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Evaluation of management of hypertension in response to the receptor tyrosine kinase inhibitor, E7080: a modeling and simulation approach

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Background: E7080 is a once-daily, orally administered, receptor tyrosine kinase inhibitor with anti-angiogenic and anti-proliferative activity. Anti-tumor activity has been reported in phase I studies. Treatment-emergent hypertension has been reported in about 47% of subjects. A pharmacokinetic–pharmacodynamic (PK−PD) model for hypertension was constructed, which was used to investigate possible hypertension intervention strategies for implementation in future clinical trials.
Methods: E7080 PK and systolic and diastolic blood pressure (BP) data were obtained from 106 subjects enrolled in two phase I studies, investigating qd and bid dosing at increasing dose levels. The PK-BP model was developed using NONMEM and quantifies the effect of E7080 on BP through indirect effect models. Prescription of anti-hypertensive medication was accounted for in the model as an estimated effect on input rate, using defined daily dose equivalents (DDDE) to account for diversity in anti-hypertensive medication. In simulations, hypertension intervention strategies were evaluated over the first 120 days of treatment at the maximum tolerated dose of 25 mg qd. Hypertension was defined as increased diastolic BP of $\geqslant$ 20 mmHg. Development of grade 4 hypertension resulted in discontinuation of E7080 and grade 3 resulted in treatment interruption until BP normalization. For grade 2 hypertension, two interventions were tested with the aim of maximized sustained dose intensity: (A) anti-hypertensive treatment alone, (B) anti-hypertensive treatment followed by dose-reduction (consecutively 25 to 20, to 14, to 10 mg).
Results: With scheme A, 67% of subjects did not have to stop E7080 treatment due to hypertension, with 36% and 27% of thosesubjects requiring 1 and 2 DDDE anti-hypertensive medication, respectively. With scheme B, 90% of subjects did not have to stop E7080 treatment due to hypertension, with 27% and 46% of subjects requiring 1 and 2 DDDE anti-hypertensive medication, respectively. Overall, 72% of subjectscontinued at dose levels of 25 mg, 13% at 20 mg, 8% at 14 mg, and 7% at 10 mg.
Conclusions: Simulation studies evaluated two effective methods of managing hypertension associated with E7080 treatment. By combining anti-hypertensive treatment prior to dose reductions, 90% of subjects could remain on E7080 treatment. The PK-BP model will be refined as additional data become available.

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Dolichol phosphate N-acetyl-glucosamine-1 phosphate transferase activity in dermal fibroblasts as a marker of chemotherapy skin toxicity in cancer patients

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Background: Skin reactions caused by chemotherapeutic agents are not rare. In breast cancer they can mimic metastases and infections. The recent results are in favour of the idea that N-glycoprotein synthesis is limited by Dolichyl Phosphate Cycle (DPC), which is a target for chemotherapy and essential in maintaining mucocutaneous resistance and immunity. This dual role is very important in prediction and prevention of chemotherapy-induced skin disorders. With focus on a risk group marker for cutaneous side effects of cancer chemotherapy, the present study was carried out to estimate Dolichol (Dol) metabolism in patients with breast cancer treated with cytostatic agents.
Materials and Methods: The samples obtained from 316 patients with breast cancer before and during treatment with cisplatin, cyclophosphamide, docetaxel, doxorubicin and trastuzumab. Dol in urine was assayed by HPLC method (Turpeinen, 1986), dolichol phosphate N-acetyl-glucosamine-1 levels. phosphate transferase (GPT) activity was defined in dermal fibroblasts by metaboling labeling (ML) method with [2-(3)H]-mannose.
Results: The normal amounts of Dol in healthy donors urine (n = 250) are 6.0−10.0 mkg/mmol. During the period of observation 92 (20.2%) of cancer patients were presented with different skin reactions, including flushing, urticaria, dermatitis, erythema, pruritus and acne. From this group of patients 76 (82.6%) have had elevated urinal Dol excretion (>20.8 mkg/mmol) 2 weeks before chemotherapy and 87 (94.6%) during and 2 weeks after chemotherapy. ML of cultured dermal fibroblasts from these patients revealed lowered incorporation of radiolabel into full-length dolichol-linked allele oligosaccharides and glycoproteins. sGPT activity was reduced to approximately 88.6–99.8% of normal levels.
Conclusion: There is a reason to suggest that reduced GPT activity, lowered N-glycoprotein synthesis and elevated urinary Dol detected in this group of patients may evidence of the disorders of DPC and possible susceptibility to the development of chemotherapy-induced cutaneous reactions. Elevated urinary Dol is one of the first manifestations of this disorder which could be prevented by breast cancer patients selection and DPC regulation.

