the biomarker response (PD) with the pharmacokinetic (PK) data showed that the maximum biomaker response occurs after the T_{max}.
Conclusions: A robust, sensitive and quantitative Western blot assay for measuring AcH3 in tissues has been developed to evaluate the target efficacy of SB939 and is currently being used to study PK/PD relationships in the ongoing Phase I clinical trials. Preliminary data show that the excellent PK/PD relationships observed in pre-clinical models are translated to the clinic.

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Development of predictive markers of responsiveness to the MEK 1/2 Inhibitor AZD6244 in Colorectal Cancer (CRC)

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Background: The ERK pathway is constitutively activated in a several types of human cancers including CRC. The MAPK/ERK kinase, MEK, occupies a central role in the ERK pathway and therefore, MEK inhibitors constitute a promising class of novel targeted cancer therapies. AZD6244 (ARRY-142886) is a potent and selective MEK1/2 inhibitor with nanomolar potency against cell lines and activity in several in vivo models of CRC. In a phase I trial of AZD6244, inhibition of ERK phosphorylation was noted in peripheral blood mononuclear cells and tumor biopsies. Despite the enthusiasm for this class of agents in tumors with a high incidence of RAS or BRAF mutation, clinical results indicate that improved patient selection strategies are needed. The goal of this study was to identify predictive markers of sensitivity or resistance to AZD6244 in order to develop a rational basis for patient selection and combination therapy in CRC.
Methods and Results: A panel of 30 CRC cell lines was exposed to varying doses of AZD6244 (0.078 μM-5.0 μM) and analyzed for inhibition of proliferation using the sulforhodamine B (SRB) assay. Cell lines were designated sensitive (S) or resistant (R) based on IC50's less than (S) or greater than (R) 1 μM. Cell lines were assessed for pre-drug and post-drug levels of pERK by immunoblotting, indicating no association between responsiveness to AZD6244 with either baseline or post-treatment activation of the MAPK pathway. Therefore, to identify predictive markers, five S and R cell lines, differing in IC50 by 10-fold, were subjected to gene array analysis. ANOVA comparison of gene expression profiles between R and S cell lines revealed over 100 differentially expressed genes with a global p-value of <0.04, whereas 64 transcripts met the $p < 0.001$ criteria. We selected three genes, FZD2 (frizzled-2), TDGF-1 (teratocarcinoma-derived growth factor) and AKR1C3 (aldo-keto reductase 1C3) based on their relevance to cancer biology and high level of differential expression between S and R cell lines. Next, we silenced FZD2 expression in S and R cell lines using stably transfected shRNAs and examined effects on the S or R phenotype. We initially examined the effects of the FZD2 gene knock-down in the R cell line, SW480 and a nearly complete reduction of FZD2 was achieved, as confirmed by immunoblot. These cell lines were exposed to AZD6244 and assayed by SRB. We observed more than 50% reduction in the IC50 to AZD6244 compared to wild-type and scrambled shRNA controls. These results indicate that FZD2 may be a predictive marker, that when modulated, increases responsiveness to AZD6244.
Conclusions: Potential predictive biomarkers for sensitivity and resistance to AZD6244 were identified through gene array analysis. Specific knock-down of the FZD2 resulted in rendering the resistant cell line SW480 more sensitive to the AZD6244. These biomarkers will be further validated in pre-clinical xenograft models.

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Pharmacodynamic and efficacy relationship of MLN4924, a novel small molecule inhibitor of Nedd8-activating enzyme, in human xenograft tumors grown in immunocompromised mice

