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Pediatric Preclinical Testing Program (PPTP) evaluation of the anti-CD19-DM4 conjugated antibody SAR3419

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Background: SAR3419 is composed of the humanized anti-CD19 antibody huB4 conjugated with the potent cytotoxic maytansinoid, DM4, a tubulin polymerization inhibitor. SAR3419 has shown preclinical in vivo activity against human B-cell lymphomas, and was selected for evaluation against the Pediatric Preclinical Testing Program (PPTP) in vitro and in vivo panels of B-lineage acute lymphoblastic leukemia (ALL), which express high levels of cell surface CD19.
Methods: The PPTP includes a molecularly characterized in vitro panel of leukemia cell lines (n = 7) and in vivo panel of ALL xenografts (n = 10), representing the common subtypes of pediatric ALL. SAR3419 was tested in vitro against the RS4;11 (CD19+) and MV4;11 (CD19−) cell lines at concentrations from 0.01 nM to 10 nM, and against the PPTP in vivo panel (n = 6) at a dose of 10 mg/kg administered weekly $\times 3$ via intraperitoneal injection to NOD/SCID mice. Three measures of antileukemic activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) proportion of human CD45+ cells in the murine peripheral blood (%huCD45+) at day 21; and (3) a time to event (25% huCD45+ cells) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C >2, and high activity additionally required a net reduction in the %huCD45+ cells at the end of the experiment).
Results: SAR3419 was ineffective against MV4;11 cells, but potently killed the RS4;11 cell line with IC50.

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Preclinical evaluation of the marine compound PM00104 within the ITCC pediatric tumor cell line panel in vitro and in vivo

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Background: The European consortium Innovative Therapies for Children with Cancer (ITCC) aims to develop new anticancer agents for the treatment of pediatric malignancies. The ITCC is composed of 35 clinical pediatric oncology centres and 9 laboratories for preclinical evaluation in six European countries. PM00104 (Zalypsis®) PM00104 is a new synthetic alkaloid related to Jorumycin and the Renieramycins with in vitro growth-inhibitory properties in the low nanomolar range as well as anti-tumor activity in vivo against several adult cancers. PM00104 affects cell cycle, displays DNA binding properties and transcriptional inhibition. PM00104 is being evaluated in 4 phase I clinical trials with different schedules.
Methods: In vitro cytotoxicity of PM00104 was screened by MTS-assay on a panel of 24 pediatric tumor cell lines (CL), composed of 4 CL of the following tumor types: Ewing sarcoma, acute lymphatic leukemia, medulloblastoma, neuroblastoma, osteosarcoma, and rhabdomyosarcoma. Cells were exposed for 72 h to PM00104 at 1.4 fmol/L to 14 nmol/L. Experiments were performed in triplicate. GI50 was considered as proof of growth inhibition and LC50 represents cytotoxicity. Anti-tumor activity was evaluated against an advanced subcutaneous rhabdomyosarcoma xenograft model in athymic mice.
Results: PM00104 significantly reduced growth of all CL in a dose dependent manner. The most sensitive CL in terms of growth inhibition were within the group of neuroblastoma, rhabdomyosarcoma and ALL with GI50s below 1 nmol/L (0.5–1.0) for 2, 3 and 2 out of 4 CL respectively. The mean$\pm$SD LC50 values were 14.0$\pm$8.1 nmol/L in Ewing sarcoma, 9.0$\pm$5.9 nmol/L in ALL, 15.5$\pm$12.7 nmol/L in medulloblastoma, 7.3$\pm$6.5 nmol/L in neuroblastoma, 13.2$\pm$2.3 nmol/L in osteosarcoma and 13.1$\pm$14.3 nmol/L in rhabdomyosarcoma, respectively. In RD rhabdomyosarcoma xenografts, PM00104 administered i.v. at 0.8 and 1.0 mg/kg q7dx4 resulted in 100% tumor regression (7 complete and 1 partial/8 tumors and 9/9 complete, respectively) and significant tumor growth delay in time to reach 5 times initial tumor volume of 38.3 and 41.7 days compared to controls ($p < 0.001$; Kruskal Wallis test).
Conclusions: PM00104 exhibits cytotoxic activity against most pediatric CL in vitro, particularly neuroblastoma, ALL and rhabdomyosarcomas, and significant anti-tumor activity against rhabdomyosarcoma xenograft model.

