RXRγ were constitutively expressed in the ovaries. 4-HPR and OCP used alone had a small induction of retinoid receptor expression. However, the combination of 4-HPR with OCP had a stronger effect on induction of all retinoid receptors except RXRβ expression. OCP induced expression of ERβ, but the combination had a stronger effect, suggesting that there was a synergistic effect between the two drugs on hormone receptor expression. 4-HPR alone at the equivalent of 200mg/day induced apoptosis in monkey ovaries. Overall the combination showed more modulation on all the markers than the other 3 groups ($p<0.004$). Erβ was upregulated in the combination group ($p<0.04$), EGF was also upregulated and approached statistical significance ($p<0.06$). ERβ, but not ERα was upregulated in the combination group, but did not reach statistical significance.
This primate study suggests that the combination of 4-HPR and the oral contraceptive can induce apoptosis and upregulate some retinoid receptors and ERβ more than either drug alone, providing some clues to their mechanism. Although 4-HPR is thought to be receptor independent, in combination with OCP may in fact, act through the retinoid receptors and may be more effective in combination than either drug alone.
(Supported by DAMD17–99–990:MB and OCRF-ACCAC03:CZ).

583 POSTER

Serum proteomic biomarkers of a natural product in a prospective randomized placebo-controlled clinical trial in patients at risk for lung cancer

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Smoking, asthma, and chronic obstructive pulmonary disease (COPD) are known risk factors for lung cancer. The disease may be preventable, but many potential chemopreventive agents have not shown clinical activity in individuals at risk for lung cancer (Van Zandvijk et al, Lung Cancer 2003, 42:S71). A novel natural product LP01 demonstrated preclinical preventive and anticancer activities, and induced time-and dose-dependent changes in serum kallikreins and proteomic patterns in human lung cancer xenograft models (Baek et al, Proc AACR/NCI/EORTC 2003). The present study evaluated LP01 in a prospective, randomized, triple-masked, placebo-controlled, parallel-group clinical trial. In this study, lung cancer risk (1–5) was assessed based on length of addiction, asthma, and COPD, for a group of former long-term smokers (smoked >20 years, quit >1 year). This group, comprised of sixty men and women ages 35–70, received oral daily doses of 3,650 mg LP01 or placebo for 6 months. Peripheral blood serum specimens were obtained at the baseline and after drug treatment for 2 weeks, 1 month, 2 months, 4 and 6 months. Serum proteins were resolved on IMAC3/Cu metal affinity ProteinChip arrays and analyzed by surface-enhanced ligand desorption/ionization (SELDI). There were no adverse clinical effects of the therapy. The patients were stratified by the low risk (1 to <3) and high risk for lung cancer (⩾3), both in the drug and placebo groups for the analysis of SELDI proteomic patterns. Statistically significant differences ($p<0.05$) were observed between the drug and placebo groups in the cluster of small proteins <10,000 mass/charge (M/Z). The drug effects on select biomarkers were similar in the low and high risk for lung cancer. The findings warrant identification and characterization of potential biomarkers of risk for lung cancer and the efficacy of LP01.
Supported by Jiang Jing, Inc.

Clinical methodology

584 POSTER

Phase II trial design for radiation (XRT) modifiers: Efficient evaluation of safety, XRT modifier effect, post-XRT cytostatic effect and relevant molecular markers

