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

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

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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

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

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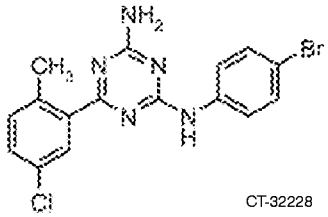
Lysophosphatidic acid acyltransferases (LPAATs) are a family of intrinsic membrane enzymes that catalyze the de novo biosynthesis of phosphatidic acid (PA), a co-factor required for raf and mTOR activity. Immunostaining with an isoform-specific monoclonal antibody to LPAAT-b showed strong expression 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.

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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

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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

the PD efficacy in the 3 different schedules. Plasma EMD72000 concentrations are determined at sequential time-points by a validated ELISA assay. PD studies include determination of EGFR expression in archived tumor material, saturation of EGFR in skin after treatment, inhibition of signaling pathways in skin and tumor before and after treatment (total and activated EGFR and MAPK; Ki67 and p27 expression; pAkt expression and TUNEL apoptosis assay). The skin and tumor tissue samples were obtained on day 0 (before treatment) and on day 43. A total of 13 p with EGFR+ tumors have been included: colon (7), gastric (3), ovarian (2) and renal (1), with a median Karnofsky index of 90% (range: 80%-100%), a median age of 57 (range: 39-75), and receiving a median of 3 prior chemotherapy regimens (range: 1-11). Toxicity has been limited to grade 1-2 nausea, vomiting, rash, pruritus, headache and diarrhea with no DLTs observed. In 9 p evaluable for response there have been 2 not confirmed PRs in p with colon cancer and 2 stable disease (>15 weeks) in a renal cell and in a colon cancer p. These responses were observed among the three different schedules. EMD 72000 treatment demonstrated an almost complete inhibition of the activated EGFR and MAPK with a decrease in the Ki67 expression and an increase in the p27 expression in skin samples in all the three different schedules. Moreover, the typical pattern of epidermal changes observed after EGFR blockade have been observed in the three cohorts of p. Complete PK and skin and tumor PD profiles will be presented. In summary, the preliminary efficacy and PD data suggest that more convenient every 2-3 weeks administration schedule may be feasible.

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Sustained activation of JNK cascade and apoptosis induced by rapamycin is suppressed by p53

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Rapamycin analogues CCI-779 and RAD001 are currently in early clinical trials for treatment of malignant disease. However, there is emerging data to support selective activity of these agents based upon genetic changes that occur during neoplastic transformation. In response to rapamycin, cells lacking functional p53 undergo apoptosis, whereas cells expressing wild-type p53 or p21Cip1 arrest in G1 and remain viable. Our studies have focused on understanding the mechanism by which rapamycins induce cell death. In rhabdomyosarcoma cells and murine embryo fibroblasts rapamycin induces rapid, sustained activation of JNK only in cells lacking functional p53, but only transient activation in cells expressing wild-type p53 or p21Cip1. Overexpression of dominant negative c-Jun (TAM67) or ASK1 (K709M) prevents rapamycin-induced phosphorylation of c-Jun, and apoptosis. Furthermore, cytoplasmic p21Cip1 interacts with ASK1, negatively regulating JNK cascade. Overexpression of p21Cip1 or p21Cip1 lacking the nuclear localization signal (DNLS-p21) also protects cells from death. The results suggest that rapamycin induces apoptosis by prolonged activation of JNK cascade, and p53 and p21Cip1 prevent cell death through inactivation of ASK1 by p21Cip1, and not through enforcing G1 arrest. These results indicate that the response to inhibition of the rapamycin target (mammalian TOR) is determined at least in part by p53 functional status, and reveals a rational basis for developing tumor-selective therapy.

