outcome of two different combination schedules. Our results were then compared against experimental data, in a single-blind test, showing our Virtual Tumour technology was able to accurately predict the experimental results.
Using the Virtual Tumour, thousands of simulations can be performed if necessary to find the best treatment regime. This allows our partners to prioritise the most effective drug combinations and the best schedules for validation *in vivo*.

566 POSTER

Frequent overexpression of Hbo1 in non-small cell lung carcinoma and its potential oncogenic role

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Background: Various histone modifying enzymes have been focused in cancer research because of their chromatin modification function by changes in acetylation or methylation status of the amino-termini of histones, which is intimately correlated to regulation of gene expression. Hbo1 is a member of MYST histone acetyltransferase family having dual functions of H4 acetylation and DNA replication licensing.
Material and Methods: To screen expression status of Hbo1, RT-PCR using fresh frozen lung cancer tissues and immunohistochemistry using tissue microarray of paraffin embedded tissue blocks were performed. Copy number profiling using array CGH and FISH were performed to identify aberration of the gene in genomic level. Using siRNA, knockdown effect of the gene in lung cancer cell line was studied.
Results: In this studies, we show that Hbo1 mRNA is frequently overexpressed in lung cancer tissues comparing normal lung tissues (9/19, 47.4%). The tendency of overexpression in cancer tissues is confirmed by immunohistochemistry (293/495, 51.2%). Its expression was correlated with histone acetylation status. Array comparative genomic hybridization assay showed frequent copy number gain at 17q21.3 region containing HBO1 gene (4/12, 33%). Knockdown of HBO1 mRNA using siRNA significantly inhibited the growth rate of Calu6 cell, in contrast to scrambled siRNA.
Conclusion: In conclusion, the histone acetyltransferase Hbo1 is frequency overexpressed in non-small cell lung cancer not only at mRNA but also protein levels. Its overexpression is supported by genomic copy number gain. Growth inhibition of tumor cell is induced by knock down of the gene. The results suggest that Hbo1 overexpression plays an oncogenic role in NSCLC and can be a potential therapeutic target.

567 POSTER

A rationale for anti-angiogenic therapy in head and neck cancer

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The clinical behavior of head and neck squamous cell carcinoma (HNSCC) is marked by a high degree of lymphatic and distant metastasis, which result in decremental decreases in overall and disease-free survival. VEGF has been demonstrated to be an important mediator of tumor angiogenesis and metastasis in HNSCC, and while anti-angiogenic therapies have been effective in other solid tumors, their role in HNSCC has not been clarified to date. Polymorphisms in the *VEGF* gene have been shown to be predictive of the clinical behaviors, treatment responses, and disease outcomes for tumors of the lung and breast. However, the impact of these mutations have not been extensively explored in HNSCC. The aims of this study were to explore the feasibility of high-throughput VEGF polymorphism analysis and to study the association between these polymorphisms and disease outcomes among patients with HNSCC.
Methods: DNA was extracted from prospectively-collected surgically-resected HNSCC tumors after IRB approval was obtained. High-throughput mutational analysis with the Sequenom® platform was performed for the following polymorphisms: *VEGF-1154G>A, VEGF-1498C>T, VEGF-634C>G, VEGF-2573C>A*. Clinical and pathological data were collected and evaluated for associations between disease outcome and tumor genotype.
Results: Genetic polymorphisms for VEGF were studied in 75 surgically-resected tumors or metastatic lymph nodes, and 58 samples (77%) were found to harbor mutations in one of the tested polymorphisms. Of these, *VEGF-634C>G* were most common, with 36/75 (48%) harboring this genotype. The *VEGF-1154G>A* and *VEGF-1498C>T* genotypes were commonly seen as well (44% and 29.3%, respectively), while the *VEGF-2573C>A* was observed in only 4 tumors. Correlation with clinical outcomes was performed, and while the presence of any polymorphism was significantly associated with death from disease ($p < 0.05$), there was no association with the presence of lymphatic or distant metastasis. When each polymorphism was analyzed independently, only the *VEGF-634C>G* genotype was associated with local-regional recurrence ($p < 0.05$) and death from disease ($p < 0.05$). Kaplan–Meier analysis revealed adverse survival among patients with any *VEGF* polymorphism ($p < 0.05$), with only 45% survival at 5 years, compared with 75% among the WT group.
Conclusions: We identified a significant percentage of patients with *VEGF* polymorphisms among surgically-resected HNSCC patients. Further, the presence of tumoral *VEGF* polymorphisms were predictive of adverse outcomes among patient with HNSCC. These data suggest that anti-angiogenic therapy may be a rational modality for selected patient with HNSCC and provide support for personalized targeted therapy in this disease. Further analysis is necessary to identify which specific polymorphisms are most predictive for both disease outcomes and treatment response.

