polymerase beta to remove the sugar and insert the proper base, and DNA ligase. Chemotherapeutic methylating agents such as Temozolomide produce high proportions of N7methylguanine, and as we have recently shown [AACR, 2005], Flutarabine induces formation of abasic sites. For this reason, we evaluated Methoxyamine, an agent that binds to the aldehyde form of the deoxyribose, as an agent that could block processing by base excision repair and potentiate cell death by either Temozolomide or Fludarabine. In both in vitro models of various human cancer cell lines – colon cancer, leukemia and gliomas – and in xenograft models of these tumor types, concurrent Methoxyamine potentiated and synergized the efficacy of Temozolomide or fludarabine. Since Methoxyamine has a short plasma halflife, continuous infusion was also an effective approach to therapeutic synergy. In addition, although some cell lines were resistant to Temozolomide due to defects in mismatch repair [MMR], they were sensitized by Methoxyamine. This indicates that the BER pathway is independent of the MMR pathway. Thus, Methoxyamine is a promising agent that is now entering clinical trial to potentiate chemotherapeutic agents that induce DNA damage, in particular, abasic sites, recognized by the BER pathway. In addition, other agents targeting this pathway may be useful to provide another mechanism based approach to cancer therapeutics.

459 INVITED

Poly(ADPribose)polymerase inhibitors – the current clinical status

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Poly(ADP-ribose) polymerase 1 (PARP-1) is a nuclear enzyme involved in base excision repair, the DNA repair pathway recognising single strand breaks. PARP-1 is activated by binding to damaged DNA; polymers of poly(ADP-ribose) are formed on acceptor proteins, including PARP-1 – signalling recruitment of other BER proteins. A number of inhibitors of PARP-1 are in early clinical development for a range of indications, both within oncology and in wider medical practice.

There are preclinical models demonstrating both radio- and chemopotentiation, particularly of alkylating agents and topoisomerase I poisons, by PARP inhibition. The first PARP inhibitor entered early oncology trials investigating this. AG014699, a potent tricyclic indole PARP inhibitor (Pfizer GD), has completed phase I and II evaluation in combination with temozolomide. The phase I study was driven by a pharmacodynamic endpoint, and a PARP Inhibitory Dose defined. Profound PARP inhibition was shown both in peripheral blood lymphocytes (PBL) and tumour biopsies. A phase II study of the combination in metastatic melanoma demonstrated an 18% confirmed CR/PR rate with 40% of patients remaining on treatment for 6 months or more. No PARP inhibitor specific toxicity was seen in either study, however it was clear that temozolomide-induced myelosuppression was enhanced, and a significant number of patients on the phase II study required a dose reduction. There are a number of other PARP inhibitors in late pre-clinical development or early clinical development centred on the use of temozolomide in malignant glioma.

There is recent compelling pre-clinical data demonstrating single agent activity in cancer cell lines which are defective in DNA double strand break repair. This has most conclusively been demonstrated in BRCA1 or BRCA 2 homozygous cell lines. It is this potentially exciting single agent application that is the focus of development of the oral agent KU-0059436 (KuDOS/AstraZeneca). Phase I data reported at ASCO 2006 demonstrated PBL PARP inhibition and activity in a patient with familial ovarian cancer and plans are established to perform the definitive phase II in this indication with AG014699

In addition to the potential applications in oncology the ability of PARP inhibition to reduce cell necrosis in situations of extensive DNA damage such as septic shock, burns and ischaemia means that range of application could be vast, however at present development in oncology leads the field.

