IMPROVING THE TOLERABILITY OF OSIMERTINIB BY IDENTIFYING ITS TOXIC LIMIT

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Background: Osimertinib is the cornerstone in the treatment of epidermal growth factor receptor mutated non-small cell lung cancer (NSCLC). Nonetheless, ±25% of patients experience severe treatment-related toxicities. Currently, it is impossible to identify patients at risk of severe toxicity beforehand. We hence aimed to study the relationship between osimertinib exposure and severe toxicity, and to identify a safe toxic limit for a preventive dose reduction.

Methods: In this real-life cohort study, patients with NSCLC treated with osimertinib were prospectively followed for severe toxicity (grade ≥3 toxicity, dose reduction or discontinuation, hospital admission, or treatment termination), progression-free (PFS) and overall survival (OS). Blood for pharmacokinetic (PK) analyses was withdrawn during every out-patient visit. To quantify individual exposure to osimertinib, a population-PK model was developed. Time to event analysis were performed using univariate and multivariate Cox proportional-hazard analysis and the Fine & Gray competing risk model. Primary endpoint was the correlation between osimertinib clearance (exposure) and severe toxicity to define a toxic limit

Results: In total, 819 samples from 159 patients were included in the analysis with median follow-up of 11.5 months for pharmacokinetic analysis and 10.3 months for severe toxicity. A one-compartment model including inter-individual variability in clearance, with CRP, thrombocyte count, haemoglobin and alkaline phosphatase as covariates explaining variability in clearance best described osimertinib pharmacokinetics. Multivariate competing risk analysis showed osimertinib clearance (c.q. exposure) to be significantly correlated with severe toxicity (HR 0.91, 95% CI 0.83 – 0.99). An ROC-curve showed the optimal toxic limit to be 259 ng/mL osimertinib. This target concentration divides the cohort in two groups: the risk of severe toxicity in the >259 ng/mL group is 34% versus 14% in the <259 ng/mL group. A 50% dose reduction in the high-exposure group - i.e. 25.8% of the total cohort - would reduce the risk of severe toxicity by 53%. Correlation of the first plasma trough concentrations in collected in the first two months of treatment revealed a similar difference in severe toxicity (31% versus 17%), when dividing the cohort in two by the toxic limit of 259 ng/mL osimertinib. Additionally, out of the 21 patients who were dose reduced to 40 mg QD in this study, only three (14%) experienced re-occurrence of severe osimertinib toxicity. Osimertinib exposure was not associated with PFS nor OS.

Conclusion: Osimertinib exposure is highly correlated with occurrence of severe toxicity. To optimize tolerability, patients above the toxic limit concentration of 259 ng/mL could benefit from a preventive dose reduction, without fear for diminished effectiveness.

NOVEL TEACHING RESOURCES FOR THE EUROPEAN OPEN PLATFORM FOR PRESCRIBING EDUCATION (EUROP^{2}E) – A NOMINAL GROUP TECHNIQUE STUDY

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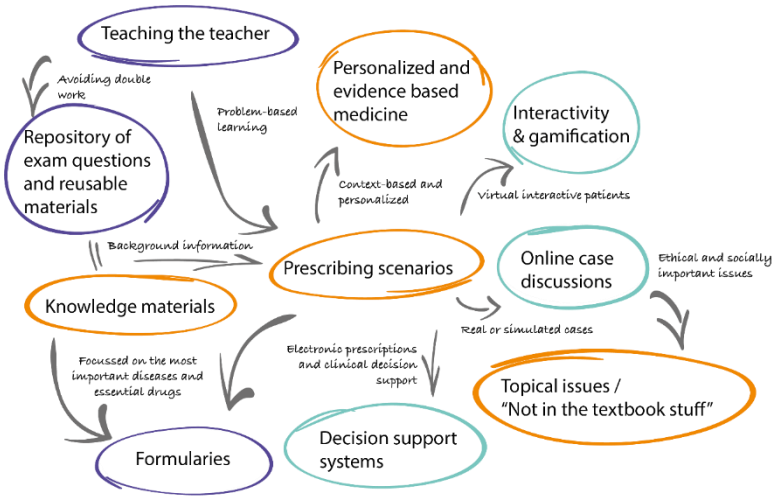
Organisations Amsterdam UMC, VUmc and the EACPT Education Working Group's EurOP^{2}E Consortium

Background The European Open Platform for Prescribing Education (EurOP^{2}E) aims to improve and harmonize European clinical pharmacology and therapeutics education by facilitating international collaboration and sharing open educational resources. The COVID-19 pandemic has forced teachers to switch to online teaching, highlighting the need for high-quality online teaching materials. The goal of this study was to establish the resources needed to sustain prescribing education during the pandemic and thereafter.

Methods A nominal group technique study was conducted with prescribing teachers from several European countries and combined with thematic analysis.

Results In four meetings, 20 teachers from 15 countries ranked 35 teaching materials. Ten themes were identified: prescribing scenarios; interactivity & gamification; re-usable materials; online case discussions; practical aspects of prescribing; teaching the teacher; knowledge multimedia; topical issues; personalized & evidence-based prescribing; and essential formularies.

Discussion/Conclusion By making teaching materials related to the learning outcomes of CPT, format of teaching and resource and faculty development openly available, EurOP^{2}E will help to make high-quality prescribing education available to all. The role of the platform will range from facilitating collaboration to educating the teachers and/or providing ready-to-use teaching materials.



CALCIUM CHANNEL BLOCKER AS A RISK FACTOR FOR PERIOPERATIVE DECLINE IN RIGHT VENTRICULAR EJECTION FRACTION IN CARDIAC SURGERY PATIENTS

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Background: A decline in Pulmonary artery catheter (PAC)-derived right ventricular ejection fraction (RVEF) is associated with both morbidity and long-term mortality after cardiac surgery.[1] Previous studies were not designed to find perioperative risk factors for developing right ventricular dysfunction. Aim of this study was to identify such risk factors.

Methods: We performed a prospective observational single centre study during a 2-year period. By protocol, all valve surgery patients and patients with a poor left ventricular (LV) function were monitored with a dedicated PAC-based computer (Vigilance II®, Edwards, USA). The following parameters were collected from the patient data monitoring system (Epic®, USA): baseline characteristics, preoperative use of cardiovascular medication, pre- and postoperative hemodynamic variables and intraoperative characteristics. For analysis patients were divided into three groups: patients with a ≥3% absolute increase in postoperative RVEF in comparison to baseline (RVEF+), patients with a ≥3% absolute decrease in postoperative RVEF (RVEF-) and patients with a <3% absolute change in postoperative RVEF (RVEF=). All parameters with a p-value ≤0.25 in the univariate analysis were included in multivariate analysis.

Results: 267 patients were included in this study. Median age was 70 (IQR 63-77], 65.5% of patients was male. Most patients underwent valve repair/replacement (42.3%) or a combination of valve surgery with CABG (37.8%). 107 patients (40%) qualified for the RVEF- group, 64 patients (24%) for the RVEF= group and 96 patients (36%) for the RVEF+ group. Based on the univariate analysis, sex, preoperative use of a calcium channel blocker (CCB), central venous peak pressure, perioperative fluid balance (FB), preoperative SvO_2, preoperative echocardiographic LV function and PAC-derived preoperative RVEF were included in the multivariate analysis. In the model patients with RVEF- were compared with RVEF= (first mentioned OR) and RVEF+ (second mentioned OR). Preoperative use of CCB (OR 3.06, 95% CI 1.24-7.54 / OR 2.73, 95% CI 1.22-6.16 (both p=0.015)), perioperative FB (OR 1.45, 95% CI 1.02-2.06 (p=0.039) / OR 1.09, 95% CI 0.80-1.49 (p=0.575)) and baseline RVEF (OR 1.22; 95% CI 1.14-1.30 / OR 1.27, 95% CI 1.19-1.35 (both p<0.001)) were identified as independent risk factors for a decline in RVEF during surgery.

Discussion/Conclusion: Apart from the impact of the perioperative FB, the acknowledgement of preoperative use of a CCB as a risk factor for perioperative reduction in RVEF is the most prominent new finding of this study. The observed association with baseline RVEF is most likely attributable to a regression-to-mean effect. We suggest further research to the potential interaction between CCB and RV function.

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USTEKINUMAB TROUGH CONCENTRATIONS ASSOCIATED WITH BIOCHEMICAL OUTCOMES IN PATIENTS WITH CROHN'S DIEASE

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Background
Ustekinumab (UST) is a monoclonal antibody which binds to the p40 subunit of interleukins-12/23 and is a treatment for patients with Crohn's disease (CD). It is currently unknown if therapeutic drug monitoring is of additional value in UST treatment. We assessed the exposure-biochemical response relationship of UST trough concentrations at week 8 in a prospective, real-world setting.

Methods
We performed a prospective study in 90 CD patients with active disease. Blood for drug levels were drawn at different time points during follow up (median follow up: 52 weeks (IQR 50-52)) with a median amount of 2 measurements (IQR 1-4) per patient. Plasma concentrations of UST were determined by means of a validated enzyme-linked immune assay. A population pharmacokinetic (PK) model was developed based on these measurements and the UST FDA review documents. Subsequently, the individual UST concentration time course during treatment were predicted using the developed model. Corticosteroid-free clinical remission and biochemical remission were assessed at week 12 and 24. Quartile analysis and logistic regression was performed to analyse if UST concentration at week 8 was associated with remission rates at week 24. An independent cohort of 34 patients was used to validate the outcomes.

Results
Median estimated trough concentrations of UST were 4.23 µg/mL (IQR 2.79–5.83) and 7.19 µg/mL (IQR 3.30–10.67) at week 8 in the primary and validation cohort respectively. Patients achieving biochemical remission at week 12 and 24 had statistically significantly higher UST levels at week 8 (P<0.01). Also, higher UST levels at week 8 were associated with better biochemical remission rates at week 12 and 24 in quartile analysis and logistic regression (fig 1). Associations of UST levels at week 8 and biochemical remission at week 12 and 24 were confirmed in the validation cohort. No UST antibodies were detected.

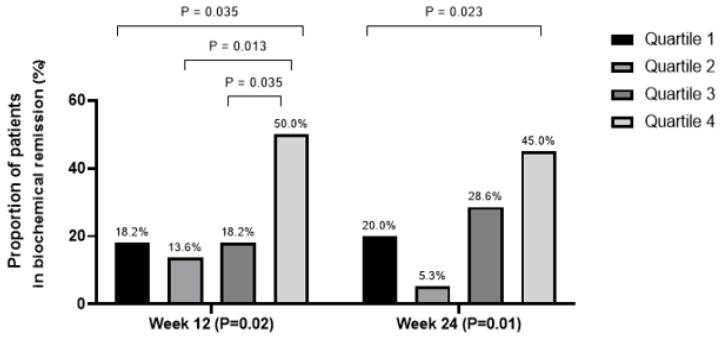

Figure 1. Quartile analysis depicting the exposure-response relationship between week 8 ustekinumab serum levels and biochemical remission rates at week 12 and 24.
Quartile 1: ≤2.8 µg/mL, quartile 2: 2.9 µg/mL – 4.2 µg/mL, quartile 3: 4.3 µg/mL – 5.8 µg/mL, quartile 4: ≥5.9 µg/mL

Discussion/Conclusion
In this real-world cohort of CD patients, ustekinumab levels of ≥5.9 µg/mL at week 8 were associated with higher biochemical remission rates at week 12 and 24.

INCREASING THE EXPOSURE OF KINASE INHIBITORS WITH RITONAVIR – A PROOF-OF-CONCEPT STUDY WITH ERLOTINIB

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Introduction: The high costs and frequent use of kinase inhibitors (KIs) in the treatment of cancer have a large impact on the healthcare budget. This warrants for effective strategies to improve dosing of these drugs. One way to increase the exposure to a drug, without increasing its dose is to inhibit its metabolic enzymes. For many KIs the cytochrome P450 (CYP)3A4 enzyme is the main enzyme responsible for its metabolism. Erlotinib is a KI which is approved for the treatment of non-small cell lung cancer (NSCLC) and pancreatic cancer in a dosing regimen of 150 mg once daily (QD). Erlotinib is mainly metabolized (~70%) by CYP3A4 to its active metabolites OSI-413 and OSI-420. Concomitant intake with ritonavir - a strong CYP3A4 inhibitor – might thus be able to boost the erlotinib concentration.

Aim: To investigate if the dose of erlotinib can be reduced by co-administration with ritonavir and to provide a rational for ritonavir-boosting for other expensive therapies.

Methods: An open-label, cross-over proof-of concept study was performed, comparing the pharmacokinetics of monotherapy erlotinib 150 mg once daily (control treatment) with erlotinib 75 mg once daily plus ritonavir 200 mg once daily (intervention treatment). The pharmacokinetic profiles at steady state of both arms were examined up to 24h after erlotinib administration. The toxicity profile of both dosing arms were compared.

Results: A total of nine patients were evaluable in this study. During erlotinib monotherapy, the systemic exposure over 24h ($AUC_{0\text{-}24h}$), the maximum plasma concentration (Cmax) and the minimal plasma concentration (Cmin) of erlotinib were 29.3 µg*h/mL (coefficient of variation (CV) 58%), 1.84 µg/mL (CV 60%) and 1.00 µg/mL (CV 62%), respectively. After intervention treatment, the $AUC_{0\text{-}24h}$ of 28.9 µg*h/mL (CV 116%, $p = 0.545$), Cmax of 1.68 µg/mL (CV 68%, $p = 0.500$) and Cmin of 1.06 µg/mL (CV 165%, $p = 0.150$) did not differ significantly with the control treatment. For the metabolites of erlotinib, statistically significant lower $AUC_{0\text{-}24h}$ were observed following the erlotinib plus ritonavir dosing ($p = 0.020$ and $p = 0.027$ for OSI-413 and OSI-420, respectively). For both dosing strategies similar safety results were found, with no grade 3 or higher adverse event reported with either treatment regime.

Conclusions: The pharmacokinetic exposure of 75 mg QD erlotinib plus 200 mg QD ritonavir is similar to 150 mg QD erlotinib. These results indicate that erlotinib exposure-boosting with ritonavir is feasible and results in a reduction of erlotinib treatment costs. In addition, it might be a promising strategy for other expensive CYP3A4 metabolized therapies.

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CAPILLARY BLOOD SAMPLING FOR THE DETERMINATION OF CLOZAPINE CONCENTRATIONS IN PSYCHIATRIC PATIENTS: ANALYTICAL VALIDATION AND PATIENT EXPERIENCES

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Background: Frequent blood tests, including Therapeutic Drug Monitoring (TDM), are a barrier to start or continue clozapine therapy for both psychiatric patients and prescribers. Capillary blood sampling based on a fingerprick, instead of venepuncture, seems to be preferred by patients and might help overcome this barrier. Therefore, the primary objective of this research is to determine if clozapine concentrations in blood collected with capillary blood sampling in the novel sampling device Hem-Col are equal to regular venepuncture. The secondary objective is to evaluate the patient experience with both methods.

Methods: This is an observational, prospective study. In- and outpatients from Reinier van Arkel mental health institute requiring regular clozapine concentration follow up were included in this study. Paired capillary and venous samples were collected. Passing-Bablok regression was performed for method comparison.
Patients were asked to rate the following items using a Visual Analogue Scale (VAS):
1) pain *(0=no pain - 0=unbearable pain)*,
2) experience *(0=pleasant - 10=very unpleasant)* and
3) preference for blood sampling method *(0=capillary blood sampling - 5=no preference - 10=venepuncture)*.

Results
Passing-Bablok analysis on 40 paired samples showed no difference between capillary and venous samples (slope 0.96 [95%-CI: 0.88-1.04], intercept -6.86 [95%-CI: -30.75-24.13]. This demonstrates that clozapine concentrations in blood obtained by venepuncture and capillary blood sampling are statistically equal.
The questionnaire was completed by 20 patients (45% outpatients, 55% inpatients). The average *pain score* for the capillary blood sampling is significantly higher than venepuncture in inpatients (6.1; SD 2.6 versus 3.3; SD 2.3; p=0.042). For outpatients there is no significant difference (2.8; SD 2.0 versus 2.6; SD 1.3; p=0.670). Concerning the *experience score*, capillary blood sampling is experienced significantly less pleasant than venepuncture by inpatients (6.9; SD 2.0 versus 3.2; SD 2.6; p=0.017). This difference is not seen in outpatients (3.2; SD 3.2 versus 2.0; SD 1.2; p=0.340). As regards to the *preference score*; inpatients significantly prefer venepuncture over capillary blood sampling (7.6; SD 2.6, p=0,023). Outpatient have no preference for blood sampling method (5.4; SD 3.5; p=0,672).

Discussion/Conclusion
Capillary blood sampling in the Hem-Col tube with a fingerprick can be used for measurement of clozapine concentrations. In contrast to outpatients, inpatients prefer venous over capillary blood sampling. The latter method could be a good alternative for outpatients and contributes to the freedom of choice with regard to blood sampling method.

IMPROVED ANTITUMOR EFFECT OF THE COMBINATION OF TAXANE AND DAROLUTAMIDE IN PROSTATE CANCER IS NOT AFFECTED BY PK

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Background Taxane efficacy in metastatic prostate cancer patients is limited due to resistance development. Preclinical data show that persistent androgen receptor (AR) pathway activity causes taxane resistance, while addition of the androgen receptor signaling inhibitor enzalutamide improves cabazitaxel efficacy. (Mout et al. EBioMed 2021) However, in clinical practice, enzalutamide causes a significant reduction of cabazitaxel plasma levels due to CYP3A4 induction. (Belderbos et al. CCR 2018) In contrast, darolutamide is not a strong inducer of CYP3A4. Therefore we aimed to investigate the preclinical efficacy of combination therapy with taxanes and darolutamide in patient derived xenograft (PDX) derived organoids and, subsequently, study the potential of pharmacological interaction of the combination of cabazitaxel and darolutamide in patients.

Methods Organoids were generated from AR positive, androgen sensitive PDXs and treated with dose ranges of docetaxel or cabazitaxel with and without darolutamide. Cell viability was assessed after 10 days of treatment using Cell Titer Glo 3D. Differences in absolute IC50 values were tested by a t-test after non-linear regression analysis.

For investigation of the pharmacological interaction, mCRPC patients were enrolled on cabazitaxel monotherapy (20 mg/m^2 Q3W) and received concomitant darolutamide (600 mg b.i.d.) from day 2 onwards for maximal 12 weeks. During infusion on day 1, and after 6 and 12 weeks of darolutamide treatment, cabazitaxel systemic exposure was measured via Area Under the Curve from 0 to 24 hours ($AUC_{0\text{-}24h}$).

Results Combination treatment of PC346C (AR+, ARV-) organoids resulted in diminished cell viability compared to cabazitaxel monotherapy (IC50 = 0.21 nM versus IC50 = 0.82 nM, p=.006). In addition, in VCaP (AR++, ARV+) organoids, combination treatment with docetaxel and darolutamide lead to diminished cell viability compared to docetaxel monotherapy (IC50 = 0.04 nM versus IC50 = 0.69 nM, p<.0001)

Cabazitaxel systemic exposure in 18 patients after 6 weeks of darolutamide was not significantly different compared to prior to darolutamide treatment ($AUC_{0\text{-}24h}$: -4%; 95%CI -19 – +13%; p = 0.58). Also, after 12 weeks of darolutamide treatment, cabazitaxel systemic exposure was unaltered ($AUC_{0\text{-}24h}$: +4%; 95%CI -10 – +20%; p = 0.54).

Conclusion In AR positive, androgen responsive organoids, taxane treatment plus darolutamide is more effective than taxane monotherapy. From a pharmacokinetic perspective cabazitaxel and darolutamide do not have an interaction. Our findings pave the way for testing the efficacy of this promising combination in an era of combination regimens for prostate cancer.

