respectively. As anticipated, the PSA variants were activated to a greater extent by the PSA positive LNCaP cell line (LNCaP>DU145>COS-1). Data demonstrating the maximum tolerated dose and efficacy in mouse prostate cell xenograft models is presented.

579 POSTER

Design and synthesis of difluoromethylphosphonamidates for prodrugs of non-hydrolyzable phosphotyrosine peptidomimetics

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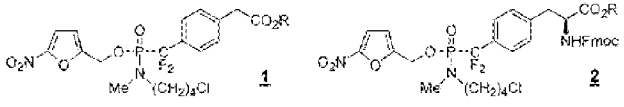
Our laboratory has reported previously a prodrug strategy to achieve intracellular delivery of nucleotides and phosphotyrosine peptidomimetics. However, the potential therapeutic benefit of such prodrugs is likely to be diminished by intracellular nucleotidases/phosphatases that can cleave the phosphate group from the drug species released in the cell. This difficulty can be circumvented for the phosphotyrosine peptidomimetics by incorporating the non-hydrolyzable difluoromethylphosphonate moiety in place of the phosphate group. We have now extended our prodrug strategy to the synthesis of aryl(difluoromethyl)phosphonate prodrug compounds **1** and **2** (R = H) that are suitable for incorporation into non-hydrolyzable phosphotyrosine peptidomimetics. The synthetic approach is based upon assembly of the analogous diethyl phosphonates and subsequent conversion of these intermediates to the nitrofuryl N-methyl-N-chlorobutyl phosphoramidates. The prodrug activation chemistry was verified for these difluoromethyl phosphonate analogs by hydrogenolysis of a model compound (**1** where the nitrofuryl group is replaced by benzyl) to the corresponding phosphonamidic acid. This intermediate was then dissolved in buffer and its conversion to the phosphonic acid monitored by ^{31}P nmr; a half life of 44 minutes (37ºC, pH = 7.4) was observed for this conversion, confirming the feasibility of this prodrug approach for the delivery of difluoromethyl phosphonates. The application of this chemistry to the synthesis of non-hydrolyzable phosphotyrosine peptidomimetics will be described, and approaches to the synthesis of analogous non-hydrolyzable phosphoserine peptidomimetics will also be presented.



580 POSTER

In vitro release of 17-demethoxy-17-allylaminogeldannamycin from its prodrugs

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Methods: In vitro stability procedure was established for both standard curves and actual plasma samples. A small amount of acetonitrile was used to help solublize prodrugs. The plasma samples were incubated at 37ºC over 24-hour period. Excess acetonitrile was used to stop possible enzymatic reactions in plasma at preset time points. Each prodrug was studied individually by HPLC to monitor the concentrations of both the remaining prodrug and the accruing 17-AAG at each time points. The conversion of each prodrug was investigated in mouse plasma, recovered, and fresh human plasma.

R	Name	NSC
-H	17-AAG	330507
-CO-(CH2)3NH2,HCL	11-(4'-amino)-butanoate HCl	683201
-CO-CH2NH2,HCL	11-(2'-amino)-acetate HCl	683661
-CO-CH2N(CH3)2,HCL	11-(2'-N,N-dimethylamino)-acetate HCl	683662
-CO-(CH2)2NH2,HCL	11-(3'-amino)-propionate HCl	683663
-CO-(CH2)2N(CH3)2,HCL	11-(3'-N,N-dimethylamino)-propionate HCl	683664
-CO-(CH2)4NH2,HCL	11-(4'-N,N-dimethylamino)-butanoate HCl	697886
-CO-(CH2)3N(CH3)2,HCL	11-(5'-amino)-pentanoate HCl	697866

Results: It was found that each individual prodrug has a comparable degradation pattern in three different plasmas. The 17-AAG is the major degrading compound that was observed for all seven prodrugs. In all plasma samples, NSC-683662 and NSC-683664 were the least stable: about 50% of these prodrugs released 17-AAG in the first five hours. Note that both of these prodrugs have a tertiary amine on the end of acyl chain. Though the NSC-697886 is similarly structured, its release rate is far slower: the release of 17-AAG was only about 10% after 24 hours. It appears that the number of $-CH_2-$ group affects the degradation rate: there are three $-CH_2-$ groups on the acyl chain in the NSC-697886 while only one in the NSC-683662 and two in the NSC-683664. It also appears that the prodrugs that have side chains with terminal primary amino group degrade to a lesser extent: the NSC-683201 almost has no degradation over 24 hours. In the first 6 hours NSC-683661, NSC-683663, and NSC-683666 only degrade marginally.