460 INVITED

ATM and DNA dependent protein kinase inhibitors

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An important determinant of the susceptibility of cancer cells to DNA damaging anti-cancer therapeutics is the ability of the cells to repair the DNA damage inflicted upon them. It has therefore been proposed that inhibition of DNA repair processes could lead to the potential therapeutic endpoints of radio- and chemosensitization. Since tumor cells in general are genomically unstable and have defects in the responses to DNA damage it has been argued (and proven in certain cases) that targeting DNA repair pathways may lead to a therapeutic index in tumor cells over "normal" cells. Two key kinases involved in the detection, signaling and repair of DNA double strand breaks (DSBs) are ATM (ataxia-telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase). The serine/threonine protein kinase ATM responds to DNA DSB damage by signaling, *via* phosphorylation events, to key cell cycle and DNA-repair components. Mutation of *ATM* occurs in the human autosomal recessive disorder ataxia-telangiectasia (A-T), which is characterised by a hypersensitivity to ionising radiation (IR) and aberrant cell cycle control. The structurally related enzyme DNA-PK also responds to DNA DSBs and is intimately involved in the repair of DNA DSBs by the process of non-homologous end joining (NHEJ). Inhibition of ATM or DNA-PK activity could therefore lead to cellular radio- and chemo-sensitisation. Screening of a combinatorial library based on the non-specific PI 3-kinase inhibitor LY294002, has identified a small molecule ATP competitive inhibitor of ATM, 2-morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU-55933) and an ATP competitive inhibitor of DNA-PK, termed NU7441. Both molecules show low nanomolar activity and are highly specific for the respective kinases. KU-55933 inhibits DNA damage signalling pathways and potentiates the cytotoxic effects of ionizing radiation and other classes of DNA DSB inducing agents. Similarly, the DNA-PK inhibitor also acts to potentiate the effects of IR and topoisomerase II inhibitors *in vitro* and *in vivo*. Our results to date support the further *in vivo* evaluation of these novel classes of molecules as chemo- or radiosensitizers prior to clinical evaluation.

Friday 10 November

Poster Sessions

Antimetabolites

461 POSTER

Early phase experience with pralatrexate (10-propargyl-10-deazaaminopterin [PRX]), a novel antifolate with high affinity for the reduced folate carrier, in patients with chemotherapy refractory lymphoproliferative malignancies

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Pralatrexate is an antimetabolite designed to have a much greater affinity for RFC-1. RFC-1 is the major transporter of both natural folates and folate analogs, and is an oncofetal protein whose expression is markedly increased by oncogenes like H-ras and c-myc, theoretically improving its therapeutic index. A single agent phase 1 and 2 study of pralatrexate has been conducted since 2001. Based on data in lung cancer, a dose of 135 mg/m^2 given on an every other week (w) schedule was used to treat 16 patients, including patients with Hodgkin's Disease (HD; n = 5), aggressive B-cell lymphoma (LBCL; n = 8), mantle cell lymphoma (n = 2) and one patient with peripheral T-cell lymphoma (TCL). A higher incidence of stomatitis (6 of 16 patients with Grade 3 or 4 stomatitis) was seen in patients with marked elevations in pre-treatment homocysteine (Hcy) and methylmalonic acid (MMA). Comparatively little to no increase in stomatitis occurred in patients with Hcy and MMA less than 10 microM and 200 nM respectively. Patients with elevated Hcy and MMA who developed stomatitis with pralatrexate did not develop advanced grade stomatitis after normalization of their Hcy and MMA with folic acid and vitamin B_{12} supplementation. In this experience, none of 15 patients with B-cell lymphoma experienced a remission, while one of 16 patients with T-cell lymphoma attained a PET negative CR after one dose. Population pharmacokinetic (PK) modeling established the importance of the nutritional covariates (Hcy and MMA) and PK parameters on toxicity, leading to a weekly phase I study. The dose escalation study started at 30 mg/m^2 weekly (w) $\times$3 every 4 w; progressing to 30 mg/m^2 w $\times$ 6 every 7 w, then increasing by 15 mg/m^2 on the 7 week schedule. The MTD was defined as 30 mg/m^2 w $\times$6 weeks on an every 7 week basis. To date, 31 patients have been accrued to the amended study, including patients with HD (n = 3); DLBCL (n = 6) and 22 patients with various sub-types of TCL. In total, including patients from the every other week and weekly treatment schedules, 42 patients with relapsed or refractory lymphoma have been treated. Of these, 8 complete remissions (CR) and one partial remission (PR) have been recorded, with all 8 CR occurring in patients with TCL (of which 11 of 22 are evaluable at this time [RR= 72%]), and the one PR in a patient with DLBCL (RR= 4%). The longest durations of response for the TCL patients have been 9 (panniculitic), 12 (T-cell ALL) and 16+ months (HTLV-1 ATLL). Normalization of Hcy and MMA pre-treatment with vitamins has completely abrogated the stomatitis, the major dose limiting toxicity (DLT). Little hematological toxicity (grade 2 thrombocytopenia in 2

patients) has been observed. Pralatrexate has achieved a remarkably high CR rate among patients with select forms of NHL. The goals of this ongoing trial are to identify the ORR in patents with B- and TCL, and to initiate an international registration study for patients with TCL.