MORPHINE RECEPTOR OCCUPANCY BASED ON AGE, GENDER, AND CHRONIC MORPHINE TREATMENT CONDITIONS IN RATS

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Background Chronic pain is impacting quality of life of 20% Europeans with inadequate pain relief in ~60% [1,2], with an annual economic burden of approximately € 300 billion [3]. The EU consortium QSPainRelief is developing an in-silico model platform that will predict optimal combinations of existing CNS active drugs to enhance analgesia and reduce side-effects for stratified chronic pain patient groups. A strong correlation exists between mu-opioid receptor occupancy (RO) and analgesic effects [8]. Indeed, receptor binding kinetics (BK) plays a key role in the interaction between the receptor and drug and may alter the magnitude and duration of therapeutic effects and side effects [5]. Importantly, multiple studies found gender and age differences in mu-opioid receptor expression [6] [7], which may affect RO and therefore analgesic effects. We investigated these factors for most used opioid morphine in rats, and will be translated to humans in future.

Methods: We define a differential equation-based model for BK of morphine to the mu-opioid receptor. Morphine concentrations were simulated using the LeiCNSPK3.0 physiologically based pharmacokinetic model [9,10]. Literature data was used to select morphine association and dissociation rate constants (K_{on}, K_{off}) [11] for the mu-opioid receptor. Mu-opioid receptor expression in different brain regions of rats, along with role of age, gender, and long-term morphine induced changes were studied alone and in combination to determine the impact of these factors on RO.

Results: We find that K_{on} is the major determinant for morphine RO, in line with the earlier findings [13-16]. Evaluation of the impact of identified variability in receptor expression due to gender (-10 to +65%), chronic morphine treatment (- 47% to +28%), or age (15-70%) did not result in RO differences, either alone or in combination.

Discussion/Conclusion: Changes in receptor expression caused by age, gender, and long-term morphine treatment in rats were too small to result relevant RO differences. This finding is contradicted by Positron Emission Tomography (PET) studies in humans which reports RO differences of the opioid ^{11}C-carfentanil [7]. The current simulations were performed assuming no role of endogenous ligands, receptor turnover and non-specific binding to brain tissue. Based on the simulation results, we hypothesize that endogenous ligands concentration and/or receptor turnover may explain this difference and will be considered as a next step in our analyses.

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PREDICTIVE PERFORMANCE OF PHARMACOKINETIC-GUIDED PROPHYLACTIC DOSING OF FACTOR CONCENTRATES IN HEMOPHILIA A AND B

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Background

Pharmacokinetic(PK)-guided dosing is used to individualize factor VIII (FVIII) and factor IX (FIX) therapy in hemophilia prophylaxis. The aim of the study was to investigate the predictive performance of PK-guided prophylactic dosing of factor concentrates in hemophilia A and B patients.

Methods

In this multicenter, prospective cohort study, hemophilia patients of all ages on prophylaxis with standard half-life (SHL) and extended half-life (EHL) factor concentrates received PK-guided dosing. Treating physicians set individual target levels. During 9 months follow-up, at least four measured FVIII/FIX activity levels per patient were compared to corresponding predictions obtained by Bayesian forecasting. Predictive performance was adequate when ≥80% of the measured FVIII/FIX levels were within ±25% of the prediction. Bias and accuracy were calculated using mean error (ME) and mean absolute error (MAE), respectively. During post-hoc analysis, predictive performance was assessed allowing maximal difference of 1 (trough), 5 (mid) and 15 (peak) IU/dL.

Results

Fifty patients were included (median age 19 years [IQR:11-30], 68% hemophilia A). So far, 27 patients completed the study (January 2022); 189 FVIII/FIX levels were available. Median targeted FVIII/FIX trough level was 2 IU/dL [IQR 1-4]. Sixty-six percent of levels (54% trough, 77% mid, 72% peak) were within ±25% of prediction. MAE was 0.7 (95%CI: 0.5-0.9, trough), 2.6 (95%CI: 1.6-3.6, mid) and 10.5 (95%CI: 7.3-13.7, peak) IU/dL. In the post-hoc analysis, 77% (trough), 92% (mid) and 78% (peak) of levels were within set limits. Patients who completed the study had a median number of total and spontaneous bleeds of one (IQR 0-3) and zero (IQR 0-1), respectively.

Discussion/Conclusion

The prespecified predictive performance target was not achieved, particularly due to high measurement error in trough levels. In our opinion, the predictive performance of PK-guidance is better represented by MAE and post-hoc analysis, as these evaluate clinically relevant absolute errors.

USE OF THE LIVERPOOL HIV INTERACTION CHECKER BY HEALTHCARE PROVIDERS FROM THE NETHERLANDS

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Background: Drug interaction management is one of the standard activities of HIV care providers in the Netherlands. Interaction signals with advice are generated when prescribing and dispensing HIV medication, and healthcare providers can check) in advance for drug-drug interactions (DDIs using various sources. A frequently used website is the HIV Interaction Checker from the University of Liverpool (www.hiv-druginteractions.org). However, it is unknown how much use is made of this tool in the Netherlands, and which drug combinations lead to most concerns. The aim of this study was to analyse usage data of the HIV Interaction Checker by Dutch healthcare providers.

Methods: Usage data was requested from the University of Liverpool via the Mixpanel program and Google Analytics from the period December 1, 2020 to December 1, 2021. From the IP address it could be recorded from which region in the Netherlands the user came from. Although the website is freely accessible in the public domain, for this analysis we assume that mainly health care providers are the users of the website. The number of persons living with HIV (PLWHIV) per province were derived from the annual report of the HIV Monitoring Foundation.

Results: An average (range) 2,769 (2,317-3,585) requests for interaction assessments were made per month; only a small proportion went through the mobile app (12.2%). In the 12 months of observation, a total of 12,732 sessions were performed from an IP address that could be related to the Netherlands (note: in one session multiple interaction assessments can be requested).

There was a highly significant correlation between the number of sessions (Y) and the number of PLWHIV per province (X): $R^2 = 0.953$; $p < 0.001$; $Y = 183 + 0{,}412*X$.

The top 10 co-medications searched via the website consisted of (in descending order): pantoprazole, rosuvastatin, omeprazole, dexamethasone, rifampicin, metformin, calcium supplements, atorvastatin, quetiapine, and diclofenac; this is consistent with queries globally.

In 68.0% of the requested drug combinations, there was no DDI; in contrast, 5.5% had a contraindication. In the remainder, a dose adjustment or additional monitoring was required before a combination could be used safely.

Discussion/Conclusion: Healthcare providers from the Netherlands use the Interaction Checker in approximately one in two PLWHIV that are in care at the HIV treatment centres. The fact that approximately 1/3 of the requested combinations requires an action, and 1/20 is a contraindication, shows that the HIV Interaction Checker is an indispensable tool to detect and prevent potentially clinically relevant DDIs in people living with HIV.

SEMI-MECHANISTIC MODELING OF HYPOXANTHINE, XANTHINE AND URIC ACID METABOLISM IN ASPHYXIATED NEONATES

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Background: The ALBINO study[1] investigates the effect of allopurinol in addition to hypothermia (TH) for hypoxic-ischemic encephalopathy (HIE) neonates. Previously, we developed a pharmacokinetics-pharmacodynamics (PK-PD) model of allopurinol, oxypurinol, hypoxanthine, xanthine, and uric acid in this population.[2] High initial hypoxanthine, xanthine and uric acid levels were observed as a result of hypoxia. However, the full dynamics of these biomarkers could not be elucidated since data from asphyxiated neonates without treatment with allopurinol was missing. With additional data from the ALBINO placebo group, the current study aimed to describe the metabolism of purine bases (hypoxanthine, xanthine and uric acid) in HIE neonates.

Methods: Neonates from the ALBINO study who received allopurinol (verum) or mannitol (placebo) were included. An extension of our previous population PK-PD model was provided using nonlinear mixed effects modeling (NONMEM®, v 7.5). The PK part of the model was fixed to previous estimates. [2] The degradation of purine bases via xanthine oxidoreductase (XOR) was described by a turnover of hypoxanthine to xanthine and subsequently to uric acid. The salvage of hypoxanthine to purine nucleotides was included. This salvage pathway was regulated by an empirical compartment representing a finite amount of precursors with a resulting net decline in amount over time. The initial level of hypoxanthine, xanthine and uric acid was a combination of endogenous turnover and high initial disease-related amounts. The effect of allopurinol and oxypurinol on XOR inhibition was described by an E_{max} function.

Results: In total, data from 20 neonates from the allopurinol group and 17 neonates from the mannitol group were included in this analysis. The clearance of hypoxanthine to xanthine and hypoxanthine salvaging was 0.21 and 0.54 1/h, respectively. The clearance of xanthine to uric acid and uric acid elimination was 2.67 and 0.03 1/h, respectively. The baseline production of hypoxanthine and xanthine was 0.53 and 0.22 mg/L*h, with a reduction of 0.4% per hour as a consequence of precursor exhaustion. Half-maximal XOR inhibition was achieved with a combined allopurinol and oxypurinol concentration of 0.68 mg/L, which was below all observed concentrations in the first 72 hours. This suggested almost a full inhibition of XOR during 24 hours post dose.

Conclusion: This extended PK-PD model provided adequate description of the complex purine metabolism in asphyxiated neonates treated with allopurinol. The purine salvage pathway and the impact of hypoxia on hypoxanthine, xanthine and uric acid initial levels were quantified.
Reference: [1] Maiwald et al. 2019 (ALBINO is funded under the Horizon 2020 Framework EU Program, H2020-PHC-2015-two-stage, grant 667224); [2] Chu et al. 2021

RAC1/PSTAT3 EXPRESSION IN T LYMPHOCYTES: A POTENTIAL PHARMACODYNAMIC MARKER FOR THIOPURINES IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE PATIENTS?

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Background

The thiopurines, azathioprine (AZA), mercaptopurine (MP) and tioguanine (TG), still remain standard treatment of Inflammatory Bowel Diseases (IBD). Their immunosuppressive mechanism is primarily based on blocking the Ras-related C3 botulinum toxin substrate 1 (Rac1) causing T-cell apoptosis by inhibition of the phosphorylated downstream transcription factor signal transducer and activator of transcription 3 (pSTAT3). A pharmacodynamic marker in T-cells may be useful to predict therapeutic outcome of thiopurine therapy. The aim of this study was to compare Rac1 and pSTAT3 expression in T lymphocytes of patients on thiopurine monotherapy with the other patient groups and with healthy subjects.

Methods

We evaluated Rac1 and pSTAT3 expression in T-cells in 6 parallel IBD groups: patients with active disease(1) and patients in remission on AZA/MP(2), TG(3), infliximab (IFX)(4), thiopurine and IFX(5) or without medication(6) to assess feasibility. Data of healthy subjects were used as reference values. Rac1 and pSTAT3 expression of patients in remission on AZA/MP or TG were compared with all other IBD groups and healthy subjects.

To investigate possible differences in Rac1 and pSTAT3 expression, unpaired t-tests and Mann-Whitney U tests were used for comparison of normal distributed and non-normal distributed variables, respectively. Because of the Bonferroni-correction for five independent comparisons, a corrected p-value of ≤0.01 was considered statistically significant for these statistical tests.

Results

Absolute Rac1 expression in the IBD patient groups in remission with thiopurine monotherapy on AZA/MP (1.18 arbitrary units (AU), IQR 1.11 – 1.31) or TG (1.36 AU, IQR 1.06 – 1.61) did not differ from Rac1 expression in IBD patients with active disease (0.94 AU, IQR 0.66 – 1.43, p>0.01). A trend in reduction of absolute pSTAT3 expression in the IBD groups in remission with monotherapy AZA/MP (0.86 AU, IQR 072 – 1.24) or TG (0.99 AU, IQR 0.69 – 1.34) was observed compared to IBD patients with active disease (1.50 AU, IQR 1.06 – 1.93, p=0.024 and p=0.054, respectively). Rac1-corrected pSTAT3 expression in IBD patients groups on thiopurine monotherapy AZA/MP (0.66 AU, IQR 0.58 – 0.89) or TG (0.68 AU, IQR 0.58 – 0.86) was reduced compared to IBD patients with active disease (1.33 AU, IQR 0.89 – 1.86, p<0.01).

Conclusion

Rac1-corrected pSTAT3 expression in T-cells was significantly decreased in IBD patients in remission on thiopurine monotherapy compared to IBD patients with active disease, resulting in comparable values to healthy subjects. Rac1-corrected pSTAT3 expression may therefore serve as a class specific pharmacodynamic marker to assess therapeutic efficacy of thiopurine monotherapy or as a marker to explain thiopurine resistance.

EUROPEAN LIST OF ESSENTIAL MEDICINES FOR MEDICAL EDUCATION: A MODIFIED DELPHI STUDY

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Background
Having sufficient prescribing knowledge and skills is essential to prescribe safely and effectively in clinical practice. However, due to the expanding drug arsenal, and increasing amount of patients with polypharmacy, prescribing medicines has become an increasingly complex task. To prepare junior doctors for this difficult task, (inter-)national projects have been developed to improve the undergraduate teaching in clinical pharmacology and therapeutics (CP&T). However, a European list of medicines that junior doctors should be able to independently prescribe safely and effectively without direct supervision is lacking. Such a list can be used for the European Prescribing Exam (EuroPE^{+}) and could also form the basis for country specific lists with the aim to harmonize the teaching in CP&T in Europe. Therefore, the aim was to reach consensus on a list of medicines that are widely prescribed and available in Europe and which junior doctors working in Europe should be able to prescribe safely and effectively without direct supervision.

Methods

This is a modified Delphi study to research consensus among CPT teachers, medical specialists, pharmacists and junior doctors working in Europe. In the first part, an extensive list of available medicines (≥80% of the European countries) was compiled. In the second part, two Delphi rounds were carried out. In each round the participants had to indicate whether a medicine should be included in the final list (5-point Likert scale). Medicines on which ≥80% of all respondents agreed or strongly agreed were included in the final list.

Results

In total, 187 (41%) participants with a diverse background and from 24 European countries completed the study. Of the 416 medicines on the initial list, a total of 98 medicines are included in the final list.

Discussion/Conclusion

This is the first Delphi consensus study among European experts working in varies fields to form a list of medicines that junior doctors should be able to prescribe safely and effectively without direct supervision. The European List of Essential Medicines for Medical Education contains 98 medicines. The list will be an extra step towards harmonization of CP&T education in Europe.

EFFECTIVENESS AND TOLERABILITY OF ORAL VERSUS SUBCUTANEOUS METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction
Although parenterally administered methotrexate (MTX) seems to have a favorable pharmacokinetic profile and possibly better efficacy compared to oral MTX in patients with rheumatoid arthritis (RA), this is based on limited and mostly low-quality trial data (Bujor et al, 2019), and effects of dosages higher than 15mg, strategy effects using a treat-to-target approach and DMARD comedication have not been researched. The aim of this study was to compare the effectiveness and tolerability of a strategy starting with oral versus subcutaneous MTX in a large group of patients with RA in a real-life setting.

Methods
This was a retrospective cohort study. Patients with a diagnosis of RA who started with either oral or subcutaneous MTX treatment at a dose of at least 15mg/week (with or without hydroxochloroquine [HCQ]) were included. The primary outcome was between group difference in ΔDAS28CRP after 3-6 months treatment compared to baseline. In case of non-superiority, this was followed by non-inferiority analysis (NI margin 0.6). Secondary outcomes included differences in adverse events, MTX dose, proportion of patients stopping treatment and comedication.

Results
In total, 640 patients were included of whom 259 started with oral and 381 with subcutaneous MTX (75% also with HCQ). There was no significant between group difference in ΔDAS28CRP after adjusting for confounding, 0.13 (95%CI -0.14 to 0.40), and indeed oral MTX strategy was also non-inferior to subcutaneous strategy. Mean MTX dose was lower in the oral strategy group (18.0 vs. 19.9mg, p=0.002), and was accompanied by lower cumulative incidence of adverse events (41 vs. 52%, p=0.005). The number of patients stopping with oral MTX during follow up was not significantly different than those stopping subcutaneous MTX (8.1 vs 12.3%, p=0.09). There was no difference in use of comedication.

Conclusion
To start with oral MTX up to 25mg in patients with RA using a treat-to-target approach and combination treatment is non-inferior to subcutaneous treatment regarding control of disease activity. As the tolerability of oral MTX was also better, this supports a strategy of starting with oral MTX.

References
Bujor et al. Comparison of oral versus parenteral methotrexate in the treatment of rheumatoid arthritis: A meta-analysis. PLoS One. 2019;14(9):e0221823.

ADAVOSERTIB IN COMBINATION WITH CARBOPLATIN IN ADVANCED *TP53* MUTATED PLATINUM RESISTANT OVARIAN CANCER

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Background

Ovarian cancer is globally the second most common cause of death among women with gynecologic malignancies. Despite high initial response rates, the overall prognosis of this patient population remains poor. The majority of advanced ovarian cancers will become platinum resistant defined by recurrence within six months after completion of platinum therapy. In the first part of the current phase II study, the combination of carboplatin and the Wee1 inhibitor adavosertib (AZD1775), showed to be safe and effective in patients with *TP53* mutated platinum resistant ovarian cancer. The aim of this additional cohort is to gain more information about the safety and efficacy of the combination and to explore predictive biomarkers for resistance and response to adavosertib.

Methods

In this additional cohort 29 evaluable were treated with carboplatin AUC 5 mg/ ml·min and adavosertib 225 mg BID for 2.5 days in a 21-day cycle. The anti-tumor activity was assessed according to RECIST 1.1.

Pre-, on- and post-treatment biopsies were obtained to explore genetic determinants of drug resistance and response to adavosertib.

Results

A total of 32 patients with a median age of 62 years (39-77 years) were enrolled in this cohort. All patients had carboplatin/paclitaxel as first line therapy. Six patients received a second-line non-platinum containing regimen. Median platinum free interval was 5.8 months (range 1.7 – 11.9). Twenty-nine patients were evaluable for efficacy. Grade 1-2 bone marrow toxicity, nausea, vomiting and fatigue were the most common adverse events. Twelve patients showed PR as best response, resulting in an ORR of 38% (95% CI 21%-56%). The median PFS was 5.6 months (range 1.1-32 months, 95% CI 4.2-7.0) and median duration of response was 5.3 months (95% CI 0.13-10.5).

Discussion/Conclusion

Adavosertib 225 mg BID for 2.5 days and carboplatin AUC 5 in a 21-day cycle could be safely combined and shows promising anti-tumor efficacy in patients with platinum resistant ovarian cancer. Bone marrow toxicity remains the most common reason for dose reductions and dose delays. Translational biomarker results (CCNE1 analysis as potential predictive marker for response and resistance and WGS) to better understand the anti-tumor activity of the combination are pending.

HIGH-DOSE MTX INTOXICATION TREATED WITH DOSE-CAPPED GLUCARPIDASE

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Background: High dose methotrexate (HDMTX) is an essential part of pediatric oncology treatment. HDMTX-associated acute kidney injury due to delayed MTX clearance is a serious toxicity and linked to an excess in MTX induced toxicities. Glucarpidase is a recombinant enzyme that rapidly hydrolyzes MTX into two non-toxic metabolites, DAMPA and glutamic acid. The recommended dose is 50 IE/kg, however no formal dose-finding studies were performed as part of the authorization application (FDA authorization 2012; EMA authorization jan 2022). In our institution patients are treated with a capped dose of 1000 IE, resulting in doses <50 IE/kg. The enzyme activity of glucarpidase together with several case reports highly suggest that lower doses of glucarpidase might be equally effective in lowering MTX levels. Here we assessed the effect of dose-capped dosing of glucarpidase on MTX levels and kidney function.

Methods: Twelve patients (the majority of which were leukemia patients, HDMTX 5 g/m^2 in 24 hours) with toxic MTX levels following HDMTX were treated with glucarpidase 1000 IE (median 25 IE/kg, range 13-53 IE/kg). Creatinine levels together with MTX levels (immunoassay) prior and post (≥ 48 hours) glucarpidase administration were retrospectively assessed.

