

Scientific Spring Meeting
Friday March 20, 2015

Dutch Society for Clinical Pharmacology and Biopharmacy
Nederlandse Vereniging voor Klinische Farmacologie en Biofarmacie

PROGRAMME of the SCIENTIFIC MEETING
Friday March 20, 2015
Antoni van Leeuwenhoek, Amsterdam

SCIENTIFIC MEETING OF THE DUTCH SOCIETY FOR CLINICAL PHARMACOLOGY AND BIOPHARMACY (NVKFB)

09.00 h **Welcome & coffee**

ORAL PRESENTATIONS

- 09.30 h *J. Boonstra, P. Nannan Panday, J. Arends, B. Span, B. Sinha, D. Touw, T. van der Werf, J. Zijlstra, J-W. Alffenaar (Groningen): Fluconazole and caspofungin dosing by an antifungal stewardship team to optimize treatment of invasive *Candida albicans* infections in the ICU*
- 09.45 h *R. van Geel, J.C. Bendell, A. Spreafico, M. Schuler, T. Yoshino, J-P. Delord, Y. Yamada, M. Lolkema, J.E. Faris, F. Eskens, S. Sharma, R. Yaeger, H-J. Lenz, Z.A. Wainberg, E. Avsar, A. Chatterjee, S. Jaeger, T. Demuth, R. Bernards, J. Tabernero, J. Schellens (Amsterdam): Combined targeted therapy for the treatment of patients with advanced BRAF^{v600} mutated colorectal cancer*
- 10.00 h *J. Hambleton, L. Zhou, S. Rogers, S. van Marle, T. van Iersel, J. Zanghi, E. Masteller, K. Baker, B. Wong (San Francisco/Groningen): A phase 1 study of FPA008, an anti-colony stimulating factor 1 receptor (anti-CSF1R) antibody in healthy volunteers and subjects with rheumatoid arthritis (RA): preliminary results*
- 10.15 h *A.C. Baakman, E. 't Hart, D.G. Kay, J. Stevens, E. Klaassen, A. Maelicke, G.J. Groeneveld (Leiden): First in human study with a prodrug of galantamine: indications for fewer side effects and improved cognitive effects*

10.30 h **Highlights of clinical pharmacology in 2014**

Prof. dr. J.H.M. Schellens: Personalized therapy in cancer

11.15 h **Coffee and Tea Break**

11.30 h Lecture of the winner of the ‘**NVKFB**’-**Education Award 2014**: *Prof. dr. Th.P.G.M. de Vries*

12.00 h **LUNCH and POSTER SESSION**

1. *A.E. Kip, J.H.M. Schellens, J.H. Beijnen, T.P.C. Dorlo* (Amsterdam): Quantification of macrophage markers neopterin and chitotriosidase activity to monitor visceral leishmaniasis treatment response
2. *E.M.J. van Brummelen, A.D.R. Huitema, E.D. van Werkhoven, J.H. Beijnen, J.H.M. Schellens* (Amsterdam): The performance of model-based versus rule-based phase I trials in oncology
3. *A.M.I. Saktiawati, M.G.G. Sturkenboom, Y. Stienstra, Y.W. Subronto, Sumardi, J.G.W. Kosterink, T.S. van der Werf, J.-W.C. Alffenaar* (Groningen): Food impacts on the pharmacokinetics of the first-line anti-tuberculosis drugs in TB-patients
4. *S. Leijen, R. van Geel, G.S. Sonke, D. de Jong, E.H. Rosenberg, S. Marchetti, D. Pluim, E. van Werkhoven, J.H. Beijnen, J.H.M. Schellens* (Amsterdam): Phase II study with Wee1 inhibitor AZD-1775 (MK-1775) plus carboplatin in patients with p53 mutated ovarian cancer refractory or resistant (<3 months) to standard first line therapy
5. *B. Milojkovic Kerklaan, D. Pluim, M. Bol, I. Hofland, J. Westerga, H. van Tinteren, J.H. Beijnen, W. Boogerd, J.H.M. Schellens, D. Brandsma* (Amsterdam): Sensitivity and specificity of circulating tumor cell detection by flow cytometry in cerebrospinal fluid for the diagnosis leptomeningeal metastases
6. *V.A. de Weger, F.E. Stuurman, S.L.W. Koolen, M. Mergui-Roelvink, E. Harms, B. Nuijen, A.D.R. Huitema, J.H. Beijnen, J.H.M. Schellens, S. Marchetti* (Amsterdam): A phase I dose-escalation trial of weekly oral docetaxel as ModraDoc001 or ModraDoc006 in combination with ritonavir

7. *T. Schutte, J. Tichelaar, M.O. Reumerman, R. van Eekeren, L. Rolfes, M.C. Richir, E. van Puijenbroek, M.A. van Agtmael* (Amsterdam): A pilot study of the student-run pharmacovigilance project
8. *F.J. Warnders, S. Waaijer, M.N. Lub-de Hooge, M. Friedrich, A.G.T. Terwisscha van Scheltinga, P. Deegen, S.K. Stienen, P.C. Pieslor, H.K. Cheung, J.G.W. Kosterink, E.G.E. de Vries* (Groningen): Tumor targeting and tissue distribution of solitomab (AMG 110; anti-EpCAM BiTE[®]) in human EpCAM-positive tumor bearing mice
9. *N. van 't Boveneind –Vrubleuskaya, T. Seuruk, J.G.W. Kosterink, T.S van der Werf, D. van Soolingen, A. Skrahina, J.W.C Alffenaar* (Den Haag): Pharmacokinetics of levofloxacin in M(X)-DR tuberculosis patients
10. *E.W. den Haak, R.J. Zaal, B.C.P. Koch, A.G. Vulto, P.M.L.A. van den Bemt* (Utrecht): Physicians' acceptance of pharmacists' interventions in a Dutch university hospital
11. *J. Berkhout, J.A. Stone, K.M.C. Verhamme, B.H.C. Stricker, M.C.J.M. Sturkenboom, M. Danhof, T.M. Post* (Rotterdam): Systems pharmacology modeling describing osteoporotic disease progression in a population of postmenopausal women receiving placebo or alendronate
12. *R.J. van Unen, T. Schutte, J. Tichelaar, Th.P.G.M. de Vries, M.C. Richir, M.A. van Agtmael* (Amsterdam): A need for structured therapeutic discussions in case reports? Pharmacotherapeutic reasoning assessed in a case report review
13. *R. Rissmann, J. Messayeh, J.H.F.M. Pinckaers, A.C. Cohen, J.L. Hay* (Leiden): Curriculum-wide user assessment of the e-learning pharmacology repository TRC
14. *L.M. Andrews, P.J. Puiman, H. van der Sijs, I.M. van Beynum* (Rotterdam): Accidental digoxin overdose due to medication error in a child
15. *D. Meulendijks, L.M. Henricks, B.A.W. Jacobs, A. Aliev, M.J. Deenen, N. de Vries, H. Rosing, E. van Werkhoven, A. de Boer, J.H. Beijnen, C.P.W. Mandigers, M. Soesan, A. Cats, J.H.M. Schellens* (Amsterdam): Pre-treatment serum uracil concentrations are superior to *DPYD* and *TYMS* genotyping to identify patients at risk of severe fluoropyrimidine-associated toxicity
16. *B.A.W. Jacobs, M.J. Deenen, J.H. Beijnen, J.H.M. Schellens, A.D.R. Huitema* (Amsterdam): Population model for circadian variability in human uracil and dihydrouracil plasma levels suggesting rhythmicity in dihydropyrimidine dehydrogenase activity

17. *C.E.S. Hoogstins, Q.R.J.G. Tummers, A.F. Cohen, C.J.H. van de Velde, A.L. Vahrmeijer, J. Burggraaf* (Leiden): A novel tumor-specific agent for fluorescence guided surgery: a translational study
18. *P. Okkerse, J.L. Hay, G. van Amerongen, M.L. de Kam, G. J. Groeneveld* (Leiden): A two part, randomised, double-blind, placebo-controlled, four-way cross-over, single dose study to pharmacologically validate a pain model battery suitable for early phase clinical drug development
19. *K.M.S. Kanhai, R.G.J.A. Zuiker, W. Gladdines, J. Stevens, I. Stavrakaki, G.J.Groeneveld* (Leiden): Doubleblind, placebo and comparator-controlled study and an openlabel study in healthy subjects, to assess safety, pharmacokinetics and –dynamics of 2B3-201
20. *D. Pluim, B. Milojkovic Kerklaan, D. Brandsma, J.H. Beijnen, J.H.M. Schellens* (Amsterdam): Quantification of circulating melanoma cells in peripheral blood and cerebrospinal fluid by positive immunomagnetic enrichment and multi-parameter flow cytometry
21. *A.M. Burylo, V.A. de Weger, J.H.M. Schellens* (Amsterdam): Flow cytometric method for quantification of circulating endothelial cells in human peripheral blood
22. *H. Yu, J.J.M.A. Hendriks, J.H.M. Schellens, J.H. Beijnen, A.D.R. Huitema* (Amsterdam): Pharmacokinetic-pharmacodynamic modelling of the effect of ritonavir on intratumoural docetaxel metabolism and anti-cancer efficacy in a mouse model for hereditary breast cancer
23. *A.C. Esselink, L.M. Bril, R. Langenhuijsen, A. Bilos, G.A. Rongen* (Nijmegen): Effect of sodium chloride intake on blood pressure response to caffeine containing coffee in humans
24. *T.F. Kröpelin, D. de Zeeuw, J. Hoekman, H.J. Lambers Heerspink* (Groningen): Reducing albuminuria confers renoprotection: a systematic review and meta-analysis of randomized controlled trials
25. *L.M. Henricks, D. Meulendijks, C.A.T.C. Lunenburg, J.J. Swen, A.J. Gelderblom, J.L. Severens, A. Cats, H.J. Guchelaar, J.H.M. Schellens* (Amsterdam): Confirmative study of safety, feasibility and cost-effectiveness of genotype-directed individualized dosing of fluoropyrimidines and exploration of additional phenotyping
26. *C.M. Nijenhuis, H. Rosing, J.H.M. Schellens, J.H. Beijnen* (Amsterdam): Simultaneous quantification of dabrafenib and trametinib using HPLC-MS/MS in plasma of melanoma patients

27. *T.B.Y. Liem, T.G. Krediet, A. Fleer, A.C.G. Egberts, C.M.A. Rademaker* (Utrecht): Comparison of antibiotic dosing recommendations for neonates from established textbooks: paving the way for e-prescribing standards
28. *V. de Weger, C. Massard, M. Mergui-Roelvink, A. Varga, N. Pham, M. Brunaud, M. Langenberg, M. de Jonge, B. Demers, J. Schellens, E. Deutsch* (Amsterdam): Preliminary results of a phase I study of combination therapy with SAR405838 and pimasertib in patients with advanced cancer
29. *S.I. Petrykiv, D. de Zeeuw, F. Persson, P. Rossing, H-H. Parving, R.T. Gansevoort, H.J. Lambers Heerspink* (Groningen): Variability in response to anti-albuminuric drugs: *true or random?*
30. *S.C. Goulooze, K.L. Franson, A.F. Cohen, R. Rissmann* (Leiden): Clinical pharmacology research internships for (bio-)medical students
31. *E.J.B. Derissen, H. Rosing, J.H.M. Schellens, J.H. Beijnen* (Amsterdam): Measurement of the intracellular 5-fluorouracil nucleotide concentrations during capecitabine treatment
32. *M. Wilhelmus, A.B. Smit, B. Drukarch* (Amsterdam): Tissue transglutaminase: a novel therapeutic target in Alzheimer's disease?
33. *B.C.P. Koch, S.R. Zabirowa, B.C.H. van der Nagel, L.M. Hanff* (Rotterdam): Development and validation of a HPLC-UV assay to quantify plasma levels of sulfametrol: a preferential antibiotic in children
34. *D. Meulendijks, A. Aliev, B.A.W. Jacobs, D. Pluim, E. van Werkhoven, M.J. Deenen, J.H. Beijnen, A. Cats, J.H.M. Schellens* (Amsterdam): Association between the G>C single nucleotide polymorphism in the first 28 BP tandem repeat of the thymidylate synthase 2R allele and risk of severe fluoropyrimidine-associated toxicity
35. *B.A.W. Jacobs, H. Rosing, N. de Vries, D. Meulendijks, L.M. Henricks, J.H.M. Schellens, J.H. Beijnen* (Amsterdam): Development and validation of an UPLC-MS/MS method for determination of uracil and dihydrouracil in human plasma
36. *R. de Haan, D. Pluim, B. van Triest, M. Verheij, J.H.M. Schellens, C. Vens* (Amsterdam): Ex vivo irradiation improves pharmacodynamic assay for PARP inhibitors
37. *B.A.W. Jacobs, N. Snoeren, M. Koopman, H. Rosing, N. de Vries, M.J. Deenen, J.H. Beijnen, J.H.M. Schellens, R. van Hillegersberg* (Amsterdam): Changes in dihydrouracil:uracil plasma ratio after partial liver resection in patients with colorectal liver metastases

38. *G. van Amerongen, T.L. Beumer, J. Killestein, G.J. Groeneveld* (Leiden): Individualized dosing of a novel oral Δ^9 -THC formulation improves subjective spasticity and pain in patients with progressive multiple sclerosis
39. *A.C. Kruithof, I.M.C. Kamerling, D. Mallikarjuna Rao, R. Kumar, J. Burggraaf* (Leiden): Effect of food on the pharmacokinetics of two formulations of a new CETP inhibitor in healthy volunteers.
40. *S.A.W. van Moorsel, M. Meurs, N. Bevers, L. van Rossum, P.M. Hooymans, D.R. Wong* (Sittard): Successful azathioprine treatment of a paediatric TPMT deficient patient using therapeutic drug monitoring
41. *R.B. Verheijen, S. Bins, B. Thijssen, H. Rosing, L. Nan, J.H.M. Schellens, R.H.J. Mathijssen, M.P. Lolkema, J.H. Beijnen, N Steeghs, A.D.R. Huitema* (Amsterdam): Development and clinical validation of an LC-MS/MS method for the quantification of pazopanib in dried blood spots
42. *B.H. Schievink, D. de Zeeuw, H-H. Parving, P. Rossing, H.J. Lambers Heerspink* (Groningen): Intra-individual variability in multiple parameters in response to angiotensin receptor blockers determines ultimate renal outcome
43. *S. Wassenaar, Z. Brkic, D. Dos Reis Miranda, N.G.M. Hunfeld, B.C.P. Koch* (Rotterdam): Toxic tobramycin levels after tobramycin intake via selective decontamination of the digestive tract (SDD)
44. *M. Moerland, K. Malone, M. Dillingh, W. Grievink, J. Reijers, J. Burggraaf* (Leiden): Exploration of the role of TNF α in TLR4/NLPR3 inflammasome-driven inflammation

13.30 h

GENERAL MEETING of the ‘NVKFB’

14.15 h

Lecture of the winner of the ‘NVKFB’-Thesis Award 2014: *Dr. Q. Fillekes*

14.45 h

Lecture of the winner of the ‘NVKFB’-TOP Publication Award 2014: *Drs. A.M. Thijs*