462 POSTER

Research and identification of the polymorphisms of the thymidylate synthase gene in the human tumor cell lines panel of the National Cancer Institute (NCI)

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Background: Thymidylate synthase (TYMS) is the target enzyme for 5-fluorouracil (5-FU). It has been shown that TYMS expression is inversely correlated with the activity and/or toxicity of 5-FU in cancer patients. On the other hand, TYMS expression is dependent on TYMS gene polymorphisms (PM). Three distinct PMs have been identified: the 2R/3R PM, consisting of the presence of 2 or 3 tandem repeats of a 28 bp sequence in the gene promoter; the 3C/3G PM, consisting of a C>G SNP in the second repeat of 3R alleles; and the 6ins/6del PM, consisting of the deletion of a 6 bp sequence in the 3′ untranslated part of the gene.
Methods: DNA was extracted from the cell lines of the NCI panel and TYMS PMs were identified using PCR-RFLP techniques. TYMS catalytic activity was evaluated in cell cytosols using a radioactive substrate.
Results: In the NCI panel, the allele frequency of the 2R allele is 53% (19 3R/3R, 17 2R/3R and 23 2R/2R cell lines). Among the 3R allele-containing cell lines, 7 with 2R/3R and 10 with 3R/3R genotype present at least one copy of the 3G allele (allele frequency: 18%). Finally, the allele frequency of the 6del variant is 32% (32 6ins/6ins, 16 6ins/6del and 11 6del/6del cell lines). We have looked for relationships between 5-FU cytotoxicity, as extracted from the NCI database, TYMS expression and catalytic activity in the cell lines of the NCI panel, and the presence of TYMS gene PMs. 5-FU cytotoxicity is significantly related to none of the PMs, and is not related either with TYMS expression or activity. However, the presence of 3G alleles is significantly associated to high enzyme expression and activity ($P = 0.03$), especially in cell lines with mutated p53 ($P = 5 \times 10^{-5}$). There is a linkage disequilibrium between the PMs, the 3G allele being significantly associated with the 6del allele and the 2R allele with the 6ins allele. In addition, there is a deviation from the Hardy-Weinberg distribution, with a smaller than expected proportion of heterozygous cell lines for any PM. This can be attributed to loss of heterozygosity occurring in tumor cell lines.
Conclusion: The NCI panel offers an interesting model for the establishment of relationships between gene PMs and pharmacological data. The absence of relationship between in vitro 5-FU cytotoxicity and TYMS gene expression, activity and polymorphisms could be due to the fact that 5-FU cytotoxicity was measured in the absence of optimal amounts of the cofactor of TYMS.

463 POSTER

Phase I study of sapacitabine, an oral nucleoside analogue, in patients with refractory solid tumors or lymphomas

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Background: Sapacitabine (CYC682, CS-682) is a rationally designed 2'-deoxycytidine-type nucleoside analogue that can be administered orally. Compared with other nucleoside analogues, sapacitabine is unique in its ability to induce G2 cell cycle arrest and cause single-strand DNA breaks that are irreparable by ligation. Following oral administration, sapacitabine is converted by amidases and esterases in the gut, plasma, and liver to its major active metabolite (CNDAC). Sapacitabine had potent anti-tumor activity in animal studies and was superior to gemcitabine or 5-FU in a mouse liver metastasis model. Previous phase I studies had evaluated once daily dosing (qD) × 3 or 5 days/week for 4 weeks every 6 weeks. To maximize drug exposure, this phase I study evaluates twice daily dosing (b.i.d.) × 7 or 14 days every 21 days, using body surface area (BSA)-based or fixed dosing.
Methods: Eligible patients had incurable advanced solid tumors or lymphomas and adequate organ function. At least 3 patients were enrolled at each dose level. Maximum tolerated dose (MTD) was the dose level at which at least 2/3 or 3/6 patients experienced DLT in the first cycle. The recommended phase II dose (RD) was the dose level immediately below MTD. Pharmacokinetic (PK) sampling was performed after administration of sapacitabine with and without food.
Results: 37 patients were treated, 28 on the b.i.d. × 14 days schedule and 9 on the b.i.d. × 7 days schedule. The most common tumor types were non-small cell lung (n = 7), colon (n = 5), breast (n = 5) and ovary (n = 4). The MTD for the14 day-schedule is 40 mg/m^2 b.i.d. (RD = 33 mg/m^2 or 50 mg b.i.d.). The MTD for the 7 day-schedule is 100 mg b.i.d. (RD =75 mg b.i.d.). DLTs were reversible myelosuppression. One patient treated at the MTD of 40 mg/m^2 b.i.d. died of candida sepsis in the setting of grade 4 neutropenia and thrombocytopenia. Non-hematological adverse events (all grades, regardless of causality) were mostly mild to moderate and included nausea, vomiting, fatigue, diarrhea, constipation and anorexia. PK data are being analyzed. The best response to sapacitabine was stable disease in non-small cell lung (n = 3), ovary (n = 3), colon (n = 2), breast, gastrointestinal stromal tumor and parotid adenocarcinoma (n = 1 for each).
Conclusion: The RD of sapacitabine for the b.i.d. × 14 days schedule is 33 mg/m^2 b.i.d. or 50 mg b.i.d. and that for the b.i.d. × 7 days schedule is 75 mg b.i.d. The DLT was myelosuppression.