Results: All patients experienced HDMTX associated acute kidney injury (median increase in creatinine levels at 48 hours after HDMTX administration compared to baseline of 311 %, range 144-623 %) and showed toxic MTX levels (median 15 µmol/L range 8,3-140 µmol/L) before glucarpidase administration. Glucarpidase was administered 41-54 hours (median 50 hours) after HDMTX initiation. MTX levels decreased to levels < 0,25 µmol/L by 216 hours (range 209-253 hours) after HDMTX start (i.e. 169 hours after glucarpidase administration, range 156-212 hours). Creatinine levels were ≤ 1,5 times baseline value within 223 hours (range 164-356 hours).

Discussion/Conclusion: For glucarpidase there are no data that allow for an assessment of the relationship between exposure and efficacy of MTX conversion. As such the minimal effective dose is unknown. Based upon in vitro enzyme activity (1 unit of glucarpidase activity catalyses the hydrolysis of 1 µmol of MTX in 1 ml in 1 minute) the recommended dose seems to be more than sufficient even taking into account redistribution of MTX. Our patients were all treated with 1000 IE and time to MTX < 0,25 µmol/L was comparable between patients. MTX levels were measured by immunoassay which does not allow for a distinction between DAMPA and MTX. Alternative approaches to monitor MTX concentrations after glucarpidase administration, such as HPLC, are required in order to better evaluate the effectiveness of glucarpidase at lower doses than recommended. Glucarpidase is an expensive drug. By limiting the dose to 1000 IE we required 20 vials less than otherwise required (savings 460.000 €).

THE CORTISOL GENE SENSITIVITY RESPONSE OF HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED ENDOTHELIAL CELLS TO STUDY CENTRAL SEROUS CHORIORETINOPATHY

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Background

Both endogenous and external use of corticosteroids are the major risk factors in central serous chorioretinopathy (CSC), which is a common posterior eye disease with high male predisposition. However, the pathogenesis of CSC is still unclear, including the response of choroidal endothelial cells (CECs) in choroidal vascular to corticosteroids exposure. Here, we aim to assess the cortisol-induced gene sensitivity response of human induced pluripotent cell-derived endothelial cells (hiPS-ECs) from healthy individuals, in comparison with primary choroidal endothelial cells (CECs) from post-mortem donors and primary human umbilical vein endothelial cells (HUVECs).

Methods

We compared the cortisol gene response between endothelial cells (ECs) models: primary CECs, primary HUVECs, and hiPS-ECs. Primary CECs were isolated from human post-mortem donors. The ECs were independently differentiated from healthy male derived hiPS lines and isolated with magnetic beads anti-CD31. The cells were cultured to confluency and treated with a range of cortisol doses (1 nM – 1 µM) for 4 hours. The dose-response curves were generated to measure the cortisol gene responses (*FKBP5*, *PER1*, *ZBTB16*). The sensitivity parameter, EC_{50}, was also determined.

Results

Cortisol target genes expressions were increased in a dose-dependent manner in all EC types. The fold change of *FKBP5* gene expression showed comparable across EC types. In contrast, hiPS-ECs exhibited lower induction of *PER1* and *ZBTB16* than primary ECs. The lower response of hiPS-ECs to cortisol was also manifested in EC_{50} values when compared with primary ECs. Three independent differentiation of hiPS-ECs showed comparable EC_{50} (25,7 nM; 37,3 nM; 36,9 nM, respectively), while primary HUVECs had higher EC_{50} value of 10 nM.

Conclusion

hiPS-ECs from the healthy male can be used as a model to study the endothelial cortisol sensitivity response. However, more hiPS-EC lines from healthy male individuals and CSC patient-derived hiPS-ECs are required to reliably assess the difference in the gene response and sensitivity.

GENE SURGERY AS A POTENTIAL TREATMENT OPTION FOR NEPHROPATHIC CYSTINOSIS

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Background

Cystinosis is a rare, monogenetic disease caused by mutations in the *CTNS* gene. In this project we aim to develop a novel gene repair strategy for the most predominant 57kb deletion of *CTNS* using CRISPR-Cas9 gene-editing technology. We use the homology-independent targeted integration (HITI) approach which allows for the delivery of a large *CTNS* repair template into a specific location in the genome.

Methods

For this study we used conditionally immortalized proximal tubule epithelial cells (ciPTEC). *CTNS*$^{-/-}$ ciPTEC cells have been derived using CRISPR/Cas9. For the delivery of the Cas9-guideRNA ribonucleoprotein (RNP) complex a novel non-viral peptide-mediated delivery system was used. The repair construct for *CTNS* (3.2 kb) contains the *CTNS* promotor and the first 10 exons of the *CTNS* gene, as well as a fluorescent reporter gene (mCherry).

Results

After transfection of the repair construct we achieved a ~5% mCherry positive cell population in *CTNS*$^{-/-}$ ciPTEC, indicating these cells had successfully taken up and inserted the repair template into their genomic DNA. Further analysis of these cells showed a >80% reduction of cystine levels, indicating that in a majority of the cells the *CTNS* function was indeed restored.

Discussion/Conclusion

In conclusion, these preliminary data show that the *CTNS* repair template can be precisely inserted into the genome, leading to the translation of a functional cystinosin transporter, which consequently restores the lysosomal cystine accumulation. Although the efficiency should be improved, eventually this gene repair system may offer a potential curative therapy for cystinosis, as well as a system for the *in vitro* restoration of several other genes involved in monogenic diseases.

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FEASIBILITY OF THERAPEUTIC DRUG MONITORING OF SORAFENIB IN PATIENTS WITH CANCER

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Background

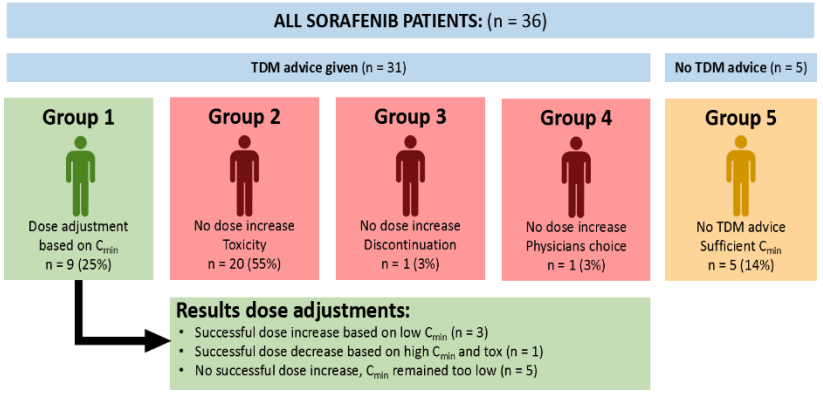
Sorafenib is a tyrosine kinase inhibitor approved for the treatment of renal cell carcinoma, hepatocellular carcinoma and thyroid carcinoma. As interindividual variability in exposure is high, there is a rationale for therapeutic drug monitoring (TDM) of sorafenib. Hence, we investigated the feasibility of therapeutic drug monitoring in a cohort of sorafenib patients, and tried to identify sub-groups in whom pharmacokinetically (PK) guided-dosing might be of added value.

Methods

We investigated the cohort of sorafenib patients included in a large, prospective study (the DPOG-TDM study, NTR: NL6695) on therapeutic drug monitoring of 24 oral targeted antineoplastic agents. Sorafenib plasma trough levels (C_{min}) were measured at pre-specified time-points. A dose escalation was advised if the measured C_{min} was <3750 ng/mL and toxicity was manageable. The target exposure was based on the mean plasma trough level of the approved dose. All patients treated with sorafenib who started with the standard dose of 400 mg BID or who started with a step-up dosing schedule were included.

Results

A total of 150 samples were collected in 36 patients. Thirty-one patients (86%) had a sorafenib exposure below the predefined target at a certain time point during treatment. In 20 patients, the dose could not be escalated because of severe toxicity. In 9 patients, the dose was adjusted based on the TDM advice. In only 4 patients, the dose adjustment was successful. In 5 other patients, the escalation did not result in an exposure above the target exposure. **Figure 1** shows the results of TDM for sorafenib.



Discussion/Conclusion

Unfortunately, TDM for sorafenib is not of added value in daily clinical practice, because in most cases toxicity restricts the possibility for dose escalations. TDM could, however, be valuable for specific sub-groups, such as patients with excessive toxicity, patients with inevitable drug-interactions, or in patients with low exposure at progression of disease. Further research on the use of PK-guided dosing in these subgroups and the confirmation of the target level for sorafenib is required.

ADDRESSING THE IMPACT OF MULTIPLE DOSING AND NONLINEAR BBB TRANSPORT IN MORPHINE CNS DISTIRBUTION

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Background Morphine is a strong analgesic and is often used for chronic pain treatment. Morphine has to cross the blood brain barrier (BBB) to reach its target at the brain extracellular fluid (ECF). It has been known that morphine is passively and actively transported by multiple efflux transporters[1–4] and an unidentified saturable influx transporter[2,5] at lower concentrations at the BBB. The extent of drug distribution across BBB is described by the unbound partition coefficient ($K_{p,uu,BBB}$). The extent of BBB transport is often studied for a single dose administration, without considering the clinically relevant long-term treatment, and often also not in a dose dependent manner. Here, we study how nonlinear active transport of morphine across the BBB affects the morphine distribution in brain ECF during chronic morphine dosing.

Methods A previously developed nonlinear pharmacokinetic model for BBB transport of morphine5 was used to derive Kp,uu,BBB based on unbound steady state concentrations in plasma, which was then incorporated in a physiologically-based pharmacokinetic model for the CNS, the LeiCNS PK3.0 model[6]. Morphine intravenous administrations at low (1mg/kg) and high (10mg/kg) doses and different frequencies (4-6 times a day) were simulated for the rat. To study the effect of multiple dosing and nonlinearity of morphine BBB transport, peak-to-trough ratios were calculated at steady state levels and peak concentrations in plasma and brain ECF were compared.

Results For different chronic dosing regimens, an increase in dosing frequency results in a decrease in peak-to-trough fluctuation in plasma and brain ECF concentrations. In contrast, nonlinear morphine BBB transport makes that the low and high morphine dosing results in different plasma-brain ECF partition coefficient ($K_{p,uu,BBB}$).

Discussion/Conclusion Here we show that multiple dosing and nonlinearity of BBB transport should be taken into account for relevant chronic dosing regimens of morphine. However, to more accurately predict the morphine brain ECF distribution in rats, the low capacity (saturable) influx transporter should be taken into account and understood better. The next step is to use this as translational factor for improved human brain ECF PK predictions. Since morphine brain ECF concentrations are facing the mu-opioid receptor, it is anticipated that steady-state fluctuations in brain ECF will also lead to changes in mu-opioid receptor binding. This is important to be considered for improved understanding of the pharmacokinetic-pharmacodynamic (PK-PD) relationships of morphine.

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A STRATEGY FOR THERAPEUTIC DRUG MONITORING OF VINCRISTINE IN A PAEDIATRIC ONCOLOGY POPULATION.

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Background Vincristine is a well-established chemotherapeutic drug within paediatric oncology. Recent studies have reported ethnic difference in vincristine exposure leading to the hypothesis of sub-therapeutic dosing of vincristine in African children. A strategy for therapeutic drug monitoring (TDM) is necessary to identify sub-therapeutic exposure to vincristine and optimise individual treatment.

Aims We aimed to developed a strategy for TDM of vincristine in children, which met the following criteria: (1) Identify children with low exposure with high sensitivity, (2) function on a limited sampling strategy of three samples, and (3) function with a limited sampling period of 4 hours after administration.

Methods We performed a simulation study to evaluate two strategies for TDM: a Bayesian approach and a pharmacometric nomogram. The pharmacometric nomogram was an empirical approach that included weight bands and ratios of individual plasma concentrations and the population median plasma concentration. A difference in exposure of 20% compared to the median exposure of the population was deemed clinically relevant and was, therefore, used as definition of low exposure. A sampling design with samples collected at 1, 1.5, and 4 hours after administration of a 5 minute push injection was considered clinically feasible. This sampling design was optimized with the above-described criteria taken into account. A sensitivity analysis was performed to investigate the influence of missing samples, erroneous sampling times, and different boundaries for the weight bands.

Results With the Bayesian approach, 56.7% of the estimated exposure values had a prediction error ±20% due to significant shrinkage. The performance of the Bayesian approach did not improve with alternative sampling designs. However, the pharmacometric nomogram was able to identify patients with low vincristine exposure with a sensitivity, specificity, and accuracy of 74.3%, 76.7%, and 75.8%, respectively. The pharmacometric nomogram performed similarly with different weight bands. Missing samples caused a maximal change in sensitivity of -12%. However, sensitivity was always over 50%. Lastly, the samples should be collected within 15, 5, and 5 minute intervals of 1, 1.5, and 4 hours after administration for acceptable performance of the nomogram.

Discussion/Conclusion The pharmacometric nomogram was capable to identifying paediatric patients with low vincristine exposure with high sensitivity, specificity, and accuracy. This pharmacometric nomogram will be used as a TDM strategy for vincristine in children and for dose optimisation in non-Caucasian populations.

QUANTIFYING INDIVIDUAL VARIABILITY IN TELMISARTAN PLASMA EXPOSURE, RECEPTOR OCCUPANCY AND ALBUMINURIA RESPONSE USING [^{11}C]TELMISARTAN PET IMAGING IN T2DM PATIENTS

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Background: Renin-angiotensin system inhibitors (RASi) are recommended for slowing progressive kidney function loss in all patients with diabetic kidney disease. However, the response variability is large and a substantial proportion of patients does not respond to RASi and remains at high risk for kidney failure. Positron emission tomography (PET) imaging allows *in vivo* quantitative assessment of pharmacological processes at kidney tissue level, which may provide insight in underlying mechanisms of response variation. Therefore, we developed [^{11}C]Telmisartan, a PET tracer analog of the angiotensin receptor blocker telmisartan, retaining its original molecular structure. In type 2 diabetes (T2DM) patients, we assessed the relation between plasma telmisartan exposure and urinary albumin creatinine ratio (UACR) response, and whether systemic telmisartan exposure corresponds to kidney exposure using [^{11}C]-Telmisartan PET imaging.

Methods: In 10 T2DM patients, treated 4 weeks with 80mg telmisartan, 9 pharmacokinetic (PK) samples were collected over a 24h period, and change in UACR was calculated from start to end of treatment. In, another, 5 T2DM patients, 2 90-min dynamic PET scans with diagnostic IV [^{11}C]Telmisartan dosing were obtained. 1 Hour before the second PET scan, patients received 20, 80 or 120mg oral telmisartan. After telmisartan administration, 9 PK samples were collected. [^{11}C]Telmisartan kidney distribution was quantified by the apparent volume of distribution (V_t). The difference between V_t of both scans per patient, was used to determine receptor occupancy (RO).

Results: Individual exposure to telmisartan varied largely, with a mean $AUC_{0-\infty}$ of 4204.0 ng·h/mL (95% CI 747-9576 ng·h/mL) and tended to correlate with UACR response (p=0.08). The PET imaging study was discontinued after 5 patients due to difficulties with [^{11}C]Telmisartan synthesis. In 1 patient, the second PET scan was performed without arterial sampling and therefore the RO could not be obtained. In another patient, the V_t of the second PET scan was unexpectedly higher, resulting in a negative RO. 3 Patients received 120mg telmisartan and showed a reduction in V_t of the tracer on the second PET scan, indicating angiotensin receptor blockage by telmisartan (Fig 1). The RO values were 5.5, 44 and 59% and showed a trend towards maximum plasma telmisartan concentrations (15.5, 524 and 323 ng/mL).

Conclusion: Telmisartan plasma exposure varied largely between individuals and tended to correlate with UACR response. In the first feasibility study assessing telmisartan tissue distribution in T2DM patients *in vivo*, we demonstrated a relationship between plasma exposure and RO. A larger study is required to assess if the albuminuria response correlates with intra-renal receptor occupancy of telmisartan.

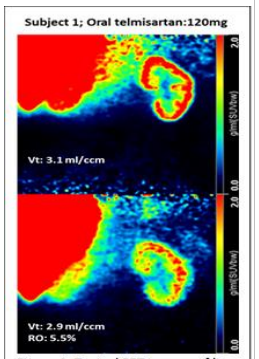

Figure 1. Typical PET images of liver and kidneys of [^{11}C]Telmisartan before and after receptor blocking with 120 mg telmisartan

ASSOCIATION BETWEEN GENETIC VARIANTS AND PERIPHERAL NEUROPATHY IN PATIENTS WITH NSCLC TREATED WITH FIRST-LINE PLATINUM-BASED THERAPY: A CANDIDATE-GENE APPROACH

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling and often long-lasting side effect, which is frequently observed in non-small cell lung cancer (NSCLC) patients treated with platinum-based therapy. There is increasing evidence for associations between single nucleotide polymorphisms (SNPs) and susceptibility to CIPN.
Aim of this study was to further explore genetic risk factors for CIPN by investigating previously reported genetic associations in an independent cohort of NSCLC patients treated with first-line platinum-based chemotherapy.

Methods: A multicentre prospective follow-up study (PGxLUNG study[1], NTR number NL5373610015) in NSCLC patients (stage II-IV) treated with first-line platinum-based (cisplatin or carboplatin) chemotherapy was conducted. Clinical evaluation of neuropathy was performed at baseline, before each cycle of chemotherapy and, at three and six months after treatment initiation, using the CTCAE v4.03 for peripheral sensory neuropathy. SNPs were selected using a candidate-gene approach. The relationship between 35 SNPs in 27 genes and any grade (grade ≥1) and severe (grade ≥2) CIPN was assessed in an univariate and multivariate setting, in both a dominant and recessive model, with logistic regression modelling. Patient and disease characteristics, concomitant chemotherapeutic agents, number of cycles of chemotherapy and performance status were taken into account as potential confounding factors. The false discovery rate was used for correction in multiple testing based on the Benjamini-Hochberg procedure.

Results: In total, 320 patients were included of which 26.3% (n=84) and 8.1% (n=26) experienced any grade and severe CIPN respectively. Median age was 65 years and 10% had diabetes. The GG genotype (rs879207, A>G) of *TRPV1*, a gene expressed in peripheral sensory neurons, was found to be associated with an increased risk of severe neuropathy (OR 5.2, 95%CI 2.1-12.8, adjusted p-value 0.012). The allele frequency for the G-allele was 0.32. In multivariate logistic regression analysis statistically significant associations between concomitant use of paclitaxel (ORadj 7.2, 95%CI 2.5-21.1), the GG genotype of rs879207 (ORadj 4.7, 95%CI 1.8-12.3) and severe CIPN were observed.

Conclusion: The present study suggest that genetic predisposition of *TRPV1 (*rs879207) could be important in the development of severe neuropathy in patients treated with platinum-based therapy. Future studies should be aimed to explore the clinical utility of these findings, which may contribute to individualization of platinum-based therapies.

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THE IDENTIFICATION OF DEAMIDATION AND OXIDATION SENSITIVE SIGNATURE PEPTIDES OF PEMBROLIZUMAB IN THE COMPLEMENTARY BINDING REGIONS AND THE STABILITY OF THESE PEPTIDES *IN VITRO* USING A QUALITATIVE LC-QTOF-MS METHOD

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Background: The monoclonal antibody pembrolizumab is used in the treatment of several cancer types. Chemical degradation of monoclonal antibodies occurs at the amino acid level, but is difficult to detect due to the large size of these drugs. Degradation of amino acids in the complementary determining regions (CDRs) could lead to loss of therapeutic effect due to impaired binding of pembrolizumab to its target PD-1. Our aim was to develop a liquid chromatography-Quadrupole Time-of-Flight mass spectrometry (LC-QTOF-MS) method to detect degradation susceptible pembrolizumab CDR peptides, and to apply this method to pembrolizumab expired reconstituted vials (Keytruda®).

