

We treated mice bearing a moderately sensitive tumor with a clinically relevant schedule (120 mg/kg every 3 days), to develop *in vivo* resistance to gemcitabine. Mice bearing this tumor, Colon 26A (T/C=0.25) had to be treated for more than one year with gemcitabine. The most resistant tumor was used for transplantation, finally resulting in the completely resistant tumor Colon 26G (T/C=0.96). Initial studies focused on resistance mechanisms known from *in vitro* studies. However, in Colon 26G dCK activity was only 1.7-fold decreased, dCDA was not changed and DNA-polymerase activity was only marginally (1.7-fold) decreased. Although Colon 26A accumulated 1.5-fold more gemcitabine triphosphate 2 hr after a gemcitabine injection, these changes were considered insufficient to explain resistance. Therefore a microarray was performed on single stranded cDNA synthesized from mRNA of both Colon 26A and Colon 26G. A striking 1.996 ± 0.095 -log increase (mean \pm SD of 3 separate arrays) in expression of the RNR M1 subunit gene was found in Colon 26G, which was highly significant ($p < 0.001$). The differences in expression of other genes involved in gemcitabine metabolism were smaller and not significant. The expression of both RNR M1 subunit protein and mRNA were >10 -fold increased, as measured by western blotting and real time PCR. In conclusion: this is the first model with *in vivo* induced resistance to gemcitabine. In contrast to most *in vitro* studies, dCK activity was not the most important determinant of gemcitabine resistance *in vivo*. This is also the first *in vivo* evidence for a key role for RNR in gemcitabine resistance. Thus, RNR should be included in selection of tumors for gemcitabine treatment.

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Rationale for combination of SDX-102 with Alimta in MTAP-negative tumors

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INTRODUCTION: SDX-102 (L-alanosine), a potent inhibitor of de novo purine biosynthesis, is being tested in Phase II clinical trials in selected patients with tumors that do not express methylthioadenosine phosphorylase (MTAP), a critical enzyme required for the purine salvage pathway. Alimta (pemetrexed) is a multitargeted antifolate that acts on a number of folate-dependent enzymes, including thymidylate synthase, dihydrofolate reductase, glycinamide ribonucleotidyltransferase (GARFT), and aminimidazole carboxamide ribonucleotide formyltransferase (AICARFT). GARFT and AICARFT are critical enzymes in de novo purine and pyrimidine biosynthesis. **AIMS:** The aims of this study were to: (1) measure the ATP-lowering activity of Alimta in cell lines from non-small cell lung cancer (NSCLC), mesothelioma and pancreatic cancer; (2) investigate the effect of engagement of the MTAP pathway on Alimta's activity; (3) test the anti-tumor efficacy of the combination of SDX-102 and Alimta in MTAP-negative tumor cells. **RESULTS:** Alimta was shown to lower intracellular ATP levels in several cell lines. Treatment with Alimta caused a 50% reduction of intracellular ATP after 72 hours incubation at concentrations ranging from 80 nM (NCI-H2452) to 5 μ M (BXP3). Activation of the purine salvage pathway, using an MTAP-substrate, was sufficient to block the Alimta-induced ATP depletion in the MTAP-expressing cells, but not in the MTAP-deleted cells. In MTAP-positive cells (HS-766T, A-427, and NCI-H226) treated with Alimta, ATP levels were restored to more than 85% of control cells by addition of an MTAP substrate. The MTAP substrate was able to fully rescue HS-766T cells from loss of viability induced by Alimta as measured by the MTT assay. Finally, low concentrations of SDX-102 was shown to enhance the cytotoxic activity of Alimta in several MTAP-deleted cell lines. In the mesothelioma cell line NCI-H2452, incubation with 200 nM SDX-102 shifted the IC75 of Alimta from 200 nM to 15 nM. **CONCLUSIONS:** These results suggest that Alimta treatment can lead to a reduction of the intracellular ATP levels in the cancer cell lines tested. In addition, engagement of the purine salvage pathway can protect MTAP-positive, but not MTAP-negative cells, from Alimta effect on ATP levels or cell viability. These results suggest that the combination of SDX-102 and Alimta in MTAP-negative tumors should be further pursued in pre-clinical experiments.

