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

R. Plummer. *Northern Institute for Cancer Research Paul O'Gorman Building, Newcastle, United Kingdom*

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

G.C.M. Smith. *KuDOS Pharmaceuticals Limited, Cambridge, United Kingdom*

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

O. O'Connor[1], P. Hamlin[1], E. Neylon[1], C. Moskowitz[1], C. Portlock[1], A. Noy[1], F. Sirotnak[1], A. Zelenetz[1], D. Mould[2], S. Horwitz[1]. [1]*Memorial Sloan Kettering Cancer Center, Medicine, New York, USA;* [2]*Projections Research, Inc., Pennsylvania, USA*

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