was evaluated after three weeks of cultivation. An aliquot of the same tumor specimens was shock-frozen immediately after removal from the patient. Subsequently, total RNA was isolated and reversely transcribed to cDNA followed by real-time multiplex PCR experiments based on Taqman technology. Results from these gene expression experiments were normalized against β-actin transcripts.
Results: A total of 29 tumor samples was collected from a variety of solid tumors. Samples were investigated for gene expression of FPGS and GARFT. No clear difference in FPGS gene expression was observed between P-sensitive and P-resistant specimens (average±SD: 428±138 versus 884±994; R=0.15, n=25). In contrast, GARFT transcripts were expressed at low levels in P-sensitive specimens (117±52 versus 318±235; n = 13). Our data indicate that GARFT expression levels and clonogenic survival after ALIMTA exposure are correlated (R=0.7810). This correlation may help identify potentially clinically sensitive tumors and supports the design of subsequent clinical trials.
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VX-944: an inosine monophosphate dehydrogenase inhibitor with unique anti-cancer activity

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Inosine monophosphate dehydrogenase (IMPDH) is an essential rate-limiting enzyme in the de novo guanine nucleotide biosynthetic pathway required for cell proliferation, and hence an attractive anti-cancer target. VX-944 is an orally bioavailable, uncompetitive and non-nucleoside IMPDH inhibitor. We have previously shown that VX-944 is the most potent cellular IMPDH inhibitor described thus far, with IC50 values ranging from 20–200 nM in AML patient samples and immortalized cell lines derived from hematological malignancies. We have also shown that VX-944 induces apoptosis synergistically with Fludarabine and Doxorubicin. Notably, VX-944 potency is not affected by MDR pumps (Jain et al, Blood 2002: 100, ibid 2003: 243). In studies reported here, we demonstrate that VX-944 also inhibits proliferation of cell lines derived from human colon, breast, lung, pancreatic, melanoma and other solid tumors with an IC50 value range of 25–250 nM. VX-944 was 3–40-fold more potent than mycophenolic acid, another IMPDH inhibitor. Complete inhibition of anchorage-independent colony formation was observed at 200–800 nM VX-944 in many of these cell lines. Activating mutations in BRAF, Ras or p53 oncogenes did not appear to alter the sensitivity to VX-944. VX-944 induced apoptosis, correlating with caspase activation, PARP cleavage and a decrease in cell viability. The induction of caspases and apoptosis was blocked by guanosine addition, consistent with the specificity of VX-944 for IMPDH. The anti-proliferative activity of VX-944 was confirmed using surgical explants derived from colon, melanoma and pancreatic cancer patients. VX-944 demonstrated dose-dependent growth inhibition using the Extreme Drug Resistance (EDR®) Assay, with median IC50 values of 250 nM for pancreatic (n=14), 330 nM for melanoma (n=21), and 500 nM for colon cancer specimens (n=15). The pancreatic, melanoma and colon specimens were tested with standard chemotherapeutics to establish their sensitivity to gemcitabine, temozolomide or 5FU combined with leucovorin respectively. VX-944 was equally active in tumor samples sensitive or resistant to these agents. Many colon specimens sensitive to VX-944 were observed to be resistant to Irinotecan, Mitomycin C, Carmustine or Topotecan. Our results, demonstrating the potent anti-cancer activity of VX-944, combined with its desirable drug-like properties, indicate that VX-944 may be an attractive new agent for the treatment of patients with aggressive cancers, particularly with refractory cancers.

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5,10-methylenetetrahydrofolate decreases 5-fluorouracil systemic toxicity without concomitant loss of antitumor activity