Conclusions: Stability profiles indicate that for each individual prodrug the degradation pattern is comparable in three different plasmas with 17-AAG as the major product. The NSC-683662 and NSC-683664 are least stable, thus are good candidates for further development.

Chemoprevention

581 POSTER

Lycopene, alone or combined with vitamin E, reduces orthotopic growth and plasma PSA release of PC-346C prostate tumors

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Background: Epidemiologic and clinical studies have indicated that high intake of lycopene or vitamin E is associated with a reduced prostate cancer risk, but no firm conclusions about protective effects can be drawn from these studies. The current study was undertaken to investigate whether lycopene or vitamin E, alone or in combination, may suppress orthotopic prostate tumor growth and whether blood PSA levels may serve as a surrogate marker for antitumor-efficacy.

Materials and Methods: The androgen responsive, PSA-releasing, human prostate cancer cell line PC-346C was injected into the dorsolateral lobe of the prostate of athymic nude mice. Three days after tumor inoculation, mice were supplemented on a once daily oral basis with synthetic lycopene (5 and 50 mg/kg BW), synthetic vitamin E (5 and 50 mg/kg BW), a mixture of lycopene and vitamin E (5 mg/kg BW each), or placebo. Tumor growth was followed weekly by transrectal ultrasonography of the mouse prostate and plasma was sampled for PSA analysis at 2-weekly intervals. Mice were sacrificed when tumor load exceeded 1000 mm^3 or at day 95, when the study was terminated. The prostate (including tumor) and liver were analyzed for the presence of lycopene isomers and α-tocopherol by HPLC-methodology.

Results: The low dose of 5 mg/kg BW lycopene significantly suppressed the growth of the prostate xenograft by 53% at day 42 and extended the tumor doubling time accordingly. All other single treatments, either with the high lycopene dose or with both vitamin E doses, had no significant tumor-inhibiting effect. Combined treatment with the low lycopene-vitamin E mixture gave by far the greatest tumor inhibition (73% at day 42). PSA values and PSA doubling times matched the tumor responses in all experimental groups. Vitamin E and lycopene were effectively taken up at nanogram levels in the prostate and liver. Although lycopene was mainly present in the *all-trans* conformation in the dietary supplement (90%), the lycopene in the tissues existed primarily as cis-isomers (70%), a pattern similar to that observed for humans and other species.

Conclusions: Synthetic lycopene in low doses may inhibit prostate cancer, but combining it with vitamin E may enhance its effects. The absence of a selective effect on PSA supports the usefulness of PSA as a surrogate marker for these supplements in clinical prostate cancer trials.

582 POSTER

Chemoprevention of ovarian cancer in primate model

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Ovarian cancer is the most lethal tumor among the gynecologic cancers and is associated with an extremely high mortality rate, partially due to the late stage of diagnosis and partially due to the lack of a durable response to cytotoxic chemotherapy. Primate models are ideal for developing strategies for both treatment and prevention because of the genetic similarity between primates and humans, such as hormonal regulation and menstrual cycle. 4-(N-hydroxyphenyl) retinamide (4HPR), a retinoid derivative, and the oral contraceptive (OCP) is currently being studied as chemopreventive agents for ovarian cancer but the mechanisms of their prevention activity are unclear.

We studied the effect of 4-HPR and OCP alone and in combination on the ovaries of 16 monkeys. The expression of retinoid receptors, hormone receptors, as well as apoptosis induction were tested in vivo. ERα was not detected in the primate ovaries, but ERβ, RARα and β, RXRα, and

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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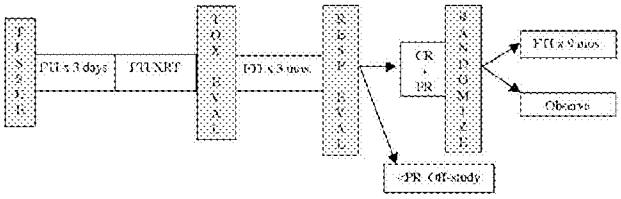
The appropriate design of trials that incorporate targeted agents with radiotherapy is critical to the development of these treatments. The principles of investigating XRT modifiers in trials have been previously reviewed (Colevas et al. JNCI, 2003). The goals of combining targeted agents with XRT are 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%.