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.

100 POSTER

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

101 POSTER

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

genes A & B are SL, then a cell missing gene A should die in the presence of a small-molecule inhibitor of protein B. Genome-wide genetic screens have been developed to simultaneously determine all the SL interactions for a given gene deletion in yeast. Preliminary screens reveal ~30 SL interactions/gene. Furthermore, each gene appears to have a unique pattern of interactions, or genetic fingerprint. Thus, a collection of sensitized strains, each containing individual deletions that are SL with a target gene, should act as a trap for specific small-molecule inhibitors of the target protein. If sensitized-strain SL interactions overlap only with the target gene, positive hits must be genetically linked to the target gene.
Using the Synthetic Genetic Array (SGA) approach, we determined the SL partner genes for a deletion of *SCH9*, a kinase involved in size control in yeast and the closest homologue to human Akt. As a proof-of-principle, we constructed an analog-sensitive (AS) *SCH9* allele that is specifically inhibited by the ATP analog NM-PP1. *AS-SCH9* strains carrying gene deletions that are SL with *sch9Δ* were inviable in the presence of NM-PP1 while the single mutants, or the AS strain alone, grew in the presence of the drug, demonstrating the validity of the approach. We then developed a high-throughput liquid comparative growth assay and screened the 50,000-molecule Maybridge library for Sch9 inhibitors using our chemical-genetic trap. The first iteration returned 77 compounds specific to the sensitized strain. Initial hits were confirmed and subjected to subsequent rounds of screening against non-overlapping sensitized strains. Confirmed hits will be analyzed in secondary phenotype screens. (DOD Breast Cancer Research Program DAMD17–03–1-0471)

103 POSTER

High-throughput screening against intractable targets: the use of target disruption gene signatures for rapid cancer drug discovery

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The human genome is full of promising disease targets that pose challenges for conventional drug screening approaches. For example, Avalon has utilized high-resolution molecular cytogenetics techniques to build a database of over 170 unique amplified chromosomal regions ("amplicons") in epithelial cancer. Follow-up studies indicate that the key amplified oncogenic driver genes within these amplicons frequently belong to conventionally "intractable" target classes including transcription factors and intracellular signaling molecules. Microarray profiling of RNAi experiments directed against such genes permits the elucidation of Target Disruption Gene Signatures that provide a molecular profile of the genome-wide consequences of the reduction in target expression levels. Small molecule libraries can be rapidly screened on a modified, high-throughput qPCR-based platform to identify active molecules that mimic the siRNA gene disruption profiles. Hit compounds can then be characterized in a number of secondary assays to confirm activity against the desired target or cellular pathway. For an initial proof-of-concept, we established and completed a Target Disruption Gene Screen against beta-catenin in colorectal cancer. Over 35,000 compounds were screened for activity against a nine-gene profile, and several active hits were recovered including two reference compounds that have been reported to selectively inhibit the beta-catenin pathway. We will also report on the results of similar screens directed against other cancer targets including AKT1 and PIK3CA and discuss the use of this screening approach against amplified oncogenic targets derived from the Avalon amplicon database. These studies demonstrate that high-throughput screening across Target Disruption Gene Signatures enables rapid drug discovery against all target classes, even those that pose challenges to conventional HTS assays.

104 POSTER

Novel drug discovery strategies based on aptamer inhibitors which are especially useful for orphan targets

