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Phase I assessment of mechanistic pharmacodynamic biomarkers for MLN8054, a small-molecule inhibitor of Aurora A kinase

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Background: The inhibition of Aurora A kinase causes defects in centrosome separation, spindle assembly and chromosome alignment, leading to transient mitotic delays in dividing cells. Despite these mitotic delays, cells lacking functional Aurora A undergo division, with deleterious consequences such as cell-cycle arrest and death. The molecular sequelae of Aurora A inhibition has been used to develop indirect mechanistic biomarkers. This study examines the application of these biomarkers to the assessment of Phase I pharmacodynamics of MLN8054, a small-molecule inhibitor of Aurora A.
Materials and Methods: Patients (pts) with advanced solid tumors were enrolled at centers in Spain and the United States. MLN8054 was administered orally in cycles of 7 to 21 days (25−80 mg/day QID) followed by 14 days of rest to cohorts of 3−6 pts. Skin biopsies (2−3 mm) and tumor biopsies were obtained before day 1 dosing and 7 days later. Skin and tumor biopsies were evaluated histopathologically using H&E staining, and assessed for mitotic counts (an indirect readout of Aurora A inhibition) by staining for the mitotic marker pSer10 histone H3. Tumor biopsies were further evaluated for morphological readouts of Aurora A inhibition by examining chromosome alignment and spindle bipolarity. Multi-focal plane images of tubulin- and DNA stained samples were acquired using an automated fluorescence microscope and 3D reconstructions of mitotic cells were generated. Multiple scorers were presented with blinded individual mitotic spindles in a random order to assess chromosome alignment and spindle biopolarity, and the majority call was assigned to each mitotic spindle.
Results: Evaluable skin biopsies pre- and post-treatment were available from 63 pts. Of these, 44 demonstrated an increase in mitotic counts after dosing relative to baseline, a change consistent with Aurora A inhibition. Evaluable tumor biopsies pre- and post-treatment were available from 10 pts. The majority of these demonstrated reduction in chromosome alignment and in spindle bipolarity after dosing relative to baseline; consistent with Aurora A inhibition. In this dataset, there was a high degree of concordance between all of the assays used, and between the result of the skin and tumor assays.
Conclusions: Taken together, these data provide compelling indirect evidence of the inhibition of Aurora A by MLN8054 in patient tumor and skin tissue.

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Expression of leptin receptor is an independent prognostic marker of Middle Eastern colorectal carcinomas

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Background: The adipokine leptin and its receptor (Ob-R) are expressed in various cancer tissues including colorectal carcinomas (CRC). High levels of leptin are seen in obesity that is a risk factor for colon cancer. Incidence of colorectal carcinomas is increasing in Saudi Arabia and a high prevalence of obesity has been reported.
Materials and Methods: We investigated the role of Ob-R and its relationship with PI3K/AKT activation in a large series of CRC tissues in a tissue micro array (TMA) format followed by in vitro studies using a panel of CRC cell lines. Immunohistochemical of Ob-R and various PI3-kinase/AKT pathway proteins on 448 CRC samples in TMA setting. MTT and flow cytometeric assays were performed to access cell proliferation and apoptosis.
Results: Ob-R over expression was associated with an early AJCC stage ($p = 0.0305$), well differentiated tumors ($p = 0.0001$), pM0 tumors ($p = 0.0396$) and histology subtype of adenocarcinomas ($p = 0.0009$). CRC with Ob-R expression showed significantly better overall survival than those with the low Ob-R expression ($p = 0.0005$) remained an independent prognostic factor in multivariate analysis. Our in vitro studies showed that Leptin increases proliferation of CRC through Ob-R that mediates the PI3-kinase/AKT signaling pathways. Leptin treatment of CRC cell lines induces activation of AKT and FOXO1 and expression of Ob-R specific siRNA prevented leptin-induced activation of AKT and its down-stream targets.
Conclusions: Leptin plays a critical role in CRC growth and survival through PI3K/AKT pathway via Ob-R. Ob-R expression is an independent prognostic marker and might represent a novel therapeutic target for CRC treatment.