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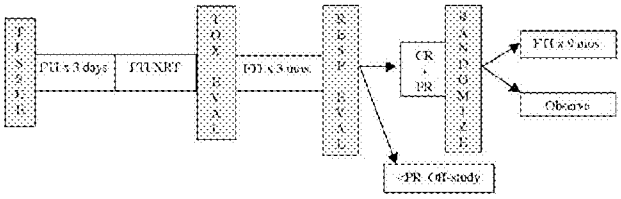
The appropriate design of trials that incorporate targeted agents with radiotherapy is critical to the development of these treatments. The principles of investigating XRT modifiers in trials have been previously reviewed (Colevas et al. JNCI, 2003). The goals of combining targeted agents with XRT are to improve efficacy and reduce combined modality toxicities, as compared to conventional chemoradiotherapy regimens. Recent preclinical studies have suggested that activation of the Ras-PI3K-Akt pathway affects XRT sensitivity, which has led to investigations of farnesyltransferase inhibitors (FTI) with XRT.
A novel bivariate phase II trial design has been proposed to evaluate FTI R115777 (NSC# 702818) and XRT in locally advanced NSCLC patients (pts). This design is well-suited to the dual aims of the trial, which are to demonstrate a reduced esophagitis toxicity (TOX) rate and similar clinical response (RESP) rate, as compared to conventional paclitaxel/carboplatin/XRT. R11577 is taken orally for 3 days pre-XRT, daily during XRT and for 3 mos. post-XRT. Randomized discontinuation in responders at 3 mos. post-XRT allows for evaluation of cytostatic effects based on time to progression (TTP) from randomization. Relevant molecular markers (*ras*, Akt, EGFr, MAPK) are examined in tumor tissue.



The Bayesian approach (MULTC99 software courtesy of P. Thall) quantifies pre-trial TOX and RESP probabilities (prob) and repeatedly updates these probs as TOX and RESP data accumulate during the trial. Early stopping rules for TOX and RESP are defined with the updated probs. FTI/XRT regimen is considered acceptable if the probs of TOX and RESP are ⩽0.25 and ⩾0.65, respectively. The trial terminates early if it is either likely (>95% chance) that the TOX goal cannot be achieved or unlikely (<10% chance) that the RESP goal can be achieved. Excellent stopping properties are demonstrated. If the true TOX and RESP probs are 0.46 (unacceptable; same as paclitaxel/carboplatin/XRT) and 0.78, respectively, then the prob of early stopping is 0.88, while for true probs of 0.25 and 0.65 (acceptable), respectively, then the prob of early stopping is <0.10.
This 80 pt trial provides precise estimates of TOX and RESP rates (posterior interval * width is 10%), 30 pts per randomized group for TTP analyses and abundant marker data for correlative analyses. Moreover, since pts are not pre-selected based on marker status, a rich co-variation in molecular markers is expected. The trial design (prior probs, stopping rules and properties) and power for correlative and TTP analyses will be fully described.

585 POSTER

Analytical and multi-center clinical performance evaluation of a diagnostic device designed to analyze the expression of 11q23/MLL abnormal fusion transcripts in acute leukemia

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Background: 11q23 abnormalities involving the *MLL* gene are highly heterogeneous. Over 50 partner genes have been described to date, and t hese molecular rearrangements are collectively associated with unfavorable prognosis in acute lymphoblastic leukemia (ALL) and an intermediate risk in acute myeloid leukemia (AML). However, the functional role and the prognostic significance of specific fusion transcripts on the progression and outcome of disease remain to be elucidated. Diagnostic testing of patients with acute leukemia includes cytogenetic analysis confirmed by FISH, RT-PCR, and/or Southern blotting. Molecular screening can be cumbersome, since identification of the fusion partner involves multiple and time-consuming analysis. We present analytical and clinical performance evaluation data for a new biochip based molecular device (*MLL* Fusion*Chip*™) designed to confirm the presence of 11q23 abnormality and identify the *MLL* fusion gene partner.
Methods: An analytical study was performed to address the robustness, precision, limit of detection, and analytical sensitivity and specificity of the *MLL* Fusion*Chip* ™ using RNA from 4 cell lines and 2 clinical samples. Clinical performance was then evaluated on a range of ALL and AML samples with known *MLL* rearrangements, identified by cytogenetics, FISH, and/or RT-PCR, in nine laboratories from seven countries. Following assessment of RNA quality, 127 *MLL* positive and 23 control samples were analysed with the *MLL* Fusion*Chip* ™. Each laboratory ran four sets of five assays including a control (positive cell line), 2 negative, and 2 positive samples.
Results: Technical validation results were: success rate 98.3%, r epeatability 100%, reproducibility 97.7%, limit of detection ⩽1%, analytical sensitivity 100%, and analytical specificity 92.3%. Nine different partners, including the rare partners AF17, AF10, MSF, and P300 were accurately identified in the clinical performance study. Furthermore, in two cases, the *MLL* Fusion*Chip*™ detected partners for which RT-PCR failed. The overall agreement between prior diagnostic analysis and the *MLL* Fusion*Chip*™ was >90%.