Methods: An Agilent AdvanceBio Peptide Plus column was used with 0,1% (v/v) formic acid in water (eluent A) and in acetonitrile (eluent B). A gradient of 2-40% (v/v) B was reached in 40 minutes. Peptides were detected with QTOF-MS after tryptic digestion of pembrolizumab expired products. Binding capacity of expired vials of reconstituted pembrolizumab was examined with an in-house anti-PD-1 ELISA.

Results: The LC-QTOF-MS method was applied to determine the presence of native and degraded CDR peptides in reconstituted pembrolizumab vials that were kept at 2-8 °C for up to 42 months. Two peptides of pembrolizumab that contain amino acids prone to oxidation or deamidation in the heavy chain CDR were identified and found after tryptic digestion of the expired vials. However, degradation did not seem to increase over storage time. No significant loss of binding capacity was found in the expired vials with ELISA.

Conclusion: An LC-QTOF-MS method to detect native and degraded peptides in pembrolizumab CDRs was developed. Application of this method suggested that these CDRs are chemically stable in reconstituted final product up to 42 months at 2-8 °C, and ELISA experiments showed that these products retain their binding capacity. To confirm these findings, a quantitative method will be developed.

Future: High clearance of pembrolizumab is associated with reduced overall survival in cancer patients. This high clearance is attributed to cancer-associated cachexia mediated protein degradation. An alternative explanation for the decreased detected pembrolizumab concentrations is degradation of amino acids in the CDRs. The presented detection method can be transformed into a quantitative method, and it can then be applied to patient samples to study any degradation of the pembrolizumab CDRs *in vivo*.

EFFECT OF A MITOCHONDRIAL ROS-INHIBITOR ON VASODILATION IN AGED MOUSE AORTA

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Background
Vascular aging, a major cause of morbidity and mortality, is marked by decreased nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling. This is partly caused by increased reactive oxygen species (ROS) levels in endothelial cells (EC), believed to be produced by mitochondria. Here, we investigated the effect of chronic treatment with SUL138, an inhibitor of reverse mitochondrial electron transfer which reduces free electron spill-over, in a model of EC-specific aging. EC-specific deletion of DNA repair endonuclease *Ercc1* in mice (EC-KO) led to accelerated vascular aging features, marked by reduced endothelium-dependent NO-cGMP vasodilation at 22 weeks of age.

Methods
EC-KO mice and corresponding littermates (LM) received either SUL138 treated or vehicle treated chow for 8 weeks (14-22 weeks of age). At the age of 21 weeks, animals were put in metabolic cages, 24 h urine was collected and snap frozen for further experiments. At the age of 22 weeks, mice were euthanized, urine, blood plasma and tissue were collected and snap frozen and albumin and creatinine levels were measured. Thoracic aorta was directly used for wire myograph experiments to assess vascular function.

Results

Endothelium-dependent (ED) relaxation was decreased in EC-KO vehicle compared to LM vehicle treated. Chronic treatment with SUL138 restored ED to LM vehicle treated level, probably driven by increased endothelium derived hyperpolarization in SUL138 treated EC-KO mice.

Vehicle treated EC-KO mice displayed a trend of proteinuria and significantly decreased creatinine filtration at the age of 21 weeks which resulted in significantly elevated albumin/creatinine ratio compared to vehicle treated LM.

Chronic SUL138 treatment improved creatinine filtration in EC-KO mice which in turn led to improved albumin/ creatinine ratio.

Discussion/Conclusion

Chronic treatment with SUL138 from 14 to 22 weeks of age restored vasodilator function. Reduced renal creatinine filtration and overt proteinuria at the age of 21 weeks in EC-KO mice were both attenuated by SUL138 treatment. Therefore, maintaining mitochondrial electron transfer might represent an effective treatment of vascular aging.

Only the title of abstract can be mentioned in the abstract book due to pending IP registration, as communicated with Tom Schirris.

DOAC PLASMA LEVELS IN BARIATRIC SURGERY PATIENTS - A CASE SERIE

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Background

Direct oral anticoagulants (DOAC) are widely used due to their favourable benefit/risk ratio. However, consequences of bariatric surgery on DOAC pharmacokinetics have been poorly investigated. Therefore, DOAC use is not encouraged in bariatric surgery patients. In the Elisabeth Tweesteden Hospital (ETH) use of a coumarin or low molecular weight heparin is preferred in these patients. However, in patients who refuse (switching to) these or with a contra-indication, a DOAC is prescribed/continued and DOAC plasma levels are measured. We aimed to report DOAC trough plasma levels, as a measure for effectiveness, in bariatric surgery patients using a DOAC.

Methods

We conducted a retrospective study including patients using a DOAC with a history of bariatric surgery (Roux-en-Y Gastric Bypass (RYGB) or gastric sleeve) and measured DOAC trough plasma levels using liquid chromatography-mass spectrometry. We collected the medical history, clinical and laboratory data.

Results

Twelve patients (6 female/6 male); mean age, 60 years [range 54–68] were included. Nine had a RYGB and three a gastric sleeve. DOACs used were rivaroxaban (n = 8), apixaban (n = 2) and dabigatran (n = 2). DOAC indications included atrial fibrillation (n=10) and deep vein thrombosis (n=2). The mean time between bariatric surgery and DOAC plasma level was 4.4 years [range 1.5–12.5]. Overall, 11/12 patients had a DOAC trough level within the reference range. In only one patient, using dabigatran, the trough plasma level was below the reference range.

Conclusion

In this study, 11/12 bariatric surgery patients had a DOAC trough plasma level within the reference range, suggesting effective DOAC therapy. Prospective studies are needed to confirm these findings.

EARLY MODEL-BASED PRECISION-DOSING AT HOME TO GUIDE ADALIMUMAB THERAPY

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Background
In this study we investigated the prediction of adalimumab levels with population pharmacokinetic model-based Bayesian forecasting early in therapy. After the first adalimumab administration future steady-state adalimumab levels were predicted. This way underexposed non-responders can possibly be identified early to optimise disease control.

Methods
A literature study was performed to identify adalimumab pharmacokinetic models. With data from a previous pharmacokinetic adalimumab study in our hospital a model was selected. A fit-for-purpose evaluation of the model was performed for rheumatologic and inflammatory bowel disease (IBD) patients with peak, trough and control adalimumab samples obtained by a volumetric absorptive microsampling technique in combination with a finger prick and administration data from an electronic needle container. Steady state adalimumab levels were predicted from peak and trough levels collected after the first adalimumab administration. Predictive performance was calculated with mean prediction error (MPE) and normalized root mean square error (RMSE). Additionally, new pharmacokinetic parameters were estimated from the prospective data

Results

Thirty-six patients (22 rheumatologic and 14 IBD) were included in our study. After stratification for absence of anti-adalimumab antibodies, the calculated MPE was -2.6% and normalised RMSE 24.0%. From clinical perspective, adalimumab serum level predictions were correct (true positive or negative) in 27 of 36 patients (75%). Three patients (8.3%) developed anti-adalimumab antibodies.

Conclusion

This study demonstrates prospectively that adalimumab levels at steady state can be predicted from early samples. This concept enables early precision dosing at home to guide therapy.

INCREASED TACROLIMUS EXPOSURE IN KIDNEY TRANSPLANT RECIPIENTS WITH COVID-19: INFLAMMATION-DRIVEN DOWNREGULATION OF METABOLISM AS A POTENTIAL MECHANISM

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Background
Kidney transplant recipients (KTRs) are at increased risk of severe COVID-19 compared to the general population. This is partly driven by their use of immunosuppressive therapy, which influences inflammatory responses and viral loads. Calcineurin inhibitors are often continued during a moderate or severe COVID-19 infection, however clinical signs of calcineurin toxicity have been described in multiple COVID-19 positive KTRs despite unchanged daily doses of tacrolimus (TAC).This study aimed, to describe dynamics in tacrolimus exposure in kidney transplant recipients that contracted COVID 19 .

Methods
In this single centre case series we describe the course of TAC exposure prior to, during, and post COVID-19 in observations from three clinical cases as well as four KTRs from a randomized controlled trial (RCT) population. Since the VOCOVID study provided an exceptional opportunity of a controlled trial population of KTRs treated with TAC. Case selection was based on the availability of patient consent and availability of TAC and C-reactive protein (CRP) levels shortly before, during and after COVID-19 infection. TAC levels obtained during COVID-19 infection were compared to recent pre-COVID TAC of the cases in combination with CRP concentrations.

Results
All cases were at least three years post-transplantation. All cases showed increased TAC levels during COVID-19 compared to prior to COVID-19 infection. Post COVID-19TAC levels decreased by 26% (range 0% - 38%). The RCT patients displayed on average a 51% (range 31%-94%) higher TAC dose-corrected area under the curve (AUC) during COVID-19 as compared to prior to COVID-19. COVID-19 infection was associated in all cases with higher TAC exposure (range 4%-794%) compared to the guideline defined target therapeutic exposure.

Discussion/Conclusion
There are several possible explanations for the observed increase in TAC exposure such as use of concurrent medication, diarrhea, inter and intra patients' variability or liver dysfunction. However, none of these factors were found in all our cases. Therefore, another explanation for the increase in tacrolimus concentrations could be inflammation-driven phenoconversion. From in-vitro studies and phenotyping cocktail studies in patients it is known that pro-inflammatory markers, such as IL-6, TNF-α and IL-1β downregulate the metabolic capacity of certain enzymes, such as CYP2C19 and CYP3A. Since COVID-19 has been found to elevate these pro-inflammatory cytokines this could lead to inflammatory driven phenoconversion. Resulting in increased exposure of tacrolimus in kidney transplant recipients, due to downregulation of CYP3A by inflammation. To mitigate the risk of tacrolimus overexposure and toxicity therapeutic drug monitoring is highly recommended in KTRs with COVID-19 both in the in- and out-patient setting.

EXTRACELLULAR VESICLES AND SOLUBLE FACTORS SECRETED BY LUNG FIBROBLASTS SUPPORT ALVEOLAR ORGANOID FORMATION

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Background

COPD is characterized by progressive and irreversible airflow limitation as a result of enhanced tissue destruction and defective tissue repair. As current therapeutics do not alter disease progression, new therapies that reactivate lung repair are needed. The secretome of fibroblasts, composed of Extracellular Vesicles (EVs) and other soluble factors (SF), has been linked to alveolar regeneration.[1] We aimed to elucidate the supportive function of lung fibroblast-derived EVs and SF on the regenerative potential of alveolar epithelial progenitor cells in an organoid model.

Methods

EVs and SF were purified using ultrafiltration and size exclusion chromatography. Mouse organoids were obtained by co-culturing 10,000 alveolar EpCAM+ cells with 2,500 lung fibroblasts, and then treated with EVs (10^9 EVs/ml) or SF (30 µg/ml). On day 14, number and size of the organoids was determined, as was the number of differentiated alveolar organoids.

Results

Single treatment with EVs or SF increased organoid count, i.e. a 29.50% ± 8.11% increase for EVs and 33.00% ± 20.34% for SF. Neither treatment with EVs nor SF affected organoid size. Immunostaining for prosurfactant protein C revealed that the alveolar organoid count was significantly enhanced upon single treatment with EVs or SF. In addition, consecutive treatment for 14 days with EVs or SF resulted in enhanced organoid count (i.e. a 58.17% ± 39.60% and 91.67% ± 33.28% increase respectively) and size (i.e. a 36.50% ± 10.46% and 37.50% ± 27.02% increase respectively).

Discussion/Conclusion

Lung fibroblast-derived EVs and SF support alveolar epithelial organoid formation, making them an interesting potential treatment to pursue for COPD.

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CLINICAL RELEVANCE OF HIGH PLASMA TROUGH LEVELS OF THE KINASE INHIBITORS CRIZOTINIB, ALECTINIB, OSIMERTINIB, DABRAFENIB AND TRAMETINIB IN NSCLC PATIENTS

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Background
the study aim was to evaluate whether high plasma trough levels of the kinase inhibitors (KIs) crizotinib, alectinib, osimertinib, dabrafenib and trametinib were associated with a higher risk of toxicity in non-small-cell lung cancer patients (NSCLC) patients.

Methods
In this retrospective cohort study, NSCLC patients treated with the selected KIs were included if at least one plasma trough level at steady state ($C_{min,ss}$) was available. The high group for each KI was defined as the 10% patients with the highest first $C_{min,ss}$. The other patients were placed in the non-high group. The frequency of dose-limiting toxicities (DLTs), defined as adverse events leading to dose reduction, dose interruption or permanent discontinuation, was compared between the two groups.

Results

547 patients were included across the different KIs. A high Cmin,ss of crizotinib, alectinib, osimertinib, dabrafenib and trametinib correlated to a Cmin,ss of ≥490, ≥870, ≥405, ≥150 and ≥25 ng/mL, respectively. In the alectinib group DLTs were more common in the high group compared to the non-high group (64% vs 29%, p = 0.036). Liver toxicity was observed in 4 (36%) patients in the high group and 4 (4%) patients in the non-high group (p = 0.004). For the other KIs, no significant differences were observed in the frequency of DLTs between the high and non-high group.

Discussion/Conclusion

For alectinib, a high $C_{min,ss}$ was correlated to a higher risk for DLT. No differences in the frequency of DLTs were observed between the high and non-high group for crizotinib, osimertinib, dabrafenib and trametinib.

ENHANCING THE PLASMA EXPOSURE OF ORALLY ADMINISTERED CABAZITAXEL BY COADMINISTERING THE CYP3A4 INHIBITOR RITONAVIR

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Background: Taxanes are widely used chemotherapeutic agents, of which cabazitaxel is used for castration-resistant prostate cancer. Recently, there is interest in the development of oral taxanes, because this is less costly and most importantly more patient-friendly. However, a huge drawback of administering taxanes orally is the low bioavailability due to their extensive metabolism by CYP3A4. Previous preclinical and phase I trials in our institute have shown enhancement of the oral availability of docetaxel (ModraDoc) after coadministration of the CYP3A4 inhibitor ritonavir [1,2]. To extend our knowledge about oral taxanes, we are interested in gaining insights into the impact of oral ritonavir on the pharmacokinetics and tissue distribution of orally administered cabazitaxel in an *in vivo* experiment.

Methods: We used male wild-type, *Cyp3a*$^{-/-}$, and Cyp3aXAV (transgenic mice with overexpression of human CYP3A4 in liver and intestine) mice between 8 and 13 weeks of age. Each group consisted of 6 individual mice. Cabazitaxel was administered at a dose of 10 mg/kg by oral gavage. Vehicle or ritonavir 25 mg/kg was orally administered 15 minutes prior to the cabazitaxel. Plasma samples and the tissues of interest were analysed, with cabazitaxel and its three active metabolites (DM1, DM2, and docetaxel) as main readout.

Results: Cabazitaxel plasma exposure was significantly enhanced by ritonavir pretreatment in wild-type ($AUC_{0\text{-}24h}$ by 13.9-fold, $P < 0.0001$) and Cyp3aXAV ($AUC_{0\text{-}24h}$ by 34.3-fold, $P < 0.0001$) mice compared to the vehicle groups of both strains. The C_{max} was 2.8-fold increased in wild-type mice pretreated with ritonavir, and 8.0-fold in Cyp3aXAV mice. In the *Cyp3a*$^{-/-}$ mice there were no significant differences in plasma exposure to cabazitaxel. The liver concentration in wild-type mice was significantly increased (12-fold, $P < 0.0001$), but not the liver-to-plasma ratio. These parameters were both increased in the Cyp3aXAV mice compared to their vehicle group, respectively 37-fold ($P < 0.0001$) and 2-fold ($P < 0.001$). The biotransformation of cabazitaxel to its active metabolites still takes place with the coadministration of ritonavir, however in the mice pretreated with ritonavir this formation process does not immediately start due to the CYP3A4 inhibition.

Discussion/Conclusion These data indicate that CYP3A is the primary limiting factor in the plasma exposure to cabazitaxel. Furthermore, these data show that cabazitaxel oral bioavailability could be enhanced by the addition of a CYP3A inhibitor, such as ritonavir. This could be of clinical relevance when an oral formulation of cabazitaxel is ready to be investigated in a small patient population.

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THE ROLE OF THIOSULFATE SULFURTRANSFERASE IN FERROPTOSIS

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Background

In neurodegenerative diseases, mitochondrial dysfunction contributes to the progressive neuronal loss. Thiosulfate sulfurtransferase (TST, EC 2.8.1.1), an enzyme located in mitochondria, has diverse cellular functions, including cyanide detoxification and transfer of sulfane and sulphur to?. Based on its structural features, TST could donate sulfur to iron-sulfur centers of mitochondria complexes III and IV, resulting in an increase of the rate of electron transport and ATP production.

Ferroptosis is a form of cell death that is linked to iron dysfunction and oxidative stress. In neurodegenerative diseases, ferroptosis has been shown to contribute to loss of neurons in brain regions involved in learning and memory processes. Due to the iron accumulation, large amount of reactive oxygen species (ROS) will be produced intracellularly, mainly on mitochondria membrane, which will lead to oxidative stress and cell death.

Our hypothesis is that TST plays regulatory functions in mitochondrial respiration and potentially has neuroprotective effects in oxidative stress conditions.

Methods

Transient transfection was performed to induce the *TST* overexpression in SH-SY5Y cells (neuroblastoma cell line). Transfected cells were then challenged with ferroptotic inducers (erastin) and oxidative stress stimuli (H_2O_2). Metabolic changes, regarded as viability of the cells were evaluated by Alamar Blue Assay. Transfection efficiency was determined by Flow Cytometry and TST overexpression was validated by Western Blot.

In collaboration with Drug Design group, a potent compound that acted as an activator of TST was synthetized. This chemical compound was tested on SH-SY5Y cell line for potential neuroprotective effects in the presence of various cell death stimuli. The activity of the compound on the mitochondrial complex activity was evaluated by Oxygraph-2k.

Furthermore, the role of TST in the mouse brain mitochondria was evaluated in WT and TST-knock out C57BL/6J mice. Various brain mitochondrial parameters were measured by high-resolution respirometry (Oxygraph-2k).

Results

In initial studies, we used the SH-SY5Y cell line to investigate whether overexpression of h*TST* and the TST activator can protect cells against ferroptosis and oxidative stress. Transient h*TST* overexpression in conditions of oxidative stress rescued cells from cell death. The TST activator increased cell metabolic levels and improved the cell viability in a dose-dependent manner.

Remarkably, the TST activator reduced basal mitochondria respiration, but enhanced mitochondrial complex II and IV respiratory levels. In isolated brain mitochondria from TST-knock mice, we observed that the KO mice had increased basal respiration and complex IV activity level compared with WT mice.

Discussion/Conclusion

The results of the high-resolution respirometry of the brain mitochondria isolated from TST KO mice revealed higher mitochondrial activity in terms of basal and Complex IV respiration compared to WT mitochondria. Our initial hypothesis was that TST KO would have reduced mitochondrial activity, however, similar results of increased mitochondrial activity have been reported in the liver mitochondria of TST KO mice. The next steps are to evaluate for potential compensatory mechanisms mediated by other mitochondrial enzymes, such as tandem-domain sulfurtransferase, mercaptopyruvate sulfurtransferase (MST), which have been reported to mediate mitochondrial effects together with TST.

Since transient TST transfection provided protection, experiments on generating a stable cell line of *hTST* overexpression gene is considered as a next step. The stably transfected cell line would allow to evaluate the extent of the novel TST activator on cell viability and further investigate the protective mechanism of TST in ferroptotic cell death pathways.

EFFICACY OF SUBCUTANEOUS VERSUS INTRAVENOUS ADMINISTRATION OF DESMOPRESSIN IN PATIENTS WITH VON WILLEBRAND DISEASE AND HEMOPHILIA A IN NEED OF COVID-19 VACCINATION

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Background
Subcutaneous desmopressin (DDAVP) can be more easily administered than intravenous DDAVP and may be an efficacious alternative for the currently unavailable intranasal DDAVP to treat mild bleedings or for minor invasive procedures in von Willebrand disease (VWD) and hemophilia A. This study was aimed to compare the one-hour response to subcutaneous and intravenous DDAVP in patients with VWD or hemophilia A.