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MLN4924 is a first-in-class, small molecule inhibitor of the Nedd8 Activating Enzyme (NAE). We investigated whether the mechanism of action of MLN4924 identified in cultured cells is also detectable in tumor xenografts from mice treated with MLN4924, and how the pharmacodynamic effects of MLN4924 in these tumors correlate with efficacy.
Inhibition of NAE leads to decreased neddylation and thereby decreased activity of the cullin-dependent ubiquitin ligases (CDLs), enzyme complexes which control the ubiquitination and subsequent degradation of proteins with important roles in cell cycle progression. In cultured cells, NAE inhibition with MLN4924 resulted in the elevation of multiple substrates of the CDLs, an accumulation of cells with increased DNA content (>4N), a DNA damage response, and induction of cell death. This phenotype (increased DNA content, DNA damage response and cell death) is similar to that induced by over-expression of Cdt-1, a critical DNA replication licensing factor which is a substrate of CDLs.
In vivo administration of MLN4924 at well-tolerated doses to mice harboring subcutaneous human tumor xenografts resulted in a dose-dependent pharmacodynamic (PD) response of Nedd8 pathway inhibition in tumors derived from lung, breast, and colon carcinoma as well as lymphoma. A single dose of MLN4924 caused inhibition of neddylated cullins and stabilization of the CDL substrate Cdt-1 in all models tested. We further explored the PD/efficacy relationship in the HCT-116 xenograft model, which showed tumor growth inhibition in response to MLN4924. A single dose of MLN4924 to HCT-116 xenograft-bearing mice resulted in decreased levels of neddylated cullins, elevation of the CDL substrates Nrf-2, cyclin D1, and Cdt-1, and the phosphorylation of Chk-1 on serine 317, an indication of ATM/ATR activation and a DNA damage response. Multiple doses of MLN4924 on a twice-daily schedule resulted in the sustained inhibition of neddylated cullins, elevation of CDL substrates, and a continued DNA damage response in the xenograft tumors, accompanied by an increase in cleaved caspase-3 levels. Histologic analysis of tumors exposed to multiple doses of MLN4924 revealed the appearance of cells with enlarged nuclei, suggesting an increase in DNA content, and an increase in the number of apoptotic cells compared to untreated xenografts. These results demonstrate that MLN4924 effectively inhibits NAE and that the resulting stabilization of CDL substrates including Cdt-1 is sufficient to activate DNA damage and apoptotic responses in HCT-116 xenograft tumors. PD markers used in the xenograft tumor experiments are being adapted for use in Phase I trials of MLN4924 to support clinical development.

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Aberrant promoter hypermethylation of DAPK gene is an independent prognostic factor in patients with diffuse large B-cell lymphomas

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Background: Diffuse large B-cell lymphoma (DLBCL) exhibits heterogeneous clinical features and a marked variable response to treatment. The aim of the study was to assess the prognostic significance of the methylation status of several tumor suppressor and related genes in DLBCL.
Materials and Methods: We investigated the methylation status of DAPK, GSTP1, P14, P15, P16, P33, RB1, SHP1, CDH1, APC, BLU, VHL, TIMP3, and RASSF1A by methylation-specific polymerase chain reaction in 46 DLBCL specimens from Tunisian patients with well known clinicopathological features including germinal center immunophenotype status. The extent of each gene methylation status on patient's overall survival (OS) and disease-free survival (DFS) was assessed using the Kaplan-Meier method and compared with the log-rank test.
Results: Hypermethylation of SHP1 was associated with elevated lactate dehydrogenase level ($p = 0.031$). P16 and VHL were frequently hypermethylated in patients with high International Prognosis Index (IPI) scores ($p = 0.006$ and 0.004). In addition, hypermethylation of P16 was significantly associated with advanced clinical stages ($p = 0.041$). Interestingly, hypermethylation of DAPK was significantly correlated with resistance to treatment ($p = 0.023$). With regard to survival rates, promoter hypermethylation of DAPK, P16, and VHL were significantly associated with shortened OS ($p = 0.003$, 0.001, and 0.017, respectively) and DFS ($p = 0.006$, 0.003, and 0.046, respectively). In multivariate analysis, hypermethylation of DAPK remain an independent prognostic factor in predicting shortened OS ($p = 0.001$) and DFS ($p = 0.024$), as well as the IPI and the germinal center status.
Conclusions: In summary, our study demonstrates that DLBCLs with hypermethylated P16, VHL, DAPK, and SHP1 commonly show a biologically aggressive phenotype and worse prognosis. Interestingly, hypermethylation of DAPK was found to be a new independent prognostic factor that may be used to predict resistance to treatment and shortened survival in conjunction with the conventional prognostic factors such as the IPI and the germinal center status. Also, our results suggest that promoter gene methylation plays an important role in the pathogenesis of DLBCLs and may be a potential interesting target for determining new appropriate treatments.