lung. Its relative high sensitivity allows confident evaluation of changes as small as 25% in a single-patient. The small estimated sample sizes needed to determine therapeutic responses of as small as 25% change make DCE-MRI a promising tool to assess tumor perfusion and permeability, particularly in the antiangiogenic/antivascular arena.

Table. Confidence levels that a clinical determination of a change in K^{trans} or $IAUC_{BN}$ values for a single patient is not due to chance.

Percentage decrease	Confidence	
	Liver	Lung
10%	70%	60%
20%	87%	71%
30%	96%	82%
40%	99%	90%
50%	100%	96%
60%	100%	99%
90%	100%	100%

134 POSTER

An adaptive phase I design for identifying a dose-outcome region for two drug combinations

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Purpose: Historically, designs for dose seeking trials using drug combinations have been geared towards finding the maximum tolerated dose of the combination, with safety as the primary outcome. With target based agents whose dose-efficacy curves are unknown and whose dose-toxicity relationships are expected to be minimal, alternative designs to identify a biologically optimal drug doses for combination regimens have become necessary.
Methods: The present approach is a natural extension of an adaptive single agent dose-finding design previously presented (Zhang, Sargent, Mandrekar, Statistics in Medicine, 2005). A generalization of the continuation ratio model to characterize the true toxicity and efficacy curves of the drug combination, allowing separate toxicity and efficacy curves for each agent to generate a dose outcome surface is used. A continual reassessment method with straightforward dose selection criterion is employed using data from all patients treated up until that time point.
Results: Our simulation studies based on 500 trials with 3 and 5 dose levels for the two agents under nine different dose-toxicity (e.g. monotonically increasing, flat) and dose-efficacy (e.g. monotonically increasing, quadratic or unimodal, monotonically decreasing) scenarios demonstrated that the proposed design has favorable operating characteristics in terms of experimentation and recommendation rates for the combination dose levels, and the average sample size. Specifically, the average sample size ranged from 19 to 43 for the different scenarios considered, with recommendation rates for the optimal dose-outcome region greater than 70% for certain combinations.
Conclusions: We believe that our present approach incorporating both toxicity and efficacy of a drug combination into the identification of a biologically optimal region in a phase I setting is novel and warrants further consideration.

135 POSTER

Development of a high-sensitive antibody-based protein array system that quantitatively detects serum Fatty Acid Synthase (sFASN) in breast cancer: correlation with Her-2/neu (erbB-2) expression and trastuzumab efficacy

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Background: A bi-directional association between the Her-2/*neu* oncogene and the lipogenic enzyme Fatty Acid Synthase (FASN) has recently been described in human breast carcinomas. While FASN is overexpressed in Her-2/*neu*-positive tissues and cell lines, FASN inhibition transcriptionally suppresses Her-2/*neu* and synergistically enhances the efficacy of the anti-Her-2/*neu* antibody (Ab) trastuzumab (Herceptin®). Here, we sought to develop a new antibody-based protein array system for the quantitative determination of serum FASN (sFASN) and to determine the clinical utility of sFASN in monitoring breast cancer patients undergoing trastuzumab-based therapies.
Materials and Methods: ECL membranes were soaked with an anti-FASN monoclonal ("capture") Ab, air-dried, and then placed on the top of a template of micro-spots generated from a computer. Through the white light, the dark spots in the template were clearly visualized and used as guide to spot solution onto the membranes. Quantities of 0.25 L of standards (affinity-purified FASN), conditioned media from cell cultures or patient's sera were manually loaded onto membranes in duplicate. Membranes were then incubated with an anti-FASN polyclonal ("detector") Ab binding to the sFASN captured on the membrane. After a short incubation, the excess Ab was washed out and goat anti-rabbit immunoglobulin G conjugated to HRP, which binds to the "detector Ab", was added. Signals were developed by an ECL system and their intensities were scanned by Scion densitometry and plotted against different concentrations of FASN standards.
Results: (a.) "sFASN" was highly detected in supernatants from cancer cells naturally overexpressing Her-2/*neu* but not in those from Her-2/*neu*-negative cancer cells. (b) Cancer cells engineered to stably overexpress Her-2/*neu* dramatically up-regulated the expression and secretion of "sFASN". (c) Trastuzumab treatment significantly impaired the appearance of "sFASN" in the supernatant of Her-2/*neu*-overexpressors. (d) Preliminary analyses of sera obtained retrospectively from 114 subjects with advanced or metastatic breast cancer revealed the existence of high levels of sFASN in patients with elevated serum Her-2/*neu* concentrations.
Conclusions: This is the first practical protein array system developed to profile sFASN expression from patient's sera with high sensitivity. The utility of sFASN as a complementary tool in predicting responses to trastuzumab-based therapies warrants further studies.