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A comparative analysis of megakaryocyte potentiating factor and mesothelin as serum markers for the detection of malignant pleural mesothelioma

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Background: An early and reliable blood test is one deficiency in diagnosis of malignant pleural mesothelioma (MPM). Megakaryocyte potentiating factor (MPF) and mesothelin variants (MSLN), members of the mesothelin gene family, have been studied as candidate serum markers for MPM. We developed a novel enzyme-linked immunosorbent assay (ELISA) system to compare the diagnostic efficacy of MPF and MSLN as serum markers for the specific detection of MPM.
Material and Methods: Serum samples were collected from 27 consecutive patients with non-resectable MPM. The patient population included 13 with epithelial type MPM, three with sarcomatoid type, five with mixed type and six with unclassified type (diagnosed by cytological analysis). For controls, we used 47 patients with lung cancer, 35 with other cancers (18 ovarian, 8 stomach and 9 colon cancers), 9 asbestos-exposed asymptomatic subjects and 38 healthy adults without a history of asbestos exposure. The serum concentrations of MPF and of the soluble form of mesothelin were measured by each specific sandwich ELISA.
Results: Statistically significant elevation of serum MPF and MSLN levels was detected in MPM patients in comparison with every control group. The area under the receiver operating characteristic curve (AUC) was calculated for differentiation of MPM, lung cancer, healthy asbestos-exposed subjects and healthy adults. While the AUC for serum MPF was 0.879, cutoff = 19.1 ng/ml (sensitivity = 74.1%, specificity = 90.4%), the AUC for serum MSLN was 0.713, cut-off = 93.5 ng/ml (sensitivity = 59.3%, specificity = 86.2%). A comparison between AUC for MPF and MSLN values showed that MPF is significantly superior to MSLN ($p = 0.025$).
Conclusions: Our study shows that MPF is a significantly more sensitive and specific serum marker for the detection of malignant pleural mesothelioma compared with MSLN.

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Potential importance of the ceramide pathway in the action of the tumour vascular disrupting agent ASA404 (DMXAA, 5,6-dimethylxanthenone-4-acetic acid)

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Background: The tumour vasculature is regarded as an important target for cancer chemotherapy and tumour vascular disrupting agents (tumour VDAs) are receiving increasing attention as components of combination chemotherapy protocols. ASA404 is a tumour VDA with a dual mode of action involving both direct effects on the tumour vasculature and indirect effects mediated by induction of cytokines. ASA404 has completed Phase II evaluation and, on the basis of promising activity, has commenced a Phase III trial in combination with carboplatin and paclitaxel for the treatment of non-small cell lung cancer. The availability of appropriate biomarkers to detect the action of tumour VDAs is of great relevance and we have previously demonstrated both in mice and in cancer patients that administration of ASA404 increases in plasma concentrations of serotonin (5-HT) and its hepatic metabolite 5-hydroxyindole acetic acid (5-HIAA). 5-HT is a platelet product and it would be advantageous to identify products of tumour tissue that increase in response to ASA404. Tumour cells, macrophages and vascular endothelial cells all respond to cytokines and other signalling molecules by hydrolysis of membrane sphingolipids to ceramides, which in turn are converted to sphingosine and released from cells. We investigated the hypothesis that treatment of mice with ASA404 led to increases in plasma sphingosine.
Methods and Materials: Liquid chromatography (LC) methods were previously developed for 5-HIAA and LC-triple quadrupole mass spectrophotometric methods were developed and validated for sphingosine. C57Bl mice, with or without Colon 38 carcinomas, were treated with ASA404 and plasma samples collected for analysis.
Results: Treatment with ASA404 at a therapeutic dose (25 mg/kg) resulted in a time-dependent increase in plasma sphingosine concentration, which was much larger in tumour-bearing mice than in non-tumour bearing