15.00 h

Coffee and Tea Break

ORAL PRESENTATIONS

- 15.15 h *V.A. de Weger, F.E. Stuurman, M. Mergui-Roelvink, E. Harms, A.D.R. Huitema, J.H. Beijnen, J.H.M. Schellens, S. Marchetti* (Amsterdam): Low-dose metronomic chemotherapy (LDMC) with oral paclitaxel formulations ModraPac001 (capsule) and ModraPac005 (tablet)
- 15.30 h *A. Lalmohamed, I. Bartelink, L. van Reij, C. Dvorak, R. Savic, J. Zwaveling, R. Bredius, A. Egberts, M. Bierings, M. Kletzel, P. Shaw, C. Nath, G. Hempel, M. Ansari, M. Krajinovic, T. Gungor, R. Wynn, P. Veys, G. Cuvelier, R. Chiesa, M. Slatter, J. Long-Boyle, J. Boelens* (Utrecht): Studying the optimal intravenous busulfan exposure in pediatric allogeneic hematopoietic cell transplantation (alloHCT) to improve clinical outcomes: a multicenter study
- 15.45 h *R.W.F. van Leeuwen, F.G.A. Jansman, P.M.L.A. van den Bemt, F. de Man, F. Piran, I. Vincenten, A. Jager, A.W. Rijnveld, J.D. Brugma, R.H.J. Mathijssen, T. van Gelder* (Rotterdam): Drug-drug interactions in patients treated for cancer: a prospective study on clinical interventions
- 16.00 h *E. van Maarseveen, C. Rogers, J. Trofe-Clark, F. Lghoul, M. Wong, A. van Zuilen, T. Mudrikova, B. Barin, P. Stock, L. Frassetto* (Utrecht): Tacrolimus exposure and its relation with clinical outcomes in HIV infected kidney transplant recipients on antiretroviral agents: an AUC driven analysis
- 16.15 h *H.J.C. Buiter, A.D. Windhorst, A.A. Lammertsma, E.L. Swart, J.E. Leysen* (Amsterdam): Agonist PET ligands for 7-transmembrane receptors in the central nervous system
- 16.30 h *J.T.H. Nielen, B. van den Bemt, A.E.R.C.H. Boonen, P.C. Dagnelie, P. Emans, A. Lalmohamed, A. de Boer, F. de Vries* (Utrecht): Use of glitazones and the risk of elective hip or knee replacement: a population based case-control study
- 16.45 h Closure and drinks**

FLUCONAZOLE AND CASPOFUNGIN DOSING BY AN ANTIFUNGAL STEWARDSHIP TEAM TO OPTIMIZE TREATMENT OF INVASIVE *CANDIDA ALBICANS* INFECTIONS IN THE ICU

Jasper Boonstra¹, Prashant Nannan Panday¹, Jan Arends², Bart Span³, Bhanu Sinha², Daan Touw¹, Tjip van der Werf^{4,5}, Jan Zijlstra⁶, Jan-Willem Alffenaar¹

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Fluconazole (FLZ) is an anti-fungal agent that has been used for the treatment of invasive candidiasis for more than twenty years. The increase of less susceptible *Candida* species necessitates the use of new anti-fungal agents. Currently, echinocandins are considered the first-line antifungal agents for the (empirical) treatment of invasive candidiasis in critically-ill patients. FLZ can still be useful for invasive *C. albicans* infections, if dosed adequately, due to its low costs and favorable safety profile. The objectives of this study were to evaluate FLZ and caspofungin therapy for the treatment of *C. albicans* infections in daily practice and to detect potential suboptimal therapy useful for an antifungal stewardship team to optimize treatment.

Patients with proven invasive *C. albicans* infections were included in this study. Demographics and medical data were collected from the patients' charts. For each patient treatment and dosing, route of administration and pathogen susceptibility were registered. The primary outcome was the global response defined as the clinical and microbiological response and the secondary outcome was suboptimal therapy

defined as too low dosing compared to the licensed dose or in relation to MIC .

From January 2009 to August 2014, 41 patients had a proven *C. albicans* infection. Patient characteristics and clinical variables were not significantly different between patients on FLZ (n=17) or caspofungin (n=18). All isolates were susceptible for FLZ (median MIC 0.5 (0.25-0.75) mg/L) and caspofungin (median MIC 0.125 (0.094-0.19) mg/L). FLZ was prescribed in a dose ranging from 100-400mg and caspofungin in a dose of 50-70mg. A successful global response was achieved in 10 (58.9%) patients on FLZ and 12 (66.7%) patients on caspofungin. After dosage evaluation 7 (41.7%) patients received an appropriate FLZ dose and 13 (72.2%) patients received an appropriate caspofungin dose. Suboptimal therapy was observed in patients with a higher bodyweight or in patients on dialysis. Appropriate FLZ dosage or inappropriate FLZ dosage in relation to MIC was associated with a successful global response respectively in 8 (80.0%) and 2 (40.0%) patients. Two patients died while receiving a too low dose in relation to MIC and one patient died while receiving a presumed adequate dose in relation to MIC.

FLZ is an acceptable and cheaper alternative for echinocandins for the treatment of invasive *C. albicans* infections in severely ill patients if adequately dosed. Personalized dosing in case of both FLZ and caspofungin may help to optimize treatment in heavier patients and patients on dialysis. This study provides information for the antifungal stewardship team to focus on individualized dosing in critically ill patients.

A PHASE 1 STUDY OF FPA008, AN ANTI-COLONY STIMULATING FACTOR 1 RECEPTOR (ANTI-CSF1R) ANTIBODY IN HEALTHY VOLUNTEERS AND SUBJECTS WITH RHEUMATOID ARTHRITIS (RA): PRELIMINARY RESULTS

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Background:

Activation of CSF1R via IL34 or CSF1 results in activation, differentiation, and survival of monocytes, macrophages and osteoclasts. CSF1R pathway activation produces inflammatory cytokines responsible for joint destruction, thus pathway inhibition may provide a therapeutic benefit to RA patients (pts). FPA008 is a humanized IgG4 anti-CSF1R antibody that blocks the binding of IL34 or CSF1 to CSF1R, and has shown preclinical activity in animal models of arthritis. This study was designed in 3 parts to study safety, pharmacokinetics (PK) and pharmacodynamic (PD) biomarkers in healthy volunteers, and clinical and radiographic efficacy parameters in RA patients.

Methods:

This is a double-blind, randomized, placebo-controlled first-in-human trial. In Part 1, 8 subjects were randomized (3:1) to receive a single intravenous infusion of FPA008 or placebo, per dose cohort of 0.2, 1, 3, or 10 mg/kg. In Part 2, 8 subjects were randomized (3:1) to receive 2 doses of FPA008 administered 14 days apart, at 1 or 3 mg/kg, with the option for additional dose cohorts. Dose escalation decisions were based on the incidence of dose limiting toxicities (DLTs), taking into account adverse events (AEs) beyond the DLT period when FPA008 was still measurable in plasma. PK, bone turnover markers, CSF1 and IL34 serum concentrations,

and non-classical CD16+ monocytes were assessed. Part 3 consisted of an open-label evaluation of 3 dose levels in RA pts whose disease was not responding to methotrexate.

Results:

Parts 1 and 2 (up to 1 mg/kg, two doses) were completed through the DLT period. No DLTs were reported.

Frequently reported AEs were Grade 1 or 2 pruritis, headache and periorbital edema. Dosing in the 10 mg/kg cohort was associated with moderate periorbital edema, facial and finger swelling, and mild, transient blurred vision outside the DLT period. Dose-dependent elevations of CK and LDH were noted at 1 mg/kg and above; AST elevation occurred at 3 mg/kg and above; and mild ALT elevation occurred at 10 mg/kg in one subject. These elevations were not associated with clinical signs/symptoms or abnormalities in total bilirubin, CK isoenzymes or troponin, were reversible as drug levels cleared, and were expected due to FPA008-mediated inhibition of Kupffer cells responsible for removing these enzymes.

Non-linear PK was observed, with exposure increasing greater than dose proportionality from 0.2 to 3 mg/kg, suggesting target mediated clearance. Full suppression of non-classical CD16+ monocytes, decreased bone turn-over biomarkers (CTx, Trap5), and dose-dependent increase in serum CSF1 and IL34 concentrations were observed.

Conclusions:

FPA008 is well tolerated up to 3 mg/kg. AEs persisted outside of DLT period at 10 mg/kg coincide with the prolonged PK exposure. Pathway inhibition was noted at dose levels tested. PD effects of full suppression of non-classical CD16+ monocytes and decrease of bone turnover biomarkers may track with clinical benefit in RA patients.

FIRST IN HUMAN STUDY WITH A PRODRUG OF GALANTAMINE: INDICATIONS FOR FEWER SIDE EFFECTS AND IMPROVED COGNITIVE EFFECTS

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Introduction: Memogain is a prodrug of galantamine. Due to its small size and lipophilic nature, it preferentially enters the brain, where it is cleaved into active galantamine. Memogain is expected to have fewer peripheral side effects than other cholinesterase inhibitors, with a comparable or improved efficacy. The aim of this study was to assess safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of increasing doses of Memogain in comparison with galantamine and donepezil.

Methods: this was a first-in-human single ascending dose study of intranasally administered Memogain in healthy young (N=16, 18-65 yrs, 5.5 and 11 mg) and elderly (N=42, >65 yrs, 22, 33 and 44 mg) men, compared to oral administration of galantamine 16 mg, donepezil 10 mg (elderly subjects only) and matching placebos. Safety assessments included incidence and severity of adverse events (AEs) and abnormalities or changes in laboratory measurements, vital signs and ECG. Standard PK parameters were derived from non-compartmental analysis. For PD the NeuroCart, a computerized test-battery of CNS tests designed for repeated measurements, was used. CNS domains tested included attention, episodic and working memory, executive functioning as well as pharmaco-EEG, eye movements, pupillometry, body stability and visual analogue scales (VAS) for mood and drug effects.

Results: administration of Memogain was well tolerated and safe. All AEs were either mild or moderate and self-limiting. The most prevalent AE was nausea. VAS nausea values were only elevated after administration of oral galantamine 16 mg and Memogain 44 mg. Based on the NCA of the plasma Memogain concentrations, a dose dependent increase in exposure was observed up to 33 mg. Memogain was rapidly absorbed into the systemic circulation with a C_{max} after approximately 15 min after Memogain 5.5 mg, up to approximately 45 min after Memogain 44 mg administration. PD effects of Memogain were seen on attention and memory. The adaptive tracking test, a psychomotor test that has proven very sensitive to changes of vigilance/arousal, showed an improved performance of +3.47% after administration of 11 mg Memogain (95%CI 0.52-6.42) and after administration of 33 mg Memogain (+1.79%, 95%CI 0.07-3.52). Improved short term memory was evident from the direct word recall variable yielded by the Visual Verbal Learning test, which was enhanced in older men after administration of 22 mg (2.67 more words, 95%CI [0.17-5.16) and of 44 mg (2.57, 95%CI -0.05-5.18) compared to placebo. Administration of galantamine did not lead to improvements on any cognitive test.

Conclusion: Memogain nasal spray was well tolerated and found to be safe in young and elderly men in the dose range investigated. A dose dependent increase in plasma exposure was observed up to a dose of 33 mg. The PD effects of Memogain, an improvement of (short term) memory and an improvement in vigilance/arousal, were generally larger and more consistent than those of galantamine.

QUANTIFICATION OF MACROPHAGE MARKERS NEOPTERIN AND CHITOTRIOSIDASE ACTIVITY TO MONITOR VISCERAL LEISHMANIASIS TREATMENT RESPONSE

AE Kip^{1,2}, JHM Schellens^{2,3}, JH Beijnen^{1,2}, TPC Dorlo^{2,4}

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Introduction Pharmacodynamic (PD) biomarkers are potentially useful to monitor treatment response in visceral leishmaniasis (VL), but have not been identified yet. The *Leishmania* parasite replicates within host macrophages, thereby increasing the overall macrophage biomass, which decreases again with waning parasitic infection. Neopterin and chitotriosidase activity are markers of macrophage activation and we evaluated their potential use as a PD marker in visceral leishmaniasis in this interim analysis.

Methods EDTA plasma samples were collected from VL patients in Sudan and Kenya, receiving 2 different treatments: (i) combination therapy (AmBisome® + miltefosine) and (ii) miltefosine monotherapy. Neopterin was quantified by ELISA (133 samples from 31 patients) and chitotriosidase activity by an enzymatic fluorescent assay employing 4-methylumbelliferyl-(4-deoxy) chitobiose (116 samples from 29 patients), both during and after treatment. All values are reported as mean \pm 95% CI.

Results Neopterin levels were elevated at baseline in VL patients (90.6 \pm 11.4 nmol/L) compared to normal (<10 nmol/L), and decreased during treatment to 35.9 \pm 13.1 nmol/L

for the combination therapy and 23.3 \pm 8.05 nmol/L for the monotherapy. On treatment day 7, neopterin concentrations halved for the combination therapy, while for the miltefosine monotherapy they remained stable and started to decrease only after day 7. For the combination treatment, the mean neopterin concentration one day after AmBisome® injection was significantly higher for cured patients (124 \pm 30.1 nmol/L) compared to relapsed patients (79.3 \pm 14.3 nmol/L, P<0.01).

Mean chitotriosidase activity level at baseline was 317 \pm 169 nmol/mL/hr, with large between-subject variability. Previously reported normal mean chitotriosidase activities in African populations ranged between 68.8-84.4 nmol/mL/hr. Chitotriosidase activity levels decreased in response to both treatments. At 1-month follow-up, activity increased significantly in relapsing patients (n=10), while cured patients (n=9) retained stable low activity levels (P<0.001). Interestingly, a chitotriosidase deficiency-rate of 26.3% was found in Kenyan patients, compared to ~5% in Caucasians.

Conclusion Neopterin levels were elevated in VL patients and decreased with waning parasite infection in both treatment arms. Additionally, neopterin levels exhibited a prognostic value for the combination therapy. The initial surge in neopterin in cured combination therapy patients, could imply an instant immunomodulatory effect of AmBisome® on the Th1 response. The delayed PD effect of miltefosine on neopterin might indicate concentration dependency. Chitotriosidase activity, though increased in relapsed patients at 1-month follow-up, might lack sensitivity as a biomarker due to the high prevalence of chitotriosidase deficiency in Kenya.

THE PERFORMANCE OF MODEL-BASED VERSUS RULE-BASED PHASE I TRIALS IN ONCOLOGY

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Aims In clinical development of oncologic agents, phase I trials provide crucial information on tolerability, safety and dosing. Classically, rule-based designs have been used for this purpose but since several years there is a trend towards the use of model-based phase I trial designs. Model-based designs are considered to have several advantages over rule-based designs such as allowing rapid dose-escalation and avoiding suboptimal treatment (Iasonos *et al.*, 2014, Le Tourneau *et al.* 2009). However, convincing evidence for better performance is still lacking hindering evidence-based decision making on trial design. Based on a systematic literature review we provide a quantitative comparison on the performance of rule-based versus model-based phase I trials in oncology. We aim to investigate whether or not model-based designs should be considered superior to the classical rule-based designs in terms of efficiency and patient safety.

Methods A total of 172 phase I trials were included after performing a literature search on PubMed on phase I trials investigating monotherapy or combinations of molecularly targeted small molecule anticancer drugs over the last 2 years. All publications were classified as rule-based or model-based trials and outcome data were extracted including the number of patients included, the number needed to determine the recommended phase 2 dose (RP2D), trial duration, the number of patients treated at suboptimal doses, dose-limiting toxicity

(DLT) rates and study characteristics including the number of dose escalations, schedules, study type and investigational drug(s).

Results Model-based trials (n=11) needed on average 34 patients to determine the RP2D with a median duration of 26 months, whereas rule-based trials (n=161) needed 36 months ($p = 0,23$) and on average only 26 patients ($p = 0,07$). The shorter trial duration of model-based trials was accompanied by a slightly higher percentage of patients treated at or above the MTD (60% vs. 55%) and an equal toxicity rate of 13%.

Conclusion We detected a non-significant but clinically relevant difference in trial duration between model-based and rule-based designs while, paradoxically, slightly more patients were needed to determine the RP2D. Considering 11 months of time gain, acceptable toxicity rates and minimization of suboptimal treatment we provide evidence to encourage the use of model-based designs.

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Phase II study with Wee1 inhibitor AZD-1775 (MK-1775) plus carboplatin in patients with p53 mutated ovarian cancer refractory or resistant (<3 months) to standard first line therapy

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Introduction:

AZD -1775 is a potent and selective inhibitor of Wee1, a kinase that phosphorylates CDC2. Phosphorylation of CDC2 inactivates the CDC2/cyclin B complex and is therefore essential for normal G2 checkpoint function. As most p53-deficient tumors lack a functional G1 checkpoint, they rely on the G2 checkpoint for cell cycle arrest in response to DNA damage. G2 checkpoint abrogation, using a Wee1 inhibitor may therefore sensitize p53 deficient tumor cells to DNA-damaging anti-cancer agents. In a phase I study the maximum tolerated dose (MTD) of AZD-1775 in combination with carboplatin demonstrated target engagement.

