Results: This module has some major advantages: it has an intuitive work-flow based Graphical User Interface compliant with GCP requirements; provide some alerts for relevant prescribing problems as the excess/suboptimal dose or the renal impairment; has some basic statistical functions; it could be accessed on the Internet and Intranet (using a virtual server or FileMaker Pro Server™); protected and secure connection with dedicated login and password. A capture is presented in Figure 1. The most powerful quality is the capacity to provide direct, on-site and instant information about the dose-intensity for each product (function of the administered dose and the delay between two consecutive cycles). A demonstration is planned to be performed at the congress.
Conclusions: RDBMS are helpful tools in our efforts to ameliorate the efficiency of prescribing modern treatments in oncology. They are a "must" for those interested to provide a highly qualified exercise, especially in clinical research.

195 POSTER

Pharmacogenomic analysis of the peripheral blood cell transcriptome in patients with advanced solid tumors treated with the mTOR inhibitor deforolimus (AP23573; MK 8669) in phase Ib studies

A. Rinaldi[1], I. Kwee[1], F. Bertoni[1], L. Viganò[2], D. Hess[3], N. Coceani[4], C. Sessa[1], V.M. Rivera[5], C.L. Bedrosian[5], C.V. Catapano[1]. *[1]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [2]Istituto Nazionale per la cura dei Tumori, Milan, Italy; [3]Kantonsspital St. Gallen, St. Gallen, Switzerland; [4]Southern Europe New Drug Organization (SENDO), Milan, Italy; [5]ARIAD Pharmaceuticals Inc., Cambridge, MA, USA*

Background: Inhibitors of the mammalian target of rapamycin (mTOR), a serine/threonine kinase that integrates multiple signaling pathways and cellular processes, are undergoing extensive clinical investigation as anticancer drugs. Deforolimus (DEF) is a potent, specific, non prodrug mTOR inhibitor that is currently being investigated in a phase 3 trial in patients with metastatic sarcomas. The immediate targets of mTOR (e.g., phospho-4E-BP1) are used as biomarkers to monitor drug effects and select the optimal biologically effective doses in phase I studies. This assay, however, does not provide information on downstream cellular pathways that might be relevant to antitumor activity. Furthermore other targets, not interrogated by the assay, might contribute to the clinical activity of mTOR inhibitors. Here, we investigated in the context of two phase Ib studies with DEF (SENDO-S045AP2301−02) whether its administration was associated with specific changes in the peripheral blood transcriptome (PBT). We hypothesized that this genomic analysis could better capture the complexity of the downstream effects of mTOR inhibitors and identify more robust biomarkers for this class of drugs.
Methods: Blood samples for PBT analysis were taken from patients receiving 12.5, 37.5, 50, or 75 mg of DEF IV on day 0 and 1 of cycle 1 prior to any other therapy. Affymetrix U133 2.0 GeneChip arrays were done in a total of 16 patients (3 to 5 per dose level). Real time RT-PCR was performed to validate selected genes.
Results: We found a set of genes that were consistently modulated 24 h after administration of DEF at doses $\geqslant$37.5 mg in $\geqslant$70% of patients and up to 100% of cases. The number of commonly affected genes increased with the dose, peaking at 50 mg. At this dose, 83 and 10 transcripts were, respectively, down- and up-regulated in $\geqslant$75% of patients, with 33 transcripts down-regulated in 100% of cases. The degree of down- and up-regulation of most genes increased with the dose, showing evidence of a dose-related response at $\geqslant$37.5 mg. This was in contrast with the phospho-4E-BP1 assay in PBMCs that showed complete inhibition already at the lowest dose. Among down-regulated genes at doses $\geqslant$50 mg there was a prevalence of genes in pathways that might be functionally connected to mTOR activity (e.g., apoptosis, NK cell-mediated cytotoxicity, MAPK, insulin and Toll-like receptor signaling).
Conclusion: The PBCT can be a powerful source of information to monitor drug effects and identify robust and stringent biomarkers in phase I trials. Here, we identified genes that were consistently modulated after DEF, showed evidence of dose-dependence, and may represent clinically useful biomarkers of mTOR inhibition. These findings need to be validated in larger clinical trials. In addition, further analysis of the biological functions associated with the genes identified in PBT may reveal important aspects of the mTOR inhibitor activity.