568 POSTER

A permutation-based confidence interval for treatment effect in the identified subset of sensitive patients in biomarker-adaptive threshold design

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Background: Difficulty in prediction of clinical outcome, efficacy and toxicity, is hallmarks of most anticancer drug. In the era of molecular and individualized medicine, molecular predictive biomarker is playing an increasingly important role. However, reliable predictive biomarkers are rarely available in designing phase of a trial, and then regulatory authorities recently encourage the co-development of drug and biomarker. Jiang et al. proposed the biomarker-adaptive threshold design for situations where a candidate biomarker, which is originally measured on a continuous or ordered categorical scale, e.g. expression levels of HER2 or epidermal growth factor receptor, is available at the start of the trial but a cutoff value is not established for converting the biomarker to a binary classifier to separate sensitive from insensitive patients. This design incorporates both the identification and the internal validation procedures of a cutoff value, with protection of type I error and only a minor increase in sample size. However, estimation method for treatment effect in the identified subset of sensitive patients has not been proposed, although it is especially valuable to aid the interpretation of trial results, and the CONSORT statement requires confidence intervals for treatment effect.
Material and Methods: We develop a permutation-based confidence interval for the parameter representing treatment effect in the identified subset of sensitive patients in the framework of the biomarker-adaptive threshold design. Simulation based on models conforming to several practical situations was performed.
Results: A permutation-based confidence interval was derived, and it is consistent to the design and statistical analysis formulated by Jiang et al. Simulation results showed favorable tendency that proposed method can produce the correct confidence interval, *i.e.* it can reduce bias in parameter estimation and maintain nominal level.
Conclusions: We can construct the permutation-based confidence interval for a co-development design of drug and biomarker, and it can provide us with the valuable information about subset treatment effect.

569 POSTER

Whole-genome sequencing and analysis of an ovarian cancer patient

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Background: Ovarian cancer is the fifth leading cause of cancer death for women in the U.S. and the seventh most fatal worldwide. Although ovarian cancer is notable for its initial sensitivity to platinum-based therapies, the vast majority of women eventually recurs and succumbs to increasingly platinum-resistant disease. To elucidate somatic genetic changes of an individual tumor, we recently completed the sequencing of the tumor genome from an ovarian cancer patient as well as her germline genome using the Illumina Genome Analyzer IIx®.
Material and Methods: Genomic DNA was sheared into segments approximately 400bp long and we generated 180 bp paired-end reads. When mapped back to the genome, this provided an average coverage greater than 15-fold for both cancer and germline genomes. In order to identify single nucleotide variations (SNV) between germline and cancer,