Methods:

Patients with p53 mutated ovarian cancer refractory or resistant (< 3 months) to standard first line therapy (carboplatin plus paclitaxel) were re-exposed to carboplatin (AUC 5), plus 5 bidaily doses of 225 mg of AZD-1775 in a 21 day cycle (MTD). p53 mutation status was analyzed by both sequencing analysis (TP53 exons 2-10) and AmpliChip TP53 array (TP53 exons 2-11). Response evaluation was performed according to RECIST 1.0, volumetric tumor measurement (enhanced RECIST) and CA-125 blood levels.

Results:

Bone marrow toxicity, fatigue, diarrhea, nausea and vomiting were the most common adverse events. Out of 24 patients enrolled, 22 patients were evaluable for study endpoints. As best response, 6 patients (27%) showed confirmed partial response (PR) with a median progression-free survival (PFS) of 10.9 months. Nine patients (41%) had stable disease and 7 patients (32%) had progressive disease as best response, with a median PFS of 5.3 and 1.3 months, respectively.

Conclusions:

AZD-1775 is a first in class Wee1 inhibitor that in combination with carboplatin is well tolerated and shows promising anti-tumor activity in p53 mutated ovarian cancer refractory or resistant (< 3 months) to standard first line therapy.

SENSITIVITY AND SPECIFICITY OF CIRCULATING TUMOR CELL DETECTION BY FLOW CYTOMETRY IN CEREBROSPINAL FLUID FOR THE DIGNOSIS LEPTOMENIGEAL METASTASES

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Abstract

Introduction: Low sensitivity of the standard methods of MRI and cerebrospinal fluid (CSF) cytology results in at least 25 % of false negative diagnoses of leptomenigeal metastases (LM) and postponing the start of therapy.

The aim of this prospective clinical study is to determine the diagnostic value of cytology versus flow cytometry of circulatory tumor cells (CTC) of cerebrospinal fluid (CSF) in patients with solid tumors suspected of having LM.

Methods: During a diagnostic lumbar puncture at least 5 ml of CSF was obtained for cytology, the same volume for the CTC assay and 2 ml for biochemical parameters and leukocyte

count. Furthermore, simultaneously whole blood samples were drawn for the CTC assay. CTCs were detected by multi parameter flow cytometry using antibodies against epithelial cell adhesion molecule (EpCam) and melanoma chondroitin sulfate proteoglycan (MCSP).

Results: In total 47 patients with clinically suspected LM were enrolled. Thirty of them had a primary tumor of an epithelial origin, previously shown to be sensitive to EpCam staining, and 16 patients had a melanoma or glioblastoma, sensitive to MCSP staining. The prevalence of definitive LM, based on either positive CSF cytology or progressive neurological symptoms compatible with LM with or without positive MRI, was 0.62. The EpCam CTC assay showed 100% sensitivity and 100% specificity for diagnosing LM, while sensitivity of CSF cytology was only 65%. The MCSP CTC assay also showed a high sensitivity and specificity, but the confidence interval was wide due to the small sample size. In 14 out of 25 patients with LM, CTCs were found in whole blood samples. Elevated total protein levels in CSF were found in 85%, decreased CSF-serum glucose ratios in 88% and elevated CSF leucocyte counts in 48% of patients with LM.

Conclusion: The EpCam-based CTC assay is superior to CSF cytology in patients with epithelial tumors and LM for the diagnosis LM. Therefore, we recommend after confirmation of our results, the use of the CTC assay in CSF next to CSF cytology in patients with a primary tumor of epithelial origin and a clinical suspicion of LM.

A phase I dose-escalation trial of weekly oral docetaxel as ModraDoc001 or ModraDoc006 in combination with ritonavir.

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Background: Docetaxel is a micro-tubule stabilizing anticancer drug administered intravenously (IV). Oral administration could have advantages since it is less invasive and might reduce the number of hypersensitivity reactions.

Docetaxel uptake from the gastro-intestinal tract is limited by intestinal P-gp and CYP3A4. In pre-clinical studies the uptake of docetaxel after oral administration is improved significantly by co-administration of ritonavir, a CYP3A4 inhibitor. The primary aim of this trial was to determine the maximum tolerated dose (MTD) and the recommended phase two dose (RP2D) of the oral docetaxel capsule (ModraDoc001) and tablet (ModraDoc006) formulations.

Patients/Methods: Patients with metastatic malignant disease, ≥ 18 years old, and with a WHO performance status ≤ 2 were included. ModraDoc001 and ModraDoc006 were co-administered with 100 or 200 mg ritonavir once weekly. Dose-escalation was performed using a classic 3+3 design. Pharmacokinetic sampling was performed during the first two weeks of treatment for up to 48 hours after study drug administration. Safety was evaluated. The dose-limiting toxicity (DLT) period was defined as the first four weeks of treatment. Anti-tumor activity was assessed every 6 weeks by CT or MRI.

Results: Forty-three patients were treated at doses ranging from 40-80 mg ModraDoc001 with 100-200 mg ritonavir. Dose-limiting toxicities (DLT) seen were grade 4 dehydration and neutropenia; grade 3 diarrhea, vomiting, nausea, elevated AST/ALT, gastritis, mucositis, fatigue and anorexia. A total of 7 patients experienced one or multiple DLTs.

ModraDoc006 was administered to eleven patients at doses ranging from 60-80 mg with 100 mg ritonavir. DLTs observed were grade 3 mucositis and neutropenic fever, grade 2 diarrhea, nausea and vomiting resulting in inability to restart treatment in 2 patients.

The most common adverse events observed with both ModraDoc001 and ModraDoc006 were nausea, vomiting, diarrhea and fatigue most often grade 1-2. No hypersensitivity reactions and neither grade 4 neutropenia were observed.

The MTD for ModraDoc001 was determined to be 60 mg with 200 mg ritonavir, however the RP2D was 60 mg ModraDoc001 with 100 mg ritonavir.

The currently predicted MTD/RP2D for ModraDoc006 is 60 mg with 100 mg ritonavir.

The area under the plasma concentration time curve (AUC) for ModraDoc001 at the RP2D is 1184 ± 1073 ng*h/ml and for ModraDoc006 at the predicted RP2D 892 ± 489 ng*h/ml.

Four partial tumor responses were reported and 21 patients had stable disease as best response. Median response duration was 18 weeks (5-72 weeks)

Conclusion: Oral administration of docetaxel as ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir once weekly is feasible. The RP2D for both formulations is 60 mg ModraDoc with 100 mg ritonavir. Anti-tumor activity is promising.

Tumor targeting and tissue distribution of solitomab (AMG 110; anti-EpCAM BiTE[®]) in human EpCAM-positive tumor bearing mice.

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Background: Bispecific T-cell Engagers (BiTE[®]) belong to a class of single-chain bispecific antibodies with dual target binding specificities. AMG 110 combines in one polypeptide chain the single-chain variable regions directed against the epithelial cell adhesion molecule (EpCAM) and the epsilon chain of the T cell receptor/CD3 complex. EpCAM is abundantly expressed by epithelial tumors and cancer stem cells. By labeling AMG 110 with Zirconium-89 (⁸⁹Zr) we aimed to study the tumor targeting and tissue distribution of AMG 110 in human tumor bearing mice.

Material and methods: AMG 110 was conjugated with desferal for ⁸⁹Zr labeling and for biological activity evaluation *in vitro* prior to *in vivo* use. ⁸⁹Zr-AMG 110 was injected into nude mice bearing subcutaneously implanted xenografts with high EpCAM expressing HT-29 human colorectal adenocarcinoma. MicroPET imaging was performed at 0.5, 3, 6, 24, 48 and 72 h after injection (n=6). Tissue was collected at 6, 24 and 72 h. To examine the impact of dose on ⁸⁹Zr-AMG 110 tumor

uptake and biodistribution, 0, 20 and 480 µg of unlabeled AMG 110 were tested in different cohorts of mice in combination with 20 µg of ⁸⁹Zr-AMG 110 (n=3-5). A non-EpCAM binding BiTE[®], Mec14, was evaluated as negative control in HT-29 xenograft imaging and biodistribution studies (n=6).

Results: *In vitro* analysis of desferal conjugated-AMG 110 showed biological activities comparable to unmodified AMG 110. MicroPET imaging revealed specific tumor uptake of 20 µg ⁸⁹Zr-AMG 110 in HT-29 tumors, maximally at 6 h after tracer injection, and prolonged tumor retention up to 72 h. Biodistribution analyses showed a dose- and time-dependent ⁸⁹Zr-AMG 110 tumor uptake in HT-29 tumors. The highest tumor uptake was observed in the 40 µg dose group with tumor % ID/g at 6 h, 24 h & 72 h ranging from 5.4 +/- 0.2, 5.3 +/- 0.3 to 2.7 +/- 0.4, respectively, and tumor-to-blood %ID/g ratio escalating from 2 +/- 0.2, 33 +/- 3 to 58 +/- 12, respectively. ⁸⁹Zr-Mec14 control BiTE[®] (40 µg) did not accumulate to appreciable level in HT-29 xenografts (0.7 +/- 0.1% ID/g), and unlabeled Mec14 had no impact on HT-29 xenograft accumulation of ⁸⁹Zr-AMG 110.

Conclusions: This study shows for the first time that PET imaging can be used to investigate ⁸⁹Zr-AMG 110 BiTE[®] antibody tumor targeting and tissue distribution non-invasively *in vivo*. Our data support using this approach to assess the distribution of a ⁸⁹Zr-labeled BiTE[®] in clinical trials.

PHARMACOKINETICS OF LEVOFLOXACIN IN M(X)-DR TUBERCULOSIS PATIENTS

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Background: Levofloxacin (LFX) belongs to the third generation of fluoroquinolones (FQs). LFX has a high *in vitro* and *in vivo* bactericidal activity against *Mycobacterium tuberculosis*. In earlier studies the *f* AUC/MIC ratio was identified as the best predictor pharmacokinetic (PK) parameter for efficacy of LFX. However, it can be questioned whether *f* AUC/MIC ≥ 100 is reached in patients infected with *M. tuberculosis* isolates with higher MIC values. The objective of our study was to determine LFX concentrations and assess pharmacokinetic variability in M(X)-DR-TB patients and its potential for AUC/MIC ratios.

Methods: Patients with pulmonary M(X)-DRTB received LFX as part of their treatment regimen at a dose of appr. 15 mg/kg once daily (750 or 1000 mg) based on breakpoint

using BACTEC MGIT960 system and testing a single critical concentration 2 mg/L. Blood samples were obtained at steady state before and 1, 2, 3, 4, 7, and 12 hours after administration of the dose. Clinical data were collected from medical records. The values of the PK parameters were calculated in non-compartmental analysis.

Results: 20 patients with a mean age of 31 (27-35) years, including 8 woman en 12 men, were enrolled between November 2012 and March 2013. The median AUC₀₋₂₄, C_{max} and C_{min} were respectively 98,815 mg/h/L (IQR 84,825-159,6), 10,05 mg/L (IQR 8,43-16,2) and 1,2 mg/L (IQR 0,85-4). The multiple linear regression analysis showed a significant correlation of the C_{max} with age (increases 0.13 (95%CI 0.00-0.25) per year) and gender with adjusted R-square of the model of 0.276. The MIC median value for LFX was 0,5 (IQR 0,5-0,25 mg/L) and the median *f*AUC₀₋₂₄/MIC ratio 109,5 (IQR; 48,52-399,36). In 4 of the 20 patients the value was below the target value of ≥ 100 . Considering a breakpoint of 0.25 mg/L, 19 out of 20 patients exceeded the target value of 100. When a MIC of 0.5, 1.0 and 2.0 mg/L were used, 18, 3 and none patients, respectively, had a *f*AUC/MIC ratio that exceeded 100.

Conclusion: We observed a large variability in AUC and C_{max} values. Target *f*AUC₀₋₂₄/MIC of ≥ 100 was only observed in case MIC values for LFX were 0,25-0,5 mg/L. The identification of new susceptibility breakpoints for LFX or dosages exceeding 15mg/kg needs consideration. Evaluation of (un)bound AUC₀₋₂₄/MIC is needed to optimize the treatment and prevent development of drug resistance.

PHYSICIANS' ACCEPTANCE OF PHARMACISTS' INTERVENTIONS IN A DUTCH UNIVERSITY HOSPITAL

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Background: Checking medication orders, reviewing results of therapeutic drug monitoring (TDM) and subsequently proposing interventions to the physician to resolve potential drug related problems, is part of the daily routine of clinical pharmacists. However, knowledge on the physicians' acceptance rate of these interventions and determinants for acceptance, which is important to optimize central pharmacy services, is limited. Therefore, a study was performed to determine the physicians' acceptance rate of pharmacists' interventions in routine daily practice and to identify determinants for acceptance.

Methods: A retrospective case-control study was performed in adult patients admitted to a university hospital in the Netherlands. Pharmacists' interventions regarding drug-drug interactions, drug dosing in patients with renal failure and TDM results that were recorded in the electronic medical record from January 2012 until June 2013 were extracted. The primary outcome was the proportion of accepted interventions, which was assessed by reviewing the computerized physician order entry system and electronic medical records. Univariate and multivariate logistic regression analyses were performed to identify determinants for physicians' acceptance as secondary outcome. Characteristics of the intervention (type and date of intervention, number of days since problem arose, the pharmacotherapeutical group of the drug involved,

continuation of pre-admission treatment), patient characteristics (age, gender, length of stay, number of medication orders at day of intervention, presence of renal failure), characteristics of the pharmacist (age, gender, resident versus specialist, department, working experience) and medical specialty of the prescribing physician were included in the analysis as potential determinants for acceptance.

Results: A total of 1098 interventions relating to 709 patients were included. Interventions were most frequently proposed for drug-drug interactions (35.5%), suprathreshold dosages (30.2%) and subtherapeutic dosages (10.7%). 807 interventions (73.5%) were accepted and 236 (21.5%) were not accepted by the physician. Acceptance could not be assessed for 55 (5.0%) interventions. After multivariate logistic regression analysis only the number of medication orders was significantly associated with acceptance (adjusted odds ratio 1.045; 95% confidence interval 1.017-1.075, meaning that for each additional medication order the probability for acceptance increases with 4.5%).

Conclusion: The physicians' acceptance rate of pharmacists' interventions is 73.5% and the probability for acceptance increases for patients with an increasing number of medication orders. To optimize central pharmacy services further insight into the physician's reasons for non-acceptance is necessary.

A NEED FOR STRUCTURED THERAPEUTIC DISCUSSIONS IN CASE REPORTS? PHARMACOTHERAPEUTIC REASONING ASSESSED IN A CASE REPORT REVIEW

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Background

Reading case reports is a method to train clinical reasoning in general, however they seem to be focused at diagnostics rather than therapeutics. Doctors in training indicate they experience a deficit in education in pharmacotherapeutic reasoning (Tobaiqy *et al.*, 2007;Heaton *et al.*, 2008;Prince *et al.*, 2004) . To determine the educational value of case reports in therapeutic reasoning, we analyzed to what degree pharmacotherapeutic reasoning was discussed.

Methods

Review of clinical cases published in two high impact medical journals (BMJ and Lancet). For every drug therapy started in these case reports, information regarding the choice and argumentation was assessed. We used a score form based on the WHO 6-step, a method used in medical schools to train students therapeutic reasoning in a step-by-step approach (de Vries *et al.*, 1995).

Results

PubMed database was searched for articles classified as case report and published in the first half year of 2014. We identified 58 articles, 44 of which we qualified as clinical case report. In 24 of these reports a total of 43 drugs were started. The drug name was mentioned in 65% and in <10% general drug information (contraindications, adverse effects and interactions) was given. In <3% the presence/absence of contraindications/interactions and suitability for the patient was discussed.