196 POSTER

Intron 1 CA repeat polymorphism is associated with the sensitivity to EGFR TKIs in NSCLC patients with wild type EGFR

J.Y. Shin[1], J.O. Kim[1], S.J. Lim[2], H.S. Won[3], T.H. Kim[3], C.H. Lee[4], M.S. Kim[5], J.H. Kang[3]. *[1]The Catholic University of Korea, Oncology Laboratory, Seoul, South Korea; [2]Kangnam St. Mary's Hospital The Catholic University of Korea, Hemopoetics stem cell unit, Seoul, South Korea; [3]Kangnam St. Mary's Hospital The Catholic University of Korea, Oncology, Seoul, South Korea; [4]School of Medicine Yensei University, Clinical Genetics, Seoul, South Korea; [5]Hanmi Research Center Hanmi Pharm. Co. Ltd., Drug Discovery Team, Seoul, South Korea*

Background: The epidermal growth factor receptor (EGFR) plays a key role in carcinogenesis and progression in various solid tumors by its activation by over-expression, mutation, and autocrine ligand production etc. Clinical outcome of EGFR TKIs is mainly affected by the mutation status of EGFR TK domain, histology, gender, smoking status and ethnicity. Recently, it has been reported that some genetic variants of EGFR gene including CA repeat polymorphism in intron 1 modulate its transcriptional activity.
We investigated the allelic frequency of three genetic variants on EGFR gene in Korean population and analyzed the genetic variants, EGFR mutations, and the sensitivity to EGFR TK inhibitors in vitro and NSCLC patients.
Methods: Genomic DNA was extracted from peripheral blood in 221 healthy volunteers and 20 NSCLC patients receiving EGFR TK inhibitors. PCR products that were amplified for promoter region, intron 1, and exon 18 21 were sequenced in a 3730XL DNA analyzer and GeneScan. For in vitro experiment, thirteen NSCLC cells and A431 epidermoid carcinoma cells (as control) were used to measure the drug sensitivity to gefitinib using the SRB assay.
Results: In healthy Koreans, the most frequent EGFR CA repeat genotype was 20/20 (32.6%) repeats followed by 16/20 (22.1%), 15/20 (8.6%) and the allelic frequencies of −216G>T and −191C>A were 95% and 99%, respectively. Among thirteen NSCLC cell lines, the most sensitive cells to gefitinib were PC9 and HCC-827 (IC50: <5 months), the sum of CA repeats was 39 40 with no mutations in EGFR.
Conclusion: Not only the distribution of CA repeat genotype but also the allelic frequency of −216G>T and −191C>A in Korean population were quite different from those of Caucasian. It is obvious that the mutations in tyrosine kinase domain of EGFR gene are the major determinant to anti-tumor efficacy of EGFR TKIs. Our results suggest that CA repeat polymorphism in intron 1 be another predictive biomarker of EGFR TKIs in NSCLC patients with wild type EGFR. Further study using sufficient human tumor samples is underway to support this preliminary results.

197 POSTER

Pathway determinants of 5-fluorouracil activity

W.L. Tan[1], D.F. Dong[2], L.S. Wong[2], R.A. Soo[3], I. Fichtner[4], R. Soong[5]. *[1]National University of Singapore, Pathology, Singapore, Singapore; [2]National University of Singapore, School of Computing, Singapore, Singapore; [3]National University Hospital, Haematology Oncology, Singapore, Singapore; [4]Max Delbrück Center for Molecular Medicine, Berlin-Buch, Berlin, Germany; [5]National University of Singapore, Oncology Research Institute, Singapore, Singapore*

Background: Response to 5-fluorouracil (5-FU) varies considerably among individuals, making it desirable to identify determinants of its activity. However, findings thus far have been inconclusive. This is possibly because most studies have focused on only a few components of entire pathways of 5-FU pharmacology or studied in vitro or in vivo models in isolation. In this study, we took a pathway based approach to identify candidate determinants of 5-FU activity in cell lines and xenografts.
Materials and Methods: Total RNA was extracted from 18 colorectal cancer cell lines and 14 human colorectal cancer xenografts before 5-FU treatment. RNA levels of 91 genes involved in folate metabolism, 5-FU transport, metabolism, activity and downstream mechanisms were quantified in these samples using real-time PCR low density array analysis. Sensitivity to 5-FU was defined by IC50 values for cell lines and extent of tumor shrinkage for xenografts. Chi-square, information gain ratio, OneR and Cfs subset were used to rank genes which were differentially expressed between the sensitive and resistant cases.
Results: Five cell lines and 8 xenografts were classified as resistant to 5-FU and 16 cell lines and 6 xenografts as sensitive. In cell lines, beta-ureidopropionase (UPB1) was the most differentially expressed by all 4 statistical tests. In xenograft samples, cytidine triphosphate synthetase II (CTPS2) was ranked the most differentially expressed in 3/4 tests. In combined analysis of cell lines and xenografts CTPS2 was the top ranked

gene in 3/4 tests. In a cross validation of this gene in cell line and xenograft datasets, its overall predictive accuracy was 77% and 86% respectively.
Conclusions: UPB1 and CTPS2 are promising novel candidate determinants of 5-FU activity. On-going studies are incorporating gene combination analyses, and gene modulation and human tissue investigations.