Methods
Patients with hemophilia A (FVIII ≤10 IU/dL) or VWD (VWF activity ≤10 IU/dL) whose treatment plans include DDAVP and who were to receive a COVID-19 vaccination were eligible to participate. For COVID-19 vaccination, FVIII or VWF activity target levels of >10 IU/dL were pursued according to international guidelines (ISTH). DDAVP was administered subcutaneously 1.5 hours before vaccination. FVIII (in hemophilia and VWD) and VWF activity levels (in VWD) were determined prior to (t = 0) and 1 hour after DDAVP (t = 1). All patients had a positive historical routine challenge test with intravenous DDAVP. For each participant, absolute and relative changes of FVIII and VWF activity levels 1 hour after subcutaneous and intravenous DDAVP (both 0.3 µg/kg) were compared.

Results
Eleven patients were included: six with hemophilia A, three with VWD type 2M and two with VWD type 2A. Both intravenous and subcutaneous DDAVP increased FVIII and VWF activity levels in all patients. In hemophilia patients, intravenous and subcutaneous DDAVP increased FVIII levels by an average of 3.8-fold and 3.4-fold respectively. Peak FVIII activity levels at t = 1 ranged from 25-62 IU/dL and 29-51 IU/dL. In VWD patients, intravenous and subcutaneous DDAVP was associated with a 11.4-fold and 5.1-fold mean increase in VWF activity levels respectively. Corresponding peak VWF activity levels ranged from 18-100 IU/dL and 28-74 IU/dL. No bleeding after vaccination was reported.

Discussion/Conclusion
Subcutaneous DDAVP appears to be an effective alternative for intravenous DDAVP. Moreover, like intranasal DDAVP, subcutaneous DDAVP allows the possibility of self-administration at home.

THE EFFECT OF FERROPTOSIS ON MITOCHONDRIAL MOVEMENT: ESTABLISHING A NEW BRAIN-ON-A-CHIP MODEL FOR NEURODEGENERATION

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Introduction: Ferroptosis is an iron accumulation-dependent and lipid peroxidation-driven type of programmed cell death, which is typically manifested by depletion of glutathione and the inactivation of glutathione peroxidase 4. Ferroptosis contributes to progression of many degenerative diseases including Alzheimer's disease (AD), the most prevalent form of dementia. Mitochondrial dysfunction is widely recognized as one of the early events of AD pathogenesis and is responsible for the cellular bioenergetics deficiency preceding neurodegeneration-related symptoms. Emerging evidence shows the occurrence of elevated mitochondrial oxidative damage and altered mitochondrial morphology during ferroptotic cell death. Mitochondrial fragmentation, shrunken mitochondria and rupture of mitochondrial outer membrane were previously observed in ferroptosis. However, little is known about how ferroptosis affects the mitochondrial dynamics and movement. Further dissection of mitochondrial dysfunction in relation to ferroptosis is needed and could lead to potential development of new therapeutic strategies for AD.

Methods: Dynamic transport of axonal mitochondria allows for the clustering of mitochondria on sites with increased energetic demand. Apart from mitochondrial morphology, the velocity and directionality of mitochondrial movement can be affected in pathological conditions. To analyse whether ferroptosis could alter mitochondrial movement in axons, we established a platform of axonal isolation in microfluidic devises. We used mouse primary cortical neurons and treated them on DIV11-14 with different concentrations of Erastin (a classical inducer of ferroptosis) and ferrostatin-1, as an inhibitor of ferroptosis. Mitochondria were stained using MitoTracker Deep Red FM and microscopes SP8 confocal and ZEISS Celldiscoverer 7 with time-laps function were used to image fixed and live cells, respectively.

Results: This novel 'brain-on-a-chip' platform allowed us to differentiate mouse primary cortical neurons for 2 weeks, after which they showed mature neuronal network with extensive neuronal outgrowth. We were able to isolate cell body from the axonal structures in two separate compartments (somatodendritic vs axonal compartment) and visualize axons passing through interconnecting microchannels. Erastin-induced ferroptosis-induced disintegration of the neuronal network in the axonal compartment and damage of cell bodies in the somatodentdritic compartment. Ferrostatin rescued this effect in both compartments. Our setup allowed the visualization of mitochondrial movement in live cells challenged by localised treatments of ferroptosis inducers. Acute challenge with Erastin, showed increased retrograde vs anterograde movement which was previously linked to turnover of aged organelles through lysosomal degradation in cell body.

Conclusion: Our newly established 'brain-on-a-chip' model will help us investigate the ability of anti-ferroptotic drugs to prevent AD-related effects on mitochondrial movement, which could have a great potential for the development of new therapeutic strategies for AD.

TRANSCRIPTOMIC LANDSCAPES OF ALZHEIMER'S DISEASE, A FOCUS ON MITOCHONDRIAL-RELATED PATHWAYS

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Introduction: Alzheimer's Disease (AD) is the main cause of dementia and it is defined by cognitive decline coupled to extracellular deposit of amyloid-beta protein and intracellular hyperphosphorylation of tau protein. Historically, efforts to target such hallmarks have failed in numerous clinical trials. In addition to these hallmark-targeted approaches, several clinical trials focus on other AD pathological processes, such as inflammation, mitochondrial dysfunction, and oxidative stress. Mitochondria and mitochondrial-related mechanisms have become an attractive target for disease-modifying strategies, as mitochondrial dysfunction prior to clinical onset has been widely described in AD patients and AD animal models. Mitochondrial function relies on both the nuclear and mitochondrial genome. Findings from omics technologies have shed light on AD pathophysiology at different levels (e.g., epigenome, transcriptome and proteome). Most of these studies have focused on the nuclear-encoded components.

Methods: This study provides an updated overview of the mechanisms that regulate mitochondrial gene expression and function in AD. We have focused on published findings and datasets that study AD pathology and delineated mitochondrial-related pathways.

Results Membership and enrichment analyses for excitatory neurons and for inhibitory neurons in the no-pathology versus pathology comparison demonstrated dysregulation of mitochondrial-related pathways, such as mitochondrial gene expression, oxidative phosphorylation and mitochondrial transport. Some of the dysregulated genes corresponded to the mitochondrial ribosomal proteins, mitochondrial complex I subunits such as NDUFA1, NDUFA5, and complex V. Analysis of glial cells such as astrocytes and oligodendrocytes showed that they are able to withstand metabolic insult better than neurons in early stages of disease than in late stages. The transcriptomic signature of metabolic pathways of the amyloid-β-associated microglia is similar to the inflammatory subpopulation.

Conclusion: Several studies have indicated that mitochondrial dysfunction leads to neuronal loss, microglial activation, and the onset of the pathological hallmarks of AD. It is well established that the mitochondrial genome, epigenome, transcriptome, and proteome are implicated in AD progression. Whether these genes and processes are not only biomarkers but whether they represent therapeutic targets able to modulate AD etiology remains to be investigated. Despite all progress made in the field of cell-specific mitochondrial gene expression and function, many areas and topics require further study: *i.e.* integration of multiple mitochondrial omics data sets. The access to rapidly evolving omics technologies and tools for analysis represents a novel avenue to delineate disease mechanisms in AD and in other neurodegenerative diseases as well.

DISCONTINUATION OF INFLIXIMAB TREATMENT IN IBD PATIENTS: A COMPARISON BETWEEN PATIENTS WHO RETRANSITIONED AND THOSE WHO REMAINED ON BIOSIMILAR

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Background

Many patients in clinical care with inflammatory bowel disease (IBD) transitioned from originator to an infliximab biosimilar. Studies demonstrate that on average 7% of patients who transition to biosimilar, retransition to infliximab originator (i.e. stop biosimilar and reinitiate originator), mainly due to (perceived) loss of effect or adverse events. Whether this unsatisfactory treatment response is related to the product or to the patient and his/her disease is unclear. This study aimed to compare the risk of infliximab discontinuation between patients who retransition to originator and those who remain on biosimilar.

Methods

IBD patients who transitioned from infliximab originator to a biosimilar between January 2015 and September 2019 were eligible for inclusion. Patients who retransitioned to infliximab originator (index date) were included in the retransitioning cohort and matched with up to 3 patients who remained on biosimilar (biosimilar remainder cohort). Patients were matched on: hospital, transitioning date and duration of biosimilar treatment up to index date. Patients were followed from index date until discontinuation of infliximab (>16 weeks no administration, or switch to another biological). Risk of infliximab discontinuation (overall discontinuation, switching to another biological or discontinuation without switching) was compared using a conditional Cox proportional hazards model, adjusted for age, gender, duration of infliximab originator use prior to transitioning and the number of other biologicals used before infliximab.

Results

Baseline characteristics of the retransitioning cohort (n=42, 59.9% females, median age 45.0 years) and the biosimilar remainder cohort (n=120,55.0% females, median age 43.0 years) were similar, but dosing interval was shorter in the retransitioning cohort (44 versus 56 days). Infliximab treatment discontinuation after 12 months was 25.0% in the retransitioning cohort and 8.8% in the biosimilar remainder cohort. Retransitioned patients had a twofold increased risk of discontinuing infliximab (adjusted HR 2.2, 95% CI 1.1-4.3) compared with patients remaining on biosimilar. The risk of switching (adjusted HR 8.1, 95%CI 0.9-71.1) was higher than that of discontinuing without switching (adjusted HR 1.7, 95%CI 0.8-3.8).

Discussion/Conclusion

Retransitioned patients have a twofold increased risk for infliximab discontinuation, including both switching to another biological or discontinuing without switching. Our results indicate that retransitioning is mainly patient or disease related and less likely to be product related.

PHARMACOKINETICS OF PREDNISOLONE PROPHYLAXIS AFTER PEDIATRIC HEMATOPOIETIC CELL TRANSPLANTATION: AN INTERIM ANALYSIS

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Background: In the hematopoietic cell transplantation setting, prednisolone is used either as prophylaxis or as therapy for graft-versus-host disease (GVHD). Regardless of the high dose systemic corticosteroids used, GVHD develops or progresses in approximately 30% of patients resulting in high morbidity and mortality rates in this population. Because of this, GVHD is considered one of the most concerning complications after pediatric hematopoietic cell transplantation. We hypothesize that the inter-individual exposure to prednisolone between patients is high, suggesting why some patients are successfully treated with prednisolone, where others fail.

Methods: Patients received prednisolone intravenously 1 mg/kg/day divided into two doses. A concentration-time curve was sampled using a limited sampling strategy: pre (through level) and approximately at 0.5h, 2h, 4h and 7h post prednisolone administration, and analysed using a sensitive LC-MS/MS method. Pharmacokinetic parameters Cmax, AUC_{0-12} and dose-normalized Cmax and AUC_{0-12} (AUC_{0-12}/dose and Cmax/dose) of total and unbound prednisolone were measured.

Results: Seven pediatric patients aged 0.2-15.4 years received 1 mg/kg/day with a median (range) dose of 9 mg (2-22 mg) prednisolone intravenously as prophylactic treatment after cord blood transplantation. The median (range, coefficient of variation %) Cmax of total and unbound prednisolone were 277 (202-385, 20.8) µg/L and 28.5 (22.3-53.0, 38.1) µg/L, respectively. The median AUC_{0-12} of total prednisolone was 1260 (558-1630, 31.2) µg*h/L and of unbound prednisolone 88.0 (49.9-121, 29.2) µg*h/L. Median dose-normalized parameters Cmax and AUC_{0-12} of total prednisolone were 38.5 (12.6-101, 63.7) µg/L and 163 (73.8-297, 50.2) µg*h/L, respectively. For unbound prednisolone, median Cmax was 5.2 (1.3-11.8, 72) µg/L and median AUC_{0-12} 12.1 (4.7-26.5, 60.9) µg*h/L. GVHD developed in 3 patients and those patients had prednisolone concentrations among the highest, middle and lowest values of Cmax and AUC_{0-12}.

Discussion: In this interim analysis, we measured the pharmacokinetic parameters Cmax, AUC_{0-12} and dose-normalized parameters (AUC_{0-12}/dose and Cmax/dose) of prednisolone after 1 mg/kg/day as prophylaxis for GVHD. As hypothesized, there is a large inter-individual variability in exposure to prednisolone between patients. This was also seen in the patients who developed GVHD, suggesting there is more involved to assess the relationship between the pharmacokinetics and pharmacodynamics. However, the sample size of this interim analysis is very small and there were no covariates taken into account. Full sample size and PK analysis is necessary to assess why some patients are successfully treated with prednisolone, where others fail.

VALIDATION OF A PREDICTIVE ALGORITHM FOR THIOPURINE INDUCED HEPATOTOXICITY IN IBD PATIENTS

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Background

The thiopurines, azathioprine (AZA) and mercaptopurine (MP), are effective and remain standard treatment options in steroid sparing and maintaining remission in patients with inflammatory bowel disease (IBD). Approximately 25% of patients discontinue within three months after treatment initiation due to adverse events, of which about half due to hepatotoxicity. We hypothesise that identification of patients with an increased risk of adverse events and timely treatment optimisation can prevent treatment failure.
The primary objective of this prospective observational multicentre study was to optimize and validate a proposed hepatotoxicity predictive algorithm in IBD patients starting AZA or MP therapy.

Methods

Inclusion criteria were adult thiopurine-naive IBD patients initiating AZA or MP treatment. Subjects were treated according to guidelines and followed for 12 weeks. The primary study outcome was hepatotoxicity within 12 weeks, defined as ALAT > 2x ULN or a R factor ((patient's ALAT/ULN ALAT) / (patient's AP/ ULN ALP)) ≥ 5.
The hepatotoxicity- and no-hepatotoxicity groups were compared using the Mann-Whitney U-test.
We adapted an algorithm to predict the risk of developing hepatotoxicity in AZA/MP treatment using multivariable logistic regression and a ROC curve. The determinants age, BMI and 6-MMPR-concentration one week after start of treatment (T=1) were inserted as continuous variables, sex as a dichotomous variable.

Results

Out of 229 patients 21 (9%) developed hepatotoxicity. 128 patients had to be excluded for analysis due to various reasons. 93% of patients received MP with a median dose of 0.7 mg/kg (95%CI 0.3-1.4 mg/kg).
There was a difference between the hepatotoxicity and no-hepatotoxicity group in BMI (27.6 versus 24.2, p=0.022) and 6-MMPR/6-TGN ratio at T=1 (16.3 versus 8.5, p=0.027). In Table 1, the number of true positives, true negatives, false positives and false negatives are presented. A specificity of 76.5% (95%CI 65.8-84.7%) and a sensitivity of 50.0% (95%CI 25.5-74.5%) was obtained.

Table 1 Cross tables for thiopurine-induced hepatotoxicity of algorithm

	Hepatotoxicity	No hepatotoxicity	Totals
Test positive	8	21	29
Test negative	8	64	72
Totals	16	85	101

Discussion/Conclusion

In conclusion, the designed algorithm does not accurately predict hepatotoxicity in this cohort, with low-dose treatment of MP. A lower starting dose seems to result in less cases of hepatotoxicity. We confirmed an association between higher BMI and thiopurine-induced-hepatotoxicity.

MODELLING THE PHARMACOKINETIC INTERACTION BETWEEN FLUCLOXACILLIN AND TACROLIMUS

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Background: Tacrolimus is a calcineurin inhibitor with a narrow therapeutic window and is used for the prevention of rejection of organ transplants. Due to the immunosuppressive action of tacrolimus, transplant recipients are prone to infections. A common antibiotic given to cure infections is flucloxacillin. An earlier study has reported that flucloxacillin decreases the blood trough concentration of tacrolimus (2). The aim of this study is to examine the influence of flucloxacillin on the pharmacokinetics of tacrolimus using population pharmacokinetic (popPK) modelling. Besides, it is established whether the target concentration of tacrolimus can still be met with the current dosing regimens.

Methods: An already published popPK model of Kassir *et al.*(1) was selected to quantify the interaction between tacrolimus and flucloxacillin. The model with allometric scaling was reproduced in NONMEM® 7.4 and extended with this interaction. The most important clinical characteristics collected were gender, age, weight, type of transplant, blood trough concentrations of tacrolimus, type of preparation (Prograft®, Envarsus®, Advagraf®, Modigraf®, Adport® or individual preparation) and doses of tacrolimus and the period and route of administration of flucloxacillin treatment. Next, 250 simulations were performed with a patient that started with the administration of tacrolimus. After 14 days, treatment flucloxacillin started for 14 days. Then, tacrolimus was with administered for another 14 days.

Results: 445 blood trough concentrations of tacrolimus were collected from 32 patients (156 samples before, 116 samples during and 173 samples after flucloxacillin treatment). The median blood trough concentration of tacrolimus was 7.5 µg/L [2.5 – 29.4 µg/L] before treatment with flucloxacillin, 6.0 µg/L [1.0 – 26.1 µg/L] during and 7.0 µg/L [1.0 – 22.5 µg/L] after treatment with flucloxacillin. The model of Kassir *et al.*(1) overpredicted the bioavailability (F) of our population. Therefore, F was fixed at 65%. The model estimated an increase in the apparent clearance of 24% when flucloxacillin was administered. Based on this result, tacrolimus should be dosed 25% higher to meet the target concentration while administering flucloxacillin.

Conclusion: Flucloxacillin has a significant influence on the blood trough concentration of tacrolimus by increasing clearance. The target concentration of tacrolimus will not be met after starting treatment with flucloxacillin. It is important to anticipate in advance with a dose adjustment of 25%. Due to the high variability, close monitoring is mandatory.

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PERFORMANCE OF A TRIGGER TOOL FOR DETECTING ADVERSE DRUG REACTIONS IN PATIENTS WITH POLYPHARMACY ACUTELY ADMITTED TO THE GERIATRIC WARD

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Introduction: Adverse drug reactions (ADRs) account for 10% of acute hospital admissions in older people and are not always recognised by physicians (1,2). The Dutch geriatric guideline recommends using an ADR trigger tool to screen all acutely admitted older patients with polypharmacy (3). The ADR trigger tool comprises ten triggers and associated drugs frequently causing ADRs. This study investigated the performance of this tool and the recognition by usual care of ADRs detected with the tool.

Methods: A cross-sectional study was performed in patients ≥70 years with polypharmacy acutely admitted to the geriatric ward of the University Medical Centre Utrecht. Electronic health records (EHRs) at admission were screened for trigger-drug combinations listed in the ADR trigger tool. Two independent appraisers assessed causal probability with the WHO-UMC algorithm (4) and screened EHRs for recognition of ADRs by attending physicians. Performance of the tool was defined as the positive predictive value (PPV) for ADRs with a possible, probable or certain causal relation.

Results: In total, 941 trigger-drug combinations were present in 73% (n=253/345) of the patients. Fall (32.4%), delirium (24.0%), renal insufficiency/dehydration (16.2%) and hyponatraemia (13.5%) were the most frequent clinical events covering 86.3% of all identified trigger-drug combinations. The overall PPV was 41.8% (n=393/941) according to the appraisers (κ=0.76). The PPV for individual triggers ranged from 0–100%. The top three drug classes most frequently associated with the ADRs were diuretics (35.4%), agents acting on the renin-angiotensin system (13.5%) and analgesics (11.2%), covering 60% of all drugs that caused an ADR. Usual care recognised 83.5% of ADRs, increasing to 97.1% when restricted to possible and certain ADRs.

Conclusion: The ADR trigger tool has predictive value (PPV 41.8%). However, implementation of this tool is unlikely to improve the detection of unrecognised ADRs in older patients acutely admitted to our geriatric ward because the majority of ADRs were recognised by usual care. Future research is needed to investigate the tool's clinical value when applied to older patients acutely admitted to non-geriatric wards.