Conclusion

Although case reports could play a role in developing clinical reasoning skills, this opportunity is not fully utilized for pharmacotherapeutic reasoning. Drug choices were frequently not described properly and argumentation for these choices was hardly mentioned. We propose a more detailed description of pharmacotherapeutic reasoning in case reports, e.g. by using some steps of the WHO-6-step method.

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Curriculum-wide user assessment of the e-learning pharmacology repository TRC

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Aim

The free pharmacology Teaching Resource Centre (TRC) was created in the 1990s with a unique symbol language to illustrate drug action in the (patho)physiological context in a consistent way. First it was published as hardcopy binder and quickly this traditional pharmacology book was upgraded to an e-learning repository that led to widespread use by students and educators both nationally and internationally. Studying the TRC also clearly has positive effect on pharmacological learning outcomes as TRC study time correlated with an increase in course grades for the individual student [1]. However, with the introduction of the tablets and smartphones Leiden students demanded increased accessibility of pharmacological knowledge, i.e. the TRC. TRC apps were released for iPad (June 2012), iPhone (November 2012) and android (November 2013). Objective of the study was to investigate usage frequency, differences in usage and user-friendliness of the apps compared to the website in the most relevant user population, i.e. undergraduate medicine students.

Methods

A pilot survey was constructed and sent out via email to medical students at Leiden University Medical Center. As an incentive two iPad mini's were given to randomly selected students who filled out the survey. The questions were mostly answered in a multiple choice format with exceptions of some checkboxes and open-ended questions. The responses were then recorded and analyzed.

In total, 484 medical undergraduate students responded.

Results

In more than 30 months since its initial release, the apps have been downloaded more than 100,000 times throughout the world. The TRC website is still frequently used with up to 5,000 hits per day before exams. The website is the most visited portal for pharmacological e-learning with the TRC. The mean overall assessment of the resource on a 10 point numerical rating scaling (0-very weak, 10-excellent) is similar. The iPad app and the website are rated the highest (7.9) while iPhone app, smartphone app and tablet app are assessed slightly lower, 7.4, 7.1 and 7.2, respectively. Interestingly, most users using a mobile app continue to use also the website for their study.

Conclusion

The free pharmacology app is readily available for all health care students and professionals worldwide. The TRC app is more accessible and used differently in respect to learning situations compared to the website. Due to the success of the apps, further extension of the content, e.g. inclusion of .

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ACCIDENTAL DIGOXIN OVERDOSE DUE TO MEDICATION ERROR IN A CHILD

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Introduction

Accidental poisonings or overdoses occur often in children. Recognition of poisonings in children is difficult because a small child cannot communicate what his symptoms are. This means specific symptoms can be missed, which can delay the diagnosis of the poisoning.

Case description

A 5 month old boy was started on heart failure treatment. After transfer to another hospital, accidentally a tenfold dose of digoxin was given for 5 days. He developed feeding problems, vomiting, weight loss, elevated urea (14,4 mmol/L) and creatinine (80 µmol/l), hyponatremia (129 mmol/L), hyperkalemia (6,4 mmol/l) and ECG abnormalities. After five days the patient was transported to our hospital and admitted to the pediatric intensive care. At that point the digoxin plasma concentration was 7,6 µg/L. The patient met multiple criteria for the administration of digoxin antibodies (ECG abnormalities due to digoxin poisoning, hyperkalemia and ingestion of 0,3 mg/kg by a child). The patient was administered 30 mg digoxin antibodies, resulting in digoxin concentration of < 0,3 µg/L. Twelve hours later the digoxin plasma concentration was 3,1 µg/L due to redistribution. Two days after the administration of digoxin antibodies the plasma concentration was within the therapeutic range.

A failure analysis was performed which showed six issues:

- Medication reconciliation. The discharge letter was correct, but unfortunately it was incorrectly entered in

the electronic prescribing system. This error wasn't noticed by the pharmacist.

- Little expertise with digoxin given to small children in the general hospital. It is a drug with a narrow therapeutic index, meaning that the overdose was toxic.
- Children. In this case the accidental digoxin poisoning was caused by a medication error. Studies have shown that medication errors occur in children in 13,2% of the medication orders, versus 2% in adults. The reason for this is that doses for children generally have to be calculated on body weight.
- More than three, call the pharmacy. In this case the patient received 3 ml instead of 0,3 ml digoxin, which is within the volume limit of <5 ml advised by the European Medicines Agency for children younger than 5 years of age.
- Electronic prescribing system with clinical decision support was inadequate. Recent studies have shown that by implementing electronic prescribing with clinical decision support medication errors can be reduced.
- Recognition of poisoning in small children is difficult, partly because children can't communicate their symptoms. A specific symptom for digoxin poisoning is xanthopsia. Not all patients develop it, but if they do it is very specific for digoxin.

Conclusion

Medication overdoses due to medication errors can and should be prevented. Hospitals should implement different strategies to prevent them. The (hospital) pharmacist plays an important role in pediatric medication error reduction.

POPULATION MODEL FOR CIRCADIAN VARIABILITY IN HUMAN URACIL AND DIHYDROURACIL PLASMA LEVELS SUGGESTING RHYTHMICITY IN DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY

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Introduction: The fluoropyrimidine drugs 5-fluorouracil (5-FU) and its orally available prodrug capecitabine are commonly applied for the treatment of solid tumours. Approximately 85% of 5-FU is metabolized into inactive metabolites by the enzyme dihydropyrimidine dehydrogenase (DPD). Earlier, we showed that DPD activity has a circadian rhythm in human peripheral blood mononuclear cells (PBMCs), characterized by peak activity during the night. Therefore, clearance of 5-FU is hypothesized to have a circadian rhythm. Another biomarker for DPD activity, which is correlated to systemic clearance of 5-FU, is the ratio between the endogenous DPD substrate uracil (U) and the metabolite dihydrouracil (DHU) in plasma (Gamelin *et al.* 1999). The aim of our study was to explore circadian variability in U and DHU levels in healthy volunteers using a population modeling approach.

Methods: An observational study in healthy volunteers was performed in which plasma samples were collected every 4 hours during a 24-hour period. U and DHU concentrations were determined by UPLC-MS/MS. Mixed effect modeling was performed using NONMEM (v.7). Interindividual variability (IIV) for individual parameters was estimated using

log normal models. Residual unexplained variability (RUV) was modeled using an additional error model. Model management and diagnostics were done using R (v.3.1), Piraña (v.2.9), Xpose (v.4) and PsN (v.4.2).

Results: U and DHU plasma concentrations were determined in 23 volunteers (12 females) with a median (range) age of 27 (20-49). Circadian variability in U and DHU plasma concentrations was adequately described by 1 cosine function with a period of 24 hours. In this function, the circadian rhythm of DHU was the inverse of the rhythm in U plasma concentrations. The rhythm-adjusted mean (baseline) U and DHU were 10.3 ng/mL (IIV = 23.7%) and 92.1 ng/mL (IIV = 17.4%). IIV of baseline U and DHU were significantly correlated ($\rho = 0.81$). The amplitude of the circadian rhythm for U and DHU was estimated to be 0.08 times the baseline plasma concentrations. Time of peak (phase shift) U and trough DHU concentrations was at 2:06 h. The additional residual errors were 2.1 and 16.4 ng/mL for U and DHU, and showed some correlation ($\rho = 0.4$).

Discussion: A model for circadian rhythmicity in U and DHU was developed. The estimated phase shift, suggesting lowest U metabolism, was at 2:06 h. This is opposite of what we found for DPD activity in PBMCs. Possibly, time of peak DPD activity varies among body tissues or other enzymes upstream or downstream of DPD might drive circadian variability in U and DHU. More research is required to explore these options.

Reference: Gamelin *et al.* J Clin Oncol;17(4):1105-1110.

A NOVEL TUMOR-SPECIFIC AGENT FOR FLUORESCENCE GUIDED SURGERY: A TRANSLATIONAL STUDY

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Aims Intra-operative fluorescence imaging of primary tumor and metastases potentially results in better patient outcomes.

OTL38 ((S)-2-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzamido)-3-(4-(((E)-2-((E)-2-(3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)-3H-indol-1-ium-2-yl)vinyl)-6-((E)-2-(3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)oxy)phenyl)propanoate heptahydrate Tetrasodium) is an imaging agent that specifically binds to the folate receptor α (FR α), which is overexpressed by various carcinomas. FR α -positive cells retain the agent, making them detectable with near-infrared (NIR) fluorescence. In this first-in-human study OTL38 was investigated in healthy volunteers and ovarian cancer patients.

Methods Four different iv doses were studied in healthy volunteers in a single ascending dose, randomized, placebo-controlled study in which tolerability, pharmacodynamics (PD; defined as fluorescent signal in the skin) and pharmacokinetics (PK) were assessed. The optimal doses were subsequently explored in 5 patients thus far, with an emphasis on tolerability, number of suspected lesions detected with fluorescence and concordance between fluorescence and FR α -status on histopathology.

Results Low dose OTL38 was without any clinically significant adverse effects, but at the highest doses levels hypersensitivity reactions were observed. The plasma concentration-time profile showed bi-phasic elimination (elimination half-life: 26-160min) and a possible non-linear increase in AUC. In OTL38-treated volunteers a dose-dependent fluorescent signal was observed in the skin, showing rapid distribution of OTL38 to tissue with a low clearance rate. These data allowed definition of the optimal doses and time window for intra-operative imaging. Preliminary analysis of the study in patients shows accumulation of OTL38 in FR α positive tumor and successful intra-operative NIR fluorescence imaging with detection of multiple lesions not identified by inspection/palpation.

Conclusions Low doses of OTL38, the first tumor-specific agent in the NIR spectrum, were successfully used for intra-operative fluorescence imaging of FR α -positive tumors. The preliminary data suggest that our approach using healthy volunteers and PK/PD modelling appear to be extremely useful in the development of tumor-specific imaging agents.

A TWO PART, RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, FOUR-WAY CROSS-OVER, SINGLE DOSE STUDY TO PHARMACOLOGICALLY VALIDATE A PAIN MODEL BATTERY SUITABLE FOR EARLY PHASE CLINICAL DRUG DEVELOPMENT

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Introduction Pharmaceutical science continues to search for suitable biomarkers that can assist in predicting the therapeutic potential of analgesic medication and its efficacy in the target population. No single experimental model can replicate the complex nature of clinical pain. Although a single experimental pain model can be used to demonstrate the pharmacological mechanism of action of a compound, one single model can not reliably mimic clinical pain. The aim of this study was to investigate the ability of a battery of pain models to detect analgesic properties of commonly used analgesics in healthy subjects. This was the first time that an integrated battery of experimental pain models was executed in combination with the administration of different analgesics.

Methods The test battery consists of a sequence of tests eliciting cutaneous electrical-, mechanical (pneumatic)-, and thermal (cold pressor)-pain, and measuring pain detection threshold (PDT), pain tolerance thresholds (PTT) and area under the VAS-time curve (AUC). Furthermore, the battery included the UVB model to measure hyperalgesia, comparing heat pain detection thresholds in UVB exposed skin compared to normal skin, the thermal grill illusion and a paradigm to measure conditioned pain modulation (CPM). In part I of the study, subjects received fentanyl 50 µg/kg, phenytoin 300 mg, (S)-ketamine 10 mg or placebo (sodium chloride 0.9%) as an intravenous infusion over 30 minutes. In part II, subjects were administered imipramine 100 mg, pregabalin 300 mg, ibuprofen 600 mg or placebo capsules as a single oral dose. Following a training session, pain test measurements were

performed at baseline (twice), 0.5, 1, 2, 3, 4, 6, 8 and 10 hours. In each part, subjects received all four treatments. Pharmacodynamic outcome variables were analysed using a mixed model analysis of variance.

Results 22 (Part I) and 17 (Part II) healthy subjects participated. 16 subjects completed all treatments periods in each part (8 females in each part). The PTT for electrical stimulation was increased compared to placebo for (S)-ketamine (+10.1%, p=0.044), phenytoin (+8.5%, p=0.019), and pregabalin (+10.8%, p=0.012). The PTT for mechanical pain was increased by pregabalin (+14.1%, p=0.005). The cold pressor PTT was increased by fentanyl (+17.1%, p=0.023) and pregabalin (+46.4%, p=<.0001). Normal skin heat PDT was increased by (S)-ketamine (+3.3%, p=0.004), fentanyl (+2.8%, p=0.002) and pregabalin (+4.1%, p=0.005). UVB treated skin PDT was increased by fentanyl (+2.6%, p=0.0006) and ibuprofen (+4.0%, p=0.0006). Thermal grill unpleasantness AUC decreased after administration of fentanyl (-34.3, p=0.011). No differences in CPM were observed. Adverse events reported in the study were all mild or moderate in severity and in line with their known pharmacological profile.

Conclusion This validation study shows that these pain models are able to detect changes in pain detection and pain tolerance thresholds after administration of different classes of analgesic compounds in healthy male and female subjects. The analgesic compounds all showed a unique profile in their effects on the pain tasks administered. These profiles were in most cases compatible with the expected pharmacology. This battery of pain models can be used to benchmark analgesic properties of new drugs against established analgesics in early phase clinical studies in healthy subjects.

DOUBLEBLIND, PLACEBO AND COMPARATOR-CONTROLLED STUDY AND AN OPENLABEL STUDY IN HEALTHY SUBJECTS, TO ASSESS SAFETY, PHARMACOKINETICS AND –DYNAMICS OF 2B3-201

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Background: Intravenous high-dose methylprednisolone (MP, 500- 1000 mg daily for 3 to 5 consecutive days) has for long been the mainstay of relapse treatment in MS. However, it is inconvenient and its acute side effects, including insomnia, depression, agitation are undesirable. Both the dose and the dosing frequency can be reduced by incorporating MP in (PEGylated) liposomes, creating a slow-release formulation with reduced systemic toxicity but with similar peripheral efficacy. Moreover, by adding glutathione to the PEGylated liposomes (2B3-201) an enhanced delivery of MP into the brain is achieved, thereby potentially enhancing central activity. Preclinical studies in animal models of MS showed that 2B3-201 had fewer behavioral side effects and a superior efficacy compared to free MP.

Objectives: This first-in-human study was designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of 2B3-201, including its immunosuppressive effects, as compared to free MP and placebo.

Methods: In this double-blind, 3-way cross over study, 18 healthy male subjects were divided over 3 cohorts and received ascending doses of 2B3-201, active comparator (free MP) or placebo (5% dextrose). MP plasma concentrations, lymphocyte counts, ACTH and fasting glucose were determined, as well as other standard safety parameters at days 0 to 3 and day 7 following each dose.

In addition, neurocognitive tests were performed at regular intervals. This part of the study was followed by part 2 of the study: an open-label infusion of 2B3-201, in different dosages, with and without antihistaminics, with different infusion schedules and also a cross over study in females (450 mg 2B3-201 versus 1000 mg methylprednisolone).

Results: 2B3-201 was shown to have a plasma half-life of 23 h, compared to a half-life of 3 h for free MP. 2B3-201, at doses of 150 mg, 300 mg and 450 mg, resulted in a similar reduction in the lymphocyte count as 1000 mg of free MP. This effect was sustained considerably longer after 450 mg 2B3-201 administration to >74 h. Similar patterns were observed for a decline in ACTH and a rise in fasting glucose (measured up to 48h). All pharmacodynamic outcome measures had returned to baseline at 7 days after dosing. Furthermore, no signs of CNS side effects or serious AEs were observed with 2B3-201. The AEs were generally mild and self-limiting. Results of part 2 are not final yet and will be presented at the NVKFB meeting.

Conclusions: 2B3-201 at doses up to 450 mg was considered safe. In addition, 2B3-201 shows a long plasma half-life (23h) and immunosuppressive effects that last for at least 3 days. This supports continued development of 2B3-201 as a safe single dose treatment of acute relapses in MS.