198 POSTER

Genetic polymorphisms associated with adverse events in childhood acute lymphoblastic leukaemia treated with SHOP-2005 protocol

E. Lopez[1], I. Martin-Guerrero[1], J. Ballesteros[2], M.A. Piñán[3], A. Navajas[4], A. Garcia-Orad[1]. *[1]University of the Basque Country, Genetics Physic Antropology and Animal Physiology. Faculty of Medicine and Odontology, Leioa Vizcaya, Spain; [2]University of the Basque Country, Neurosciences. Faculty of Medicine and Odontology, Leioa Vizcaya, Spain; [3]Hospital of Cruces, Hematology and Hemotherapy, Barakaldo Vizcaya, Spain; [4]Hospital of Cruces, Paediatric Oncology, Barakaldo Vizcaya, Spain*

Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer, and still the most important cause of cancer-related death in children. Although the introduction of treatment protocols has improved survival, interindividual differences in drug responses are an important cause of resistance to treatment and adverse drug reactions. Pharmacogenetic studies are providing a rational base for further treatment efficacy and reduction of complications.
The aim of the present study was to determine if there was a correlation between genetic polymorphisms and toxicity and/or outcome during therapy in paediatric ALL patients treated according to the SHOP-2005 protocol (high-dose methotrexate [MTX] and 6-mercaptopurine [6-MP]).
We analyzed 12 polymorphisms of 9 genes in 21 paediatric ALL patients: 3 genes of the MTX pathway (MTHFR, RFC1 and ABCB1), 1 gene of the 6-MP pathway (TPMT) and 5 genes involved in xenobiotic detoxification (CYP1A1, NQO1 and the GSTs GSTM1, GSTT1 and GSTP1). Then, data were analyzed by using the Fischer exact test.
Several associations were found, such as that of the MTHFR C677T and A1298C polymorphisms and minimal residual disease (predictor of relapse in ALL), and the association between the MTHFR A1298C polymorphism and vomiting, as well as that of the GSTM1 null genotype and diarrhoea. Moreover, when the genes involved in the MTX pathway and the GST genotypes were analyzed together, they predicted diarrhoea even better than GSTM1 alone.
Our results indicate that several polymorphisms of the MTX-related genes and GST genes may be useful as predictors of gastrointestinal toxicity and outcome of the SHOP-2005 treatment protocol.

199 POSTER

Influence of PXR haplotype variants on paclitaxel pharmacokinetics and pharmacodynamics in Asian cancer patients

B. Chowbay[1], E. Sandanaraj[1], S. Lal[1], Z.P. Wang[1], T. Kulkarni[1], W.T. Lim[2], C.S. Ang Peter[2], E.H. Tan[2]. *[1]National Cancer Centre Humphrey Oei Institute of Cancer Research, Clinical Pharmacology Laboratory, Singapore, Singapore; [2]National Cancer Centre Humphrey Oei institute of Cancer Research, Department of Medical Oncology, Singapore, Singapore*

Background: Paclitaxel is primarily metabolized by CYP3A4 and CYP2C8 and transported by ABCB1, which are downstream targets of the pregnane X receptor (*PXR*) gene. The objective of this exploratory study was to investigate the influence of *PXR* genetic variants on the pharmacokinetics and pharmacodynamics of paclitaxel in Asian cancer patients.
Materials and Methods: A total of 25 Asian cancer patients receiving intravenous infusions of paclitaxel either as a weekly (80 mg/m^2, N = 11) or three weekly (170 mg/m^2, N = 14) dosage regimens were recruited. Pharmacogenetic and pharmacokinetic data were available for all the patients and pharmacodynamic data was available for 12 patients. Paclitaxel pharmacokinetic parameters were estimated using non-compartmental analysis (WinNonlin) and Mann-Whitney *U* test was used to assess genotypic-phenotypic correlations.
Results: Two main *PXR* haplotype groups were identified, *PXR*1B* and non-*PXR*1B* haplotype groups. The *PXR*1B* haplotype group was tagged by the IVS6−17C>T and 2654T>C SNPs. Patients harbouring the *PXR*1B* haplotypic constitution had significantly lower clearance [CL/dose ($mL \times h^{-1} \times mg^{-1}$), median: 94.0; range: 45.3–207.2] and significantly higher exposure levels of paclitaxel [$AUC_{0-\infty}$/dose ($hr \times \mu g \times mL^{-1}$): median: 52.8; range: 35.5–89.0] compared to patients belonging to the non-*PXR*1B* haplotype group [CL/dose ($mL \times h^{-1} \times mg^{-1}$), median: 229.50; range: 65.5–624.3, (P = 0.03) and $AUC_{0-\infty}$/dose ($hr \times \mu g \times mL^{-1}$): median: 27.2; range: 12.5–53.9, (P = 0.007), respectively]. Patients carrying the *PXR*1B* haplotype group also had significantly higher C_{max} levels of paclitaxel [C_{max}/dose ($\mu g \times mL^{-1}$): median: 17.6; range: 12.1–40.9] compared to patients belonging to the non-*PXR*1B* [C_{max}/dose ($\mu g \times mL^{-1}$): median: 11.03; range: 1.3–22.5, P = 0.03] haplotype group. Pharmacodynamic analysis revealed that patients carrying the *PXR*1B* haplotypic constitution had 2.1- and 1.7-fold lower absolute neutrophil counts and platelet counts when compared to the patients bearing the non-*PXR*1B* haplotypic constitution.
Conclusion: The *PXR*1B* haplotype group was found to be associated with significant alterations in the pharmacokinetics of paclitaxel and a non-significant trend towards decreased ANC counts and thrombocytopenia. This exploratory study suggests that *PXR* haplotype constitution may be important in influencing interindividual variations in the disposition of paclitaxel.