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Background: The antimetabolite 5-fluorouracil (5-FU) is the standard treatment for numerous cancer types, in particular colorectal cancer. Despite its antitumor activity, 5-FU can cause dose-limiting side effects, including decreased white blood cell and platelet counts, which can impair its efficacy. To increase 5-FU antitumor activity, it is commonly used in combination with folinic acid (leucovorin). However, leucovorin can also increase the severity of 5-FU side effects. Furthermore, leucovorin must be intracellularly converted into its active metabolite 5,10-methylenetetrahydofolate (CoFactor™), potentially limiting the full anti-tumor activity of this drug combination. In contrast, CoFactor supplies 5,10-methylenetrahydrofolate directly and has demonstrated antitumor activity in combination with 5-FU in phase I/II clinical trials for colon and breast cancer. To further investigate the activity of CoFactor in comparison to leucovorin, we examined both the systemic toxicity and antitumor activity of these drugs in combination with 5-FU using *in vivo* mouse models.
Methods: For tumor studies, nude mice were inoculated subcutaneously with HT-29 colorectal tumor cells. After tumors reached approximately 50mm^3 in volume, mice were treated with combinations of 5-FU, CoFactor, and leucovorin by intraperitoneal injection for 7 consecutive days (0.6mg/mouse/drug). Tumor volumes were calculated every 2 to 3 days. For toxicity analysis, BALB/c mice were injected with the same schedule and dosage of drugs. Complete blood cell counts were analyzed pre-treatment and eight days after treatment initiation. Simultaneously, survival was followed for 15 days.
Results: In BALB/c mice, treatment with either 5-FU alone or combination 5-FU/leucovorin caused 100% mortality within 12 days of treatment initiation. In contrast, significantly more ($p<0.05$, Logrank test) CoFactor/5-FU treated mice survived (38%) beyond this time. Blood analysis revealed significantly more white blood cells in 5-FU/CoFactor treated mice than 5-FU/leucovorin treated mice ($p<0.05$, Student's t test). Specifically, we observed significantly more platelets and neutrophils in the 5-FU/CoFactor treated group. In contrast to the lower systemic toxicity profile of CoFactor/5-FU, this drug combination still maintained its antitumor activity in the HT-29 nude mouse model. Compared to the mean tumor volume in mice treated with only 5-FU (368.5±63.7, mean±SEM, n=10), we observed significant inhibition ($p<0.05$, Student's t test) of tumor growth with combination CoFactor/5-FU (225.4±32.0, n=12). Furthermore, CoFactor/5-FU treated mice had smaller tumor volumes than leucovorin/5-FU treated mice (262.0±36.5, n=11).
Conclusions: This study suggests CoFactor can reduce 5-FU mediated hematological toxicity while simultaneously increasing its antitumor activity in comparison to leucovorin.

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Synergistic combination of SDX-102 with docetaxol or 5-fluorouracil in pre-clinical models of lung and pancreatic cancer

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INTRODUCTION. SDX-102 (L-alanosine), a selective inhibitor of de-novo purine biosynthesis, is being tested in clinical trials in patients with tumors defective in the purine salvage pathway. These tumors do not express methylthioadenosine phosphorylase (MTAP), a critical enzyme of purine salvage from the polyamine catabolic pathway. Docetaxol is a microtubule-stabilizer anti-neoplastic agent, structurally related to paclitaxel, widely used in several indications, including non-small cell lung cancer (NSCLC). Fluorouracil (5-FU) is an anti-metabolite also frequently used in several cancer indications, including pancreatic cancer and NSCLC. AIMS. The aims of this study were to: (1) compare the activity of docetaxol and 5-FU in MTAP-positive and MTAP-negative NSCLC, pancreatic cancer and mesotheliomas cancer cell lines; (2) test the anti-neoplastic efficacy of the combination of SDX-102 with docetaxol or 5-FU in in-vitro and in in-vivo human xenograft models. RESULTS. The effect of docetaxol or 5-FU alone or in combination with SDX-102, on proliferation and survival in MTAP-negative cells was measured by MTT assay 72 hours post-treatment. Docetaxol and 5-FU, used alone, displayed a range of activity in several MTAP-negative cell lines (IC50 range 0.2–4.63 μM for 5-FU; 1–5nM for docetaxol) comparable to the IC50s values reported in the literature for MTAP-positive cells from the same tumor type. In vitro combinations of SDX-102 with 5-FU or docetaxol in MTAP-negative cells demonstrate additive to synergistic interactions when analyzed using the combinatorial index (CI) analysis method of Chou and Talalay (CI range: 1.1–0.5). In vivo, the combination of docetaxol (10 mg/kg) and SDX-102 (50 mg/kg) was superior to either single agent in a mesothelioma xenograft model (H-Meso-1) in SCID mice. Treatment was initiated at a tumor volume of 100 mm3. Thirty-one days following treatment the mean tumor volume of the combination group was 217 mm3 compared to 655 mm3 and 1035 mm3 for taxotere and SDX-102-treated groups, respectively, while control tumors were 1272 mm3. CONCLUSIONS. These results suggest that studies of SDX-102 in combination with either docetaxol or 5-FU should be further explored in preclinical models.