Phamacogenomics

194 POSTER

How to prescribe standard chemotherapy or targeted-therapy using a fully featured relational database

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Background: Treatments in oncology should be ordered using relational database management systems (RDBMS). This is very useful in both standard clinical practice and clinical trials. Electronic prescribing and computerized drug management can improve the safety, quality and cost-effectiveness of prescribing.
Material and Methods: This module was constructed using a RDBMS as FileMaker Pro™ (FileMaker Inc, Santa Clara, CA, USA). It has two different parts: the input and the output section. (a) Variables that are included as "input": gender, weight, height, anatomic location of cancer, protocol of treatment, number of cycle, performance status (ECOG), creatinine, presence of anxiety, alcohol abuse, pain intensity (visual analogic scale), date of start of the actual chemotherapy and the previous cycle, area under the curve (for carboplatin). (b) Variables that are calculated as the "output": age, body surface area (du Bois formula), body mass index, obesity class (the International classification), the theoretical time interval and delay duration between two consecutive cycles of treatment, the score of patient and protocol-related emesis with an associated global emesis category risk (as defined by Grunberg), estimated creatinine clearance rate (Cockcroft-Gault and MDRD formula), categories of renal dysfunction (National Kidney Foundation), dose of carboplatin (Calvert formula), pain category, date of the next cycle, efficiency of dose intensity (DI).

Date 15.07.2007

CHEMOTHERAPY PRESCRIPTION
"If what you are doing good, keep doing it" - Loeb's rules of therapeutics

Time 19:21

First name	Xxx	Identification	5707
Last name	Dddd	Year of file	1998
Sex	female	Age	48 years
Date of birth	12.10.1959	Body surface area (BSA)	1,82 m^2
Weight (kg)	75	Body mass index	27,5 kg/m^2
Height (cm)	165	Cycles interval	28 days
Anatomic location	Breast cancer	Delayed	5 days
Protocol	AVASTIN + PACLITAXEL	Emesis score (patient)	5
Cycle number	2	Emesis risk (patient)	High risk
Creatinine	123 µmol/l	Emesis score (protocol)	2
PS ECOG	1	Emesis (global score)	High risk
Alcohol abuse	○ Yes ◉ No	Clearence of creatinine	58,8 ml/min/m^2
Anxiety	○ Yes ◉ No	Moderate chronic renal insufficiency - stage 3 KDOQI	
Pain grade (VAS)	2	Pain class WHO	Mild pain
Date of start	20.06.2007	Blood group	0 I
Date of previous cycle	18.05.2007	Date of the next cycle	18.07.2007
Oncologist	Banu Eugeniu	Clinical trial	
AUC		*Efficiency of dose intensity*	78 %

Day		Drug	Value		Theoretical	Real dose	Observation	% DI
1	20.06.2007	Bevacizumab	10	mg/kgc	750	700	iv infusion, 90 min	79
1	20.06.2007	Paclitaxel	90	mg/mp	164	150	iv, 3h, in 500 ml SF PVC	78
8	27.06.2007	Paclitaxel	90	mg/mp	164	150	iv, 3h, in 500 ml SF PVC	78
15	04.07.2007	Paclitaxel	90	mg/mp	164	150	iv, 3h, in 500 ml SF PVC	78
15	04.07.2007	Bevacizumab	10	mg/kgc	750	700	iv infusion, 60 min	79

VAS: Visual Analogic Scale PS ECOG: Eastern Oncology Cooperative Group Performance Status WHO: World Health Organisation
National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) Emesis score: Grunberg and col. (*Support Care Cancer 2005*)

Figure 1.

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.

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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

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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.

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

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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.

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Pathway determinants of 5-fluorouracil activity

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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