136 POSTER

Discovery of DNAa hypermethylation targets associated with bladder cancer progression using CpG island microarrays

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Purpose: CpG islands arrays represent a high-throughput epigenomics discovery platform to identify global promoter hypermethylation events associated with bladder cancer progression. We investigated the role of CpG island hypermethylation in silencing targets identified using CpG arrays and their clinical/prognostic relevance.
Patients and Methods: Hypermethylation patterns of DNA obtained from ten pairs of bladder tumors were profiled versus their respective normal urothelium using differential methylation hybridization on custom-made arrays, containing 12,288 CpG island clones. Promoter CpG island methylation status of identified genes was analyzed by bisulfite genomic sequencing and methylation-specific polymerase chain reaction (MS-PCR) in bladder cancer cells (n = 12), and primary bladder tumors (n = 100). SOX9, PMF1 and CMKOR1 expression was assessed by oligonucleotide arrays, RT-PCR, and Western blot.
Results: Among the identified epigenetic signatures associated with bladder cancer progression, 84 clones showed up as hypermethylated in at least 70% of the samples. Bisulfite sequencing and MS-PCR confirmed Promoter CpG island methylation in bladder cancer cells for identified targets such as SOX9 (J82); PMF1 (TCCSUP); and CMKOR1 (RT4). The presence of hypermethylation was associated with gene expression loss, being restored *in vitro* by a demethylating agent. In primary tumors, hypermethylation and transcript levels of SOX9, PMF1 or CMKOR1 was associated with tumor stage and overall survival ($P < 0.05$).
Conclusion: Epigenetic silencing of the SOX9, PMF1 and CMKOR1 genes was identified by CpG island promoter hypermethylation using CpG arrays. The association of hypermethylation to low transcript levels also supported the role of methylation at silencing these critical genes in bladder cancer progression. Their association with poor survival in bladder tumors, suggested their important clinical implications at stratifying clinical outcome of bladder cancer patients and as therapeutic targets.

137 POSTER

Use of semilog plots and nonlinear regression analysis of survival curves to identify prognostically distinct patient subgroups

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Background: Kaplan-Meier survival curves are often multiphasic. Biological processes often follow first-order kinetics, in which semilog plots yield straight lines. In other biological processes (eg, pharmacokinetics [PK]), inflection points on semilog plots arise from distinct factors that

affect the process, and nonlinear regression is useful in defining curve characteristics. For example in PK, inflection points define different phases or "compartments" of drug disappearance, and segment slope defines the T1/2 for that compartment. We propose to apply similar analyses to survival data to assess if different "compartments" derived from semilog curves identify prognostically distinct subpopulations. If so, nonlinear regression analysis would permit characterization of each prognostic "compartment".
Materials and Methods: We reviewed the literature and replotted selected progression-free and overall Kaplan-Meier survival curves as semilog plots, then initiated curve-stripping/nonlinear regression analyses to assess the feasibility of this approach.
Results: In preliminary analyses, survival curves and progression-free survival (PFSC) curves for patients with non-small cell lung cancer (NSCLC) treated with erlotinib & gefitinib were at least biphasic, suggesting at least 2 prognostically-distinct subpopulations, while curves were uniphasic for placebo-treated patients. In other analyses, PFS curves for patients treated with surgery alone for stage I NSCLC were biphasic, while those for stage IIIA disease were triphasic, with an initial steep-slope compartment, then 2 "compartments" with slopes matching those of the stage I curve.
Conclusions: These preliminary analyses suggest converting Kaplan-Meier plots to semilog plots may potentially be useful in delineating prognostically distinct patient subpopulations. We will be applying this method widely to published clinical data to more fully assess its validity (eg, assessing relative impact on erlotinib on curves for patients with EGFR mutations vs others). Preliminary analyses of stages I vs III NSCLC suggest: (a) poor prognosis of stage III may be driven by a subpopulation with particularly rapid tumor growth, while other stage III prognostic "compartments" correspond to those seen in stage; (b) patients with stage I who relapse may come from a prognostically distinct stage I compartment in which tumor cell characteristics match those in the stage III intermediate "compartment".

138 POSTER

Validation of a histological sample transport medium preserving histoarchitecture and total and phospho-activated proteins