Conclusions: Analytical performance validation shows compatibility of this oncodiagnostic device with its intended use. The results of the multi-centre clinical performance evaluation demonstrated that the *MLL* Fusion*Chip*™ gave reproducible and reliable results in a range of clinical laboratories, and provided accurate results when compared with those obtained by more conventional methods. Further studies are necessary to evaluate the clinical utility of the molecular classification of acute leukemia, and whether this tool will facilitate optimised use of molecular targeted-based therapeutics for the different *MLL* partners, based on their unique molecular targets.

586 POSTER

Response assessment classification: effect of multiple measurement criteria and parameters

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Background: To evaluate the response assessment classication in patients with metastatic oesophageal cancer using unidimensional and bidimensional criteria. For unidimensional criteria the impact of short axis, rather than long axis measurement will also be assessed. Tumor eccentricity, a new parameter, of response and change was also assessed.
Material and Methods: 22 patients with metastatic oesophageal cancer involved in a phase II trial were included in this study. Ninety-three lesions were assessed at baseline and followed on serial CT scans. Response assessment was calculated with unidimensional and bidimensional tumor measurements. To measure the eccentricity of tumor shape (the degree of divergence from a perfect sphere), a new parameter, "EF", was calculated (EF = $\sqrt{}$) where LPD = largest perpendicular diameter, and MD = maximal diameter).
Results: There was a 27.3% disagreement rate in the best overall response categorization between unidimensional and bidimensional measurements. The average change in lesion EF was 0.45 for patients with agreement and 0.8 for patients with disagreement between unidimensional and bidimensional measurements. This difference was statistically significant ($p<0.001$). By utilizing the short axis for lymph node measurement there was no disagreement between bidimensional and unidimensional short axis measurement.
Conclusion: There is a significant difference in response assessment between both measurements methods which may be due in part to the change in eccentricity of tumors measured over time with EF. The greater the change in eccentricity the greater the discordance. The short axis measurement better predicts the tumor response when compared to the bidimensional response. This factor could be critical to the assessment of overall tumor response on any therapy.

587 POSTER

Development of proximity based assay to detect and quantify erbB (or Her) receptor dimerization in formalin fixed-paraffin embedded tissue sections

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The EGFR, ErbB2, ErbB3 and ErbB4 are members of the Type I receptor tyrosine receptor kinase family (also known as Her or ErbB family). Overexpression of these receptors found in a number of cancers (e.g. breast, colon, ovarian, Lung) has aggressive phenotype with poor prognosis. However, recent clinical trials have shown that overexpression of erbB receptors alone is not sufficient to predict patient response. A thorough analysis of the activation status in the erbB pathway will likely achieve better prognosis.
Immunohistochemistry (IHC) is the most commonly employed method used to evaluate the expression of receptors in formalin fixed-paraffin embedded (FFPE) clinical samples. Although IHC provides valuable information about the relative level of expression and subcellular localization of a particular target, it is not quantitative. The scoring of results is also very subjective and prone to error among independent observers. Consequently, there is a need to develop assays to circumvent these issues. Here, we report the development of novel proximity based assays to detect and quantify various Her dimers in formalin fixed-paraffin embedded (FFPE) samples. In this assay, the sample was first deparaffinized and rehydrated by regular xylene/ethanol/water protocols. After antigen retrieval, the sample was incubated with a mixture of erbB specific antibodies conjugated either with reporter etag ™ or a chemical scissor. The reporter etag were then released based on its proximity to the scissors. The released etags were separated by capillary gel electrophoresis and quantified by etag-informer ™ software. Assays were developed to quantify the levels of EGFR/EGFR, EGFR/Her2, Her2/Her2 and Her2/Her3 homo- and hetero-dimers in the FFPE sample. Tublin was used as an internal reference control for the total cellular content. The assays were first developed using ligand (heregulin or EGF) stimulated tumor cell line pellets in FFPE sections. The assays were then used to detect and quantify Her dimers in various xenograft models and clinical patient tissue samples. The data demonstrated the simultaneous detection and quantification of Her receptor expression, dimerization and phosphorylation in a single tissue section. Inter- and intra-assay reproducibility was 8–20% (n=8). The validity of the detection and quantification of Her dimers was independently confirmed by etag assay analysis of the Her dimers in FFPE sections and cell lysates from the same samples.
We conclude that the etag assays are simple, sensitive and provide a quantitative assessment of various Her dimers from the same sample. They can be used to determine the activation status of erbB/Her receptor in clinical sample for the correlation with disease prognosis or response to targeted therapies.