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In vitro and in vivo tools to assess the myelotoxicity of anti-cancer drugs

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Background: About 50% of oncology drugs, including those with target-specific mechanism of action, induce myelosuppression as a dose limiting toxicity in patients. The benefits of these drugs, as the reduction of the solid tumor mass, are produced at the expense of serious injury to the immune system, as the decrease of multipotent cells in the bone marrow of patients, inducing neutropenia and leucopenia, conditions that can lead to infections and fever.
In vitro and in vivo PK/PD approaches can be adopted during the different phases of drug development to test and select new anticancer compounds devoid of myelosuppression effects preserving the therapeutic efficacy.
Material and Methods: Anticancer compounds are tested *in vitro* using the Hemotoxicity Assay via Luminescence Output (HALO) method that measures the increase in intracellular ATP as a result of the proliferative process in stem cells.
The same compounds are also tested in animals and the *in vivo* toxic effects are evaluated using a semi-mechanistic PK/PD model, characterized by a dynamical system with non-linear feedback. The aim is to describe leukocytes or neuthrophils peripheral concentrations during and after the treatment in order to predict the minimum concentration (nadir), and the time necessary to reach that concentration (time-to-nadir).
Results: The IC_{50} values evaluated *in vitro* are correlated with the *in vivo* PK/PD end-points. *In vitro* and *in vivo* preclinical results are used to predict the concentration-toxicity relationship of the anticancer compounds in patients. In addition, the *in vivo* results are indicative of the myelotixicity mechanism and are used to rank order different compounds.
The *in vivo* dose sampling is optimized by *a posteriori* simulation.
Conclusions: The results of *in vitro* myelotoxicity testing are shown to correlate with the myelotoxic effects observed *in vivo*. A combined *in vitro* and *in vivo* PK/PD approach is then proposed to:

- assess possible myelotoxic effects of new anticancer drug candidates,
- improve the safety margin by maximizing efficacy at the most acceptable toxicity,
- provide consistent savings in time and resources.

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Comparative pharmacokinetics and intermediary metabolism of of 4-demethyl-4- cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN)

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DM-CHOC-PEN is a cholesteryl carbonate derivative of 4-demethyl-penclomedine (DM-PEN), a metabolite of penclomedine (PEN) – an active anticancer agent screened through phase I by NCI. PEN induced a dose limiting cerebellar neurotoxicity. 4-Demethylpenclomedine (DM-PEN) is a non-neurotoxic metabolite of PEN that has been modified by DEKK-TEC for anticancer trials. DM-CHOC-PEN is an active and stable member of a large series of carbonates and carbamates prepared (AACR 48, abst. 5614, 2007). DM-CHOC-PEN *vs* DM-PEN has improved activity (% LTS/CR) in intracerebrally (IC) implanted human xenograft models − U251 glioma: +54/20 *vs* 17/0, resp. and MX-1 breast cancer: +20/17 *vs* 0/0, resp (CCP, 64,829, 2009).

Acute toxicology: In mice, single IV dosing with DM-CHOC-PEN resulted in a $LD_{10/50}$ of 136/385 mg/kg (sexes combined; with 95% conf. limits); oral − LD_{50} >2 g/kg in mice (sex combined). In rats, single IV doses of DM-CHOC-PEN − 100 to 300 mg/kg were well tolerated but produced a transient DLT of hypercholesterimia − 2nd to release of cholesterol from the carbonyl residue. LDL cholesterol was significantly increased 30 fold in the 200 & 300 mg/kg groups which returned to normal (predominatnt HDL varient) by Day 15. Plasma DM-CHOC-PEN and DM-PEN (a metabolite) were assayed by HPLC. In dogs, single dose IV studies were conducted in adult Beagle dogs @ 10, 20, 30 mg/kg concentrations and no toxiicty or deaths were observed. No microscopic nor macroscopic pathology could be identified in any of the animals. No seizures or CNS toxicity noted in any studies. Total rat and dog brain examinations for cellular necrosis were negative.

