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

M. Stumm[1], M. Walker[1], C. Fux[1], N. Hanoteau[1], U. Wagner[2], T. O'Reilly[1]. *[1]Novartis Institute of BioMedical Research, Oncology Research, Basel, Switzerland; [2]Viollier AG, Basel, Switzerland*

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

K. Stiede[1], J. Eder[2], S. Anthony[3], P. Conkling[4], L. Fayad[5], D. Petrylak[6], E. Sausville[7], C. Verschraegen[8], G. Bhat[1]. *[1]PRAECIS Pharmaceuticals INC, Clinical Operations, Waltham, USA; [2]Dana Farber Cancer Institute, Phase I Clinical Director, Boston, USA; [3]US Oncology, Cancer Care Northwest, Oncology, Spokane, USA; [4]US Oncology, Virginia Oncology Associates, Oncology, Norfolk, USA; [5]UT M.D. Anderson Cancer Institute, Lymphoma/Myeloma, Houston, USA; [6]Columbia-Presbyterian Medical Center, Oncology, New York, USA; [7]University of Maryland, Oncology, Baltimore, USA; [8]University of New Mexico, Oncology, Albuquerque, USA*

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)

C.S. Ng[1], D.L. Raunig[2], E. Ashton[3], E.F. Jackson[4], F. Kelcz[5], R. Kurzock[6], R. Evelhoch[7], C. Charnsangavej[1], T.M. McShane[2]. *[1]M.D. Anderson Cancer Center, Radiology, Houston, USA; [2]Pfizer, Global Technology, Groton, USA; [3]VirtualScopic Inc, Rochester, USA; [4]M.D. Anderson Cancer Center, Imaging Physics, Houston, USA; [5]University of Wisconsin, Radiology, Madison, USA; [6]M.D. Anderson Cancer Center, Experimental Therapeutics, Houston, USA; [7]Amgen, Thousand Oaks, USA*

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

manual method, 5.2% and 8.1%; 2D automated, 7.6 % and 8.1%; and 3D automated, 8.2% and 8.8%, respectively.

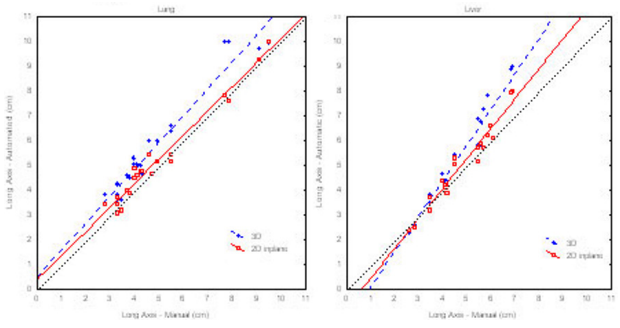

Fig. 1. Correlation of automated 2D *vs*. manual inplane long-axis measurements for lung (left) and liver (right) tumors (black dotted line represents perfect correlation).

Conclusions: Scan-rescan measurements of tumor size can be made with reproducibility in the range 5–9%, with no significant differences between manual and automated methods. There were no significant differences in assessments of size between inplane manual and 2D automated methods, but 3D derived measurements were significantly larger, which, for liver lesions, showed divergence from manual and automated inplane 2D.

141 POSTER

Effect of population and gender on chemotherapeutic agent-induced cytotoxicity

R.S. Huang[1], E.O. Kistner[2], W.K. Bleibel[1], S.J. Shukla[3], M.E. Dolan[1].
[1]University of Chicago, Department of Medicine, Chicago, IL, USA;
[2]University of Chicago, Department of Health Studies, Chicago, IL, USA;
[3]University of Chicago, Department of Human Genetics, Chicago, IL, USA

Large inter-individual variance is observed in both response and toxicity associated with chemotherapy. Our goal is to identify factors that contribute to chemotherapy-induced toxicity. To this end, we used EBV-transformed B-lymphoblastoid HapMap cell lines derived from thirty Yoruban trios (African descent) and thirty CEPH trios (European descent) to evaluate population and gender specific differences regarding cytotoxicity of carboplatin, cisplatin, daunorubicin and etoposide using a high throughput, short-term alamarBlue™ assay. The IC_{50} was compared for population and gender specific differences for the four drugs. We observed large inter-individual variance in IC_{50} values for carboplatin, cisplatin, daunorubicin and etoposide for both Yoruban and CEPH populations (range from 8- to 433-fold). Statistically significant differences in carboplatin and daunorubicin IC_{50} were demonstrated when comparing Yoruban cell lines ($n = 89$) to CEPH cell lines ($n = 87$) ($p = 0.002$ and $p = 0.029$, respectively). This population difference in treatment induced cytotoxicity was not seen for either cisplatin or etoposide. In the Yoruban population, cell lines derived from females were less sensitive to platinating agents than males [median carboplatin IC_{50} 29.1 vs 24.6 M ($p = 0.012$); median cisplatin IC_{50} 7.0 vs 6.0 M ($p = 0.020$) in female and male, respectively]. This difference was not observed in the CEPH population. These results demonstrate that population and gender may affect risk for toxicities associated with certain chemotherapeutic agents.

