flattened mean steady state plasma concentrations (25−35 μM) across all dose levels. GLI1 was down-modulated >2-fold in surrogate tissue from a majority of pts tested thus far.
Conclusions: The Hh antagonist GDC-0449 was evaluated at 3 dose levels. The unusual pharmacokinetic profile of this oral agent reflects the accumulation of high stable plasma concentrations of GDC-0449. GDC-0449 at 150 mg/day demonstrates clinical efficacy and is tolerated for extended periods of continuous dosing.

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MLN4924, a potent and novel small molecule inhibitor of Nedd8 activating enzyme, induces DNA re-replication and apoptosis in cultured human tumor cells

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The initial step in the pathway for conjugation of the ubiquitin-like protein Nedd8 to its cellular targets requires the activity of the Nedd8 Activating Enzyme (NAE). Nedd8 conjugation is required for the proper function of mammalian cullin-dependent ubiquitin ligases (CDLs). These CDLs in turn control the timely ubiquitination and subsequent degradation of many proteins with important roles in cell cycle progression and signal transduction. Inhibition of NAE leads to decreased activity of the CDLs impacting cellular processes relevant to tumor cell growth and survival thereby providing a rationale for targeting NAE as an anti-cancer strategy. MLN4924 is a first-in-class, potent and selective small molecule inhibitor of NAE. This molecule was used to explore the consequences of inhibiting the Nedd8 pathway in cultured human cancer cells.
MLN4924 specifically inhibits Nedd8-cullin formation leading to stabilization of direct CDL substrates by preventing their ubiquitination and degradation through the proteasome. One such CDL substrate is the critical DNA replication licensing factor Cdt1. Over-expression of Cdt1 has been reported to induce DNA re-replication in cells resulting in cell cycle arrest, DNA damage and genomic instability. Immunoflourescence and western blot analysis of HCT-116 cells treated with MLN4924 demonstrated an increase in the nuclear localization and stabilization of Cdt1 preventing its normal cell cycle regulated turnover. Furthermore, cells treated with MLN4924 accumulated in S-phase of the cell cycle with an apparent increase in nuclear size and DNA content coupled with increased BrdU incorporation reflecting over replication of DNA. This aberrant phenotype was associated with the activation of a DNA damage checkpoint response through the ATM/ATR pathway assessed by the expression of elevated levels of phospho-p53 (S15), phospho-Chk1(S317) and phospho-H2AX ultimately leading to cell death through apoptosis. Synchronized cells released into MLN4924 were prevented from progressing into mitosis as demonstrated by a loss of pH3 signal. The continued increase in DNA content >4N and analogous BrdU incorporation associated with the loss of pH3 signal demonstrated that re-replication of the DNA was occurring within the same cell cycle.
The gross accumulation of re-replicated DNA and DNA-damage resulting from re-replication leads to cell cycle arrest and apoptosis demonstrates a novel mechanism of action for MLN4924 in cultured human tumor cells.

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Effects of 5-fluorouracil (FUra)/leucovorin(LV)-induced DNA damage on the Wnt signaling pathway and downstream targets in human colon carcinoma cell lines (cc)

