the reduction of 17AAG by NQO1 is relatively stable to autooxidation and formation of the 17AAG hydroquinone may allow for increased levels of 17AAG intracellular drug accumulation via a slow autooxidation process. Another implication of the data is that the 17AAG hydroquinone may represent an alternative conformation for binding to HSP90 (supported by CA51210).

99 POSTER

Para-amino benzoic acid (PABA) modulation of chemotherapy – evolution from preclinical work to a Phase I study. A report of early clinical activity including pharmacokinetic and pharmacodynamic studies

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Background: B16F10 melanoma cells cultured in media containing PABA resulted in a rapid reduction in pigmentation compared to cells grown PABA deficient media. *In-vivo* murine studies with a B16F10 melanoma xenograft model treated with daily PABA IP at 50 mg/kg and paclitaxel (P) treatment alone or in combination showed that the PABA + P combination was significantly different from the control ($p = 0.016$) and P groups. Patients (pts) with metastatic melanoma who had failed first line therapy with dacarbazine routinely receive platinum and/or taxane agents as second line therapy. We have translated these findings into a Phase I study of combination PABA, P and carboplatin(C) in metastatic melanoma patients who had failed first-line therapy.
Methods: Both C and PABA dose were fixed (AUC 5 mg/ml, IV; 2 g/d, po) with P escalated over 4 dose levels ranging from 100 to 175 mg/m2. PABA was administered on days 1–10 with chemotherapy on day 6. Three patients were enrolled at each dose level. Toxicity was assessed at day 21 of the 1st cycle. Correlative studies include the measurement of PABA, P and C PK, monitoring for shed collagen cryptic epitopes, molecular pathology assessment on accessible metastatic lesions, and documentation for vitiligo development. We plan to correlate these findings with the clinical response of these pts.
Results: Fifteen pts have been enrolled to date with 14 eligible for assessment. All pts at DL I treated with only PABA and C showed no toxicities or response. For DL II and III, (P 100 and 125 mg/m2), no toxicities have been seen. Three of 6 pts have shown clinical responses. The second pt on DL IV (P 150 mg/m2), developed Grade III neutropenia. DL IV was expanded to 6 pts. 4 evaluable pts had no toxicity and 1 not yet evaluated. Two of 5 pts on DL IV, had clinical responses. We have observed inflammatory responses in responding cutaneous lesions. PABA PK for DL I-IV showed a mean Cmax of 14 μg/ml ± 7.9 μg/ml at 0.5 hour, and an elimination HL that ranged from 0.2–13 h. In 6 out of 11 pts, a mean 4-fold increase of the AUC was observed 24 h following the administration of P. Assessments of the lack of toxicity with P PK are ongoing. Data on the C AUC, shed cryptic epitopes, and histopathologic markers for antibodies to β1 integrin, BRCA-2 and CDC-25A are in progress.
Conclusions: This regimen demonstrates activity with low toxicity in refractory melanoma pts. A phase II efficacy trial is planned.

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A peptide inhibitor of t(4;11) leukemia

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Despite considerable progress in the treatment of leukemias, particularly in children, acute lymphocytic leukemia (ALL) in infants remains highly resistant to treatment. Infant ALL is characterized by reciprocal chromosomal translocations involving the mixed-lineage leukemia (*MLL*) gene at locus 11q23 and results in the in-frame fusion of *MLL* to a variety of different genes. In each case, the translocation leads to the expression of a chimeric protein with transforming properties. Investigations of the MLL fusion proteins and their interacting partners could provide reasonable targets of study to gain insights into mechanism of transformation. In particular, AF4 is a prime target as the MLL-AF4 fusion protein is expressed in most cases of ALL in infants.
We find that a small domain of AF4 interacts with the carboxy-terminus of another, relatively less common MLL fusion partner, AF9. The binding domains of AF4 and AF9 are maintained in leukemia-associated MLL fusion proteins, suggesting that AF4 and AF9 are capable of interacting in their native form and/or as MLL fusion proteins. The physical interaction of MLL-AF4 with AF9 may be important in leukemogenesis in cells with t(4;11)(q21; q23) translocations characteristic of infant leukemia and may serve as an important target in the development of a treatment for this disease. To this end, our lab has developed a small synthetic peptide based on the 14 amino acid residues of AF4 that are essential for the interaction with AF9. The ability of this peptide to disrupt the binding of AF4 to AF9 has been established using both *in vitro* and *in vivo* assays. Furthermore, we have shown that it specifically inhibits proliferation of t(4;11) leukemia cell lines and that this inhibition is mediated through apoptosis. Importantly, the peptide does not affect the proliferative capacity of hematopoietic stem cells as evidenced by methylcellulose-based assays to determine colony forming potential. Here we examine the relationship between AF4-AF9 binding and its resulting leukemogenesis potential. Future studies with this peptide or its derivatives could lead to the development of novel therapies specifically targeted to infant leukemia or other leukemias with t(4;11) rearrangements