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Aptamers are short single-stranded nucleic acid ligands that fold into well-defined three-dimensional conformations capable of binding almost any protein target with low nano- to picomolar affinities and exceptional specificities. They are isolated from combinatorial libraries comprising up to 10^{15} different molecules by in vitro selection or SELEX. Due to their high inhibitory potential, aptamers provide an excellent means for the functional validation of protein targets and the subsequent identification of pharmaceutical lead compounds. Aptamers mimic the effect of a small molecule drug in terms of binding a focused site and inactivating a distinct functional epitope on a protein. The aptamer technology is therefore an excellent counterpart to RNA interference approaches since it provides valuable, additional data by simulating a drug's action at the protein level. Importantly, effective aptamers can be directly employed as surrogate ligands in competitive high-throughput screening assays to identify functionally equivalent small molecule leads. Therefore, the approach is especially useful for orphan targets when no natural ligand or substrate is known and no structural information is available.
Aptamers have been used to design screening assays based on fluorescence polarization, fluorescence intensity, and fluorescence lifetime. Low nanomolar concentrations of targets and aptamer probes were used in screening experiments with libraries of natural products and synthetic organic compounds. Compounds that were able to compete with the aptamer for binding to the protein target were further evaluated for biological activity.
Aptamer-based screening assays proved to be very robust and reliable with *Z'* factors between 0.7 and 0.85. No labeling of target or compound was necessary to generate appropriate signals. Development times are short and the consumption of reagents is very low making aptamer-based assays very cost-effective. Significantly, compounds that exhibited the same activity as the aptamer in biological assays, e.g. inhibiting an activator protein of leukocyte adhesion, were isolated at a high frequency.
Thus, the unique features of aptamers enable new strategies to circumvent a bottleneck in the drug discovery process by directly linking target validation with HTS and above all, are generally applicable for every genomic protein target.

105 POSTER

High throughput screening of a natural product library for telomerase inhibitory agents using target-defined model systems and bioinformatics

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The telomerase holoenzyme complex, telomeres and their regulatory proteins are attractive targets for cancer drug development owing to the fact of the essential function of telomerase during cellular immortalisation and tumorigenesis. Several concepts for inhibiting enzyme activity are currently under preclinical investigation. However, small molecule inhibitory agents that can specifically interfere with the telomerase reverse transcriptase catalytic subunit (hTERT) or prevent activity of the holoenzyme are very rare.
To identify novel lead structures, we have screened a natural product library comprised of 5.000 defined, pure compounds of fungal or bacterial origin. A panel of 33 permanent human tumor cell lines were characterized for TRF length (Telomere Restriction Fragment length) by Southern blotting, endogenous hTERT expression by immunostaining, and telomerase activity (TA) using the TRAP assay. 12 lines expressing high and low levels of telomerase, and with long or short telomeres were chosen for high throughput robot screening. Cells were seeded into 96-well plates and treated with drug in 2 concentrations (0.3 and 3 μg/ml) for 4 days. Antiproliferative effects were assessed by the fluorescence based propidium iodide assay. Results expressed in terms of 50%, 70% or 90% inhibition of tumor cell growth *versus* controls were entered into a database. 350 agents showing activity $\leqslant 3$ μg/ml were further tested in isogenic MCF-7 subclones transfected with empty vector or dominant negative −/− hTERT. MCF-7 cells expressing −/− hTERT have critically short telomeres (1.9kb) and only residual telomerase activity. 44 agents possessed antiproliferative activity with a differential profile dependent on high or low telomerase/telomere values in the cell line panel and the genetically engineered MCF-7 clones. They were then evaluated in clonogenic assays of 12 human tumor xenograft tissues for extended tumor type testing and COMPARE analyses. The COMPARE algorithm was programmed to enable comparison of chemosensitivity in the propidium iodide and clonogenic assays to hTERT expression, telomerase activity and TRF-length. 10 agents were found COMPARE positive (Spearman correlation coefficient >0.7) for a particular telomerase target. Two, avilamycin C and LAKVC9, were confirmed to induce hallmarks of telomerase inhibition and appear to have a therapeutic window in mice. E.g. avilamycin C, COMPARE positive for hTERT, inhibits TA activity in the TRAP assay at IC_{50} = 35 nM and induces cellular senescence at 20 nM, both are concentrations below the IC_{50} for acute cytotoxicity (80 nM).
Our data indicate that target tailored, cellular HTS can successfully identify novel lead compounds (telomerase hit rate 0.2%) and that natural products are a rich source for drug discovery. Preclinical studies assessing the therapeutic potential of avilamycin C and LAKVC9 are warranted.