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Sporadic and familial mutations in the APC gene and aberrant Wnt signaling have been demonstrated in the initiation and progression of human colorectal cancer. Activation of Wnt results in the dissociation of the 'B-catenin destruction complex' thereby stabilizing cytosolic B-catenin and facilitating its nuclear translocation. Nuclear B-catenin is subsequently responsible for the transcription of Wnt target genes including cyclin D1 and c-myc. In the absence of Wnt signal or in presence of wtAPC, B-catenin is sequentially phosphorylated by CK1 and GSK3B leading to its ubiquitinylation and subsequent proteosomal degradation. The goal of this study was to determine the effect of FUra/LV treatment on components of the Wnt signaling pathway in the presence of wtAPC or mtAPC and to further evaluate the effect of FUra/LV on survival of these cells.
In a panel of cc, data demonstrated expression of wtAPC in RKO and GC3/c1 and expression of mt APC in HT29. In these cc, FUra/LV caused DNA damage-induced inhibition of clonogenic survival with an IC50 of ~0.5–2.5 uM, reversible by dThd. WtAPC expression was induced in RKO and GC3/c1, while expression of mtAPC was downregulated in HT29 by FUra/LV. Dab-2, a negative regulator of Wnt signaling, demonstrated low expression in RKO, that was upregulated by FUra/LV treatment. Phosphorylation of GSK3B (p-GSK3B) leads to its inactivation, thereby inhibiting B-catenin degradation. Basal levels of p-GSK3B in RKO cells were relatively high and were downregulated by FUra/LV. Further, attenuation in the levels of B-catenin, cyclin D1 and c-myc was also observed, and Wnt signaling was inhibited. These data suggest that FUra/LV elevates the expression of wtAPC and Dab2 and stabilizes the B-catenin destruction complex in RKO cells, thereby causing B-catenin degradation and resultant inhibition of Wnt signaling.
In HT29 cells stably expressing wtAPC in presence of ZnCl2 (HT29-APC), FUra/LV increased the expression of wtAPC and attenuated the levels of B-catenin, cyclin D1 and c-myc. Further, survivin and c-FLIP expression was also reduced and cells were sensitized to FUra/LV-induced apoptosis. No changes were observed in the expression levels of Bcl-xL, cIAP-1 or c-IAP2.
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18F-FLT-PET for the evaluation of MEK inhibitor AZD6244

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Background: AZD6244 (ARRY-142886) is a potent, selective, uncompetitive inhibitor of MEK 1/2 being tested in phase II trials for a number of solid tumours. The RAF/MEK/ERK protein kinase signal cascade is a key intracellular pathway regulating cellular proliferation and survival. A solid oral formulation, incorporating the Hyd-Sulfate salt of AZD6244, is currently being investigated in a phase I study. To assess the effect of AZD6244 on proliferation in tumours, we used the 18F-FLT-PET. 3′-deoxy-3′-[18F] fluorothymidine (18F-FLT), a thymidine analogue PET tracer, which is phosphorylated by the enzyme thymidine kinase (TKI) and is subsequently trapped intracellularly. 18F-FLT trapping is a surrogate marker for proliferation. 18F-FLT-PET was used in this study to investigate if this method can be used as early predictor for patients (pts) on therapy with AZD6244.
Methods: 18F-FLT-PET scans were performed baseline and after two weeks of treatment with AZD6244 Hyd-Sulfate. In this phase I study pts received 25 mg, 50 mg, 75 mg, or 100 mg bid. FLT-uptake in tumours was analyzed qualitatively and quantitatively by measuring SUVmax, SUV50 and SUV70 in regions of interest (ROI). 18F-FLT-PET scans were compared to CT-scans (baseline and after 8 weeks), which were evaluated using the RECIST criteria.
Results: In four pts a baseline and follow up 18F-FLT-PET was performed. One pt with a melanoma showed a qualitative and quantitative decrease of FLT uptake (mean SUVmax baseline 4.6, follow up 2.30). This was followed by a decrease in RECIST of 11% at the CT scan after 8 weeks. Unfortunately, at the second CT evaluation the pt had PD. In one pt with colorectal cancer (CRC) a visual increase of FLT-PET combined with an increase of SUVmax from 1.81 to 4.19 was observed. This pt had SD after the first CT evaluation (RECIST +3.8%) but had PD at the second CT evaluation. The other two pts (one melanoma, one CRC) did not show significant changes in FLT uptake; mean SUVmax baseline 6.46, follow up 5.32 for melanoma pt, mean SUVmax 2.30, follow up 1.37 for CRC patient and had PD (at the first CT evaluation for the melanoma pt or after the second evaluation for the CRC pt).
Conclusion: This is the first report of 18F-FLT-PET to assess the effect of AZD6244, a MEK inhibitor. Changes in SUVmax could be measured. As yet the number of pts is too limited to assess if there is a correlation between the changes in SUVmax and RECIST measurements. Further investigation is warranted.

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A role for tumor associated macrophages in tumorigenesis

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Immunocells are known to infiltrate tumor microenvironments and, is the case for most cancers, promote tumorigenesis through various pathways, the best studied being tumor-angiogenesis promoted by tumor associated macrophages (TAMs). However, various intra- and intercellular signaling