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A RETROSPECTIVE STUDY OF THE ANTI-XA ACTIVITY IN RENALLY IMPAIRED NON-ICU AND ICU PATIENTS RECEIVING REDUCED OR NOT-REDUCED THERAPEUTIC DOSES OF DALTEPARIN

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Background: Renally impaired (RI) patients have an increased potential to accumulate dalteparin and consequently an increased risk of (major) bleeding events. To reduce this risk, guidelines advice dalteparin dose reduction with anti-Xa monitoring. However, clinical experience has shown that dalteparin has a minimal tendency to accumulate in RI patients.

Objectives: The objective of this study was to compare the anti-Xa activity between a 75% and 100% weight-based therapeutic dose. Furthermore, we aimed to investigate the association between anti-Xa activity and the occurrence of bleeding.

Methods: This study was a multicentre, retrospective observational study including non-intensive care unit (non-ICU) and ICU patients (≥18 years) with an estimated glomerular filtration rate (eGFR) <60 mL/min who received therapeutic doses of dalteparin (≥ 7500 IU daily). Anti-Xa levels were eligible for inclusion when they were sampled at peak concentrations during steady state. Primary outcome was the median anti-Xa level. The Wilcoxon test was used for analysis of the primary outcome, comparing 75% with 100% weight-based therapeutic dose. The data was stratified for different patient groups, dose frequencies and dose reduction. The secondary outcome was the occurrence of bleeding.

Results: The median anti-Xa levels of non-ICU and ICU patients who received 75% or 100% weight-based therapeutic dose were mainly around or below the lower limit of the therapeutic ranges, as shown in table 1. In group A median anti-Xa levels of 75% dose were significantly lower than in 100% dose. In the other groups no significance was found between the median anti-Xa levels of 75% and 100% dose.

Table 1 Median anti-Xa level at 75% or 100% weight-based therapeutic dose in non-ICU and ICU patients. The therapeutic range for once daily is 1.0 – 2.0 IU/mL and for twice daily is 0.6 – 1.0 IU/mL: N/A = not applicable

Group	Ratio Dose (n)	median Anti-Xa level (IU/mL) (IQR)	Therapeutic range (n)
A. non-ICU patients, eGFR <30 mL/min, once daily	75% (10)	0.80 (0.60 – 0.95)	Below lower limit (8) Within range (2)
	100% (21)	1.2 (1.0 – 1.34)	Below limit (5) Within range (15) Above upper limit (1)
B. non-ICU patients, eGFR 30-60 mL/min, once daily	75% (4)	0.89 (0.86 – 0.91)	Below lower limit (4)
	100% (3)	0.66 (0.22 – 1.30)	Below lower limit (2) Within range (1)
C. non-ICU patients, eGFR <30 mL/min, twice daily	75% (21)	0.60 (0.40 – 0.90)	Below lower limit (10) Within range (7) Above upper limit (4)
	100% (24)	0.60 (0.40 – 0.90)	Below lower limit (10) Within range (11) Above upper limit (3)
D. non-ICU patients, eGFR 30-60 mL/min, twice daily	75% (2)	N/A	Below lower limit (1) Within range (1)
	100% (7)	0.70 (0.40 – 0.90)	Blow lower limit (3) Within range (4)
E. ICU patients, eGFR <30 mL/min, twice daily	75% (15)	0.39 (0.30 – 0.60)	Below lower limit (11) Within range (4)
	100% (18)	0.50 (0.36 – 0.69)	Below lower limit (12) Within range (5) Above upper limit (1)
F. ICU patients, eGFR 30-60 mL/min, twice daily	75% (8)	0.44 (0.36 – 0.50)	Below lower limit (7) Within range (1)
	100% (10)	0.53 (0.40 – 0.80)	Below lower limit (6) Within range (3) Above upper limit (1)

Preliminary data: Seven bleeding events occurred in groups A and C combined. Three events (9,7%, all minor) occurred in patients receiving 75% of the weight-based therapeutic dose. Four events (8,9%, one major, three minor) occurred in patients receiving 100% of the weight-based therapeutic dose. No bleeding events occurred in patients of group B and D.

Conclusion: Dose reduction according to our guideline in non-ICU and ICU patients predominantly results in lower anti-Xa activity than the recommended ranges in the guidelines. So far, our findings shows that the occurrence of bleeding is similar when dosed 75% and 100%. We therefore recommend that initial dose adjustment is not necessary in RI patients. However, preventing high anti-Xa levels requires monitoring.

TOPICAL ALLYL ISOTHIOCYNATE AS A MODEL OF TRPA-1 MEDIATED VASODILATION IN HUMANS

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Background
The transient receptor potential cation channel A1 (TRPA1) plays a role in the sensory neuron system and may be involved in the pathophysiology of a variety of disorders including neuropathic pain, inflammatory bowel disease, lung fibrosis and asthma, making TRPA1 a target for the development of pharmaceuticals. Previous research observed that allyl isothiocyanate (AITC) induces a temporary increase in dermal blood flow (DBF) after topical administration through TRPA1-activation [Andersen et al, 2017; Joseph et al, 2021]. This study aimed to validate the AITC skin challenge in healthy subjects as model to quantify pharmacodynamic activity of drugs targeting TRPA1 and to evaluate its tolerability and safety.

Methods
In this open-label interventional study, 25µL of 15% AITC in mineral oil was topically applicated within an O-ring to the forearm of healthy volunteers. Baseline DBF prior to AITC application and post-application maximal DBF were measured in arbitrary units (AU) using laser speckle contrast imaging. Subjects classified as non-responders were excluded from statistical analysis conform prior research [Joseph et al, 2021]. Application site reactions were assessed and intensity was measured using an 11-point numeric rating scale (NRS). Data are presented as mean ± standard deviation, unless stated otherwise.

Results
A total of 12 healthy male subjects were included in this study. The baseline DBF was 28.8 ± 2.9 AU in the full flare area of responders (n = 9). This increased after AITC application to a maximal DBF of 52.1 ± 11.0 AU. When the perfusion was measured in the O-ring, the DBF increased from 32.3 ± 3.2 AU to 111.9 ± 29.2 AU. The flare area was 1408.0 ± 556.6 mm^2 with a DBF of 85.0 ± 10.11 AU. No systemic adverse reactions were observed. Temporary local adverse reactions consisted of pain and pruritus at the AITC-application site. Pain was reported by 8 subjects with a duration ranging from 14-64 minutes (median 27.5) and a peak NRS ranging from 1-7 (median 4.0). Pruritus was observed in 2 subjects, they both reported a NRS of 1 with a duration of 13 and 69 minutes respectively.

Conclusion
AITC induces an increase in DBF and was tolerable and safe, without systemic side effects. Our data suggest that AITC can be used as a pharmacological challenge model to evaluate TRPA1 antagonists in healthy subjects.

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DURABILITY OF IMMUNE RESPONSES AFTER BOOSTING AD26.COV2.S-PRIMED HEALTHCARE WORKERS

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Background

The Janssen vaccine (Ad26.COV2.S), approved as a single-shot regimen, is effective against severe coronavirus disease-2019 (COVID-19). However, this vaccine induces lower severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S)–specific antibody levels than messenger RNA (mRNA)–based vaccines. There is evidence that heterologous boosting (with BNT162b2 or mRNA-1273) of Ad26.COV2.S primed individuals resulted in higher (S)-specific antibody levels than homologous boosting (with Ad26.COV2.S). Because of the emergence of SARS-CoV-2 variants, questioning arises on the long-term protection of homologous and heterologous vaccination strategies after Ad26.COV2.S primed individuals.

Methods

The SWITCH trial is a single-blind, multicenter, randomized, controlled trial among ±450 healthcare workers (HCW) and investigated the immunogenicity and reactogenicity of a homologous (Ad26.COV2.S) or heterologous (BNT162b2 or /mRNA-1273) vaccination strategy among Health Care Workers (HCW) who were primed with Ad26.COV2.S. We focused on development of short (1-month)- and long-term immune responses (up to 5 months post booster).

Objectives

The primary end-point is the level of S-specific binding antibodies, and the secondary end points are the levels of neutralizing antibodies and S-specific T-cell responses.

Results

Homologous or heterologous booster vaccination resulted in higher levels of S-specific binding antibodies, neutralizing antibodies, and T-cell responses than a single Ad26.COV2.S vaccination. Responses were significantly higher with heterologous regimens compared to homologous booster; mRNA-1273 was most immunogenic. Antibody and T-cells measured in whole blood waned at 5 months post booster vaccination; however, levels were still significantly higher after BNT162b2 or mRNA-1273 booster vaccination, compared to Ad26.COV2.S booster. When assessing cross-reactivity of neutralizing antibodies with the emerging Delta and Omicron variants, participants that were vaccinated and boosted with Ad26.COV2.S, had relatively low levels of neutralizing antibodies to Delta and could not cross-neutralize Omicron. Participants boosted with an mRNA vaccine had high titers to Delta, and cross-neutralized Omicron.

Discussion/Conclusion

We showed that mRNA booster vaccination after Ad26.COV2.S priming induces strong humoral and cellular immune responses, which are detectable up to 5 months after booster vaccination. However, although waning was observed and cross-neutralization of emerging variants is less likely, the fact that immune responses were detected in almost all study participants early and later after booster vaccinations indicates that immunological memory was properly performed. Our results do not directly lead to a recommendation for a second boost within 5 months after the first boost, provided there are no alarming variants of concern.

BRAIN AND LUMBAR CSF PK PREDICTION IN ALZHEIMER'S DISEASE VERSUS THE IC50: TARGET SITE MATTERS

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Background: Brain pharmacokinetic (PK) information in Alzheimer's disease (AD) patients are lacking. Limited PK data measured at the cerebrospinal fluid of the lumbar subarachnoid space (CSF-SAS) of AD patients are available and are linked to the observed drug effect. CSF-SAS does not provide an accurate reflection of the drug concentration in the CNS target sites in the brain extracellular fluid (brain-ECF) and brain intracellular fluid (brain-ICF). The goal of this project is to predict and compare the CNS PK of AD patients against IC_{50} values of AD drugs and to assess the impact of AD and aging on CNS PK.

Methods: The CNS PBPK LeiCNS-PK3.0 model has been demonstrated to predict the PK profiles of brain-ECF and CSF-SAS of healthy adults within the two-fold error limit. LeiCNS-PK3.0 parameters were adapted to the CNS physiology of AD and aging populations based on an extensive literature search. Model simulations were performed for: donepezil, galantamine, memantine, rivastigmine, and semagacestat. Rivastigmine is a dual acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitor, donepezil and galantamine are AChE inhibitors, memantine is an N-methyl-D-aspartate antagonist, and semagacestat is a gamma-secretase inhibitor. The predicted PK profiles in brain-ECF, brain-ICF, and CSF-SAS following repeated dosing were compared to the in vitro IC_{50} values and between healthy adults, elderly, and AD patients.

Results: The predicted CNS PK profiles were generally above the IC_{50} values with some exceptions. Rivastigmine PK profile was below the IC_{50} value of AChE at brain-ECF and brain-ICF, but above that of BuChE at brain-ICF. Semagacestat brain-ECF and brain-ICF PK were below the IC_{50} of gamma-secretase about half of the interdose interval, unlike CSF-SAS PK profiles that were consistently above IC_{50}. Memantine PK profiles were above the IC_{50} at the brain-ECF and brain-ICF, but below it in the CSF-SAS. In addition, the PK profiles of all drugs differed between these CNS compartments regarding plateau levels and fluctuation (C_{max}:C_{min}). Interestingly, aging and AD had a little (if any) impact on CNS PK profiles, when compared to those of healthy individuals.

Discussion/Conclusion: This study provides, for the first time, insights into the brain-ECF and brain-ICF PK profiles in AD patients and also on the relation between CNS compartments PK profiles, including target sites. LeiCNS-PK3.0 predictions have shown the importance of studying the target sites PK in relation to drug effect. CSF-SAS remains an inaccurate surrogate of the CNS target sites. Furthermore, despite extensive changes observed in BBB and brain properties in AD, model simulations have shown a remarkably small impact of AD pathology on CNS PK, which implies that the brain PK of healthy adults might represent that of the AD and aging populations.

PREDICTING TREATMENT RESPONSE TO VANCOMYCIN USING BACTERIAL DNA LOAD AS A PHARMACODYNAMIC MARKER IN PREMATURE AND VERY LOW BIRTH WEIGHT NEONATES: A POPULATION PKPD STUDY.

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Background
Late-onset sepsis (LOS) has a high risk of morbidity and mortality among premature and very low birth weight (VLBW) newborns. Whilst positive blood cultures are the gold standard for the diagnosis and subsequent treatment of sepsis, this is time-consuming and therefore results in suboptimal antibiotic treatment regimens. The objective of the present study was to investigate whether treatment response to vancomycin could be quantified using bacterial DNA loads (BDL) based on multiplex real-time quantitative polymerase chain reaction (RT-qPCR).
Methods
VLWB and premature neonates with suspected late-onset sepsis were included in a single-centre, prospective, observational study. Serial blood samples were collected for measurement of BDL and vancomycin concentration (t=0, t=1, r=2, t=4, t=8, t=12, t=24 and t=48 after inclusion). BDL were measured with RT-qPCR, whereas vancomycin concentrations were measured using LC-MS. A population pharmacokinetic-pharmacodynamics model was developed with NONMEM software.
Results
28 patients with (suspected) LOS that were treated with vancomycin were included. A total of 94 vancomycin concentrations and 103 BDLs levels were available. A one-compartment model with post-menstrual age (PMA) and serum creatinine was used to describe vancomycin pharmacokinetics. In 12 patients there was no decrease in BDL over time. Close inspection of the clinical records of these patients explained the underlying mechanism of the lack of bactericidal effect. In 16 patients time profiles of BDL were adequately described with a pharmacodynamic turnover model. The relationship between vancomycin concentration and the increase in first-order BDL elimination was described with a linear effect model. The slope of this model increased with rising PMA.
Discussion/Conclusion
BDLs determined through RT-qPCR were adequately described and predicted with the population PKPD model. Our findings demonstrate that using RT-qPCR, treatment response to vancomycin may be evaluated as early as 4 hours after treatment initiation, allowing early assessment of efficacy of vancomycin in LOS.

EFFECTS OF DAPAGLIFLOZIN ON VOLUME STATUS AND SYSTEMIC HEMODYNAMICS IN PATIENTS WITH CKD: RESULTS FROM DAPASALT AND DIAMOND

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Background
Sodium-glucose cotransporter 2 (SGLT2) inhibitors reduce blood pressure and confer kidney protection in patients with CKD potentially by inducing natriuresis. We assessed the effect of dapagliflozin on natriuresis, blood pressure and volume status in patients with CKD without diabetes from two clinical trials, DAPASALT and DIAMOND.

Methods
We performed a mechanistic open-label study (DAPASALT) to evaluate the effects of dapagliflozin on 24-hr sodium excretion, 24-hr blood pressure (BP), extracellular volume, and markers of volume status during a standardized sodium diet (150 mmol/day) in six patients with CKD. In parallel, in a placebo controlled double-blind cross-over trial (DIAMOND), we determined the effects of 6-weeks dapagliflozin vs. placebo on markers of volume status in 53 patients with CKD.

Results
In the DAPASALT trial, dapagliflozin did not change 24-hr sodium and volume excretion during 2 weeks of treatment (mean 24-hr sodium change from baseline at day 4 and 14, -23.2 [95% CI -56.3, 9.8] mmol/24-hr and -35.0 [95% CI -74.7, 4.7] mmol/24-hr). Dapagliflozin was associated with a modest increase in 24-hr glucose excretion at day 4 (55.7 [95%CI -4.9, 116.3] mmol/24-hr) which persisted at day 14 and reversed to baseline after discontinuation. Mean 24-hr systolic BP decreased by -9.3 (95% CI -19.1, 0.4) mmHg after 4 days and sustained at day 14 and wash-out. Renin, angiotensin II, urinary aldosterone and co-peptin increased from baseline. In the DIAMOND trial, compared to placebo dapagliflozin increased plasma renin (38.5 [95%CI 7.4, 78.8] %), plasma aldosterone (19.1 [95% CI -5.9, 50.8] %), and plasma co-peptin (7.3 [95%CI 0.1, 14.5] pmol/L).

Discussion/Conclusion
During a standardized sodium diet, dapagliflozin decreased blood pressure but did not increase 24-hr sodium and volume excretion. The lack of increased natriuresis and diuresis may be attributed to activation of intra-renal compensatory mechanisms to prevent excessive water loss.

CONCOMITANT INTAKE OF COCA-COLA TO MANAGE THE DRUG-DRUG INTERACTION BETWEEN VELPATASVIR AND OMEPRAZOLE STUDIED IN HEALTHY VOLUNTEERS

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Background

Velpatasvir (VEL) is a lipophilic weak base with pH-dependent solubility. Proton pump inhibitors increase gastric pH resulting in a decrease in VEL absorption. We aimed to evaluate the effect of the acid beverage Coca-Cola on the pharmacokinetics of velpatasvir (VEL) when given with omeprazole.

Methods

This was an open-label, randomized, crossover trial in 11 healthy adults. A single dose of sofosbuvir/velpatasvir (SOF/VEL) 400/100 mg was administered alone (reference) or with omeprazole 40 mg once daily with water (intervention I); in the intervention II arm, omeprazole 40 mg was combined with 250 mL of Coca-Cola. Geometric mean ratios (GMRs) were calculated for VEL area under the concentration-time curve from zero to infinity ($AUC_{0\text{-}inf}$) and maximum plasma concentration (C_{max}).

Results

VEL exposure was reduced by 26.7% when SOF/VEL was coadministered with omeprazole (intervention I) compared with reference. GMRs (90% confidence interval (CI)) were 73.3% (55.6–96.8) and 69.1% (52.3–91.2) for $AUC_{0\text{-}inf}$ and C_{max}, respectively. Intake of SOF/VEL with Coca-Cola (intervention II) compensated for this drug-drug interaction with omeprazole, and resulted in a higher VEL exposure. GMRs (90% CI) were 161.6% (122.4–213.3) for $AUC_{0\text{-}inf}$ and 143.9% (109.0–190.0) for C_{max}. No serious adverse events were reported during the trial.

Discussion/Conclusion

Concomitant intake of a glass of Coca-Cola can be used to overcome the clinical relevant drug–drug interaction between SOF/VEL and omeprazole.

INCREASE IN BNP IN RESPONSE TO ENDOTHELIN-RECEPTOR ANTAGONIST ATRASENTAN IS ASSOCIATED WITH INCIDENT HEART FAILURE

Authors

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Background

The endothelin receptor antagonist atrasentan reduced the risk of kidney failure in patients with type 2 diabetes mellitus and CKD in the SONAR trial, although with a numerically higher incidence of HF hospitalization.

Methods

Participants with type 2 diabetes and CKD entered an open-label enrichment phase to assess response to atrasentan 0.75 mg/day. Participants without substantial fluid retention (>3 kg body weight increase or BNP increase to >300 pg/mL), were randomized to atrasentan 0.75 mg/day or placebo. Cox proportional hazards regression was used to assess the effects of atrasentan versus placebo on the pre-specified safety outcome of HF hospitalizations.

Results

Among 3668 patients, 73 (4.0%) participants in the atrasentan and 51 (2.8%) in the placebo group developed HF (HR 1.39 (95%CI 0.97, 1.99; p=0.072)). In a multivariable analysis, HF risk was associated with higher baseline BNP (HR 2.32; 95%CI 1.81, 2.97) and percent increase in BNP during response enrichment (HR 1.46; 95%CI 1.08, 1.98). Body weight change was not associated with HF. Exclusion of patients with at least 25% BNP increase during enrichment attenuated the risk of HF with atrasentan (HR 1.02; 95% CI 0.66, 1.56) while retaining nephroprotective effects (HR 0.58; 95% CI 0.44, 0.78).