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A phase I study of pemetrexed supplemented with folic acid (FA) and vitamin b12 (VB12) in Japanese patients with solid tumors

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LY231514 (ALIMTA®; pemetrexed, N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl]-L-glutamic acid) is a novel multitargeted antifolate antimetabolite. The antitumor activity of this agent likely derives from inhibition of several key folate-requiring enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). In initial phase I study d1, q21d without vitamin supplementation, maximum tolerated dose (MTD) was determined to be 600 mg/m². However, life threatening toxicities were observed in the subsequent phase I or II studies, in which a multivariate analysis suggested baseline homocysteine plasma concentration was a statistically significant predictor for the toxicities. In addition, in a phase III trial (CDDP+/-LY231514 for malignant pleural mesothelioma: MPM), the results indicated that supplementation of FA and VB₁₂ reduced the incidence of neutropenic fever and other side effects. LY231514 has recently been approved for MPM in combination with CDDP in US and is also under development for a wide range of human cancers. Based on those results mainly obtained from pts in Western population, we planned another phase I trial of LY231514 with FA and VB₁₂ in advanced cancers for Japanese pts. Primary endpoints were determination of MTD and recommended dose (RD). The starting dose was 300 mg/m² and the dose level has escalated up to 1200 mg/m². In total, 31 pts (NSCLC:19, MPM:7, thymoma:2, rectal cancer:1, alveolar soft part sarcoma:1, unknown:1) were administered. Dose limiting toxicities (DLT) included ALT elevation at 700 mg/m², infection and skin rash at 1200 mg/m² (each one patient). As consequence of the observation that the incidence of DLT was for 2/6 at 1200 mg/m², the MTD and RD were assessed at 1200 mg/m² and 1000 mg/m², respectively. Hematological toxicities (G3 leukopenia: 3 pts, G3 neutropenia: 4 pts, G3 lymphopenia: 1 pts) and non-hematological toxicities (G3 ALT elevation: 2 pts, G2 AST elevation: 2 pts, G3 skin rash: 1 pts, G2 mucositis: 2 pts) were observed in cycle 1. Dose-normalized plasma LY231514 concentrations in Japanese pts following administration of LY231514 with FA and VB₁₂ were similar to those in Western pts without vitamin supplementation. LY231514 pharmacokinetics in Japanese pts did not appear to be overtly different from those in Western pts. 27 of 31 enrolled were evaluable for antitumor activity to date and 5pts achieved PR (NSCLC: 4 pts, thymoma: 1 pts). 4PR/16 evaluable pts were observed in previously treated advanced NSCLC. 4 pts are too early to evaluate. As a result of this study, the chemotherapy of LY231514 with FA and VB₁₂ supplementation expressed tolerable toxicity profile and the MTD/RD of LY231514 was assessed at 1200 mg/m²/1000 mg/m². This study also showed potent anti-tumor activity of LY231514 against advanced NSCLC.

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Correlations of *in vitro* chemosensitivity of solid tumors to Pemetrexed (P, ALIMTA®) and target gene expression

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Background: The new antifolate ALIMTA® (Pemetrexed, P) is clinically active in various solid tumors and has recently been approved for the treatment of mesothelioma. Main targets include key enzymes in purine and pyrimidine biosynthesis including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). P is one of the best substrates presently known for folyl polyglutamate synthetase (FPGS). Polyglutamated P has a substantially higher potency for inhibition of GARFT and TS. The specific aim of the present study was to correlate FPGS gene expression with *in vitro* chemosensitivity of freshly explanted human tumor specimens. **Methods:** Freshly biopsied tumor cells (solid tumors, pleural effusions, or ascites) were used for soft-agar cell cloning experiments. Cells were exposed to several concentrations of P and clonogenic tumor growth