QUANTIFICATION OF CIRCULATING MELANOMA CELLS IN PERIPHERAL BLOOD AND CEREBROSPINAL FLUID BY POSITIVE IMMUNOMAGNETIC ENRICHMENT AND MULTI-PARAMETER FLOW CYTOMETRY

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INTRODUCTION:

Evidence is mounting for the importance of circulating melanoma cells (CMCs) as biomarker for overall survival of melanoma patients. Leptomeningeal metastasis (LM) is frequent in melanoma patients resulting in a median overall survival of only 4 – 6 weeks. New systemic targeted anticancer therapies are now developed in metastatic melanoma, especially with BRAF inhibitors (vemurafenib and dabrafenib) and immunotherapy (ipilimumab), with promising results. Therefore, early diagnosis of leptomeningeal metastasis (LM) is needed to improve the quality of life and the overall survival of these patients. However, investigations are hampered by a lack of thoroughly validated protocols that miss sensitivity and specificity, are uneconomical, and lack objectivity due to reliance on CMC identification by human observation.

METHODS:

A flow cytometry method was developed and validated for the enumeration and classification of CMCs based on DNA content for blood and cerebrospinal fluid (CSF). CMCs were enriched by melanoma-associated chondroitin sulfate

proteoglycan (MCSP) immunomagnetic cell sorting with subsequent detection by fluorescence-activated cell sorting (FACS) using antibodies against MCSP, CD146, CD45, and Hoechst33258 for DNA staining to distinguish apoptotic cells.

RESULTS:

The method was highly sensitive with only 0.3 ± 0.8 background events, and lower limit of quantification (LLOQ) of 2 melanoma cells per 8 ml of whole blood. We detected CMCs (mean = 9.8, range 2 - 33) in 8 ml of whole blood from 82% (11 stage 3 - 4 metastatic melanoma patients, n = 3 per patient). The CSF from 9 patients with definitively no LM contained on average 0.33 (range 0 - 1) CMCs in 7.5 ml of CSF. Seven melanoma patients with confirmed LM had median CMC counts of 902 (range 49 – 61459) per 7.5 ml of CSF. The method was successfully used for the quantification of CMCs with both low and normal-high DNA content, which may explain the higher number of CMCs detected with our method as compared to other reports. On average 72.5% (n = 33) and 12% (n = 7) of CMCs in, respectively, whole blood and CSF have a low DNA content.

CONCLUSIONS:

A method has been developed and validated for the enumeration and classification of CMCs based on DNA content for blood and CSF. The method is straightforward with long-term stability, and application of standard laboratory equipment and techniques allow wide spread use in clinical trials. The method is currently successfully validated against cytomorphological analysis in a clinical trial with melanoma patients with suspicion of LM.

Flow cytometric method for quantification of circulating endothelial cells in human peripheral blood.

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Introduction: In recent years, circulating endothelial cells (CECs) have been studied as a biomarker of vascular damage to monitor anti-angiogenic drug activity in malignant diseases[1,2]. CECs are mature vascular cells that shed from the vessel intima into the circulation and rarely occur in healthy individuals. Their numbers are, however elevated in malignant disease[3]. Quantification of CECs has been a persistent problem since the phenotype of endothelial cells is unclear and because of lack of a selective method for quantification. The primary aim of our study was to develop a reliable, selective and sensitive method to enrich CECs with magnetic micro-beads and quantify them by flow cytometry by detection of endothelial cell surface markers. The secondary aim was to validate this method for use in the clinic to measure changes of CEC levels in patients receiving anti-angiogenic therapy.

Method: *Selectivity;* Peripheral blood of healthy volunteers was drawn in 4.5 ml citrated collection tubes, erythrocytes were lysed, remaining cells were incubated with Fc receptor blocking reagent and then enriched with CD34 magnetic micro-beads. The enriched cell fraction was stained with antibodies against surface markers of the hematopoietic lineage (CD45-FITC, CD14-PEviolet770, CD15-PEviolet770) and endothelial cells (CD34-APC, CD146-PE), and intracellular Hoechst DNA staining. Endothelial cells were isolated by fluorescence-activated cell sorting (FACS) and stained with antibody against von Willebrand (vWBF) factor and

endothelial origin was confirmed by fluorescence microscopy. *Sensitivity;* Peripheral blood of healthy volunteers was drawn in 4.5 ml citrated collection tubes, FACS sorted and pre-labeled CD146-PerCp5.5 HUVEC cells were spiked in the range 3-800 mL⁻¹. Then spiked samples were processed as described for selectivity, however, vWBF staining was omitted. Spiked samples were analyzed by flow cytometry and the precision, recovery and linearity of the method was calculated in freshly processed and frozen (-80°C) samples.

Results: *Selectivity;* FACS sorted cells were of endothelial origin since anti-vWBF staining marked Weibel-Palade bodies, which are intracellular organelles storing vWBF and exclusively present in endothelial cells. *Sensitivity:* Linear correlation of reproducibility between duplicate samples of whole blood spiked with HUVEC was strong ($R^2=0.9997$, mean HUVEC recovery 79.6±16.4%, coefficient of variation (CV) 20.4%). Between-run results obtained from three experiments of freshly prepared spiked samples (3-50 HUVEC ml⁻¹) resulted in an overall-recovery of 78.5±10.1% and overall-precision CV=10.4%. Sample stability experiments were performed in frozen samples for 1, 2 and 4 weeks. Similarly to the freshly prepared samples, between-run overall-recovery of 78.9±7% and overall-precision CV=14.4% was observed in frozen samples.

Conclusion: Our technique is a reliable method for quantitative detection of circulating endothelial cells in peripheral blood, and an alternative for selective CEC enrichment with a reliable reproducibility.

References: [1]. Simkens LH, et al. Ann Oncol 2010;21:2447-2448. [2]. Bidard FC, et al. Ann Oncol 2010;21:1765-1771. [3]. Kraan J, et al. Drug Discov Today 2012;17:710-7.

PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF THE EFFECT OF RITONAVIR ON INTRATUMOURAL DOCETAXEL METABOLISM AND ANTI-CANCER EFFICACY IN A MOUSE MODEL FOR HEREDITARY BREAST CANCER

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Introduction In both mice and human we have shown that docetaxel systemic exposure is significantly increased by co-administration of ritonavir. In a mouse tumour model for hereditary breast cancer, we explored whether ritonavir has additional anti-cancer efficacy over docetaxel when combined. We demonstrated an additional anti-cancer effect of the combination of docetaxel and ritonavir compared to single docetaxel treatment, which was related to intratumoral Cyp3a inhibition (Hendriks et al. submitted). The objective of the current study was to apply pharmacokinetic (PK)-pharmacodynamic (PD) modelling on this previous study to further investigate and quantify the effects of ritonavir in co-administration with docetaxel.

Methods PK models of docetaxel and ritonavir in plasma and in tumour were developed. The effect of ritonavir on docetaxel exposure in system (Cyp3a knock-out) and in tumour (with inherent Cyp3a) was studied, respectively. Subsequently, we developed a tumour growth inhibition (TGI) model which included inhibitory effects of both docetaxel as ritonavir.

Results PK and PK/PD models were successfully built for docetaxel and ritonavir. Even in Cyp3a knock-out host, ritonavir increased docetaxel systemic exposure with docetaxel clearance estimated as 92% (relatively standard error (RSE) 0.4%) in the co-treated group compared to that in

docetaxel only-treated group. As expected, docetaxel tumour exposure was increased by ritonavir with mean area under concentration-time curve 2.5-fold as high as that of docetaxel only-treated group. Figure 1 compared the TGI models with only effect of docetaxel (panel A) or including additional anti-tumour effect of ritonavir (panel B), suggesting potential ritonavir anti-tumour efficacy. Formal testing of this potential anti-tumour effect of ritonavir proved that this effect was small but significant (p-value <0.001).

Conclusion We separately quantified the effect of ritonavir on docetaxel systemic and tumour PK and on tumour growth in this mouse model. By this, we showed that ritonavir inhibited docetaxel metabolism systemically and in the tumour. Besides, ritonavir was found to have a small contribution to the anti-cancer effect when co-administered with docetaxel.

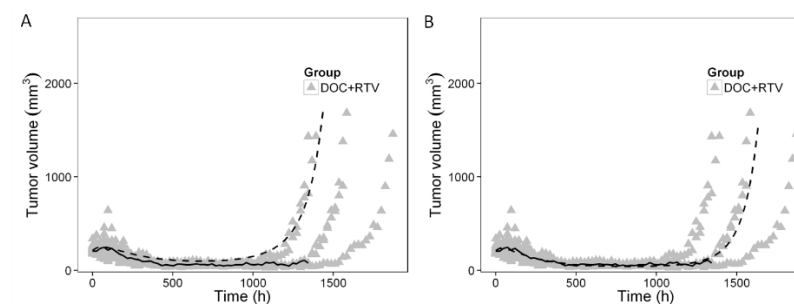


Figure 1. Comparison of prediction of tumour volumes without (A) or with (B) ritonavir anti-cancer effect in co-administration of docetaxel and ritonavir. DOC, docetaxel; RTV, ritonavir. The dots represent the observation of individual mouse; the solid lines show the median of observation; and the dashed lines represent the population prediction from pharmacokinetic-pharmacodynamic model.

Hendriks J, et al. submitted.

EFFECT OF SODIUM CHLORIDE INTAKE ON BLOOD PRESSURE RESPONSE TO CAFFEINE CONTAINING COFFEE IN HUMANS

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Objective: In rats, high sodium intake augments adenosine-induced vasodilation (Licican EL et al., 2005 and Dobrowolski L et al., 2007). This study tests the hypothesis that the acute blood pressure response to caffeinated coffee (adenosine receptor antagonist) is augmented by high sodium intake in humans.

Design and method: 13 healthy volunteers (8 male) used low (LS; 6 gram/24 hours) and high (HS; 12 gram/24 hours) salt diet in random order, each for 5 days with 4 day wash-out . At the end of each diet and after 24 hours of caffeine abstinence, subjects drank 350 ml of caffeinated coffee. Primary endpoint was the blood pressure response (Dinamap 1846 SX; Critikon, Portanje Electronica BV) to coffee.

Results: Maintenance to the diet was confirmed by urinary sodium excretion (5 ± 2 (SD) versus 12 ± 4 g NaCl/24 hours, $p < 0,0001$). Plasma caffeine concentration significantly increased from 0.2 ± 0.2 (SD) to 6.6 ± 1.8 $\mu\text{g/ml}$ ($p > 0.3$ for comparison between 2 days, paired t-test).

Table 1: Course in blood pressure (mean \pm SEM (mmHg)

Time (minutes) after coffee	0	30	60	90	120
SBP LS#	115(\pm 3)	118(\pm 3)	120(\pm 3)	121(\pm 4)	124(\pm 4)
SBP HS*#	121(\pm 4)	127(\pm 3)	126(\pm 4)	126(\pm 5)	129(\pm 4)
MAP LS #	84 (\pm 2)	91(\pm 2)	90(\pm 2)	91(\pm 2)	93(\pm 2)
MAP HS #	88(\pm 2)	94(\pm 2)	93(\pm 2)	92(\pm 2)	93(\pm 2)
DBP LS#	68(\pm 1)	73(\pm 1)	73(\pm 1)	72(\pm 1)	74(\pm 2)
DBP HS#	69 (\pm 1)	75(\pm 1)	74(\pm 1)	72(\pm 1)	73(\pm 1)

* $p < 0.05$ versus low salt; # $p < 0.01$ for increase over time. The interaction between high and low sodium diet and caffeine intake on SBP, DBP ($p > 0,3$) and MAP ($p > 0,05$) was not statistically significant (ANOVA for repeated measures). SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Blood Pressure.

Conclusion: High sodium intake does not augment the acute blood pressure response to coffee. This observation does not support a mitigating role of adenosine on the salt-associated increase in blood pressure in humans.

Dobrowolski L et al. Nephrol Dial Transplant. 2007;22(10):2805-9
Licican EL et al. Am J Physiol Renal Physiol. 2005;289(2):F386-

REDUCING ALBUMINURIA CONFERS RENOPROTECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Introduction

Albuminuria has been proposed as a surrogate endpoint in randomized clinical trials (RCT's) of renal disease progression. Most evidence is based on observational analyses showing that a treatment induced short-term change in albuminuria correlates with risk change for end-stage renal disease (ESRD). However, these studies were post-hoc analysis of RCTs and are prone to bias and residual confounding. To minimize this type of bias it is necessary to associate the placebo controlled treatment effects on albuminuria with the placebo controlled treatment effects on ESRD through a combined analysis of multiple trials.

Aims

We performed a meta-analysis of RCT's to correlate the placebo corrected drug effect on albuminuria and ESRD in order to reliably examine whether albuminuria is a valid surrogate endpoint.

Methods

MEDLINE and EMBASE were searched without language restriction for RCT's reported between 1950 and April 2014. Included RCT's had a mean follow up of at least 1000 patient years, reported ESRD outcomes, and measured albuminuria at

baseline and during follow-up. Meta-regression was performed to assess the association between drug effects on albuminuria and ESRD.

Results

Twenty-one RCT's involving 78,342 patients and 4,183 ESRD events were included. Median time to first albuminuria measurement was 6 month. Fourteen trials tested the effect of renin-angiotensin-aldosterone-system inhibitors (RAASi) and 7 trials tested other interventions. We observed a wide variability across trials in the treatment effect on albuminuria (range -1.3% to -32.1%) and ESRD (range -55% to +35% relative risk change). Meta-regression revealed that the treatment effect on albuminuria significantly correlated with the treatment effects on ESRD: for each 30% reduction in albuminuria the risk of ESRD decreased by 23.7% (95%CI 11.4 to 34.2). The association was consistent regardless of RAASi drugs or non-RAASi drugs (p interaction 0.73) or other patient or trial characteristics.

Conclusion

The significant association between drug effects on albuminuria and ESRD and the consistency across drug classes and patient characteristics suggests that albuminuria is a valid substitute for ESRD in a variety of circumstances, taking into account possible other drug effects that positively or negatively impact on renal outcomes.

CONFIRMATIVE STUDY OF SAFETY, FEASIBILITY AND COST-EFFECTIVENESS OF GENOTYPE-DIRECTED INDIVIDUALIZED DOSING OF FLUOROPYRIMIDINES AND EXPLORATION OF ADDITIONAL PHENOTYPING

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Introduction: The fluoropyrimidine anticancer drugs 5-fluorouracil (5-FU) and capecitabine are standard of care in the treatment of early and advanced breast, colorectal and gastric cancer. There is ample evidence demonstrating that variation in activity of the fluoropyrimidine-metabolizing enzyme dihydropyrimidine dehydrogenase (DPD), encoded by the gene *DPYD*, causes clinically significant differences in sensitivity to the toxic effects of 5-FU and capecitabine. DPD deficiency, occurring in $\leq 5\%$ of the population, is associated with the risk of severe, potentially lethal, toxicity. Both upfront genotype- and phenotype-directed dose-adaptation are promising strategies to improve patient safety, but have not been universally implemented as standard of care, since there is controversy whether improvement of patient safety and cost-effectiveness have been studied sufficiently.

Aims: The primary objective of this project is to confirm whether severe toxicity associated with fluoropyrimidine treatment can be significantly diminished by individualized dosing based on upfront genotypic assessment of DPD deficiency. Secondary objectives are to confirm that adaptive dosing based on genotyping is cost-effective and to determine the additional value of several phenotype-based screening methods for DPD activity.

Methods: A prospective, multicenter, non-randomized clinical trial will be performed, with a duration of 24 months. 1250 patients will be included in at least 15 participating centers.

Prospective screening for four single nucleotide polymorphisms (SNPs) in *DPYD* (*DPYD**2A c.2846A>T, c.1236G>A and *DPYD**13) will be performed using validated real-time polymerase chain reaction assays. Patients with a SNP in *DPYD* will be treated with a 25-50% reduced starting dose, depending on which SNP is identified. The dose will be titrated in subsequent cycles, to achieve maximal safe and effective exposure. In addition to the genotyping, the DPD phenotype of all patients will be determined by measuring baseline dihydrouracil/uracil ratio. In a subgroup of 300 patients other phenotyping methods will be tested: measuring the plasma levels of dihydrouracil/uracil after a test dose and a [2-¹³C]-labeled uracil breath test. To validate these phenotyping tests, results will be compared with the results of a DPD activity assay (which measures DPD enzyme activity in peripheral blood mononuclear cells and is considered the gold standard in DPD phenotyping assays). The results of the phenotyping tests will be evaluated at the end of this study and will be the basis for a subsequent study where confirmation will be sought for the combined use of upfront genotype- and phenotype based DPD-screening. The cost-effectiveness of genotype-guided dosing will be calculated.