Discussion/Conclusion

In patients with type 2 diabetes and CKD, baseline BNP and early changes in BNP in response to atrasentan were associated with HF hospitalization highlighting the importance of natriuretic peptide monitoring upon initiation of atrasentan treatment.

IS NORTRIPTYLINE MORE APPROPRIATE THAN AMITRIPTYLINE IN THE ELDERLY?

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Background

Guidelines and clinical rules advise active conversion from amitriptyline (AT) to nortriptyline (NT) in the elderly. This is because of the assumption that nortriptyline has fewer side effects than amitriptyline.
A systematic review was conducted to test this assumption, subsequently theoretical and clinical evidence was compared.

Methods

Systematic review of the literature. A search was conducted in Medline using the terms: “nortriptyline”, “amitriptyline” and “elderly” with synonyms. 1820 articles were found. In the first selection on title and abstracts, studies were excluded when not satisfying the inclusion criteria. After this first selection 97 articles were left. None of the studies compared the effect or side effects of AT versus NT for neuropathic pain in the elderly. Two studies compared the effect of AT versus NT for depression in the elderly, but full texts could not be retrieved due to their old age. Two other studies directly compared the side effects of AT versus NT for depression in the elderly.

With snowballing a network meta-analysis in the NICE guideline for neuropathic pain was found, the meta-analysis indirectly compares effects and side effects of AT versus NT in adults. No such guideline or meta-analysis was found for adults with depression. Forty studies investigated a particular side effect of AT or NT.

Results

The NICE guideline for neuropathic pain included 11 RCTs studying the effects of AT on pain in adults. Six of these RCTs report a significant positive effect on pain reduction, the other 5 had no or minimal effect. Three RCTs studied the effect of NT on pain in adults, one study showed a pain reduction of 50%, two studies report no effect on pain. Side effects of AT versus NT were comparable in this indirect comparison. Based on these results the NICE guideline recommends amitriptyline in the treatment of neuropathic pain.

A laboratory study showed AT having a stronger inhibitory effect than NT on the following receptors: muscarine-, alfa-1-, histamine 1, and 5-HT-receptors, and NT having a higher affinity with the NE- transporter than AT.

When comparing the *supposed* side effects on basis of their receptor affinity with the *actual* side effects in the clinical studies, no clear relationship is found. For example, a higher affinity of AT for the muscarine receptor is supposed to result in more complaints of a dry mouth. However, the two studies investigating this particular side effect are contradicting: one study finds a dry mouth being more frequent in AT than NT (85 versus 79%), as another study finds dry mouth *less* frequent in AT than NT (12% versus 22%)

Discussion/Conclusion

No direct evidence supports the necessity of active conversion from AT to NT in all elder patients. All patients using AT or NT should be periodically asked for specific side effects.

REAL-LIFE SAFETY OF PD-1 AND PD-L1 INHIBITORS IN OLDER PATIENTS WITH CANCER: AN OBSERVATIONAL STUDY

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Background: Due to the aging of society, the incidence rate of cancer in older patients is likely to increase in upcoming years. In spite of the current efforts to elucidate the effectiveness and safety of immune checkpoint inhibitors, our knowledge in special patient populations such as older patients remains limited due to their underrepresentation in clinical trials. The objective of this study is to compare the real-world safety profile of programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors between younger and older patients.

Methods: All patients receiving pembrolizumab, nivolumab, atezolizumab or durvalumab between September 2016 and September 2019 at Haga Teaching Hospital, The Hague, were included in this retrospective study. Immune-related adverse drug reactions (irADRs) were manually retrieved from the patient files. The cumulative incidences of irADRs were compared between younger (<65 years) and older (≥65 years) patients using a Pearsons Chi-square test.

Results: We identified 217 patients who were treated with at least one dose of PD-(L)1 inhibitor during the study period. 58% were 65 years or older at the start of immunotherapy. 183 patients (84.3%) received monotherapy PD-(L)1 inhibitors and 34 (15.7%) received chemo-immunotherapy. A total of 278 irADRs were registered. Cutaneous irADRs (53.9%), thyroid gland disorders (20.3%), and non-infectious diarrhoea/colitis (17.5%) were the most frequently reported irADRs. The majority of the irADRs were mild to moderate and no fatal irADRs were observed. 61 (21.9%) of the irADRs needed systemic treatment, of which 19 (6.8%) required treatment with corticosteroids. 18 irADRs (6.5%) were severe and resulted in hospitalisation. The cumulative incidence of cutaneous irADRs was different between the age groups: 45.7% of the patients <65 years and in 60.0% of the patients ≥65 years (p=0.036). No statistical difference was found in the cumulative incidence of other irADRs between the two age groups.

Discussion/Conclusion: The results of this study suggest that the safety of PD-1 and PD-L1 inhibitors is comparable between older and younger patients. There was however a higher incidence of skin toxicity in older patients. The majority of the reported irADRs were mild or moderate reactions. irADRs leading to death were not seen in our study. Among all the severe reactions, colitis, pneumonitis, and hepatitis had the highest frequency.
In conclusion, we demonstrated that PD-1 and PD-L1 inhibitors can be safely prescribed independent of age. Except for mild and moderately severe skin toxicity, no significant differences were found between younger and older patients.

SUPERIOR EFFECTIVENESS OF TOFACITINIB COMPARED TO VEDOLIZUMAB IN ULCERATIVE COLITIS: A NATIONWIDE DUTCH REGISTRY STUDY

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Background

Clinicians face difficulty in when and in what order to position biologics and JAK inhibitors in anti-TNF refractory ulcerative colitis (UC) patients. We aimed to compare the effectiveness and safety of vedolizumab and tofacitinib in anti-TNF exposed UC patients in our prospective nationwide Initiative on Crohn and Colitis (ICC) Registry.

Methods

UC patients who failed anti-TNF treatment and initiated vedolizumab or tofacitinib treatment, were identified in the ICC Registry in the Netherlands. We selected patients with both clinical as well as biochemical or endoscopic disease activity at initiation of therapy. Patients previously treated with vedolizumab or tofacitinib were excluded. Corticosteroid-free clinical remission (SCCAI≤2), biochemical remission (CRP ≤5 mg/L or fecal calprotectin ≤250 µg/g) and safety outcomes were compared after 52 weeks of treatment. Inverse propensity scores weighted comparison was used to adjust for confounding and selection bias.

Results

Overall, 83 vedolizumab and 65 tofacitinib treated patients were included. Propensity score weighted analysis showed that tofacitinib treated patients were more likely to achieve corticosteroid-free clinical remission at week 12, 24 and 52 compared to vedolizumab treated patients (OR: 5.87, 95%CI:3.55-9.70, P<0.01, OR: 2.96, 95%CI: 1.85-4.73, P<0.01 and OR 1.90, 95%CI: 1.18-3.07, P<0.01, respectively). In addition, tofacitinib treated patients were more likely to achieve biochemical remission at week 12 and week 24, remaining only statistically borderline at week 52 (OR: 3.09, 95%CI: 1.85-5.14, P<0.01, OR: 2.01, 95%CI: 1.22-3.29, P<0.01 and OR 1.68, 95%CI: 0.99-2.86, P=0.05, respectively). Remission rates are shown in figure 1. There was no difference in infection rate or severe adverse events.

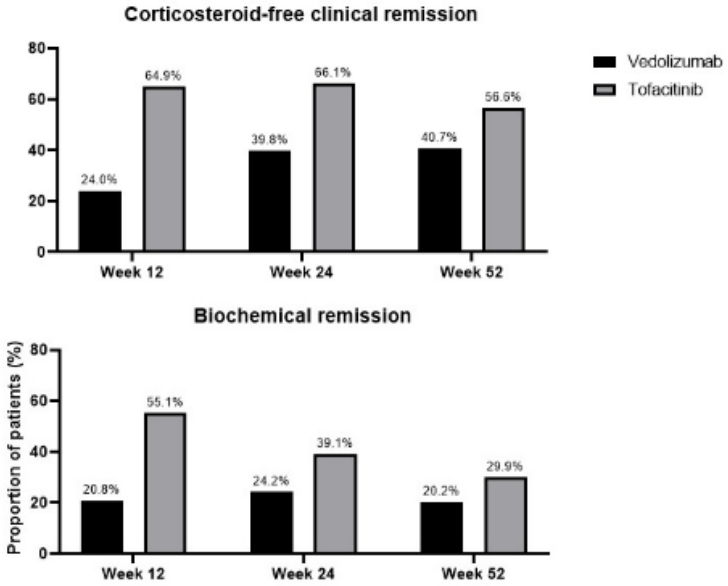

Figure 2. Clinical and biochemical rates in the weighted cohort

Discussion/Conclusion

Tofacitinib was associated with superior effectiveness outcomes compared to vedolizumab in anti-TNF refractory UC patients along with comparable safety outcomes.

A PLACEBO-CONTROLLED STUDY TO ASSESS THE SENSITIVITY OF FINGER TAPPING TO MEDICATION EFFECTS IN PARKINSON'S DISEASE

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Background
For the detection of (dopaminergic) medication effects in Parkinson's disease (PD) clinical trials, a short, rater-independent measurement would be ideal. Finger tapping has been suggested to be such a measure. However, the set-up and devices used for tapping tasks vary among studies and it is unclear which is best at quantifying medication effects in randomized placebo-controlled trials. Here, we aimed to validate three different tapping tasks by evaluating if they could differentiate between placebo and dopaminergic medication and showed a time-related response.

Methods
This was a randomized, double-blind, placebo-controlled, two-way crossover study in 20 PD patients. Patients had to withhold their own anti-Parkinson medication overnight. The next morning, levodopa/carbidopa (individualized dose) or placebo capsules were administered. Pre- and up to 3.5 hours post-dose, tapping tasks were performed. Tapping tasks included two touchscreen-based alternate finger tapping tasks (with 2.5 or 20 cm between targets) and a task using a goniometer that assesses angular movement during thumb-index finger tapping. Mixed model ANOVA was used to assess treatment effects (contrast active-placebo with 95% CI indicated under Results).

Results
The 2.5 cm-task was difficult to correctly perform for 6 of 20 patients (defined as ≥4 of 22 measurements with >70% double taps). One patient seemed unable to correctly perform and/or the device did not correctly record the task, so was excluded. For the 2.5 cm-task, a significant treatment by time interaction effect, but not overall treatment effect was found for the total number of taps. In addition, levodopa/carbidopa compared to placebo resulted in significantly more accurate tapping, i.e., a higher ratio good:total taps (0.14 (0.07 – 0.21). In the 20 cm-task, levodopa/carbidopa compared to placebo resulted in tapping that was significantly faster (total number of taps: 12.5 (6.7 – 18.2)), less accurate (total spatial error: 240 (123 – 357) mm), and with better rhythm (standard deviation (SD) of inter-tap intervals: -16.3% (-29.9% – -0.0%)). In the thumb-index finger tapping task, levodopa/carbidopa compared to placebo resulted in tapping that was significantly faster (mean opening velocity: 151 (64 – 237) deg/s), with higher amplitude (mean tapping amplitude: 8.4 (3.7 – 13.0) deg) and better rhythm (SD of inter-tap intervals: -46.4% (-63.7% – -20.9%). The endpoints showed a time-related response in line with expected levodopa pharmacokinetics.

Discussion/Conclusion
Albeit the 2.5 cm-task could differentiate between levodopa/carbidopa and placebo treatment on some endpoints, it was not always correctly performed by the PD patients and is therefore (in the current set-up) least useful. Both the 20 cm touchscreen and goniometer tasks were able to differentiate between levodopa/carbidopa and placebo treatment, and showed a time-related response, thereby validating them for use in future PD clinical trials.

Association between patient- and disease related factors and the occurrence of severe adverse events during immune checkpoint inhibitor therapy in cancer patients

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Background Immune checkpoint inhibitors (ICI) targeting the cytotoxic T lymphocyte-associated protein (CTLA-4) or the programmed cell death protein 1 (PD-1) have substantially improved the prognosis of patients with advanced cancer, but are associated with severe immune-related adverse events. With ICI entering earlier lines of treatment, severe adverse events (SAEs) may have a larger impact on quality of life. Hence, it is important to identify patients who are prone to develop SAEs upfront. We therefore explored the association between patient and disease related factors and the occurrence of SAEs during ICI therapy.

Methods Patients initiating anti-PD-1 monotherapy or anti-PD-1/ CTLA-4 combination therapy between July 2015 and Feb 2020 and prospectively included in the MULTOMAB-trial (NL6828), were followed for the development of SAEs (CTCAE grade ≥3) up to Aug 2020. Patient and disease related factors at treatment start were tested for their association with the occurrence of a first SAE using univariate and multivariate competing risk cox-regression analysis. The occurrence of death before SAE onset was regarded as a competing risk. The following factors were tested for an association with the occurrence of a first SAE: age, sex, tumour type, number of prior treatment lines, WHO performance status, anti-PD-1 antibody, number of organ sites with metastases, presence of brain metastases, histology (NSCLC only), and treatment setting and LDH level (melanoma only). The association was studied in the total cohorts (separate models for mono- and combination therapy), and for melanoma and NSCLC separately.

Results A total of 641 patients were included, of whom 550 (86%) initiated anti-PD-1 monotherapy and 91 (14%) patients received combination therapy with anti-CTLA-4. The indication was melanoma in 293 (46%) patients, NSCLC in 181 (28%), and mesothelioma, RCC or UCC in the remaining 167 patients. Overall, 106 (17%) patients developed a total of 129 SAEs during follow-up. The incidence of a first SAE was 44% (40/91) in the combination therapy group and 12% (66/550) in the monotherapy group. None of the tested factors were associated with the occurrence of a first SAE in the total cohorts. Increasing age was the only factor associated with a first SAE in the melanoma monotherapy (sHR: 1.03 [95%-CI: 1.00-1.06]) cohort. In NSCLC patients treated with anti-PD-1 monotherapy, a trend to a higher risk was seen in females compared to males (sHR: 2.12 [95%-CI: 0.96 – 4.69]).

Conclusion The risk of developing a first SAE during ICI therapy is largely independent of patient and disease related factors, though elderly (melanoma) and female (NSCLC) patients may be at increased risk. Future studies should focus on potential genetic or molecular/cellular predictors of SAEs during ICI therapy.

PRELIMINARY RESULTS: REAL-LIFE DATA OF PEMBROLIZUMAB LEVELS AND PATIENTS' PERSPECTIVE ON A PERSONALISED DOSING REGIMEN IN PATIENTS WITH NSCLC

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Background

Pembrolizumab is a monoclonal antibody, proven effective in treating multiple types of solid tumours. Currently, all patients receive a fixed dose of either 200 mg per 3 weeks (200 mg Q3W) or 400 mg per 6 weeks (400 mg Q6W). (1) However, this dosing strategy is suboptimal due to inter-individual variability, possible overdosing and high costs. (2–6) Therefore, we impose the implementation of personalised pembrolizumab dosing to optimise treatment and lower costs. Preparatory to implementing a personalised dosing regimen, we aimed to gain insight into the pembrolizumab levels and patients' perspectives towards personalised dosing via this observatory pilot study.

Methods

Adult non-small-cell lung carcinoma (NSCLC) patients receiving pembrolizumab therapy in OLVG were included from October 2020 till January 2021. Pembrolizumab levels were quantified from residual plasma by Sanquin. These levels were compared to levels defined in the literature. Also, a questionnaire focused on patients' perspectives on personalised dosing was drawn up and presented to the included patients.

Results

A total of 133 samples from 46 patients were analysed. The median pembrolizumab trough concentration (C_{trough}) was 35.55 µg/mL (SD 18.17) for the 200 mg Q3W regimen and 57.60 µg/mL (SD 15.07) for the 400 mg Q6W regimen. The high standard deviation (SD) are a result of a high inter-individual variability.

These levels were notably higher than the 10 µg/mL level, at which dose-finding studies indicate sufficient receptor occupation. (5–7) Moreover, since our C_{trough} levels comprised samples in steady-state- and non-steady-state conditions, the solely steady-state C_{trough} is expected to be even higher.

Furthermore, 22 patients completed the questionnaire. This questionnaire evinced that 82 per cent of the patients would favour a personalised dosing regimen if it does not involve any risk. 77 per cent of the patients would still prefer a personalised dosing regimen if an unknown risk occurred in the hypothetical situation. Notably, patients experiencing more side effects at the moment of filling out the questionair, were less willing to favour the personalised dosing regimen ($P = 0.024$).

Discussion/Conclusion

Despite the low number of patients, these preliminary results corroborate our hypothesis of the possible overdosing of pembrolizumab. Furthermore, the questionnaire demonstrated the support of patients for the implementation of a personalised dosing regimen. Therefore, our next step is to include the additional results of this pilot and start our multicentre, double-blind non-inferiority study to investigate the hypothesised benefits of a personalised dosing regimen.

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THE TRANSGLUTAMINASE-2 INTERACTOME IN THE APP23 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction
Amyloid-beta (Aβ) deposition in the brain is closely linked with the development of Alzheimer's disease (AD). Unfortunately, therapies specifically targeting Aβ deposition have failed to reach their primary clinical endpoints, emphasizing the need to broaden the search strategy for alternative targets/mechanisms. Transglutaminase-2 (TG2) catalyzes post-translational modifications, is present in AD lesions and interacts with AD-associated proteins. However, an unbiased overview of TG2 interactors is lacking in both control and AD brain.

Objectives
Here we aimed to identify these interactors using a crossbreed of the AD-mimicking APP23 mouse model with wild type and TG2 knock-out ($TG2^{-/-}$) mice.

Methods
APP23 mice, overexpressing human APP751 carrying the Swedish double mutation (K670M/N671L), $TG2^{-/-}$ mice generated by deletion of 1,200 base pairs, from exon 5 to intron 6, which includes exon 6 containing the active site of TG2, and C57BL/6 mice wild type (WT) were used. Crossbred of APP23 and $TG^{-/-}$ were generated. 18-month-old mice were selected for the study, consisting of APP23 (n = 8), WT (n = 6), APP23/$TG2^{-/-}$ (n = 10) and WT/$TG2^{-/-}$ (n = 5). Aβ pathology (immunohistochemistry), soluble brain levels of $A\beta_{1-40}$ and $A\beta_{1-42}$, and mRNA levels of TG family members were analyzed. Using five mice brains per animal group (WT, $TG2^{-/-}$, APP23 and APP23/$TG2^{-/-}$) quantitative proteomics and network analysis was performed as well as comparative proteomics on whole brain homogenates.

Results
We found that absence of TG2 had no (statistically) significant effect on Aβ pathology, soluble brain levels of $A\beta_{1-40}$ and $A\beta_{1-42}$, and mRNA levels of TG family members compared to APP23 mice at 18 months of age. Quantitative proteomics and network analysis revealed a large cluster of TG2 interactors involved in synaptic transmission/assembly and cell adhesion in the APP23 brain typical of AD. Comparative proteomics of wild type and $TG2^{-/-}$ brains revealed a TG2-linked pathological proteome consistent with alterations in both pathways.

Conclusion
Our data show that TG2 deletion leads to considerable network alterations consistent with a TG2 role in (dys)regulation of synaptic transmission and cell adhesion in APP23 brains.

CIGARETTE SMOKE AND AIR POLLUTION INDUCE DYSFUNCTIONAL PULMONARY MICROVASCULAR ENDOTHELIAL REPAIR

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Background: Dysfunctional microvascular endothelial cells (ECs) drive microangiopathies in respiratory diseases where tobacco smoking and air pollution are two major risk factors. We aimed to assess the effect of cigarette smoke (CS) and diesel exhaust particles (DEP) on pulmonary microvascular endothelial repair *in vitro*.

Methods: Immortalized human pulmonary microvascular endothelial cells (HPMECs) were serum-starved for 24 h followed by treatments with either control, CS extract (CSE) (1%, 2.5%, 5%) or DEP (50, 100, 200 µg/ml) for 24h/48h. Post-analyses comprised scratch wound healing and organoid assays. Lung organoids were generated by co-culturing murine epithelial progenitors (CD31-/CD45-/Epcam+), fibroblasts and HPMECs in Matrigel. Furthermore, RNA sequencing (RNA-seq) was performed on ECs (CD31+ cells) isolated from mice exposed to air or CS.

