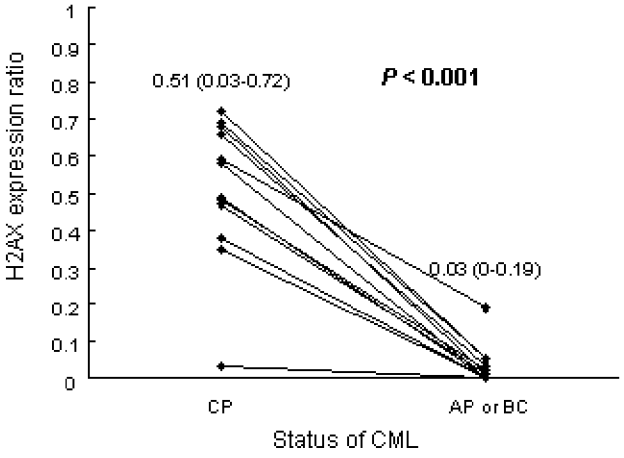
Material and methods: We evaluated the expression of γ H2AX protein in bone marrow samples by western blot from 65 CML patients and analyzed its prognostic significance.
Results: Western blot analysis revealed that the γ H2AX was undetectable in 20 (40.8%) of 49 cases in chronic phase and 12 (75.0%) of 16 cases in accelerated phase/blastic crisis. The levels of γ H2AX expression of the CML samples were significantly lower than that of the normal bone marrow controls ($P<0.001$). The levels of γ H2AX in the leukemia cells were markedly lower in the accelerated phase or blastic crisis at the initial presentation compared to that in the chronic phase ($P<0.05$, and $P<0.01$, respectively). Correlation analyses of γ H2AX levels and characteristics of patients demonstrated that the γ H2AX levels correlates well with peripheral blast counts ($P<0.05$). There was no correlation between γ H2AX levels and patient age, sex, white blood cell count, platelet count, hemoglobin, spleen size, percentage of eosinophils, or basophils. No differences in the cytogenetic response to interferon-α were observed according to the γ H2AX levels. The disease free survival or overall survival time was not significantly different according to expression levels of γ H2AX protein. Of 49 patients diagnosed as chronic phase, 12 patients progressed to the acute phase during the follow up period. In these patients, the levels of γ H2AX were markedly decreased with disease progression ($P<0.001$).



Conclusions: The γ H2AX protein was markedly down-regulated in a substantial proportion of CML. Down-regulation of γ H2AX protein was significantly associated with disease progression. These findings might lend additional insight into the molecular pathogenesis of CML.

482 POSTER

Quantitative trait locus analysis reveals two intragenic sites that influence O6-alkylguanine-DNA alkyltransferase activity in peripheral blood mononuclear cells

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The repair of specific types of DNA alkylation damage by O^6-alkylguanine-DNA alkyltransferase (MGMT) is a major mechanism of resistance to the carcinogenic and chemotherapeutic effects of certain alkylating agents. The levels of expression of MGMT in normal and tumour tissues are thus of interest in relation to the prevention and treatment of cancer. MGMT expression in a given tissue varies widely between individuals but the underlying causes of this variability are not known. Here we investigate the contribution of variation at the DNA level on intra-individual differences in MGMT activity in peripheral blood mononuclear cells (PBMC). First we use an expressed single nucleotide polymorphism (SNP) to demonstrate that the two MGMT alleles are frequently expressed at different levels in PBMC, suggesting that there is a genetic component of inter-individual variation of MGMT levels that maps close to or within the MGMT locus. Next, we show by quantitative trait locus analysis using intragenic SNPs that there are at least two sites influencing interindividual variation in MGMT activity in PBMC. One of these sites is characterized by an SNP at the 3′ end of the first intron and the second by two SNPs in the last exon. The latter two are in perfect disequilibrium and result both in amino acid substitutions; one of them, Ile143Val, affecting an amino acid close to the cysteine (145) residue at the active site of MGMT. In vitro assays did not reveal any influence of the amino acid substitutions on the activity of the protein on methylated DNA substrate, however, the Val^{143} variant was more resistant to inactivation by the MGMT inactivator O^6-(4-bromothenyl)guanine. The effect of analogue inhibitors on the variant MGMT is currently being investigated. Finally, the relationship between alleles at the two sites and MGMT expression levels allows the prediction of MGMT activity in individuals according to their genotype and we report the results from a case-control series suggesting a link between MGMT activity and lung cancer risk.