Results: CCMO approval for the study is achieved. Turnaround time of SNP-analysis is two working days. An initial network of 10 centers has been built. Currently additional sites are being selected for participation in the study and inclusion of patients is about to start.

Conclusions: A large prospective study employing *DPYD* genotyping of four SNPs and dose-adaptation has started to definitely demonstrate safety and feasibility of this approach in patients treated with fluoropyrimidines.

SIMULTANEOUS QUANTIFICATION OF DABRAFENIB AND TRAMETINIB USING HPLC-MS/MS IN PLASMA OF MELANOMA PATIENTS

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Introduction

The combination of BRAF inhibitor dabrafenib (Tafinlar®) and MEK inhibitor trametinib (Mekinist®) improved overall survival in previously untreated patients with metastatic melanoma (Robert *et al.*, 2015). To support therapeutic drug monitoring (TDM) an assay to simultaneously quantify dabrafenib and trametinib in human plasma using liquid chromatography-tandem mass spectrometry was developed and validated.

Method

Human plasma samples were collected from patients during their visit at the outpatient clinic and samples were stored at nominally -20°C. Dabrafenib and trametinib were isolated from plasma by liquid-liquid extraction, separated on a C18 column with gradient elution, and analyzed using a triple quadrupole mass spectrometer operating in the positive-ion mode. Isotopically labelled analytes were used as internal standards for the quantification.

Results

The validated assay ranges were 50-5000 ng/mL for dabrafenib and 0.5-50 ng/mL for trametinib. For all quality control samples the measured concentrations for both analytes were within $\pm 15\%$ of the nominal concentrations and precisions were $\leq 15\%$. Dabrafenib was found to be stable at room temperature in plasma for utmost 6 hours and stock solutions should be prepared in DMSO to prevent degradation. In addition 58 patient samples were analysed for TDM purposes. Only one sample had trametinib concentrations below the LLOQ (see Figure 1).

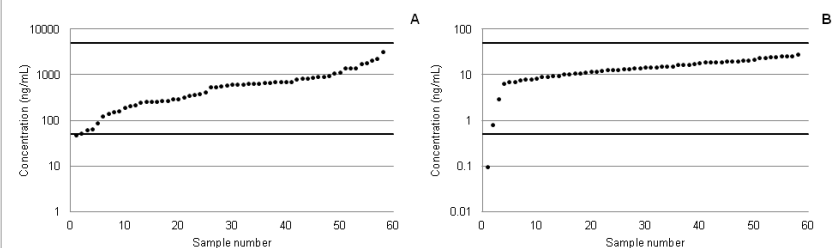


Figure 1. Plasma concentrations of dabrafenib (A) and trametinib (B) of individual samples. The solid lines show the LLOQ and ULQ of both analytes.

Conclusion

The described method to simultaneously quantify dabrafenib and trametinib in human plasma was successfully validated and applied for TDM in cancer patients treated with dabrafenib and trametinib. Sample collection for TDM purposes is still ongoing.

References

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COMPARISON OF ANTIBIOTIC DOSING RECOMMENDATIONS FOR NEONATES FROM ESTABLISHED TEXTBOOKS: PAVING THE WAY FOR E-PRESCRIBING STANDARDS

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Background and aims. Incorrect dosing is the most frequent occurring prescribing error in paediatrics (Kaushal *et al*, 2001) and antibiotics are the most frequently prescribed medicines. Computer physician order entry (CPOE) and clinical decision support systems can contribute to the reduction of medication errors (Kaushal *et al*, 2001). Although evidence-based dosing recommendations should be included in such systems, the necessary evidence is not always available and subsequently dosing recommendations mentioned in guidelines and textbooks are often based on expert opinion. The aim of this study is to compare dosage recommendations for antibiotics in neonates provided by seven commonly used and well-established international textbooks.

Methods. Neonatal daily doses for the 10 most frequently used antibiotics, classified by categories for birth weight and gestational age, were identified from 7 well-respected textbooks in paediatrics/paediatric infectious diseases, and expressed as standardized average daily dosage.

A team of experts from our children's hospital, including a paediatrician-infectious disease specialist, medical microbiologist, neonatologist and hospital pharmacist, selected the 7 established textbooks (Liem *et al*, 2010), i.e. The British National Formulary for children (BNF), Pediatric Dosage

Handbook (PDH), Red Book, Infectious Diseases of the Fetus and the Newborn Infant (Remington & Klein), Principles and Practices of Pediatric Infectious Diseases (Long & Pickering), Textbook of Pediatric Infectious Diseases (Feigin & Cherry) and Nelson's Pocket Book of Pediatric Antimicrobial Therapy (Nelson's).

Results. In total 311 dosage recommendations were included for systematic comparison. Antibiotics with a wide therapeutic window (e.g. ampicillin, benzylpenicillin, ceftazidime and cefotaxime) showed greater variation in dosage recommendations compared to those with a small therapeutic window (e.g. meropenem, gentamicin, and vancomycin). The BNF showed larger variation in standardized dosage recommendations compared to the other textbooks. In contrast, the Red Book demonstrated the least variation in standardized dosage recommendations of all evaluated textbooks.

Conclusions. Gold standard, expert opinion antibiotic dosage recommendations for neonates can be derived from important textbooks and guidelines for most, but not all antibiotics. Further exploration to overcome variation in dosage recommendations is necessary to obtain standardized dosage regimens and thus full benefit of CPOE and clinical decision support systems in neonatology.

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Clinical pharmacology research internships for (bio-)medical students

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Introduction: The Centre for Human Drug Research (CHDR) is non-profit clinical research institute at the interface between academia and the pharmaceutical industry and offers internships to undergraduate (bio-)medical students. During these internship, students get hands-on experience in clinical pharmacology research.

Aims: Assess the clinical pharmacology research internships at the CHDR using an online survey of (former) interns. Areas of interest included general perceptions of the internship and a comparison with academic research internships.

Methods: An anonymous online Google Forms® survey—open-ended and Likert-scale questions—was sent to all students that started an internship at the CHDR between 2007-2014. Data from the survey was analysed using R (R Development Core Team, 2014). A PubMed search was performed to identify co-authorships of students related to their internship project.

Results: The response rate of the online survey was high (53 students of 89, 61%). Most students gave the internship an overall rating of good (43%) or excellent (42%), and 80.5% strongly agreed that their training at the CHDR was helpful to

their career. Many students considered their internship at the CHDR to be (much) more broad (58%) and outcome-driven (50%), compared to academic research internships. Although CHDR has a more commercial setting compared to purely academic institutes, this was not considered a major distraction by 98% of the interns. A Wilcoxon rank-sum test revealed a significantly lower score of pre-Master students in both ‘Own knowledge of clinical research’ and ‘Link between internship and knowledge from their study’ when they were compared to Master students (both $p < 0.01$).

The PubMed search identified 19 published papers on which (former) interns were (co-)authors with a CHDR affiliation. Out of all interns in 2007-2014, 20% co-authored at least one of these papers.

Conclusion: The clinical pharmacology research internships at the CHDR are perceived by most students as having a good or excellent quality, and being helpful to their career. Pre-Master students might benefit from additional guidance to ensure a sufficient knowledge level for an effective internship. We aim to increase the number of (co-)authorships by interns in the future, as we believe they can benefit from this experience.

References:

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Tissue Transglutaminase: a novel therapeutic target in Alzheimer's disease?

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Background: Alzheimer's disease (AD) is characterized by amyloid-beta (A β) aggregates in the brain as senile plaques (SP) and cerebral amyloid angiopathy (CAA). Targeting A β aggregates has been one of the major approaches for AD therapies, although these attempts had little to no success so far. Therefore, novel treatment options should focus on blocking the actual formation of neurotoxic A β multimers. Evidence is accumulating that the enzyme tissue transglutaminase (tTG) plays a key role in these processes. tTG is abundantly expressed in the human brain and catalyzes post-translational modifications resulting in covalently cross-linked protein complexes. A β is a substrate for tTG cross-linking, resulting in stable and neurotoxic A β oligomers. As such, tTG activity plays a prominent role in initiating the A β cascade in AD. Therefore, inhibition of tTG activity may offer an attractive strategy to counteract A β toxicity in AD. **Aim:** Evaluation of *suitability* and *feasibility* of tTG as a therapeutic target to counteract A β aggregation and pathology in a translational AD model. **Results:** Using well-characterized post-mortem human tissue of AD cases, we found colocalisation of tTG with A β deposition in both diffuse and classic SPs. In CAA, tTG was present as halo's surrounding deposited A β . In addition, by activating the endogenous tTG enzyme, we demonstrated *in situ* of tTG in the deposited A β part of CAA. Based on these findings, we propose a two-tiered preclinical proof of concept study to investigate *suitability* and *feasibility* of tTG as a therapeutic target to counteract A β pathology. The *suitability* of tTG as a therapeutic target will be investigated using a crossbreed of the tTG^{-/-} mouse model and the AD-relevant APP23 mouse model.

The *feasibility* will be investigated by injection of a selective and irreversible tTG inhibitor, into the APP23 mouse model using surgically implanted osmotic minipumps. As a readout of the effects of both absence or suboptimal levels of tTG in crossbreed animals and the APP23 animals, the following analysis will be performed: 1) cognitive performance will be analysed and compared between all animal groups using a Morris watermaze and a contextual fear-conditioning test, 2) (immuno)histochemical analysis of presence and severity of A β pathology, tTG levels, activity and distribution, and colocalization with A β will be performed, 3) biochemical analysis of presence of (stable) tTG-induced A β multimers, tTG levels and activity in the brain will be performed. For the feasibility research plan, APP23 mice will be injected with or without the irreversible tTG inhibitor Z006 using osmotic minipumps (Alzet[®], model 2ML4, Durect; and dummy). Pumps will be subcutaneously implanted for 2 x 4 weeks, and gradually deliver inhibitor into the brain ventricles for a period of 2 months in which the AD pathology is formed. Accordingly, both treated and sham-treated APP23 mice will be analysed using the above-described parameters.

In addition, we will investigate target engagement of the tTG inhibitor *in vivo* by labelling the tTG inhibitor with radioactive tracer and use PET imaging for analysis. Initially, we will perform target engagement in the mouse model. If successful, we will use this approach to investigate the target engagement of the tTG inhibitor in humans as both a diagnostic tool as well as a disease- and/or therapy-monitoring biomarker.

Conclusion: Based on our findings in human post-mortem AD brain tissue, we designed a complete pharmacological work up for the analysis of tTG as both a *suitable* and *feasible* drug target to counteract A β pathology, neurodegeneration and cognitive decline in AD. These studies will ultimately provide insight into the use of specific tTG inhibitors as therapeutic agents in a novel disease-modifying therapy and/or as disease-monitoring PET tracers in AD.

DEVELOPMENT AND VALIDATION OF A HPLC-UV ASSAY TO QUANTIFY PLASMA LEVELS OF SULFAMETROL: A PREFERENTIAL ANTIBIOTIC IN CHILDREN

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Background:

Sulfonamides in combination with trimethoprim are frequently used antibiotics. They work synergistic. In infections with *Pneumocystis jiroveci* or *Stenotrophomonas maltophilia* higher dosages are indicated than in other infections.

Therapeutic Drug Monitoring (TDM) is warranted to assure the efficacy while limiting toxicity. Although trimethoprim in combination with sulfamethoxazole is the most common combination with established TDM reference values, the intravenous formulation is not suited for children due to its additives to increase solubility, ethanol and propylene glycol. An alternative can be sulfametrol in combination with trimethoprim. As this combination was introduced in the hospital, the need for a TDM method for sulfametrol was warranted.

Methods:

A HPLC-UV method was developed and validated according to International Conference on Harmonization (ICH) guidelines. Linearity, limit of detection (LOD), lower limit of quantification (LLOQ), recovery, process efficiency, selectivity, within run precision, inter run precision and sample stability were tested.

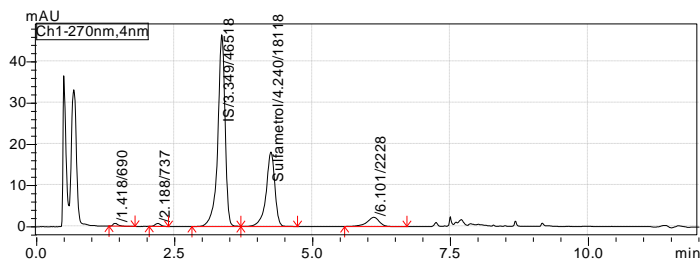
Results:

All tested parameters met the required criteria. For linearity r^2 was 0.9948, LLOQ was 10 mg/L and LOD was 6 mg/L. Recovery was 100.4% and process efficiency 94.4%. Selectivity was met with no interfering peaks at the retention time of 4.2 minutes. Inter run precision and within run precision were evaluated by replicating quality control (QC) levels. Recovery of the samples after storing 8 days was 101.9% and recovery of already tested vials was 98.8% after 48 hours.

Conclusion

A TDM method for sulfametrol in human plasma was developed and validated. The method is quick, accurate, reproducible and has a short analysis time. It is now being used in routine TDM in our clinic.

Figure 1: a patient HPLC chromatogram with sulfametrol, internal standard, metabolites and trimethoprim peaks.



DEVELOPMENT AND VALIDATION OF AN UPLC-MS/MS METHOD FOR DETERMINATION OF URACIL AND DIHYDROURACIL IN HUMAN PLASMA

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Introduction: Approximately 85% of the fluoropyrimidine drugs 5-fluorouracil (5-FU) and its oral prodrug capecitabine are metabolized into inactive metabolites by the enzyme dihydropyrimidine dehydrogenase (DPD). Clearance of 5-FU can be highly reduced in patients harboring mutations in the gene coding for DPD (*DPYD*), which puts patients at risk for developing severe and sometimes lethal toxicity. Sensitivity for detecting DPD deficiency might be improved by determining the DPD phenotype instead of *DPYD* genotype, since phenotyping enables to account for epigenetic variability. The most frequently used DPD phenotyping method is based on *ex vivo* assessment of DPD activity in cell lysate of human peripheral blood mononuclear cells (PBMCs). An alternative DPD phenotyping approach is to determine the ratio between the endogenous DPD substrate uracil (U) and the metabolite dihydrouracil (DHU) in human plasma. The aim of our study was to develop and validate an assay for quantification of U and DHU in human plasma.

Methods: An ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) assay for quantification of U and DHU was developed and validated. Plasma sample pre-treatment consisted of protein precipitation with a mixture of methanol and acetonitrile (50:50, v/v). Dried extracts were reconstituted in 0.1% formic acid before

injection into the UPLC system. Chromatographic separation was performed on a Acquity UPLC[®] HSS T3 column and a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was applied. The eluent was directed to a tandem mass spectrometer equipped with an electrospray ionization source. DHU was quantified in the positive ion mode and U in the negative ion mode. Assay validation was performed using blank plasma which was dialysed in order to remove endogenous U and DHU. Stable isotopes for U and DHU were used as internal standard (IS).

Results: The total chromatographic run time was only 5 min. Using 300 µL of human plasma, the lower limit of quantification (LLOQ) was 1 ng/mL and 10 ng/mL for U and DHU respectively. The validated concentration ranges for U and DHU were from 1 to 100 ng/mL and 10 to 1000 ng/mL. Maximum inter-assay bias and inter-assay precision for U were 2.8% and 12.4% at the tested concentrations. For DHU, the maximum inter-assay bias and inter-assay precision were 2.9% and 7.2%. Carry-over for U was 11.2% of the signal at the corresponding LLOQ and for DHU no carry-over was detected. For the IS of both analytes, carry-over did not exceed 0.2%. Stability of U and DHU in dry extract and final extract was demonstrated for at least 5 days at 4°C. The validated parameters were acceptable according to FDA criteria.