we developed a score quantifying the evidence for sequence differences at the single nucleotide level. The score is based on read coverage and sequence quality and allows us to rank variants based on their support by the read data. We classified SNVs in loss of heterozygosity (LOH), substitution and gain of heterozygosity (GOH).
Results: Using the LOH and GOH classification of SNVs we were able to identify copy-number neutral chromosomal loss of heterozygosity, complete and partial quantitative chromosome losses, focal amplifications and deletions, as well as a range of other alterations in the genome. More fine scale analysis has identified sequence mutations in a number of genes that may help explain the development and progression of the disease. Most informative were missense mutations in LOH regions, which supplied a set of novel candidate tumor suppressor genes in ovarian cancer. Additional RNA-Seq data using polyA+ RNA derived from the tumor sample provides confirmation of many of the observed genomic alterations and allele specific expression.
Conclusions: Deep-sequencing of a paired tumor and germline DNA sample from the same patient has the potential to be a valuable tool for the discovery of novel ovarian cancer mechanisms, potential molecular targets and cancer therapeutics.

Anthracyclines, antimetabolites, antimicrotubules, topoisomerase inhibitor

570 POSTER

Phase 1 study of XMT-1001, a novel water soluble camptothecin conjugate, given as an IV infusion every 3 weeks to patients with advanced solid tumors

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Background: XMT-1001 is a water soluble macromolecular conjugate of camptothecin (CPT). In this novel CPT prodrug, CPT is conjugated with a 70 kDa biodegradable hydrophilic polyacetal, poly (1-hydroxymethylethylene hydroxymethylformal). XMT-1001 has an improved therapeutic window compared with irinotecan in human tumor xenograft models, providing a compelling rationale for its clinical development.
Methods: This is a dose escalation study of XMT-1001 given as an IV infusion once every 3 weeks. The objectives are to determine the maximum tolerated dose (MTD) and assess safety as well as pharmacokinetics (PK) of XMT-1001. Three patients (pts) are entered at each dose level, with expansion to 6 pts in the event of dose limiting toxicity. Analyses of plasma and urine are performed for XMT-1001 (conjugated CPT), two major drug release products, and for unconjugated (free) CPT.
Results: To date, 63 pts have received 186 cycles of XMT-1001 at dose levels from 1.0 to 151 mg CPT equivalents (eq)/m^2. Two pts had Grade (Gr) 3 infusion reactions related to study drug. After the introduction of clinical trial material with an improved formulation, no infusion reactions suggestive of hypersensitivity have occurred (42 pts, 124 cycles). No hemorrhagic cystitis or $\geqslant$Gr 2 diarrhea related to study drug was noted at any dose of study drug. Dose limiting toxicities (DLTs) were febrile neutropenia and Gr 4 neutropenia >5 days in two of six patients dosed at 151 mg CPT eq/m^2. The MTD is initially defined as 113 mg CPT eq/m^2 and is being confirmed. A partial response (PR) was observed in a patient with small cell lung carcinoma (SCLC) at the 151 mg CPT eq/m^2 dose level. Tumor shrinkage also was observed in a patient with colorectal carcinoma dosed at 15.4 mg CPT eq./m^2 and CEA declines occurred in a patient with colorectal cancer dosed at 113 mg CPT eq/m^2. Stable disease (SD) was noted in 17 of 60 evaluable pts with prolonged SD ($\geqslant$12 weeks) in 9 patients. Dose proportional increases in C_{max} and exposure to XMT-1001 and its release products were observed and levels of free CPT recovered in urine were low.
Conclusions: 1. The MTD for XMT-1001 was initially defined; DLTs were Gr 4 neutropenia and febrile neutropenia. 2. No drug related $\geqslant$ Gr 2 diarrhea and no hematuria were noted. 3. Anti-tumor activity was observed; a patient with SCLC had a PR; CEA declines and tumor shrinkage were documented in two pts and prolonged SD ($\geqslant$12 weeks) was noted in nine pts. 4. XMT-1001 and its release products have a favorable PK profile.