Results: HPMECs rapidly closed scratch wounds in controls, yet were inhibited, both by 2.5% CSE and 100 µg/ml DEP. In the lung organoid assay, the presence of HPMECs increased the formation of alveolar epithelial organoids. This stimulatory effect was inhibited by pre-treatment of HPMECs with 2.5% CSE or DEP (100 µg/ml). RNA-seq analysis on ECs obtained from smoke-exposed mice showed several differentially expressed gene pathways including VEGFA-VEGFR2, IL-18, vitamin D receptor, circadian rhythm, and NRF2 signaling pathways, which were all upregulated in response to CS exposure.

Discussion/Conclusion: CSE or DEP exposure impairs the wound repair function of HPMECs. CS induces a wide range of transcriptomic changes on pulmonary microvascular ECs, which provide novel guidance for mechanisms and future therapeutic strategies.

PHARMACOLOGICAL ADAPTATIONS TO IMMUNOSUPPRESIVE THERAPY IN CANCER PATIENTS WITH AUTO-IMMUNE DISEASE TREATED WITH IMMUNE CHECKPOINT INHIBITORS

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Background
Prescribing immune checkpoint inhibitors (ICIs) for cancer patients with autoimmune disease (AID) is presumed to be safe provided cautious adverse event management. Practical guidance on the adaptations of immunosuppressive therapy prior to and during ICI treatment is emerging. We describe a case series to objectify the pharmacological management of AIDs and concurrent irAEs in patients with solid tumors treated with ICI therapy. In addition, a literature review of similar cases was conducted.

Methods
A single centre retrospective chart review was performed of cancer patients with AIDs treated in a tertiary university hospital with ICIs, including programmed death (ligand)-1 (PD(L)-1) antagonists and cytotoxic lymphocyte antigen-4 (CTLA-4) inhibitors, from 2016 to 2021, reviewing flare-up and irAE management. A systematic search of the PubMed database was added to identify published similar cases. Articles (1/1/2010-30/11/2021) were screened and included by two independent researchers if sufficient patient and drug-related data was provided. A third researcher was consulted upon disagreement. Demographic, disease and drug-related data were collected and summarized using descriptive statistics.

Results
Of 578 patients treated with ICI, sixteen patients (75% male; median age: 66 years) were included of which 50% had active AID. Two patients received an anti-PD-1 and anti-CTLA-4 combination. In five cases (31%), immunosuppressants were withheld before ICI initiation (TNF-inhibitors=4; methotrexate=1). Eleven patients (69%; 6 patients with active AID) experienced an episode of AID flare-up (11; median=1) and/or irAE (16; median=11). Flares were mostly treated systemically (8 patients) with corticosteroids (n=4), TNF-inhibitors (n=2), 5-aminosalicylic acid derivates (n=2) or acitretine (n=3). Two flare-ups resolved spontaneously. Of 16 irAEs, twelve required systemic treatment (in 8 cases corticosteroids, 3 hormone therapy).

In the literature review, 817 articles were eligible for inclusion. Finally, 86 reports of patient cases were identified in 38 articles (83 patients with PD(L)-1 inhibitor monotherapy). Of those with active AID (n=54), 18% had a treatment change before ICI start. Interventions on TNF-inhibitors (n=3) and azathioprine (n=5) were most frequent. A total of 66 patients (77%) experienced an AID flare-up and/or irAEs after ICI therapy initiation (n=84). Corticosteroids were the preferred treatment, prescribed in 63 episodes (37 flares; 26 irAEs); followed by hormonal therapy (n=6).

Conclusion
Limited data are available on the concomitant use of ICI and AID drug therapy, resulting in a very diverse pharmacological approach of AID flare-ups and irAEs. As every AID has its own characteristics, guidelines should be tailored to the type of AID and immunosuppressive regimen.

INCREASE OF GLUTAMATE-INDUCED HT22 CELL DEATH BY BIODEP INVOLVES FERROPTOSIS AND EPAC1 BUT NOT CASPASES AND EPAC2

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Background

Air pollution exposure is one of the important threats to human health. Diesel combustion produces diesel exhaust particles (DEP) which seem to contribute to the onset of different neurological diseases due to the induction of oxidative stress, inflammation and neuronal degeneration [1]. Underlying molecular mechanisms are ill defined. Glutamate-induced neurotoxicity has been linked to oxidative stress and neuronal cell death including the newly identified iron-dependent form of cell death ferroptosis [2]. Cyclic adenosine monophosphate (cAMP) seems to be linked to ferroptosis type of cell death in processes involving Epac (exchange protein directly activated by cAMP) [3].

Methods

Hippocampal neuronal (HT22) cells were treated with DEP and BioDEP [4] at different concentrations (up to 600 μg/mL) for 24h alone or in combination with glutamate (3 mM, 18h) at the indicated concentrations. Cell viability was measured by MTT assay, and propidium iodide (PI) staining by fluorescence microscopy. To further determine the basis of BioDEP and glutamate mediated cell death, several inhibitors (QVD, 10 μM; ferrostatin-1, 5 μM; ESI-05, 30 μM; CE3F4, 30 μM; Forskolin, 30 μM; Rolipram, 30 μM; colistamide, 30 μM) were used.

Results

HT22 cells were treated for the indicated time points and concentrations with DEP and BioDEP. Neither DEP nor BioDEP alone significantly changed HT22 cell viability. Importantly, co-treatment with BioDEP but not DEP further increased glutamate-induced cell death measured by MTT and PI staining. Neither pan-caspase inhibitor QVD nor Epac2 inhibitor ESI-05 restored cell viability after treatment of glutamate and BioDEP. In contrast, the ferroptosis inhibitor ferrostatin-1 and Epac1 inhibitor CE3F4 restored cell viability.

Discussion/Conclusion

Our current work demonstrates that co-treatment of HT22 cells with glutamate and BioDEP - but not DEP - caused a significant increase in cell death. Accelerated cell death of HT22 cells by glutamate and BioDEP seems to involve ferroptosis (as shown by ferrostatin-1), in a process independent of caspase (as shown by QVD) but dependent on Epac1 (as shown by CE3F4). Currently, we study the effect of cAMP elevations on glutamate/BioDEP-induced HT22 cell death.

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TARGETED LIPIDOMICS REVEALS PROGRESSION OF EARLY ALZHEIMER'S DISEASE IN TGF344 TRANSGENIC RATS

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Background: Alzheimer's disease (AD) is the most common cause of dementia and accounts for about two-thirds of dementia cases. Current AD diagnosis methods can only identify patients in the advanced stage of the disease, that means when the dementia is already present. This highlights the need for new approaches for the diagnosis of AD at earlier stages, especially before the symptoms appear. In addition, AD is reported to be closely linked with abnormal lipid metabolism. The objective of this study is to find blood-based lipid biomarkers to gain a more comprehensive understanding of what causes AD and its subsequent development.

Methods: A longitudinal study with different ages (12 weeks, 25 weeks, 50 weeks, and 85 weeks) was conducted in TgF344 transgenic (AD) and wild-type (WT) rats. EDTA plasma was collected to study relative quantitative lipidomic profiling. Data were processed by SCIEX OS and an in-house tool to assess data quality. All values were $\log_{10}$ transformed to ensure that the values were normally distributed.

Results: In total 123 lipids were detected, and relative concentrations were determined based on internal standards. Comparisons were made between AD and WT groups, and between different ages for WT as well as for AD groups. Changes were found for two lipid classes, being phosphatidylethanolamines (PE) and triacylglycerols (TG). Specifically:

- **Comparison of AD versus WT**

	50 weeks AD/WT	85 weeks AD/WT
PE (16:0_22:5)	1.23 (p = 0.06)	0.68 (p < 0.05)

- **Comparison of ages in WT group ($p < 0.05$)**

Fold change	25w/12w	50w/12w	85w/12w
PE(18:0_18:3)	0.50	0.59	0.59
PE (O-18:0_20:2)	0.66	0.57	0.74
PE (O-18:0_20:3)	0.74	0.69	0.83

- **Comparison of ages in AD group ($p < 0.05$)**

Fold change	25w/12w	50w/12w	85w/12w
PE (16:0_22:6)	NG	1.61	1.34
PE (17:0_22:6)	NG	1.49	1.30
PE (18:0_22:6)	NG	1.41	1.26
PE (18:1_22:6)	NG	1.51	1.64
PE (20:0_22:6)	NG	1.23	1.66
PE (P-16:0/22:6)	NG	1.27	1.77
TG (52:0)	0.65	0.67	NG

NG: Non-significant

Conclusion: Lipidomics data on longitudinal course of potential lipid biomarkers in plasma of WT and AD rats obtained so far show some effects of AD and/or ageing. It is anticipated that this information will contribute to developing lipid biomarker fingerprints for diagnosis and monitoring AD progression distinct from normal ageing.

ADDED VALUE OF *DPYD* WHOLE EXON SEQUENCING TO EXPLAIN SEVERE FLUOROPYRIMIDINE-INDUCED TOXICITY

Q. Zhai[1], M. van der Lee[1], C. A.T.C. Lunenburg[1], L. M. Henricks[1], S. Böhringer[1], F. M. de Man[2], S. Offer[4], A. Baars[*], G.-J. Creemers[*], V. O. Dezentjé[3*], H. J. Droogendijk[*], P. Hamberg[*], A. L.T. Imholz[*], R. L.H. Jansen[*], F. J.F. Jeurissen[*], M. Koopman[*], C. M.P.W. Mandigers[*], P. Nieboer[*], M. H.W. van de Poel[*], J. E.A. Portielje[1], R. H. N. van Schaik[2], H. Gelderblom[1], R. H.J. Mathijssen[2], J. H.M. Schellens[*], A. Cats[3], H.-J. Guchelaar[1], J. J. Swen[1]
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Background

Fluoropyrimidines are commonly used in the treatment of cancer. Up to 30% of patients treated with fluoropyrimidines experience severe toxicity (≥3 grade), primarily caused by a deficiency in dihydropyrimidine dehydrogenase (DPD). Prospective genotyping for four genetic variants (c.1905+1G>A, c.1679T>G, c.1236G>A, and c.2846A>T) in the gene encoding for DPD (*DPYD*) followed by individual dose reductions has been proven to reduce fluoropyrimidines-related severe toxicity. However, substantial fluoropyrimidines-induced toxicity remains, which might be attributed to rare deleterious variants in *DPYD*.

Methods

Exon sequencing (including 20bp flanking region) of *DPYD* was performed for 1,103 patients treated with fluoropyrimidines who participated in the Alpe DPD study. Carriers of one of the four clinical *DPYD* variant alleles (n=85) who received dose reductions were excluded from the analyses. The potential impact of all non-synonymous *DPYD* variants was assessed with two *in silico* tools, being *DPYD*-Varifier and MMsplice, and one *in vitro* tools, being an expression system in HEK293T/c17 cells. Variants were considered deleterious if predicted so by at least one tool. For toxicity analysis, a matched-pair analysis was performed in which, for each deleterious variant carrier, three matched *DPYD* wild-type patients were identified based on three clinical criteria, including treatment regimens, tumor type, and disease stage.

Results

In the 1,018 patients included in the primary analysis, 24 non-synonymous genetic variants in *DPYD* were found. Of these, a total of 7 variants was defined as deleterious. Five variants (c.1670C>T, c.1913T>C, c.1925T>C, c.506delC, c.731A>C), were identified as deleterious by at least the *in vitro* assay or the *DPYD*-Varifier. Two other variants (c.1740+1G>T and c.763-2A>G) were predicted deleterious by MMsplice. In total, ten patients carried one of these seven predicted deleterious variants, of whom three experienced severe toxicity such as gastrointestinal toxicity. These ten patients showed a 2.14-fold (95%CI: 0.408-11.255, p= 0.388) increased risk of severe toxicity compared to matched *DPYD* wild-type controls.

Discussion/Conclusion

Rare deleterious variants detected by exon sequencing in the *DPYD* gene might lead to an increased risk of severe fluoropyrimidine-induced toxicity. Whole exon sequencing detected an additional 1% deleterious allele variants in the *DPYD* gene. The impact of these allele variants in daily clinical practice is unsure yet, as the number of patients in this study was too small to draw definitive conclusions.

ADDED VALUE OF *DPYD* WHOLE EXON SEQUENCING TO EXPLAIN SEVERE FLUOROPYRIMIDINE-INDUCED TOXICITY

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INVESTIGATE EPAC2/GLUA3 PATHWAY IN EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE

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Background
One of the major hallmarks of Alzheimer's Disease (AD) is the accumulation of amyloid beta (Aβ). We showed that Aβ causes synaptic depression through reducing currents of GluA3-containing AMPA receptors (AMPARs), a process involving cAMP[1]. Even though the molecular mechanism through which cAMP regulates GluA3 still remains to be identified, recent studies implicate the involvement of the exchange protein directly activated by cAMP (Epac). We also showed that downregulation of Epac2 in the hippocampal CA1 area impaired memory retrieval in C57BL/6 mice[2]. However, how Epac2 activity contributes to memory retrieval and the underlying mechanisms by which it coordinates memory is still unclear. We aim to investigate whether activation of Epac2 can counterbalance the neurotoxic effect of Aβ on GluA3-containing AMPA receptors.

Methods
Pharmacological modulation of Epac2 activity was done in the presence or absence of oligomeric Aβ. A selective Epac2 activator and inhibitor as well as a pan-Epac activator were used for cell viability assays using MTT. Caspase-3 and pan caspase inhibitors were used as control. Other methods included Western blot analysis and immunofluorescence. Analyses were conducted using AD and healthy human brain samples, Aβ overexpressing transgenic (J20) and wildtype mice, and retinoic acid-differentiated SH-SY5Y cells.

Results
Epac2 activation did not protect against Aβ-induced cell death in differentiated SH-SY5Y cells. Pretreatment with Epac2 activator or pan Epac activator led to a significant increase in Aβ toxicity. In contrast, Epac2 inhibition induced significant cell death in a process involving caspases but did not reverse the Aβ-induced cell death. On protein level, treatment with Aβ decreased Epac2 and GluA3 in differentiated SH-SY5Y cells, while the Epac2 activator tend to increase total GluA3. In addition, Epac2 activation increased PICK1 while leaving GRIP1 unaffected, potentially leading to more GluA3 cycling onto the postsynaptic surface.

Discussion/Conclusion
Our results indicate a decrease in GluA3 and Epac2 in AD. Epac2 activation does not protect against Aβ but instead promotes cell death. Interestingly, Epac2 activation tend to increase total GluA3 and reduce the removal of postsynaptic GluA3 in neuronal-like cells. We propose a model in which channel activation or increased postsynaptic GluA3 abundance through Epac2 activation increase neuronal sensitivity towards Aβ. Differential stages of Epac2 activity seem to define the neurotoxic effect of Aβ, thereby potentially AD memory deficits.

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NOVEL SK CHANNEL ACTIVATORS PREVENT FERROPTOSIS AND EXCITOTOXICITY IN NEURONAL CELLS

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Background

Small conductance calcium-activated potassium (SK) channels have been implicated in many neurodegenerative diseases. The pathology of these diseases is characterized by progressive neuronal loss, that can be initiated by ferroptosis and excitotoxicity processes. Besides regulating plasma membrane excitability, SK channel activation induced neuroprotection by reducing the mitochondrial calcium uptake, and reactive oxygen species (ROS) production. Recently, we developed novel pharmacological SK2 channel openers based on compound affinity to the SK2 channel binding pocket. We have screened 7 potential SK2 channel positive modulators and selected two best compounds for their neuroprotective effects in ferroptosis and excitotoxicity models and investigate whether the novel compounds provide neuroprotective effects in ferroptosis and excitotoxicity conditions and to compare their neuroprotective potential with the classic and commercially available SK channels activator, CyPPA.

Methods

MTT assays, xCELLigence real-time impedance measurements, FACs measurements, Confocal imaging.

Results

We determined the cell viability of the novel activators of SK channels in neuroprotective studies against ferroptotic cell death. Several novel synthetized SK channel activators prevented ferroptotic cell death in a concentration-dependent manner, as detected by MTT assay and xCELLigence measurements. FACS analysis showed that these compounds attenuated mitochondrial calcium uptake, mitochondrial ROS production mediated by ferroptosis inducer, RSL3. Interestingly, these compounds performed with higher potency than CyPPA. One compound exerted mitochondrial protection at nanomolar concentrations compared to micromolar concentrations of CyPPA, in HT22 cells. Additionally, confocal imaging was used to quantify neuronal networks in primary cortical neurons. As a result, these compounds were able to rescue neuronal network degeneration from glutamate-induced excitotoxicity.

Discussion/Conclusion

These data demonstrate a novel class of compounds that open SK channels and mediate neuroprotection in conditions of cell death.

In order to guarantee substances, target specifically to SK2 channel, we are performing electrophysiology-patch clamp in SK2 overexpressed models. Surprisingly, both compounds alone decreased proliferation compared to control group in xCELLigence. So it is still interesting to see the potential role of SK2 channel in cancer cells.

USE OF MAXIMAL URINE CONCENTRATING CAPACITY MARKERS TO EVALUATE LITHIUM-INDUCED NEPHROGENIC DIABETES INSIPIDUS

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Background
Lithium is the cornerstone in pharmacological treatment of patients with bipolar disorder. A common early renal side effect of lithium treatment is nephrogenic diabetes insipidus (NDI), which refers to a decreased maximal urine concentrating capacity causing polyuria (urine production >3L per day) and polydipsia. To evaluate NDI, a water deprivation test or DDAVP test is performed to determine maximal urine concentrating capacity, but this is time consuming and therefore not feasible in clinical practice. Consequently, NDI is probably underdiagnosed in this population with risk of significant interference of patient daily routine and occupational activities and possible irreversible kidney damage.

Methods
98 patients with a mood disorder treated with lithium at the outpatient psychiatry clinics of the Canisius Wilhelmina Hospital (CWZ), Nijmegen and GGZ inGeest Mental Health Center, Amsterdam, the Netherlands, underwent a desmopressin (DDAVP) test at to determine maximal urine concentrating capacity after inclusion in 2012/2013. Subsequently plasma copeptin, a precursor of vasopressin, and urine biomarkers for tubular injury AQP-2, α1M, NGAL, KIM-1 and NAG were measured in frozen samples to study the association with maximal urine concentrating capacity. Urine urea and plasma urea were measured to calculate the urine-to-plasma urea ratio (UPU)) to study the association with maximal urine concentrating capacity as well. Urine samples for biomarker measurement were collected as spot samples and corrected for urine creatinine. Multivariable lineair regression models were used to test associations between different variables. Non lineair variables were log (LN) transformed to attain normal distribution.

Results
98 patients underwent a DDAVP test (age 51±12 years, 38% male, duration lithium use 8 [4-15] years, eGFR 85±19 nl/min/1.73m^2). 50 out of 98 patients (51%) had an impaired maximal urine concentrating capacity (urine osmolality 600-800 mOsmol/kg) and 17 patients (17%) had NDI (urine osmolality <600 mOsmol/kg). Baseline eGFR, UPU, copeptin and NGAL were significantly associated with MUCC (St. β 0.44, p<0.001, St. β 0.30, p=0.003; St. β -0.36, p<0.001; St. β -0.25, p=0.01, respectively). UPU, copeptin and NGAL remained significantly associated after adjustment for age, sex and eGFR (St. β 0.22, p=0.02; St. β -0.22, p<0.03; St. β -0.26, p<0.01). Other urine biomarkers were not associated with maximal urine concentrating capacity.

Discussion/Conclusion
UPU, copeptin and NGAL were associated with maximal urine concentrating capacity, independent of eGFR, and therefore show promising value to screen for lithium-induced NDI.