymorphisms, the first one consisting of 2 or 3 repeat of a 28 bp sequence, a further single nucleotide polymorphism (SNP) consisting in a G>C substitution within the second repeat of 3R (3RG>3RC) and recently it has been identified an additional SNP a G>C substitution at the 12th nucleotide in the first repeat of the 2R allele (2RG>2RC). These polymorphisms can influence TS expression, in particular 3R/3R genotype and the presence of 3RG alleles are associated to an increased transcriptional activity and to higher TS levels. The sequence of promoter region of colorectal cancer (CRC) samples was subjected to an in silico analysis (http://www.cbrc.jp/research/db/TFSEARCH.html) to search for all potential transcription factors binding this region. We found that Myeloid zinc finger 1 (MZF-1) binds the analyzed consensus. By the literature it is known that this factor induces invasion and in vivo metastasis in CRC, so we investigated a possible correlation between TS and MZF-1 expression in the same pathological samples.
Materials and Methods: Materials and Methods: we analyzed the distribution of these polimorphisms in a group of 68 healthy Caucasian subjects, and in the normal tissue, primary tumour and liver metastasis of 13 CRC patients. Tandem repeat length and the presence of SNP was determined by direct sequencing of genomic DNA. TS and MZF 1 expression were analyzed by immunohistochemistry.
Results: In healthy population the allele frequency was respectively 2RG(35%) 3RG (44%) 3RC (21%), in colorectal patients while both primary that normal and metastatic samples showed the same genotype: 2RG/3RG. TS and MZF-1 expression were related and gradually increased from normal tissue (negative) to the primary tumour (focally positive) in the metastases (overexpressing).
Conclusion: Conclusions: These unexpected results lead to the hypothesis of a genetic selection towards a more aggressive disease and enough suggest that regardless of genotype other factors are involved in regulation of TS expression as MZF 1, therefore the only genetic marker is not a valid predictor of eventual fluoropyrimidine response.

PP 77

Dissecting time- from tumor-related gene expression variability in the bilateral breast cancer model

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Background: Metachronous (MBC) or synchronous bilateral breast tumors (SBC) are generally distinct primaries, while pairs of primaries with local recurrences (LRC) share a common origin. Intra-pair biological variability in these three types of diseases results from combinations of time/host-related and genetic-related factors. Such clinical situation represents therefore an ideal model for trying to dissect tumor-related gene expression variability from time-related variability.
Materials and Methods: 18 pairs of synchronous, 11 of metachronous bilateral breast tumors and 10 pairs of primaries and locally recurrences were characterized with respect to gene expression profiles and similarity between pairs was measured using an intraclass correlation coefficient (ICC) computed for each gene. ICC distributions were compared for each type of tumor pairs using a Kruskal-Wallis test. No systemic treatment was administered between initial diagnosis and new disease manifestation in the subsets of women with MBCs or LRCs, whose primary tumors were all axillary node-negative.
Results: Considering all genes, the highest correlations were found for primaries and paired LRC and the lowest for MBC pairs. By limiting the analysis to the breast cancer intrinsic genes, correlations between primaries and paired LRC were enhanced while similar distribution were observed for SBC and MBC. On the opposite, using stromal-related genes there was a decrease of ICC values for MBC, which appeared significantly different from SBC.
Conclusion: Our data indicate that it is possible to dissect intra-pair gene expression variability into components associated with genetic origin or with time using specific gene subsets, in fact intrinsic genes are not influenced by the host and time, as instead happens for stromal genes.

PP 83

Gene expression profiling of circulating tumor cells in breast cancer

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Background: Enumeration of circulating tumor cells (CTC) in metastatic breast cancer predicts relapse-free survival and treatment failure while scanty data are currently available on their molecular features. Since CTCs might represent a surrogate tissue, their transcriptional characterization could likely allow to identify pathways involved in metastatic dissemination and to obtain clinically relevant information for monitoring prognosis and treatment response.
Materials and Methods: Predefined numbers (200, 100, 50) of MCF7 and MDA-MB-468 cells were spiked into blood from healthy donors captured using the AdnaTest EMT-1/Stem CellSelect kit (AdnaGen) and profiled (Illumina, DASL) in parallel with controls without cells and with RNA (100, 10, 1, 0.5 ng) from un-spiked cells. Controls were washed with PBS or with the AdnaWash buffer designed to improve leukocyte removal from captured cells.
Results: Gene expression detection rates for captured cells were around 60%. As expected detection rates dropped to lower values in control samples either washed with AdnaWash (30%) or with the standard washing buffer (45%). Samples derived from different numbers of spiked cells