Discussion: We successfully developed an accurate, precise and sensitive UPLC-MS/MS assay for quantification of U and DHU in plasma. Sample pre-treatment only consists of protein precipitation, which together with short chromatographic run time, enables fast sample analysis.

EX VIVO IRRADIATION IMPROVES PHARMACODYNAMIC ASSAY FOR PARP INHIBITORS

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INTRODUCTION:

Poly (ADP-ribose) polymerase (PARP) inhibitors such as olaparib are currently developed in clinic as anti-cancer drugs, both as monotherapy and in combination with chemotherapy and/or radiotherapy. A pharmacodynamic (PD) assay that measures PAR (poly ADP-ribose), the direct product of PARP, in tumor cells and peripheral blood mononuclear cells (PBMCs) is available for clinical trials. However, PAR levels in PBMCs are often too low for reliable detection of a PAR level reduction upon treatment with a PARP inhibitor. The aim of our study was to improve the current PD assay to reliably quantify reductions in PAR levels in PBMCs, in order to be able to use the assay for all patients in clinical trials. We hypothesized that *ex vivo* irradiation of PBMCs would improve the assay by inducing PAR levels.

METHODS:

PBMCs were isolated from healthy volunteers and *ex vivo* irradiated. Different radiation doses, incubation times, temperatures and other variables were tested to optimize the assay. Cell lysates were prepared and PAR levels were quanti-

fied using a commercially available PD immunoassay for clinical trials. To compare the current assay with the ‘improved’ assay, we incubated PBMCs of 6 healthy volunteers with clinically relevant doses of olaparib and determined PAR levels with and without the additional steps in the assay.

RESULTS:

PAR levels were strongly induced by *ex vivo* irradiation and reached a steady state level after 1 hour incubation on ice. Radiation up to 32 Gy resulted in a dose dependent linear increase in PAR levels up to a 100-fold induction. PAR levels remained stable after three freeze/thaw cycles, and after storage of lysates at -80°C for 1 year.

The addition of *ex vivo* irradiation and one hour incubation on ice strongly improved the biological response range of the PD assay. Accurate IC50 determination was only possible in half of the healthy volunteers (3/6) without these additional steps and in all healthy volunteers (6/6) with the additional steps. By improving the accuracy of IC50 determination, the additional steps in the assay allowed us to pick up significant differences in IC50 between different healthy volunteers (range 3.5 – 6.9nM), which was impossible without the additional steps. A 99% reduction in PAR levels by 10.000 nM olaparib (C-max of olaparib monotherapy) could still be accurately quantified.

CONCLUSIONS:

Ex vivo irradiation of PBMCs in the PAR pharmacodynamic assay improved the quantification of individual responses to PARP inhibitors and enhanced the potential applicability of the assay to all patients in current and future clinical trials.

CHANGES IN DIHYDROURACIL:URACIL PLASMA RATIO AFTER PARTIAL LIVER RESECTION IN PATIENTS WITH COLORECTAL LIVER METASTASES

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Introduction: About 50% of the patients with advanced colorectal cancer will develop liver metastasis. Some of these patients will undergo partial liver resection and adjuvant treatment with capecitabine-based chemotherapy. Capecitabine is the oral prodrug of 5-fluorouracil (5-FU) and is for approximately 85% catabolized into inactive metabolites by dihydropyrimidine dehydrogenase (DPD), which is highly expressed in the liver. Patients that are (partly) DPD deficient are at risk for developing severe and sometimes lethal toxicity. Phenotyping of DPD activity seems promising for assessing DPD deficiency. Most DPD phenotyping methods rely on determination of DPD activity in peripheral blood mononuclear cells (PBMCs), in which dynamic changes in systemic DPD activity will not be detected. Alternatively, determination of the ratio between dihydrouracil (DHU) and the endogenous DPD substrate uracil (U) in plasma might be used for phenotyping DPD activity. The aim of study was to assess dynamic changes in DHU:U in human plasma.

Methods: An observational study was performed in which plasma samples from patients with colorectal liver metastasis were collected before and one day after partial liver resection.

If applicable, additional plasma samples were collected before subsequent courses with capecitabine-based chemotherapy. Plasma U and DHU concentrations were determined using an ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) assay. Values below the quantification limit (LLoQ) were substituted by 0.5 * LLoQ.

Results: U and DHU plasma concentrations were determined in plasma samples from 14 patients (9 males) with a median (range) age of 68 (55-82). Mean (range) plasma DHU:U ratios were 10.6 (5.9 – 14.4) prior to partial liver resection and significantly reduced to 5 (0.3 – 10.4) one day after surgery ($p = 0.002$). Before surgery, the mean (range) plasma U and DHU concentrations were 11.3 (8.61 – 16.7) ng/mL and 116 (79.8 – 153) ng/mL, respectively. One day after partial liver resection, the mean DHU plasma concentration was significantly reduced to 45 ng/mL ($p < 0.001$) and showed variability among patients (range: 5 – 160 ng/mL). After liver surgery, the mean U plasma concentration remained approximately the same (10.4 ng/mL). A trend towards recovery of DHU plasma levels was found in plasma samples (n=4) that were collected prior to the first course of adjuvant chemotherapy (approximately 1-2 months after liver surgery).

Discussion: Dynamic changes in the DHU:U plasma ratio are found after partial liver resection and are the result of reduced DHU plasma levels. Possibly this is the result of reduced systemic DPD activity, but the role of other enzymes within the U pathway or non-specific effects of liver surgery might cause these dynamic changes.

Individualized dosing of Namisol® (a novel oral Δ^9 -THC formulation) improves subjective spasticity and pain in patients with progressive Multiple Sclerosis

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Introduction

Cannabinoids have been shown to improve symptoms of Multiple Sclerosis (MS) including muscle spasticity and pain through modulation of neuronal excitability via presynaptic cannabinoid receptors. Previous formulations of Δ^9 -THC are notorious for variable pharmacokinetic profiles, thereby demanding cumbersome uptitration. The current formulation was developed to improve clinical application of Δ^9 -THC in the treatment of spasticity and pain in MS.

Aims

The aim of the present study was to evaluate the efficacy of a novel oral formulation of Δ^9 -THC (Namisol®) to treat spasticity, pain and improve functional outcome measures in 24 patients with primary or secondary progressive MS.

Methods

This was a two-phase study consisting of a dose-finding phase utilizing pharmacokinetic-pharmacodynamic (PK-PD) modelling and a 4-week treatment phase. In the dose-finding phase, the effect of an escalating oral dose of Δ^9 -THC on pharmacodynamic outcome variables was assessed in a randomized placebo-controlled, two-way cross-over trial design. Patients visited the outpatient clinic on two occasions and received an escalating oral dose of Δ^9 -THC or placebo. Plasma concentrations of Δ^9 -THC and metabolites were

measured to generate an individual treatment regimen based on PK and PD. In the 4-week treatment phase, the individual dose was administered three times daily in a randomized placebo-controlled, parallel fashion. During the treatment phase muscle spasticity (Ashworth, EMG and subjective spasticity), pain and clinical outcomes (e.g. EDSS, 25Ft Timed walk, GNDS) were measured at baseline, week 2 and 4.

Results

Pain was significantly ($p=0.0439$) reduced when measured directly after administration of Namisol® in the clinic, but not when measured in a daily diary. The NRS least square mean (LSM) was 2.99 for active treatment versus 4.26 for placebo, LSM estimated difference -1.27 (95% CI: -2.50 – -0.04). A similar pattern was observed in subjective muscle spasticity. Other (objective) clinical outcomes were not significantly different between active treatment and placebo. Cognitive testing indicated there was no decline in cognition after 2 or 4 weeks of treatment due to Δ^9 -THC compared to placebo.

Conclusions

Namisol® appears to be effective in reducing subjective symptoms including muscle spasticity and pain in patients suffering from MS when measured immediately after dosing in the clinic. This effect could not be shown when measured retrospectively using a diary.

Acknowledgements

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Successful azathioprine treatment of a paediatric TPMT deficient patient using therapeutic drug monitoring

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Introduction

Thiopurines are indicated for the treatment of colitis ulcerosa and Crohn's disease. In the Netherlands only azathioprine (AZA) is registered for the treatment of inflammatory bowel disease in children.

Thiopurine S-methyltransferase (TPMT) plays a pivotal role in the metabolism of AZA. TPMT enzyme deficiency leads to an elevated 6-TGN metabolite formation and subsequently to a high risk of developing myelosuppression.

In order to monitor the effect and risk of the development of adverse events, the levels of thiopurine metabolites 6-TGN and 6-MMPR can be measured. High steady-state levels of 6-TGN ($>500 \text{ pmol}/8 \times 10^8 \text{ RBC}$) are associated with myelotoxicity.

Case description

A 14 year old male patient (69 kg) with colitis ulcerosa was treated with AZA 175 mg once daily. This therapy correlates with national dosage guidelines for children of 2.5 mg/kg bodyweight. Two weeks after the start of AZA therapy the patient presented with high 6-TGN levels ($2095 \text{ pmol}/8 \times 10^8 \text{ RBC}$). Leukocytes count showed no signs of myelotoxicity and the AZA dose was reduced to 50 mg once daily.

Two weeks later, 6-TGN levels were increased to $2353 \text{ pmol}/8 \times 10^8 \text{ RBC}$. TPMT enzyme activity was assessed,

which appeared to be almost absent in this patient. TPMT genotyping revealed a homozygote variant of the gene encoding for the TPMT enzyme (*3A/*3C).

AZA was discontinued and it took 6 weeks before 6-TGN levels decreased within the therapeutic range.

However, instead of discontinuing AZA completely in this patient, treatment was initially restarted with 75 mg once weekly (7% of standard daily AZA dose 2.5 mg/kg bodyweight). AZA dose was adjusted based on a weekly scheduled monitoring of 6-TGN levels, 6-MMPR levels and close haematologic monitoring.

The patient responded well and within two weeks therapeutic levels of 6-TGN were reached on a dose of 50 mg once a week, which is approximately 4% of the standard dose for this paediatric patient. 6-TGN steady-state levels were between 500 and $600 \text{ pmol}/8 \times 10^8 \text{ RBC}$ without signs of myelotoxicity. The patient is in clinical remission on this maintenance dose for over 2 years now.

Conclusion

Our case demonstrates for the first time that a TPMT deficient paediatric patient can be successfully and safely treated with AZA under close clinical surveillance, guided by therapeutic drug monitoring.

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DEVELOPMENT AND CLINICAL VALIDATION OF AN LC-MS/MS METHOD FOR THE QUANTIFICATION OF PAZOPANIB IN DRIED BLOOD SPOTS

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Introduction: Pazopanib is a multi-targeted angiogenesis inhibitor approved for the treatment of renal cell carcinoma and soft tissue sarcoma.¹ A retrospective analysis of pazopanib clinical trials showed an increased median PFS in patients with plasma trough levels (C_{\min}) ≥ 20.6 mg/L compared to patients with a lower C_{\min} (50.2 wks vs. 19.6 wks).² Developing a dried blood spot (DBS) method might be an easy and patient friendly approach to guide treatment based on the measured trough level. Therefore, we describe the development and analytical and clinical validation of an LC-MS/MS method for the quantification of pazopanib in DBS.

Methods: Pazopanib was extracted from DBS cards using formic acid and methanol and ¹³C,²H₃-pazopanib was added as internal standard. Plasma sample pre-treatment consisted of protein precipitation using methanol and centrifugation. Processed samples were injected on a C18 column and gradient elution was applied. Tandem mass spectrometry operating in the positive mode was used for the quantification (m/z 438 \rightarrow m/z 357). Paired DBS and EDTA plasma samples were obtained from patients with advanced solid tumours treated with pazopanib. The relationship between plasma and DBS concentrations was studied.

Results: Both the DBS and plasma LC-MS/MS method were validated in accordance with FDA and EMA guidelines. Influence of spot homogeneity, spot volume and hematocrit were shown to be within acceptable limits. DBS samples were demonstrated to be stable at ambient temperatures for at least 202 days.

Analysis of the first 197 paired samples showed a strong correlation between plasma and DBS concentrations (Pearson's R of 0.949) with a slope of 0.687 using a weighted Deming fit. Plasma concentrations could be calculated from DBS levels using $[\text{Pazopanib}_{\text{calculated plasma}}] = ([\text{Pazopanib}_{\text{dried blood spot}}] + 0.147) / 0.687$. Back calculated plasma concentrations were within 20% of measured plasma concentrations for 162 (82%) out of the 197 DBS samples analyzed so far. Methods for improving the calculated plasma concentration based on patient specific hematocrit, protein binding and blood cell-to-plasma partition coefficient will be investigated.

Conclusion: The developed DBS method was successfully validated and applied to paired clinical samples. A correlation between plasma and DBS concentrations has been demonstrated and the method will be used for therapeutic drug monitoring to optimize the treatment of patients undergoing pazopanib therapy.

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TOXIC TOBRAMYCIN LEVELS AFTER TOBRAMYCIN INTAKE VIA SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT (SDD)

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Background:

In our hospital Intensive Care Unit (ICU) patients on mechanical ventilation are treated for selective decontamination of the digestive tract (SDD). The purpose of SDD is to eradicate potentially pathogen micro-organisms from the oropharynx and gastro-intestinal tract of intensive care unit (ICU) patients, thereby significantly reducing mortality. In SDD tobramycin is given as mouth paste and oral suspension (through nasogastric tube), in a starting dose of 4 times a day 80 mg orally, and this dosage can be increased with the persistence of potentially pathogen micro-organisms. Tobramycin is an aminoglycoside and high trough blood levels of tobramycin can lead to nephrotoxicity and ototoxicity by means of systemic exposure. However, in SDD, tobramycin suspension is given orally and aminoglycosides are not absorbed via the gut. Therefore, SDD will, generally, not lead to systemic exposure. However, in this abstract we describe one of the cases of the ICU in which a severe ill patient with Graft Versus Host Disease (GVHD) developed toxic tobramycin levels, probably due to intestinal leakage of tobramycin.

Methods:

In this case report, we describe a 34 year old male patient, submitted to the ICU of our hospital due to respiratory insufficiency. Primarily, he was admitted to our hospital

with a recurrent Acute Myeloid Leukemia (AML). After allogeneic stem cell transplantation he developed GVHD on his skin and intestines. Blistering of skin and failure of liver and intestines were seen, amongst all leading to severe diarrhea. Previously, several ICU patients with SDD and GVHD developed systemic tobramycin exposure. Therefore tobramycin level was measured in this patient by means of EMIT Immunoassay (Architect, Abbott).

Results:

After consecutive use of 8 times a day 80 mg tobramycin orally for 30 days, his tobramycin trough level was 3,5 mg/L (reference < 0,5 mg/L, toxic above 1 mg/L).. As the patient is bedridden in ICU for a long time, renal function is difficult to assess, but creatinin was 102 umol/L and Urea was 23,1 mmol/L (increase > 20% last 4 days). Tobramycin was stopped and levels dropped to 0,87 mg/L after 2 days and 0,22 mg/L after 4 days. Tobramycin was started again in a regimen of 4 times a day 80 mg tobramycin orally, under daily monitoring of tobramycin trough levels. Creatinin and urea recovered. High tobramycin levels were contributed to systemic leakage of tobramycin via the intestines.

Conclusion: In SDD, tobramycin is normally not absorbed. However, in severe intestine GVHD, systemic absorption of tobramycin can occur. In this patient toxic tobramycin levels were combined with impaired renal function. After stopping tobramycin, levels dropped to normal levels. In ICU patients with GVHD of the intestines and frequent administration of tobramycin-containing SDD close monitoring of systemic exposure of tobramycin is recommended.