483 POSTER

Polymorphisms of DNA repair genes in the molecular pathogenesis of esophageal (Barrett) adenocarcinoma

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Background: To test the hypothesis that aberrations of DNA repair contribute to susceptibility for the progression of gastroesophageal reflux disease (GERD) to Barrett esophagus (BE) and esophageal adenocarcinoma (EADC), we studied the frequency of polymorphisms of selected DNA repair genes (XPC, XPD, XRCC1, XRCC3) in patients with GERD, BE and EADC enrolled in a 2-year prospective case-control study.
Materials and methods: Genomic DNA was extracted from blood samples (obtained with informed consent) of patients with GERD (n=126), BE (n=125), and EADC (n=56), defined according to strict clinicopathologic criteria. Controls comprised 95 healthy, asymptomatic individuals from the same geographic region. Polymerase chain reaction, restriction digestion and gel electrophoresis were used to identify wild-type and polymorphic variants of XPD (C22541A and A35931C), XRCC1 (C26304T and G28152A), XRCC3 (T18067C), and the poly (AT) insertion/deletion of XPC (PAT). Allelic frequencies were compared between cases (GERD, BE, EADC) and controls using logistic regression analysis to calculate age, gender, smoking and alcohol-adjusted odds ratios (OR) and 95% confidence intervals (CI).
Results: Genotype frequencies in controls were as predicted from Hardy-Weinberg equilibrium theory. Compared to controls, a large and statistically significant increased frequency for the XPC PAT homozygous variant genotype was seen in patients with EADC (OR 3.82; 95% CI 1.05–13.93). However, significantly reduced frequencies were seen for the XPD A35931C homozygous variant genotype in patients with EADC (OR 0.24; 95% CI 0.07–0.88), and for the XRCC1 G28152A homozygous variant genotype in patients with BE (OR 0.38; 95% CI 0.12–0.64) and GERD (OR 0.29; 95% CI 0.12–0.66).
Conclusions: 1) The contribution of DNA repair gene polymorphisms to the molecular pathogenesis of EADC is complex, with polymorphisms of nucleotide excision repair genes showing opposing effects (increased risk for XPC vs. a protective effect for XPD). 2) The protective effect of the homozygous variant of XRCC1 G28152A for GERD and BE suggests that base excision repair alterations may occur early in progression to EADC, possibly in response to endogenous oxidative or inflammatory DNA damaging processes, and suggests potential clinical application for this polymorphic marker in endoscopic Barrett surveillance programs.

Antimetabolites

484 POSTER

In vivo induction of resistance to gemcitabine results in amplification of ribonucleotide reductase as the major determinant

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Gemcitabine is a deoxycytidine analog with activity against several solid cancers. Deoxycytidine kinase (dCK) phosphorylates gemcitabine, which is required for its incorporation into DNA by DNA-polymerase. The drug can be inactivated by deoxycytidine deaminase (dCDA). The metabolite gemcitabine diphosphate, (dFdCDP) is an inhibitor of ribonucleotide reductase (RNR).
In most *in vitro* models resistance to gemcitabine was associated with a decreased dCK activity. In addition, RNR might be an important determinant of gemcitabine resistance. In all these models resistance was established using continuous exposure to gemcitabine with increasing concentrations, which is clinically not relevant.

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±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.

485 POSTER

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 ribonucleotidetide formyltranserase (GARFT), and aminoimidazole 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 μM (BXPC3). 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.

486 POSTER

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/m^2. 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 VB_{12} 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 VB_{12} in advanced cancers for Japanese pts. Primary endpoints were determination of MTD and recommended dose (RD). The starting dose was 300 mg/m^2 and the dose level has escalated up to 1200 mg/m^2.
In total, 31 pts (NSCLC:19, MPM:7, thymoma:2, rectal cancer:1, alveolar soft part sarcoma:1, unknouwn:1) were administrated. Dose limiting toxicities (DLT) included ALT elevation at 700 mg/m^2, infection and skin rash at 1200 mg/m^2 (each one patient). As consequence of the observation that the incidence of DLT was for 2/6 at 1200 mg/m^2, the MTD and RD were assessed at 1200 mg/m ^{2} and 1000 mg/m^2, 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 VB_{12} 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 VB_{12} supplementation expressed tolerable toxicity profile and the MTD/RD of LY231514 was assessed at 1200 mg/m^2/1000 mg/m^2. This study also showed potent anti-tumor activity of LY231514 against advanced NSCLC.

487 POSTER

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 neosynthesis including thymidilate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). P is one of the best substrates presently known for folyl polyglutamate synthetase (FPGS). Polyglutamted 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