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Combinatorial cancer therapy using a pharmacological inhibitor of indoleamine 2,3-dioxygenase (IDO), a target of the cancer suppression gene Bin1

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Mutations produce a dynamic substratum upon which modifier genes and the stromal microenvironment act to facilitate the development of a fully malignant state. Previous studies of Myc interactions have identified Bin1 as a candidate suppressor or negative modifier gene in cancer. Bin1 encodes a set of alternately spliced adapter proteins. Here we report that Bin1 loss can promote tumor formation by facilitating escape from host anti-tumor immunity, and ongoing mechanistic investigations have identified a unique pharmacological strategy to correct this. We have found that homozygous deletion of the Bin1-gene enhances tumor formation by Myc+Ras – transformed murine skin fibroblast and keratinocyte isografts. In striking contrast, the negative impact of Bin1 expression on tumor growth was either diminished or lost in immunocompromised mice, suggesting a cell-extrinsic effect on anti-tumor immunity. A candidate mediator of this effect, indoleamine 2,3-dioxygenase (IDO), is being investigated. IDO is an oxidoreductase that we have found to be genetically controlled by Bin1. Catabolism of tryptophan by IDO has previously been shown to locally attenuate the activation of T cells and promote immune tolerance. Consistent with the more aggressive growth of Bin1–/– isografts in immunocompetent hosts, Bin1–/– cells exhibit superinducible IDO. We have tested whether IDO blockade may have therapeutic benefit in the MMTV-neu transgenic mouse breast cancer model using the competitive bioactive IDO inhibitor 1-methyl-tryptophan (1MT). By itself 1MT had little effect on tumor growth. In contrast, 1MT elicited tumor cell death and regression when combined with cytotoxic agents that alone were inefficacious in the model. No synergy occurred in tumors engrafted into nude mice, supporting the expectation that combinatorial efficacy is immune-based. Results of additional studies examining mechanism, PK/ PD, and survival in response to treatment will be presented. Our findings 1) support the concept of Bin1 as a cancer suppression gene, 2) link Bin1 deficiency to a pharmaceutically tractable enzyme that may control anti-tumor immunity, and 3) identify IDO/cytotoxic combination therapy as a promising new strategy to treat cancer. One radical implication of this work is that combining immunotherapy and chemotherapy to treat cancer, which may seem counterintuitive in principle, may prove to be unexpectedly effective in practice.

Drug screening

102 POSTER

The synthetic lethal trap: a general approach for screening small-molecule protein inhibitors using genetic triangulation in the yeast Saccharomyces cerevisiae

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Our purpose was to develop a novel chemical-genetic screening strategy to identify small-molecule inhibitors of any non-essential protein using a surrogate synthetic lethal (SL) phenotype. Synthetic lethality is a form of genetic enhancement in which two mutations are lethal in combination, but the corresponding individual mutants are viable. We hypothesize that SL genetic interactions can be used as reporters for drug-based inhibition of any non-essential protein function. If protein inactivation using a small molecule is functionally equivalent to a loss-of-function mutation, then a specific protein inhibitor should satisfy all the genetic SL interactions associated with the corresponding gene. For example, if inactivation of