Overall, pharmacokinetic studies for DM-CHOC-PEN in rats and dogs revealed the following profile: rats (300 mg/kg) − AUC_{0-t} = 2.95 (mg, h/L), $T_{1/2}\alpha$ = 0.24 (h), $T_{1/2}\beta$ = 2.98 (h) & CL = 34.86 (mL/h) and for dogs (30 mg/kg) − AUC_{0-t} = 1.12 (mg, h/L), $T_{1/2}\alpha$ = 0.63 (h), $T_{1/2}\beta$ = 18.7 (h) & CL = 342.7 (L/h) [a two compartment model]. The AUC was linear for the doses. There were no differences between males & females. DM-CHOC-PEN could be identified in normal rat brain tissue (in 200 μg/g quantities) post single injections with 135 mg/kg, however, neither neurotoxic nor psychological changes were noted in any of the above tudies.

Summary: The release of cholesterol from hydrolysis of the cholesteryl carbonate resulting in elevated levels of LDL-cholesterol, that returns to normal values is the only DLT that could bve identified and does appear to be a limiting toxicity. Neither bone marrow nor hepatic toxicity was noted in the rat or dog models.

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Validation of a cell panel for preclinical evaluation of antitumor efficacy and toxicity of anticancer agents

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Aims: To collect and validate a cell panel for preclinical evaluation of antitumor efficacy and toxicity of anticancer drugs.

Methods: Drug activity of amsacrine, arsenic trioxide, bortezomib, cisplatin, cytarabine, doxorubicin, etoposide, 5-fluorouracil, gefitinib, imatinib, melphalan, PKC412, rapamycin and vincristine were tested in tumor cells prepared from patient samples from eleven different hematological and solid tumor diagnoses. Method validation was performed by comparing drug sensitivity in vitro with established clinical use of the drugs. For in vitro toxicity testing a normal cell panel consisting of lymphocytes (peripheral blood mononuclear cells), renal- (renal proximal tubular epithelial cells), liver- (cell line of tumor origin) and epithelial (telomeras transfected cell line) cells was used. Also, $CD34^+$ umbilical cord blood cells were used for prediction of bone marrow toxicity in the 14 days granuclocyte macrophage (GM14) assay. A GM14 index was calculated by dividing the IC_{50} from the GM14 assay with the median IC_{50} for the most sensitive tumor type for each drug. Drug sensitivity for both tumor and normal cells was measured in the non-clonogenic fluorometric microculture cytotoxicity assay.

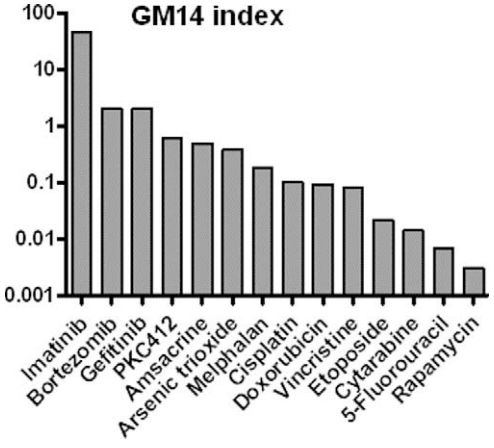

Figure 1. Index of IC_{50} from the GM14 assay and the median IC_{50} from the most sensitive diagnosis in vitro for each drug.

Results: In general, in vitro drug activity in patient tumor cells reflected known clinical activity of the drugs investigated. As an example, CML was the most sensitive tumor type for imatinib, and cisplatin and 5-fluorouracil were the most solid tumour active agents. The toxicity panel was easy to handle in a high throughput manner, and was able to detect differences in therapeutic ratios, e.g. between targeted drugs and classical cytotoxic agents, which is shown in the GM14 assay (figure 1).

Conclusion: In the preclinical stage of drug development, tumor cells from patient samples can be used for prediction of cancer diagnosis specific activity and a normal cell panel may reflect expected toxicity.