Methods: 3-stage phase II design with interim analyses planned after 10 and 20 subjects enrolled. Subjects with previously untreated stage IIIB with pleural effusion or IV NSCLC and normal organ function without hemoptysis or brain metastasis were treated with B (15 mg/kg every 21 days) plus E (150 mg OD) for 4 cycles followed by B (15 mg/kg), carboplatin (AUC 6) and paclitaxel (200 mg/m^2) every 21 days. Subjects who did not progress on initial B+E received further consolidation with B+E until progression.
Results: Twenty subjects have been enrolled thus far (11 male, 9 female; median age 61 years; 18 stage IV, 2 stage IIIB; 18 former smokers; 13 adenocarcinoma, 3 large cell carcinoma, 4 undifferentiated carcinoma; performance status 0 in 8 and 1 in 12). Sixteen subjects have completed at least 2 cycles of B+E with 3 partial responses (response rate 19%) and 8 stable disease (50%). Seven subjects have completed bevacizumab plus chemotherapy with best responses of 1 partial response and 5 stable disease. Toxicity observed during B+E has included grade 3 rash (4 subjects), grade 3 diarrhea (1 subject), grade 2 hypertension (1 subject), and grade 3 epistaxis (1 subject). Toxicity observed during bevacizumab/chemotherapy has included grade 3 neuropathy (2 subjects), grade 3 hypersensitivity reaction (1 subject), grade 4 neutropenia (1 subject), and grade 4 arterial thrombosis (1 subject).
Conclusions: The combination of B+E appears effective and well tolerated in 1st line stage IIIB/IV NSCLC. The administration of 4 cycles of B+E does not impair subsequent administration of cytotoxic chemotherapy. Accrual to a planned total of 48 subjects continues.

131 POSTER

Magnetic Resonance Perfusion Imaging predicts oligodendroglial cytogenetic subtypes and determines profiles of tumor angiogenesis

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Background: Chemosensitivity of oligodendroglial neoplasms and prolonged patient survival can be predicted based upon the loss of heterozygosity (LOH) of chromosomes 1p and 19q. Although the genes involved with oligodendroglial pathogenesis on chromosome 1p and 19q have not been identified, noninvasive characterization of the biological behavior of these tumors through advanced imaging techniques can direct the search for candidate genes. The relative tumor blood volume (rTBV) as determined by magnetic resonance (MR) perfusion-weighted imaging reflects the degree of neoplasm angiogenesis and metabolism. Therefore, the present study is aimed to correlate MR perfusion-weighted imaging data to molecular determinants of glial tumor malignancy and angiogenesis in distinct cytogenetic subsets of oligodendroglial tumors.
Materials and Methods: Thirty patients with oligodendroglial neoplasms were retrospectively selected for this study. Tumors were classified according to the current WHO histological criteria as either low-grade (II) or high-grade (III) and as either oligodendrogliomas or oligoastrocytomas. Tumors were divided into two groups: tumors with 1p or 1p/19q LOH (group 1) versus tumors with 19q LOH or intact alleles (group 2). MR studies were performed on a 1.5 T scanner using dynamic susceptibility-weighted methods (DSC) to determine rTBV. Cytogenetic tumor analyses were performed by either FISH or PCR methods. Paraffin-embedded tumor tissues were assessed for tumor expression of vascular endothelial growth factor (VEGF) using standard IHC techniques.
Results: In WHO grade II neoplasms, the rTBV was significantly greater ($p < 0.05$) in group 1 [n = 7; mean 2.63; (range 0.96–3.28)] compared to group 2 [n = 7; 1.71; (1.27–2.23)]. In grade III neoplasms, the differences between group 1 (n = 4; 2.83; (1.59–6.26)) and group 2 [n = 12; 2.88; (1.81–3.76)] were not significant. The rTBV was significantly greater ($p < 0.05$) in grade III neoplasms [n = 16; 2.88; (1.59–6.26)] compared to grade II neoplasms [n = 14; 1.99; (0.96–3.28)]. Interestingly, there was increased expression of VEGF and related angiogenic proteins in 1p/19q deleted tumors. It is possible that the genes on chromosomes 1p and 19q may include negative regulators of tumor angiogenesis and invasion.
Conclusions: Collectively, our data demonstrates the utility of advanced MR imaging in predicting molecular correlates of tumor malignancy and angiogenesis in distinct cytogenetic subsets of oligodendroglial tumors.