142 POSTER

The role of the novel apoptosis related gene BCL2L12 in prognosis and individualized treatment of breast cancer: a molecular and clinical approach

H. Thomadaki[1], M. Talieri[2], A. Ardavanis[3], A. Scorilas[4]. *[1]National and Kapodistrian University of Athens, Faculty of Biology, Biochemistry and Molecular Biology, Athens, Greece; [2]"Saint Savvas" Hospital, "G. Papanicolaou" Research Center of Oncology, Athens, Greece; [3]St. Savvas Anticancer-Oncologic Hospital, 31st Department of Medical Oncology, Athens, Greece; [4]National and Kapodistrian University of Athens, Faculty of Biology, Biochemistry and Molecular Biology, Athens, Greece*

Background: Breast cancer is a major health problem. The currently most successful approach for combating breast cancer is by early diagnosis, good prognosis and administration of effective treatment. A multitude of markers have been discovered within the last three decades, including factors related to different cell functions, such as apoptosis, with many members of the *BCL2* family of apoptosis-related genes being found to be differentially expressed in various malignancies, and some regulating cellular fate after exposure to anticancer drugs. A new member of the *BCL2* gene family, *BCL2L12*, was discovered and cloned (Scorilas et al. 2001) and it was found to be expressed in mammary gland. It maps to chromosome 19q13.3 and is localized between the *IRF3* and *RRAS* oncogene. Our objective is to investigate the novel gene *BCL2L12*, as a novel molecular biomarker for prognosis and individualized treatment of breast cancer.
Materials and Methods: In the present study, we explored the research on the prognostic value of *BCL2L12*, as a novel breast cancer biomarker. Sixty specimens from patients with, histologically confirmed, epithelial breast carcinoma were analyzed for *BCL2L12* gene expression by RT-PCR using gene specific primers. Actin was used as a control gene. Their gene expression profile was associated with clinicopathological parameters and survival analysis regarding to relapse and death were evaluated by constructing Kaplain-Meier curves and developing a Cox proportional hazard regression model.
We also studied the possible alterations in the mRNA expression of the apoptosis-related gene *BCL2L12* after cell treatment with cisplatin or carboplatin, in the breast cancer cell lines MCF7 and BT-20. The cytotoxic effect of each drug was evaluated by the MTT method and trypan blue staining, whereas the expression levels of distinct apoptosis-related genes were analysed by RT-PCR, using gene specific primers.
Results: Increased expression of *BCL2L12* gene was found in estrogen receptors positive as well as in chemotherapy responded patients. In addition, *BCL2L12*-positive patients were found to be almost 4 times less likely to relapse or die in comparison to *BCL2L12*-negative patients. Furthermore, treatment of the breast cancer cell lines, MCF-7 and BT20, with well-known chemotherapeutic drugs induces distinct alterations in the mRNA expression levels of *BCL2L12* gene, giving some preliminary information about its value in chemotherapy response prediction.
Conclusions: *BCL2L12* is involved in both breast cancer progression and in chemotherapy response, implying a possible role in individualized medicine and its application into more successful therapeutic interventions.
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143 POSTER

Comparison of cell death ELISAs applied as potential surrogate biomarkers in the clinical evaluation of AEG35156 (XIAP antisense)

J. Cummings[1], T.H. Ward[1], M. Ranson[2], N.K. Smith[1], D. Jodrell[3], L. Robson[4], E. Dean[1], C. Dive[1]. *[1]Paterson Institute for Cancer Reserach, Clinical and Experimental Pharmacology, Manchester, United Kingdom; [2]Christie Hospital NHS Trust, Department of Medical Oncology, Manchester, United Kingdom; [3]Western General Hospital, Cancer Research Centre, Edinburgh, United Kingdom; [4]Cancer Research UK, Drug Development Office, London, United Kingdom*

The protein XIAP is the most potent endogenous inhibitor of caspase function currently known, and its over-expression is associated with poor patient outcome. AEG35156 is a second generation 19-mer oligonucleotide targeting XIAP and is currently undergoing early clinical trials. One of the anticipated outcomes of AEG35156 treatment is the induction of tumour cell death. We have studied 3 different plasma ELISAs as pharmacodynamic biomarkers during a CRUK Phase I trial of AEG35156 administered as a 7 day infusion. M30 Apoptosense detects a caspase cleaved fragment of the epithelial cell protein cytokeratin 18 (CK18) as a selective marker of apoptosis. M65 detects both intact and caspase cleaved CK18 as markers of apoptotic and non-apoptotic cell death. Quantitation of circulating nucleosomal DNA (nDNA) offers a further approach to apoptosis measurement. The 3 assays were utilised to analyse plasma samples collected at multiple time points spanning the first three week treatment cycle from 20 patients who had received AEG35156 at multiple dose levels from 48 to160 mg/m^2/d, where dose limiting transaminitis was encountered. Baseline concentrations of M30 and M65 antigens exhibited a 15 fold range. Similar values to those of healthy subjects were seen in patients with non-epithelial tumours (60–300 U/L). Very high values of 1600–3200 U/L were recorded in 2 patients with breast cancer. Analysis of two independent pre-treatment samples showed only minor variations in M30 and M65 antigens (<15%), whereas greater variability was detected with the nDNA assay. Increases in M30, M65 and nDNA antigens occurred with greater frequency at the higher doses of AEG35156, normally reaching a peak during the 7-day drug infusion. Increases in M30 and M65 antigens were also detected in patients with non-epithelial tumours, suggesting that these assays may also detect toxicity in non-tumour tissues. In 50% of patients, the concentration-time profiles for all 3 assays showed close temporal agreement. In a further subset of patients good temporal agreement was observed between nDNA and M65. In conclusion, the 3 ELISA assays appear to detect drug induced changes in circulating levels of their