EXPLORATION OF THE ROLE OF TNF α IN TLR4/NLPR3 INFLAMMASOME-DRIVEN INFLAMMATION

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The role of TNF α in the pathogenesis of atherosclerosis is incompletely understood. TNF α blockade reduces the severity of various autoimmune diseases and the often related atherosclerosis. However, excessively released TNF α is only one component of the hyperactive innate immune system in such diseases. To provide more insight into the role of TNF α in the induction of inflammation, we explored the effects of TNF α blockade in human whole blood.

TLR4/NLPR3 inflammasome challenges were applied to induce an inflammatory response. For this purpose, whole blood was incubated 4 hours with LPS and aluminium hydroxide (Alhydrogel). TNF α blockade was evaluated *in vitro* (LPS/Alhydrogel challenge in whole blood of 4 healthy human subjects, +concentration range of adalimumab) and *ex vivo* (LPS/Alhydrogel challenge in whole blood of 13 healthy human subjects receiving a single subcutaneous (sc) dose of 40 mg adalimumab). Cytokine release was evaluated in culture supernatants.

In vitro, TNF α blockade strongly reduced TNF α levels detected; -97 \pm 1% at the lowest adalimumab concentration (0.3125 μ g/mL). TNF α blockade did not affect LPS/Alhydrogel-induced IL-6, IL-1 β and IL-18 release, but reduced IFN γ release; maximally -93 \pm 4% at 5 μ g/mL adalimumab. A single sc adalimumab dose in healthy subjects reduced LPS/Alhydrogel-induced TNF α levels (maximally -98 \pm 1% on day 4, and still -58 \pm 59% on day 64; versus baseline). IL-6, IL-1 β and IL-8 release were not reduced after anti-TNF α treatment. The effect of TNF α blockade on IFN γ release could not be reliably estimated due to highly variable IFN γ levels, especially between genders (baseline IFN γ levels 1248 \pm 1771 and 140 \pm 283 pg/mL, males vs females).

TNF α is a major inducer of NF κ B-driven cytokine gene transcription, but TNF α blocking did not reduce LPS/Alhydrogel-induced release of IL-1 β , IL-6, IL-8 or IL-18 by primary human cells. This suggests that primary TLR4- and inflammasome-mediated signalling is sufficient to drive secretion of these cytokines. However, *in vitro* TNF α blockade did impair IFN γ release. Since IFN γ is a key factor in atherogenesis, exerting both pro- and anti-atherogenic properties, our data warrant further mechanistic investigation of the role of TNF α and anti-TNF α therapies in atherosclerosis.

Low-dose metronomic chemotherapy (LDMC) with oral paclitaxel formulations ModraPac001 (capsule) and ModraPac005 (tablet)

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Background: Low-dose metronomic chemotherapy (LDMC) consists of chronic administration of cytotoxic drugs at relatively low doses in a continuous schedule. As supported by pre-clinical research, LDMC could represent an alternative approach for anti-cancer treatment to dose-dense chemotherapy, as the therapeutic effect relies on anti-angiogenic activity by targeting the tumor vascular endothelium. LDMC with docetaxel or paclitaxel has proven to be feasible in several mouse models. However, a clinical study performed with LDMC with paclitaxel administered as a continuous infusion was halted due to significant infusion-related toxicity.

We performed a phase I dose escalation study in order to evaluate the Maximum Tolerated Dose (MTD) of LDMC with two novel oral formulations of paclitaxel, ModraPac001 (capsule) and ModraPac005 (tablet) administered twice daily (BID) with 100 mg of the booster Ritonavir.

Methods/patients: This phase I study had a classic 3+3 dose escalation design. Patients with metastatic solid tumor, ≥ 18 years old and WHO PS ≤ 2 were enrolled. Primary endpoint consisted of evaluation of the MTD of LDMC with paclitaxel as ModraPac formulation administered bi-daily in a continuous schedule in combination with 100 mg ritonavir. The MTD was defined as the dose at which $\leq 1/6$ patients experienced a dose

limiting toxicity (DLT) in the first 3 weeks of treatment. Secondary aims included evaluation of pharmacokinetics, overall toxicity and preliminary anti-cancer activity.

Results: To date twenty-nine patients have been enrolled. Dose-escalation ranging from BID 2.5 mg to 20 mg was performed using ModraPac001 (capsule) administered in combination with 100 mg ritonavir. Further dose-escalation (from BID 20 mg to BID 30 mg) was performed with ModraPac005 (tablet).

Dose level BID 30 mg ModraPac005 in combination with 100 mg ritonavir has been considered unfeasible due to DLTs observed in 2 out of 3 patients enrolled (i.e., grade 3 nausea [2 pts] and grade 3 febrile neutropenia and grade 4 neutropenia lasting >5 days [1 pt]). Currently the cohort treated with ModraPac005 30 mg (morning dose) and 20 mg (evening dose) in combination with 100 mg ritonavir is being expanded in order to determine whether it could represent the MTD. Common treatment related toxicities were fatigue, diarrhea and nausea, most often grade 1-2.

Pharmacokinetics might be different, however no dose-level comparing the formulations is available. AUC_{inf} after oral administration of BID 20 mg ModraPac001 with 100 mg ritonavir was 217 ± 116 ng*h/ml. The AUC_{inf} for BID 30 mg ModraPac005 was significantly higher 809 ± 185 ng*h/ml. No complete or partial tumor responses were observed. Eleven patients had stable disease as best response.

Conclusion: LDMC with orally administered paclitaxel as ModraPac001 and ModraPac005 appears to be feasible. The 30 mg morning and 20 mg evening dose-level plus 100 mg ritonavir with ModraPac005 is being expanded to confirm the MTD and R2PD for further clinical testing.

STUDYING THE OPTIMAL INTRAVENOUS BUSULFAN EXPOSURE IN PEDIATRIC ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (ALLOHCT) TO IMPROVE CLINICAL OUTCOMES: A MULTICENTER STUDY

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Background: In children, the therapeutic drug monitoring (TDM) of intravenous (IV) busulfan (BU) in alloHCT can contribute to better clinical outcomes. Current therapeutic targets for exposure are however primarily based on adult studies. Therefore, this study aimed to define the optimal therapeutic target of BU in children and identify other patient specific variables to optimize outcomes following alloHCT.

Methods: This retrospective study utilized exposure-response data available from routine pharmacokinetic analysis with or without TDM of BU levels in children and young adults treated with HCT between the years of 2000-2013 from 13 different centers. Primary endpoints were event-free survival (EFS) and overall survival (OS). Secondary endpoints included treatment-related mortality (TRM), veno-occlusive disease (VOD), acute graft-versus-host disease (aGVHD) grade II–IV and cGVHD. A predictor analysis using Cox regression and multivariate Weibull models was performed.

Results: A total of 685 subjects (range 11-116 per center) treated for a variety of malignant (n=318) and non-malignant disorders (n=367), with a median age of 5.0 years (range 0.1 - 28.7) were included in the analysis. The median cumulative BU area-under-the-concentration-curve (AUC) was 77mg*hr/L (range 21-160). In all patients, three-year probability of EFS, OS and graft failure was 72%, 79%, and 5.4% respectively. BU AUC below 75 or above 100mg*hr/L (p = 0.03), “ex-vivo T cell depletion” (p = 0.02) & “in vivo T-cell depletion using serotherapy” (ATG or alemtuzumab: p = 0.02) were negative predictors of EFS. A significant U-shaped relationship between BU AUC and EFS was observed. In patients with malignant disease optimal BU AUC was lower compared to non-malignant disorders (83-95mg*hr/L vs 99-111 mg*h/L, p= 0.04). Below the optimal BU target, the incidence of graft failure and relapse (malignant only) was higher (p=0.01), while above the target TRM increased (p=0.04). BU AUC above the median and addition of melphalan were both independently associated with the risk of aGvHD (p=0.01, p=0.04), and melphalan use further increased the risk of VOD (p<0.01). No association was found with cGvHD.

Conclusion: BU AUC targeted to a narrow therapeutic range, which is indication dependent, (83-95 mg*h/L for malignant and 99-111 mg*h/L for non-malignant diseases) was found to increase EFS and OS in children. Lower BU AUC was associated with graft-failure and relapse and higher BU AUC with TRM. Our findings suggest that personalizing BU AUC by patient-specific factors may improve efficacy and reduce toxicity.

Drug-drug interactions in patients treated for cancer: a prospective study on clinical interventions

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Introduction: Drug-drug interactions (DDIs) are of major concern in oncology, since cancer patients typically take many concomitant medications. (van Leeuwen et al, 2011 and 2013) Some retrospective studies have been conducted to determine the prevalence of potential DDIs (PDDIs). (van Leeuwen et al, 2011 and 2013; Riechelmann et al 2007) However, prospective studies on DDIs needing interventions in cancer patients have not yet been performed. Therefore we investigated DDIs leading to an intervention proposed by a clinical pharmacologist in ambulatory cancer patients receiving oral and/or intravenous anticancer treatment. Potential determinants for performing interventions were also studied.

Methods: Patients starting with a new I.V. or oral anticancer treatment regimen were asked to participate. Data on demographic characteristics, use of co-medication, over-the-counter (OTC) drugs and co-morbidities were collected by the clinical pharmacologist during a structured interview with the patient. Subsequently, the patients' medication was checked

for PDDIs by using drug interaction software. An expert team of 3 clinical pharmacologists evaluated the relevance of these identified PDDIs. If a PDDI was qualified as clinically relevant, an intervention was proposed to the treating physician. Several variables were studied as potential determinants for performing an intervention, e.g. number of drugs and number of comorbidities. Descriptive statistics and uni- and multivariate logistic regression analyses were performed.

Results: In this study 302 patients were included. The drug interaction software identified 603 PDDIs. Next to the intervention proposed by the (hemato)oncologists, an additional intervention was proposed by the expert team for 42 patients (14%). The number of comorbidities (adjusted odds ratio (OR): 1.4 (95% Confidence Interval (CI): 1.0-2.0)) and the number of OTC-drugs (adjusted OR: 1.4 (95% CI 1.1-1.8)) were identified as determinants.

Conclusions: Clinical interventions on DDIs are frequently required among patients starting with anticancer therapy. Structured screening for these potentially clinically relevant DDIs, by (hemato)oncologists in close collaboration with clinical pharmacologists, should take place before the start and during anticancer treatment.

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TACROLIMUS EXPOSURE AND ITS RELATION WITH CLINICAL OUTCOMES IN HIV INFECTED KIDNEY TRANSPLANT RECIPIENTS ON ANTIRETROVIRAL AGENTS: AN AUC DRIVEN ANALYSIS

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Aims

Protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) are cornerstones of current antiretroviral therapy. PIs such as ritonavir, and NNRTIs such as efavirenz are respectively strong inhibitors and inducers of P450 cytochrome 3A, which is the main metabolizing enzyme of tacrolimus (TAC). Subsequently, TAC pharmacokinetics are affected to a large extent by the coadministration of antiretrovirals (ARVs) in human immunodeficiency virus 1 (HIV1)-infected kidney transplant recipients (KTRs).¹ Our objectives were to study tacrolimus (TAC) exposure in detail and investigate its relation with clinical outcomes in HIV infected kidney transplant recipients (KTRs) on ARVs.

Methods

Data were extracted from the prospective NIH-sponsored trial AI052748² and a rich within-population pharmacokinetic (PK) data set. HIV KTRs receiving TAC and a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) within one year posttransplant were included. PK models

were designed to estimate area-under-the-curves (AUCs). TAC levels and AUCs were compared between KTRs receiving PIs or NNRTIs. Univariate and multivariate analyses were used to test the associations between TAC levels or AUCs and the risk of acute rejection.

Results

53 KTRs on an NNRTI and 40 KTRs on a PI were included. The mean daily AUC of TAC was 44% lower in KTRs on PIs compared to NNRTIs (12.1 vs 17.4 ng*day/L, p<0.01), while their mean TAC level was higher (10.5 vs 8.5 ng/mL, p<0.01). The mean daily AUC in KTRs who experienced an acute rejection episode was lower compared to KTRs who did not (12.1 vs 16.1 ng*day/mL, p=0.026). A higher mean daily AUC (HR=0.89; 95% CI: 0.82-0.97; p=0.01) and higher TAC trough level (HR=0.92; 95% CI: 0.85-0.997; p=0.04) were each associated with a lower risk of acute rejection.

Conclusions

In HIV infected kidney transplant recipients TAC exposure is strongly affected by their antiretroviral regimen. Thereby and because of a significant AUC-rejection association, AUC monitoring should be considered in these patients.

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AGONIST PET LIGANDS FOR 7-TRANSMEMBRANE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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Introduction 7-Transmembrane receptors (7-TMRs), also known as G-Protein coupled receptors, are targets for a wide variety of therapeutic agents. Therefore, it is of interest to develop positron emission tomography (PET) ligands for these receptors. PET is the method of choice for non-invasive *in vivo* imaging of molecular targets. To date, most successful PET receptor ligands have been antagonists, likely due to their favourable pharmacological, physicochemical and/or pharmacokinetic properties. Nevertheless, agonist PET ligands are of potential interest, as they could provide differential insight in receptor function and regulation. In contrast to antagonist PET ligands that bind to the total pool of a 7-TMR, agonist PET ligands specifically bind to the functionally active 7-TMR. In this research we present a review of literature on agonist PET ligands.

Results One of the first successful PET ligands was the opioid OP₃ receptor agonist [¹¹C]carfentanil [1]. Recently, interest in the development and study of agonist PET ligands has been boosted by work performed on dopamine (D_{2/3}) [2] and serotonin (5-HT_{1A}) receptors [3]. These specific agonist PET ligands were successfully used to determine *in vivo* receptor occupancy and to assess sensitivity to changes in endogenous ligand concentrations, allowing for the assessment of neurotransmitter release. A comprehensive overview is provided of agonist PET ligands for 7-TMRs that (1) have

been labelled with carbon-11 or fluorine-18 and (2) have been evaluated for imaging 7-TMRs in the brain by PET. For each of the 7-TMRs described, a summary is provided on its biological role, together with a critical assessment of physicochemical and pharmacological properties of corresponding agonist PET ligands. Specific information on receptor function obtained with agonist ligands and advantages of agonist over antagonist ligands are provided.

Conclusion Agonist PET ligands for dopamine D_{2/3}, 5-HT_{1A}, 5-HT_{2A} and OP₃ receptors have successfully been developed and used. Studies, in particular those using D_{2/3} agonist PET ligands, clearly have shown that agonist PET ligands can provide differential information with regard to the receptor population being imaged (i.e. active state rather than total pool).

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USE OF GLITAZONES AND THE RISK OF ELECTIVE HIP OR KNEE REPLACEMENT: A POPULATION BASED CASE-CONTROL STUDY.

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Introduction: Osteoarthritis (OA) is the most common musculoskeletal condition in the elderly population. However, to date, no disease modifying drug exists for this disease. In vivo studies have shown that glitazones may be used as anti-arthritic drugs. (Kobayashi, 2005; Boileau, 2007). Therefore, we aimed to determine the risk of total joint replacement (TJR), as a proxy for severe OA, with the use of glitazones.

Methods: A population based case-control study was performed using the Clinical Practice Research Datalink (CPRD). Cases (n=94,609) were defined as patients >18 years

of age who had undergone TJR surgery between 2000 and 2012. Controls were matched by age, gender and general practice. Conditional logistic regression was used to estimate the risk of total knee (TKR) and total hip replacement (THR) associated with use of glitazones. We additionally evaluated risk of TJR in current glitazone users compared to DM patients using other antidiabetic drugs (ADs). In order to determine a dose effect relationship, we also stratified glitazone users by total number of prescriptions prior to surgery.

Results: There is no difference in risk of TKR (OR=1.11 (95% CI=0.95-1.29)) or THR (OR=0.87 (95% CI=0.74-1.02)) between glitazone users and patients not using glitazones. Furthermore, there is no difference in risk of TKR (OR=1.03 (95% CI=0.88-1.22)) and THR (OR=0.90 (95% CI=0.75-1.08)) when glitazones users are compared to other AD users. Finally, we did not find a dose response effect with increasing number of prescriptions.

Conclusion: This study did not find any evidence for an anti-arthritic effect of glitazones.

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