464 POSTER

Pharmacokinetics of talotrexin (PT-523), a novel aminopterin analogue, in patients with non-small cell lung cancer

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Background: Talotrexin, N^{α}-(4-Amino-4-deoxypteroyl)-N^5-hemiphthaloyl)-L-ornithine (PT-523) is a nonpolyglutamatable antifolate which has demonstrated improved antitumor activity in a broad spectrum of cancer models by targeting DHFR to inhibit tumor growth. Talotrexin binds more tightly (15-fold, Ki 0.35 pM) to DHFR than methotrexate (MTX). In lung cancer cell lines, talotrexin inhibits tumor cell proliferation at sub- to low-nanomolar concentrations and is more potent than MTX in all cell lines tested. We conducted a dose escalation study of talotrexin administered as a 5–10 minute infusion on Days 1, 8, on a 21-day cycle in non-small cell lung cancer (NSCLC). The primary objectives of this study were to determine the maximum tolerated dose (MTD), pharmacokinetic (PK) profile, as well as the safety and efficacy. This report describes the PK behavior of talotrexin in NSCLC patients.
Methods: Plasma samples were obtained prior to infusion, at the completion of the infusion, at 15 and 30 minutes, then at 1, 2, 3, 4, 6, 8, 10, 16, 24 and 48 hrs after completion of the infusion. A validated LC/MS/MS assay was used to measure talotrexin in plasma. PK parameters were estimated by standard noncompartmental methods.
Results: The PK of talotrexin was characterized in 25 patients with normal renal and hepatic function, and a median age of 59 years (range, 48–76 years). Data was obtained from groups of at least three patients receiving doses of 13.5, 27, 54, 90, and 135 mg/m^2. The talotrexin concentration in plasma decreased in a mono-exponential manner following a rapid distribution phase. In the 6 patients who received the MTD dose of 54 mg/m^2, the mean peak drug concentration in plasma (C_{max}) was 17.42 ng/L (13.7−22.3) and the mean plasma concentration 48 hr after dosing was 17.3 ng/ml (range, 17.8−36.7 ng/mL). The apparent biological half-life ($t_{1/2,z}$), total body clearance (CL) and apparent volume of distribution at steady-state (V_{ss}) were all independent of the dose. Mean (range) values of PK parameters for the entire cohort of 25 patients were: CL, 1.4 L/hr/m^2 (3.6−10.3), $t_{1/2,z}$, 6.6 hr (4.7−6.8) and Vd_{ss}, 8.1 L/m^2 (7.4−13.0).
Conclusions: Talotrexin exhibits linear PK with moderate interpatient variability when administered as a short IV infusion at doses of 13.5−135 mg/m^2. In future PK studies, talotrexin major route of elimination will be examined and an evaluation of whether diminished renal or hepatic function warrants dose modification will be conducted.

465 POSTER

Phase I/II study of oxaliplatin (L-OHP) in combination with S-1 (SOX) as first-line therapy for metastatic colorectal cancer (MCRC)

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Background: FOLFOXs are one of the world's established standard therapies for MCRC. S-1 is an oral dihydropyrimidine dehydrogenase (DPD)-inhibitory fluoropyrimidine consisting of tegafur which is a 5-FU prodrug activated by CYP2A6 in the liver, 5-chloro-2,4-dihydroxypyridine of the DPD inhibitor, and potassium oxonate of the orotate phosphoribosyltransferase (OPRT) inhibitor. The response rate of S-1 monotherapy for chemo-naïve MCRC was 35.7%. SOX may provide a new alternative to FOLFOX. This study was designed to determine the recommended dose (RD), to assess the pharmacokinetics (PK), and to evaluate the efficacy and safety of this combination therapy.
Methods: Patients were eligible as follows: unresectable MCRC with no prior chemotherapy, PS (ECOG) 0–1, age 20−75, measurable