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PLENARY SESSION 7

Transcription factors and related pathways

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Cooperating mutations as targets for therapy in leukemia

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AML, like other human cancers, is caused by more than one mutation. Epidemiologic evidence, and data from murine models, indicates that two broad classes of mutation cooperate to cause acute leukemia. One class of mutation provides a proliferative and/or survival signal to leukemic cells, as exemplified by activation mutations in tyrosine kinases, such as FLT3, c-KIT, BCR/ABL, or TEL/PDGFβR. A second broad class of mutations impairs hematopoietic differentiation. These include chromosomal translocations or point mutations that result in aberrant function, or loss of function, of hematopoietic transcription factors required for normal hematopoietic development. Examples include translocations involving core binding factor; the retinoic acid receptor a; HOX family members; and point mutations in AML1 and C/EBPa. Together, these two classes of mutations result in the acute leukemia phenotype of uncontrolled proliferation and impaired differentiation. Epidemiologic data supports the hypothesis that two classes of mutation cooperate to cause leukemia. For example, progression of CML to blast crisis may be associated with acquisition of a NUP98/HOXA9 fusion as a consequence of t(7;11); and FLT3 activating mutations occur in 40% of APL patients with the PML/RARa gene rearrangement. We have also developed murine models of acute leukemia to support this hypothesis, including models of cooperativity between BCR/ABL and NUP98/HOXA9; and between FLT3-ITD mutations and PML/RARalpha. Inhibition of the respective tyrosine kinase, both in humans and in murine models, has therapeutic efficacy against leukemia blasts that harbor more than one mutation. That is, agents that target the proliferative signal in acute leukemia blasts may have therapeutic activity. Furthermore, as mutations in FLT3, c-KIT and RAS account collectively for no more than 50% of leukemias, it is likely that additional as yet unidentified kinases or their effectors are mutant in AML, and may represent novel therapeutic targets. It may also be possible to achieve synergistic therapeutic effects by using agents that target the respective kinase with agents that override the block in differentiation, as in the case of ATRA therapy of APL. For example, combinations of FLT3 inhibitors and ATRA may have therapeutic benefit in a select group of APL patients. The future holds great promise for further development of molecular therapies that target these two respective classes of mutation.

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Targeting C/EBP alpha in leukemia and lung cancer

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The primary defect in acute myeloid leukemia (AML), like many cancers, is a block in differentiation. Development of new drugs which specifically target the differentiation block, such as ATRA in t(15;17) APL, are likely to be more effective and less toxic than less specific chemotherapy. Recent data from our laboratory have demonstrated that the C/EBP alpha transcription factor (1) is the critical factor for myeloid differentiation; (2) is mutated or down-regulated in many AML subtypes; and (3) restoration of expression relieves differentiation block of leukemic cells lines. Therefore, we have been working on methods to identify and characterize drugs which augment C/EBP alpha expression and/or function in order to develop novel specific therapies for AML. To date we have generated stable myeloid cell lines to serve as indicator cells for high throughput drug screening for drugs activating C/EBPa function, including the human myeloid U937 and murine myeloid progenitor 416B lines. Prior to the drug screening, all lines were tested for responsiveness to a known inducer of granulocytic differentiation of these cells, all-trans retinoic acid (ATRA). We then collaborated with Robert Shoemaker and Ed Sausville at the National Cancer Institute's Developmental Therapeutics Program. One U937 line was used for initial pilot drug screens in which 1990 compounds were tested (the NCI "Diversity Set", which was selected by computer algorithm in an attempt to represent the greater chemical diversity of the total NCI repository of almost 140,000 compounds). Subsequently 135,640 compounds were screened at a single, 1 mM concentration. 70 of the most active compounds were subsequently analyzed in 20-concentration titration experiments. After completion of the primary (single-dose) drug screen, reaching a total of 140,000 compounds, the first set of active compounds identified thus far will be subjected to additional testing in our leukemic cell line models. In addition, recent data from our laboratory has indicated that C/EBP alpha is downregulated in a significant number of patients with non-small cell lung cancer, suggesting that drugs identified in our screen using leukemic cell lines might also have usefulness in other types of cancer as well.