ity. Our group has generally taken a target-gene approach, genotyping children with ALL whose therapy has been well-documented for polymorphisms of known functional consequence and exploring whether genotypes predict drug-induced phenotypes of interest. For example, we have shown that polymorphisms in thiopurine methyltransferase (TPMT) are associated with an increased frequency of thiopurine-induced acute myelosuppression among ALL patients with at least one mutant allele for TPMT, and this has translated into reduced dosage requirements for such patients. In addition, such patients also appear to be at a higher risk of therapy-induced cancers, such as topoisomerase II-inhibitor-associated secondary AML and irradiation-induced brain tumors. A polymorphic repeat in the thymidylate synthase (TS) gene has been linked to overall event-free survival in childhood ALL. Polymorphisms in glutathione transferase have been associated with ALL outcome in some studies but not others. Although some prior studies linked polymorphisms in CYP3A4 and NQO1 with the risk of secondary myeloid leukemia, among children with ALL, we found no associations between CYP3A4*1B, CYP3A5*3, or NQO1 genotypes and the risk of secondary myeloid malignancies. Relatively modest differences in the delivery of therapy and in study design may account for some of the conflicting conclusions in the literature. Well-controlled clinical trials are required to evaluate the importance of pharmacogenetic variability to to ALL treatment outcomes.

Thursday 21 November

PLENARY SESSION 6

Proffered Papers 2

214

The role of poly(ADP-ribose) polymerase-1 (PARP-1) in the cellular response to topoisomerase I poisons

L. Smith[1], E. Willmore[2], Z. Hostomsky[3], S.E. Webber[3], C.A. Austin[2], N.J. Curtin[1]. *[1]University of Newcastle upon Tyne, Cancer Research Unit, Newcastle upon Tyne, United Kingdom; [2]University of Newcastle upon Tyne, Biochemistry & Genetics, Newcastle upon Tyne, United Kingdom; [3]AgouronPfizer GRD, La Jolla, USA*

DNA Topoisomerase I (Topo I) catalyzes the breakage, unwinding and religation of DNA, forming a transient Topo I-associated DNA strand break (cleavable complex). Topo I poisons, such as camptothecin (CPT) and topotecan (TP), stabilise the cleavable complex, resulting in persistent DNA breaks. PARP-1 is activated by DNA strand breaks and facilitates their repair. PARP-1 inhibitors enhance Topo I poison-induced cytotoxicity[1] but the underlying mechanism has not been defined. Potential mechanisms are: a) PARP-I modulates Topo I activity and b) PARP-1 participates in the repair of Topo I-induced DNA lesions. To elucidate the role of PARP-1 in Topo I poison cytotoxicity we have investigated the effect of a novel potent PARP-1 inhibitor, TBI-361 (Ki<5 nM), in combination with CPT and TP, in human leukaemia cells (K562) and PARP-1 -/- and +/+ mouse embryonic fibroblasts (MEFs). TBI-361 augmented CPT-induced growth inhibition in K562 cells (16 hour exposure): the GI_{50} (growth inhibitory IC_{50}) of 4 $\pm$ 0.6 nM for CPT alone was reduced to 2.4 $\pm$ 0.1 nM by co-incubation with TBI-361. PARP-1 -/- MEFs were 3-fold more sensitive to TP (5-day exposure) than PARP-1 +/+ MEFs; GI_{50} 21 and 65 nM, respectively. TBI-361 caused a 3-fold sensitisation of PARP +/+ cells compared to only a 1.4-fold sensitisation in PARP-/- cells. These data confirm both a role for PARP-1 in Topo I poison cytotoxicity and that the cellular effects of TBI-361 are due to PARP-1 inhibition. The level of Topo I cleavable complexes[2] formed after 30 min exposure to CPT was not significantly altered by TBI-361, and preliminary data shows that TBI-361 has no significant effect on Topo I activity. However, DNA strand breaks induced by CPT were increased by ~20% by TBI-361 after 20 hour but not 30 mins exposure. These data are more consistent with the hypothesis that PARP-1 enhances Topo I cytotoxicity by inhibiting DNA repair rather than a direct effect on Topo I activity. However, the possibility that prolonged exposure to a PARP-1 inhibitor may be necessary to modulate Topo I activity cannot be excluded and ongoing experiments are designed to address this hypothesis. Definition of the mechanism of PARP-1 Topo I interactions will be crucial to exploit fully the clinical potential of PARP-1 inhibitors in combination with Topo I poisons for cancer therapy.