571 POSTER

TLC388, a novel topoisomerase-1 inhibitor with anti-hypoxia inducible factor-1 alpha activity: a phase I and pharmacokinetic study in patients with advanced solid malignancies

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Purpose: To assess the feasibility of administering TLC388, a novel derivative of topotecan with a unique modification in the lactone ring resulting in a more potent inhibition of topoisomerase I with anti-HIF1 alpha activity.
Patients and Methods: Patients with chemotherapy-refractory advanced solid malignancies were treated with escalating doses of TLC388 as a 30-minute intravenous infusion weekly X 3, repeated every 28 days. Plasma and urine sampling were performed to characterize the pharmacokinetics of TLC388.
Results: Forty-two patients (M:F = 24:18, median age 63, range 33−80) with advanced solid malignancies (prostate 7, colorectal 5, pancreas 3, esophageal 3, kidney 3, others 20) received 83 courses (range 1−7) of TLC388 at 9 dose levels ranging from 1.5 to 40 mg/m^2/d. Anemia was the most frequently reported hematological toxicity (37.5%). Leukopenia and neutropenia were reported in <20% of patients. Two DLT grade 4 hyponatremia cases and one grade 4 thrombocytopenia were observed at the level of 40 mg/m^2. To date, 15 out of 25 evaluable patients (60%) have stable disease (SD) as best response by RECIST. Prolonged stable disease was noted in 6 patients (7+ months in one patient with chromophobe renal cell carcinoma; 6 months in sorafenib-refractory hepatocellular carcinoma, docetaxel-refractory prostate cancer, and thymoma, one each; and 5 months in anal and cholangiocarcinoma, one each). Prolonged dosing does not lead to cumulative toxicity in patients. Pharmacokinetics of TLC388 was dose-independent; mean (SD) values for the volume of distribution at steady-state and plasma clearance were 845 (986) L/m^2 for S,R-TLC388 and 1102 (1481) L/m^2 for S,S-TLC388, and 2265 (2434) L/h-m^2 for S,R-TLC388 and 2914 (3233) L/h-m^2 for S,S-TLC388, respectively. The half-life values averaged 0.57 (1.18) hours for S,R-TLC388 and 0.65 (1.25) hours for S,S-TLC388.
Conclusion: TLC388 is safe as a weekly infusion up to 35 mg/m^2/d. The DLT of grade 4 hyponatremia at 40 mg/m^2/d is possibly drug-related and being investigated. Based on preliminary clinical efficacy, further disease-directed trials of TLC388 are planned.

572 POSTER

Berubicin, a topoisomerase II poison with high CNS uptake, inhibits cell growth and induces apoptosis in diffuse large B-cell lymphomas

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Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive form of lymphoma that frequently metastasizes to the central nervous system (CNS). Current clinically used drugs for DLBCL often fail to overcome drug resistance in relapsed and refractory DLBCL, especially in CNS DLBCL. Doxorubicin (DOX), an anthracycline, is an important component of the CHOP treatment regimen. We have designed a novel class of anthracyclines with high CNS uptake and an improved molecular targeting spectrum. Berubicin (BRN), the lead drug of this class, is being clinically evaluated in brain tumor patients. It has been shown that BRN is a topoisomerase II poison and a potent inhibitor of hypoxia induced factor-1α (HIF-1α) transcriptional activity.
Materials and Methods: Because of its high CNS uptake, we tested the effects of BRN in DLBCL cell lines. In our studies, two human large cell lymphoma cell lines, DB and Toledo, were treated with BRN and compared with DOX. We analyzed the effects of these compounds on cell proliferation, apoptosis, and the cell cycle using MTS assays and flow cytometry.
Results: BRN potently inhibited the growth of both cell lines in a dose-dependent manner, with IC_{50} values of 12.4 nM for the DB cell line and 3.3 nM for the Toledo cell line. BRN was more potent in both cell lines than DOX (IC_{50s} for DOX, 31.1 nM and 10.0 nM, respectively). BRN also potently induced apoptosis and G2/M cell cycle arrest in the DB and Toledo DLBCL cell lines in a dose-dependent manner.
Conclusions: BRN showed more potent activity against DLBCL cell lines than DOX. These results are promising and consistent with BRN's