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Background: Biomarkers studies using precious tumor tissues is an essential part of oncology clinical trials. In preclinical studies, protocols providing high-quality samples preserving histoarchitecture, total and phospho-activated proteins exist. However, deviations from such protocols (eg delay in fixation, over fixation) can result in artifacts due to loss of immunoreactivity, in particular phospho-proteins. In the clinical setting, adherence to such protocols is challenging, particularly with multi-center studies. The clinical trial programme for the mTOR-inhibitor RAD001 (everolimus) uses centralized tissue/immunohistochemistry (IHC) analysis, thus requiring a reliable protocol for sample collection, preparation and transportation. We have validated a sample transport medium evaluating markers influencing the activity of mTOR, or being affected by mTOR activity, using tumor tissues from experimental animals.
Material and Methods: BT474 tumours, over expressing ErbB-2, grown subcutaneously in nude mice were excised and slices immediately placed into 4% phosphate buffered formaldehyde, pH 7.4, and fixed at 4ºC for 24h, allowing a range of $\pm$1h, prior to transfer of the samples to 70 % v/v ethanol transport medium. Slices were either directly processed into paraffin or stored in transport medium at 'room temperature' (20−27ºC) for 1, 2, and 4 weeks. Freshly cut sections from all samples were subjected to H&E staining and IHC for pAKT (S473, Cell Signaling Technology), HER-2 (Herceptest, Dako), pHER-2 (PN2A, Dako), pS6 (S235/236, and S240/244, Cell Signaling Technology), Ki-67 (Mib-1, Dako), and FISH-HER2 (PathVision, Vysis).
Results: No significant changes in either H&E or IHC/FISH-HER2 for any marker was observed when comparing stored versus immediately processed tissue. General architecture of the tumors was also maintained.
Conclusions: 70% ethanol provides a safe transport medium as compared with formaldehyde in that over fixation is prevented and stability of the sample architecture and immunoreactivity is maintained.

139 POSTER

Phase I dose escalation safety/tolerance study of PPI-2458 in subjects with Non-Hodgkin's lymphoma or solid tumors

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Methods: Patients with non-Hodgkin's lymphoma or solid tumors who failed prior treatments or are refractory to standard therapy are being enrolled in cohorts to receive escalating doses of PPI-2458. The treatment regimen being studied is an oral dose of PPI-2458 every other day (QOD) for 28 days. Subjects are enrolled for two 28 day cycles of PPI-2458. The first three cohorts studied 2, 3 and 5 mg QOD doses. Blood samples for pharmacokinetic (PK) and pharmacodynamic (PD) analyses were obtained during the first cycle of treatment on study days 1, 2, 3 and 15. The third cohort of this study is currently ongoing.
Results: To date 25 patients with a wide range of tumor types have been treated across three dose levels: 2 mg, 3 mg and 5 mg. One dose limiting toxicity (DLT) of grade 3 elevated liver transaminases was observed in Cohort 1 (2 mg). No additional DLTs have been observed to date. Preliminary PD data shows complete MetAP-2 inhibition (below lower limits of quantitation at any time point) in white blood cells in 76 % (13 out of 17) of the subjects treated to date. Preliminary PK data is being analyzed.
Conclusion: PPI-2458 administered orally QOD for 28-day cycles is safe and well tolerated at the doses tested to date. In addition PD data demonstrates MetAP-2 inhibition in white blood cells, even at initial dose levels evaluated. Tumor biopsies will be included in future cohorts to evaluate MetAP-2 inhibition in the target tissues.

140 POSTER

Morphologic assessments of tumor size: scan-rescan reproducibility of long- and short-axis measurements using manual and automated 3-dimensional assessments in liver and lung tumors using magnetic resonance imaging (MRI)

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Background: Current evaluations of therapeutic efficacy in solid tumors rely on assessments of changes in size, e.g., WHO and RECIST criteria. Conventional evaluations are limited to the plane of imaging. The aims of this study were to evaluate the differences in, and variability of, measurements of tumor size using manual and automated 2D and 3D methods.
Material and Methods: Scan-rescan MRIs were undertaken between 2 and 7 days apart, in 25 patients with malignant tumors in the liver or lung. The main inclusion criteria were: no preceding therapy >4 weeks, no inter-scan treatment, and lesions >3 cm. MRI included T_2 and T_1-weighted images without and with gadolinium, in 5 mm sections. Manual measurements of maximum inplane long- and short-axes were made from scan-rescan images and compared to automated inplane (2D) and 3D evaluations, which used a Geometrically Constrained Region Growth computer algorithm. The variances between visits were estimated using a statistical model and compared using Levene's test. *P*-values were adjusted by Tukey's method with α=0.1. The measurement methods were also compared by linear regression. Reproducibility was assessed using coefficients of variation.
Results: There were 24 evaluable patients (12 liver, 12 lung). Mean (SEM) long-axis measurements (cm) for manual, and automated 2D and 3D were: for lung lesions, 4.9 (0.43), 5.1 (0.49), and 5.8 (0.47); and for liver lesions, 4.9 (0.30), 5.1 (0.41) and 5.7 (0.48), respectively. 3D measurements were significantly longer than those obtained from 2D and manual inplane methods [$p_{adjusted} < 0.1$]. 3D and 2D automated measurements were correlated to the manual measurements, with a slopes significantly >1 for liver [$p < 0.05$], but not for lung [$p > 0.5$] (Figure 1). Short- and long-axis values were highly correlated [$p \approx 0.9$]. Scan-rescan reproducibility of long-axis measurements was not significantly different between the three methods [$p = 0.11-0.85$]: for liver and lung lesions, using