References

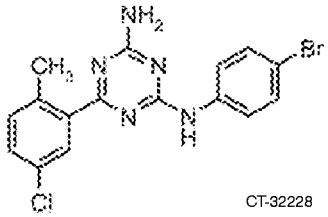
[1] Bowman et al 2001 B J Cancer 84 1 106-112
[2] Padget et al 2000 Biochem Pharm 59 629-638

215

CT-32228, a specific inhibitor of lysophosphatidic acid acyltransferase-beta (LPAAT-b) causes selective tumor cell apoptosis

J.W. Singer, L. Bonham, M. Coon, P. de Vries, D. Hollenback, D. Leung, T. White. *Cell Therapeutics, Inc., Seattle, USA*

Lysophosphatidic acid acyltransferases (LPAATs) are a family of intrinsic membrane enzymes that catalyze the de novo biosynthesis of phosphatidic acid (PA), a co-factor required for raf and mTOR activity. Immunostaining with an isoform-specific monoclonal antibody to LPAAT-b showed strong expression in lung, ovary, prostate, bladder, cervix, and brain tumors while normal tissue expression was primarily limited to endothelial, smooth muscle and inflammatory cells. Ectopic over-expression of LPAAT-b contributed to transformation of NIH/3T3 cells and its removal showed that it was required both for proliferation in low serum and tumor formation in nude mice. Cellular transformation of Rat-1 fibroblasts by Ha-ras led to increased levels of PA and LPAAT activity and increased the amount of 18:1 and 18:2 at the expense of 20:4 fatty acids in cellular lipids, a pattern also seen with LPAAT overexpression. Knockdown of LPAAT-b by RNAi blocked proliferation in DU-145 cells and induced apoptotic cell death in IM9 lymphoblastoid cells. LPAAT-b specific inhibitors were identified following screening of a chemical diversity library. Compounds that inhibited LPAAT-b but not the related housekeeping enzyme, LPAAT-a were selected for optimization. Standard medicinal chemical optimization of hits yielded highly specific structure-activity relationships. Compounds have been identified within 3 classes of related heterocyclics that inhibit LPAAT-b at less than 50 nM in both cell free and intact cell assays. CT-32228, [N-(4-bromophenyl)-6-(5-chloro-2-methylphenyl)-[1,3,5]triazine-2,4-diamine] is representative of one of these classes and is a non-competitive allosteric LPAAT-b inhibitor (Ki 47nM). CT-32228 is anti-proliferative (IC_{50} 50-100nM) and cytotoxic (100-200nM) to a broad variety of tumor cell lines whereas it is not cytotoxic to human hematopoietic progenitors at concentrations up to 2 mM.



CT-32228

In a preliminary study, treating nude mice bearing DU-145 prostate cancer or HT-29 colon cancer with CT-32228 was non-toxic and produced significant tumor growth delay. Similar results were achieved with CT-32548, a follow-on compound with enhanced solubility, in mice bearing NCI-H460 lung cancers. These data suggest that LPAAT-b activity may be critical to oncogenic signaling and potentially represents a novel and selective enzymatic target for cancer therapy that can be inhibited by low molecular weight drug-like compounds.

216

A phase I pharmacokinetic (PK) and serial tumor and skin pharmacodynamic (PD) study of weekly, every 2 weeks or every 3 weeks 1-hour (h) infusion EMD72000, an humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients (p) with advanced tumors known to overexpress the EGFR

J. Tabernero[1], F. Rojo[1], E. Jiménez[2], I. Montaner[2], L. Santomé[1], M. Guix[1], 0. Rosen[3], A. Kovar[3], I. Viaplana[1], J. Baselga[1]. *[1]Vall d'Hebron University Hospital, Medical Oncology Department, Barcelona, Spain; [2]Merck Farma y Química S.A., Barcelona, Spain; [3]Merck KGaA, Darmstadt, Germany*

EMD72000 is an humanized monoclonal antibody directed at the EGFR that has shown potent antitumor activity in preclinical studies. In prior studies EMD72000 has been administered weekly and the MTD has been established at 1,600 mg weekly (Proc. ASCO 2002;38(A378)). In terms of QoL and compatibility with standard chemotherapy schedules a prolongation of the administration interval would be desirable. Insofar, EMD72000 PKs is not linear and the half-life increases with the dose allowing for a more prolonged exposure of the drug. We are therefore conducting a phase I clinical trial of EMD72000 given as a weekly, every 2 or every 3 weeks 1,200 mg 1-hour infusion, with PK and PD assessments to determine the PK profile and