Poster Presentations

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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.

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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