588 POSTER

Prevalence of erbB/Her dimerization in breast cancer

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Background: Epidermal growth factor receptors (EGFRs) and signaling pathways activated by these receptors have been implicated in the development of breast cancer. The EGFR family includes, human EGFR-1 (Her1), human EGFR-2 (Her2), human EGFR-3 (Her3), and human EGFR-4 (Her4). It is well established that ligands like EGF and HRG bind to the extracellular region of the EGFR monomers and promote receptor dimerization. Receptor dimerization leads to increased tyrosine kinase activity resulting in uncontrolled cell proliferation and inhibition of apoptosis. Determining the dimerization patterns in breast cancer may provide useful information for the treatment of breast cancer. Hence, we have developed eTag ™-multiplexed assays, to detect and quantify the different types of erbB/Her dimers in breast cancer tissues.
Materials and Method: We have analyzed 61 snap-frozen human breast tissues comprising of 31 ductal or lobular carcinoma samples and 30 normal samples. Of these, 8 tumors and 8 normal breast tissues were matched with the same donor. Using the proximity-based multiplexed eTag assays, we determined the dimerization profiles in these tissues.
Results: ErbB/Her dimerization was detected only in tumor tissues but not in normal breast tissues, whether matched with the same donor or not. Out of the 31 tumor samples analyzed, Her1/2 dimers were detected in 19 tumor samples while 24 tumor samples had Her-2/3 dimers. We also found that all tumor samples had higher Her-2 levels compared to normal breast samples. In addition, we detected Her-2/2 homodimers in 23 out of 31 tumor samples. Our quantitative dimerization assays showed the presence of different amounts of Her-1/2 and/or Her-2/3 and/or Her-2/2 dimers in different breast cancer tissues of either ductal or lobular types.
Conclusion: As erbB/Her dimerization levels are associated with activation status of the receptor, eTag technology can be a valuable prognostic tool both, in stratifying patients with breast cancer for targeted therapy as well as for assessing the activation state of the receptor during the course of patient treatment. These assays represent the first quantitative methods that can provide receptor activation signatures for the erbB/Her family.

589 POSTER

Gene expression profiling defines new molecular classes and predicts response to adjuvant anthracycline-based treatment in breast cancer patients: development of a biochip to predict prognosis and improve clinical management of breast cancer

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Background: The significant genetic heterogeneity amongst breast cancer patients continues to be one of the primary obstacles to effective clinical diagnosis and management. Recent advances in microarray technology have contributed to enhanced understanding of the underlying diverse molecular mechanisms that drive tumorigenesis in individual patients, and emerging technologies based on gene expression profiling (GEP) may provide clinically useful tools to improve the standard of care in breast cancer. However, the translation of large-scale GEP technologies from the research to clinical setting has yet to be achieved. In this study, we describe the development of the Breast Cancer Profile*Chip* (BCPC), a device based on GEP for molecular characterization and management of breast cancer.