132 POSTER

Translation of in vitro markers of the anti-angiogenic and anti-tumor activity of the SOD1 inhibitor ATN-224 to clinical trials

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ATN-224 is a second generation analogue of the copper binding drug tetrathiomolybdate (TM) and is currently entering phase II trials in several oncology indications. ATN-224 inhibits CuZn superoxide dismutase (SOD1) having anti-angiogenic and anti-tumor effects (Juarez et al. (2006) Clinical Cancer Research, *in press*). SOD1 catalyzes the dismutation of superoxide anion into H_2O_2 and O_2. Hydrogen peroxide is a promiscuous second messenger that mediates mitogenic signaling and by inhibiting SOD1, ATN-224 suppresses the generation of H_2O_2 and down-regulates several mitogen-induced kinase cascades (VEGF, FGF-2, EGF, PDGF and IGF-1). The need for biomarkers to follow biological activity in patients or to select patients for clinical trial is obvious. Historically, the pharmacodynamics of TM have been followed by tracking ceruloplasmin (Cp), a biomarker for systemic copper. However, at least in mice, the inhibition of angiogenesis occurs before a measurable decrease in systemic copper is observed. In this study, we present the pre-clinical evaluation of three potential biomarkers and the translation of two of these in the clinic. We initially correlated the ATN-224 mediated inhibition of plasma, blood cells and tumor SOD as well as decrased in ERK 1/2 phosphorylation in animal tumor models with the inhibition of tumor growth and angiogenesis. Finally, we evaluated the effects of ATN-224 on circulating endothelial progenitors (EPCs) and circulating endothelial cells (CECs), which are established biomarkers for anti-angiogenic activity, in primates. Based on these pre-clinical studies, we evaluated the effects of ATN-224 on SOD activity in blood cells, Cp levels in plasma and CECs and EPCs in patients as part of a phase I clinical trial in advanced solid cancer (sponsored by Cancer Research UK). A dose-dependent decrease in SOD1 activity as well as CECs and EPCs was observed in ATN-224 treated patients. The inhibition of SOD activity occurred earlier than the depletion of Cp, suggesting that the inhibition of SOD activity is a more sensitive pharmacodynamic readout of ATN-224 activity than the depletion of systemic copper measured by Cp. Furthermore, changes in SOD activity were also more sensitive than Cp to ATN-224 dose adjustments. These results indicate that SOD activity and CEC/EPC measurements are useful biomarkers of ATN-224 activity and support the evaluation of these biomarkers in phase II trials for correlation to clinical benefit or tumor response.

133 POSTER

The reproducibility of perfusion parameters obtained from dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in liver and lung tumors, and implications for sample size in clinical trials using DCE-MRI

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Background: DCE-MRI is being increasingly incorporated into Phase I/II clinical trials, particularly with antiangiogenic drugs. The aims of this study were to determine the reproducibility of measurements of perfusion parameters derived from DCE-MRI, and to evaluate their impact on trial design.
Material and Methods: DCE-MRI was undertaken on two occasions (2–7 days apart) in 25 patients with malignant tumors in the liver or lung. The main inclusion criteria were: no preceding therapy for >4 weeks, no inter-scan therapy, and lesions >3 cm. Dynamic gadolinium enhanced 3D fast spoiled gradient-echo images were acquired for approximately 4 minutes. Tumor blood normalized initial area under the curve ($IAUC_{BN}$) and transfer constant (K^{trans}) parameters were computed using a semi-automated algorithm. Coefficients of variation (CoV) were determined using the logtransformed K^{trans} and $IAUC_{BN}$ data, and the pooled between-visit variance. Sample size estimates used a general estimating equation model, single-sided 5% significance, 90% power and a 25% response rate.
Results: There were 24 evaluable patients (12 liver, 12 lung lesions). The medians (inter-quartile range) across patients for $IAUC_{BN}$ and K^{trans} for liver lesions were 0.23 (0.20–0.32) and 0.065 (0.056–0.096), and for lung lesions, 0.18 (0.14–0.26) and 0.053 (0.035–0.073), respectively. The CoV values for $IAUC_{BN}$ and K^{trans} were lower for liver than for lung lesions, estimate (95% range): 9.8% (6.8−17.3%) and 10.6% (7.4−18.9%) for liver, versus 18.9% (13.1−33.9%) and 19.3% (13.4−34.5%) for lung [p = 0.07 and 0.059], respectively. Sample size calculations with these data indicate that, for a sought after 25% therapeutic response, appropriate sample sizes are 4 and 12, for liver and lung tumors, respectively. A single patient can be evaluated, with 90% confidence, for a 25% and 40% change in DCE-MRI parameters for liver and lung lesions, respectively; and a 10% response in a single patient can be determined with 70% and 60% confidence, respectively (Table).
Conclusions: The reproducibility of perfusion parameters derived from DCE-MRI are in the range 10−20%, and appear better in the liver than

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