200 POSTER

Transcriptome analysis method for in vivo mechanism of action study: IMC-D11 anti-FGFR3 +/− cisplatin in bladder cancer models

W. Wang[1], Y. Cheng[2], D. Deevi[3], H. Li[3], M. Prewett[3], R. Bassi[3], M. Malabunga[4], K. Paz[2], H. Sun[4], J. Tonra[3]. *[1]ImClone Systems Inc., Antibody Technology, New York, USA; [2]ImClone Systems Inc., Cell Biology, New York, USA; [3]ImClone Systems Inc., Preclinical Pharmacology, New York, USA; [4]ImClone Systems Inc., Protein Science, New York, USA*

Background: IMC-D11, a fully human IgG1 against the human fibroblast growth factor 3 (FGFR3), enhances the anti-tumor effects of cisplatin (CDDP) when given as a combination therapy in the RT112, RT4 and BFTC-905 bladder cancer xenograft models. The molecular mechanisms in support of this combination however have not been fully elucidated. To this end, we took a systems approach to gain further insights into the molecular networks underlying the synergistic/additive effects between IMC-D11 and CDDP in vivo.
Materials and Methods: Total RNA from RT112, RT4 and BFTC-905 derived tumors treated with IMC-D11, CDDP or the combination (n = 3 tumors per group) were subjected to a global gene expression profiling using Affymetrix Human Genome U133A array.
Results: The raw data were normalized, filtered and statistically analyzed, and the lists of genes significantly regulated by IMC-D11, CDDP or the combination treatment as compared to control groups were determined. Two levels of comprehensive bioinformatics analysis of these data were performed; at the gene level and at the pathway/network level. Combination treatment with IMC-D11 and CDDP uniquely altered the expression of many genes. In RT112 for example, DOK3, FOXP3 and hprt (1200 kb deletion mutant) were significantly upregulated only with combination treatment. However, none of the genes regulated by the combination treatment were found common to three models. We therefore further examined whether these differentially expressed genes are associated with common functions, networks, or processes using Gene Ontology (GO) annotation. The results indicate that the main GO classes found enriched in the combination group in the three models were related to the processes of cell cycle/proliferation, cell death/apoptosis, DNA replication and repair.
Conclusions: Results from these analyses, and others being performed, provide not only a molecular framework for further investigation on the mechanism by which IMC-D11 and CDDP exert their anti-tumor effects, but also crucial information that may potentially be utilized for optimizing therapeutic strategies against bladder cancer.

201 POSTER

In Mdm2 SNP309 cancer cells the small molecules nutlin-3 and MI-63 facilitate recruitment of RNA polymerase II to p53 target genes

J. Bargonetti[1], A. Polotskaia[1], S. Wang[2]. *[1]Hunter College and Graduate Center CUNY, Biology, New York, USA; [2]University of Michigan, Cancer Center, Ann Arbor, MI, USA*

Background: Mdm2 inhibits p53 transactivation in part by forming a p53-Mdm2 complex on chromatin. A homozygous single nucleotide polymorphism (T to G) in the mdm2 gene at position 309 (SNP309) results in increased Mdm2 expression and increases susceptibility to cancer. In human cancer cells overexpressing Mdm2 due to homozygous G/G SNP309, the p53-Mdm2 chromatin complex is highly stable and is not disrupted following DNA damage.
Materials and Methods: To determine how the p53 response phenotype was influenced in cells with variable mdm2 genotypes using differential activation of the p53 pathway we compared targeted p53-Mdm2 complex disruption by the small molecule inhibitors Nutlin-3 and the MI-63 in wild-type p53 human cancer cell lines with variable SNP309 genotypes and wild type p53 to etoposide DNA damage treatment. The ability of the small molecule inhibitors to facilitate increased transcription factor recruitment to the chromatin was compared to the recruitment facilitated by treatment