Abstract book

SCIENTIFIC MEETING

Dutch Society for Clinical Pharmacology and Biopharmacy (NVKFB) &
Dutch Society for Pharmacology (NVF)

Part 1:

ABSTRACTS

TAPERING BIOLOGICALS IN RHEUMATOID ARTHRITIS: SIX YEAR REAL-WORLD-DATA BRIDGING BETWEEN TRIALS AND CLINICAL PRACTICE.

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Introduction
Biological Disease-Modifying Anti-Rheumatic Drugs (bDMARDs) have significantly improved long-term outcomes for rheumatoid arthritis (RA) patients by effectively controlling inflammation and preventing joint damage. However, long-term use is associated with high costs, potential adverse effects, and concerns about overtreatment, especially in patients with sustained remission or low disease activity (LDA). As a result, international guidelines recommend tapering bDMARDs in eligible patients to minimize unnecessary drug exposure while maintaining disease control. Despite these recommendations, the real-world implementation of tapering strategies remains unclear.

Methods
This retrospective cohort study analyzed RA patients treated with bDMARDs at the Maasstad Hospital in the Netherlands between 2016 and 2021. Eligible patients LDA during at least 180 days (DAS28-CRP <3.2). Study outcomes included the proportion of patients tapering bDMARDs, time in sustained remission or LDA (resp. DAS <2.6 or DAS <3.2), and flare occurrence and timing (DAS >3.2, dose increase or bDMARD switch). Dose intensity (DI) scores quantified tapering. Data analysis included descriptive statistics, logistic regression, modified Kaplan-Meier methods (Mantel-Byar, Simon-Makuch), and Cox regression with tapering status as a time-varying covariate.

Results
Of the 264 patients eligible for tapering, 45 (17.0%) tapered their bDMARDs. Tapering was significantly more frequent in younger patients and those with longer LDA duration. The majority of patients were treated with adalimumab (n=100, 37.9%), etanercept (n=88, 33.3%), or abatacept (n=37, 14.0%). The median time to first taper was 392 days of LDA (IQR: 181-1190). The type of bDMARD had no significant effect on the likelihood of tapering. Flares occurred in 46.7% of tapering patients, compared to 39.3% in those who did not taper. Cox regression, adjusted for DI, confirmed a significant association between tapering and flare occurrence. Figure 1 illustrates time to flare in tapering and non-tapering patients. The mean DI at the first taper was 0.54 (SD 0.12). 6 patients (13.3%) had a DI <0.5 at their first taper, all of whom experienced a flare. In 18 tapering patients (40.0%) DI was not increased despite a disease flare. Multivariate logistic regression showed significant association ($p<0.05$) between DI at first taper and flare occurrence.

Conclusions
Despite international guidelines recommending bDMARD tapering in RA patients with LDA, the proportion of patients tapering in our real-world cohort remained relatively low. Younger patients and those with a prolonged period of low disease activity were more likely to initiate tapering. Tapering bDMARDs was associated with and increased risk of disease flares over time. Notably, tapering strategies varied, but an early reduction in dose intensity to <0.5 was significantly associated with a higher risk of a flare, emphasizing the need for cautious and individualized tapering approaches.

References available on request

Figure 1. Simon-Makuch plot for time to flare in patient who do an do not taper biologicals during the study period.

ADALIMUMAB DOSE REDUCTION USING THERAPEUTIC DRUG MONITORING TO MANAGE LOW DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS: A SINGLE-BLIND, RANDOMIZED CLINICAL TRIAL

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Introduction
Tapering of biological DMARDs in patients with low disease activity, is common practice. Most clinicians use disease activity scores to monitor dose tapering. Considering the inter-individual variation in serum concentration of biologics, this trial-and-error approach is inevitably associated with increased risk of flare. Reducing bDMARD doses in patients with low serum concentrations can lead to ineffective drug levels and subsequent flares. There is strong evidence that withdrawal of bDMARDs result in flares. A novel approach involves a tailored tapering strategy based on serum concentration assessment known as therapeutic drug monitoring (TDM). The concentration-effect curve of adalimumab (ADA) suggests that patients with levels above 5 mg/L are at risk of overexposure. An ADA concentration of 5 mg/L is likely necessary for an adequate clinical response in the initial treatment phase. However, to control disease activity after 28 weeks, lower concentrations might be sufficient. Our study aimed to assess whether a strategy using TDM-based dose adjustments to a target concentration of 2 mg/L was non-inferior to that with a concentration of 5 mg/L with respect to disease activity. Secondarily, superiority in reducing the amount of drug administered was assessed between both groups.

Methods
Sixty-two rheumatoid arthritis (RA) patients on ongoing ADA therapy (40 mg every other week) for at least 28 weeks, and an ADA concentration of > 5 mg/L were enrolled. Patients were randomly 1:1 assigned to dose reduction aiming a serum drug level of either 2 mg/L or 5 mg/L. The newly developed algorithm based on the PK/PD model of Ternant [1], determined the dosing interval to achieve the target drug level (Table 1). Clinical visits were scheduled at baseline, 12 weeks and 24 weeks thereafter. The primary endpoint was the mean time weighted DAS28-CRP (MTW-DAS28) after 24 weeks. Noninferiority was defined as a difference with an upper 95%CI no greater than 0.6. Secondary outcomes included number of flares and ADA dosing, expressed as number of injections used during 24 weeks.

Results
Randomization created similar study groups (Table 2). The mean ADA concentration decreased from 10.1 mg/L (SD 3.5) to 5.6 mg/L (3.0) and from 9.9 mg/L (3.8) to 3.3 mg/L (1.4) respectively for the 5 mg/L and 2 mg/L group. The MTW-DAS28 at week 24 was 0.23 lower in the 2mg/L group [95%CI –0.07;0.53] P=0.14. The odds of having a flare were 1,30 higher in the 2mg/L group, which was a nonsignificant difference (OR 1.30 [95%CI 0.31;5.38] P=0.72). The total number of injections over 24 weeks was significantly lower in 2mg/L group: 30% difference (2 [95%CI –1,31; –2.85] P< 0.001) (Figure 1).

Conclusion
TDM -based tapering strategy targeting a drug level of 2 mg/L is non-inferior to a target level of 5 mg/L with respect to disease activity and number of flares, while it saves a significant amount of medication. Moreover, it reduces patients' burden of self-injections and costs.

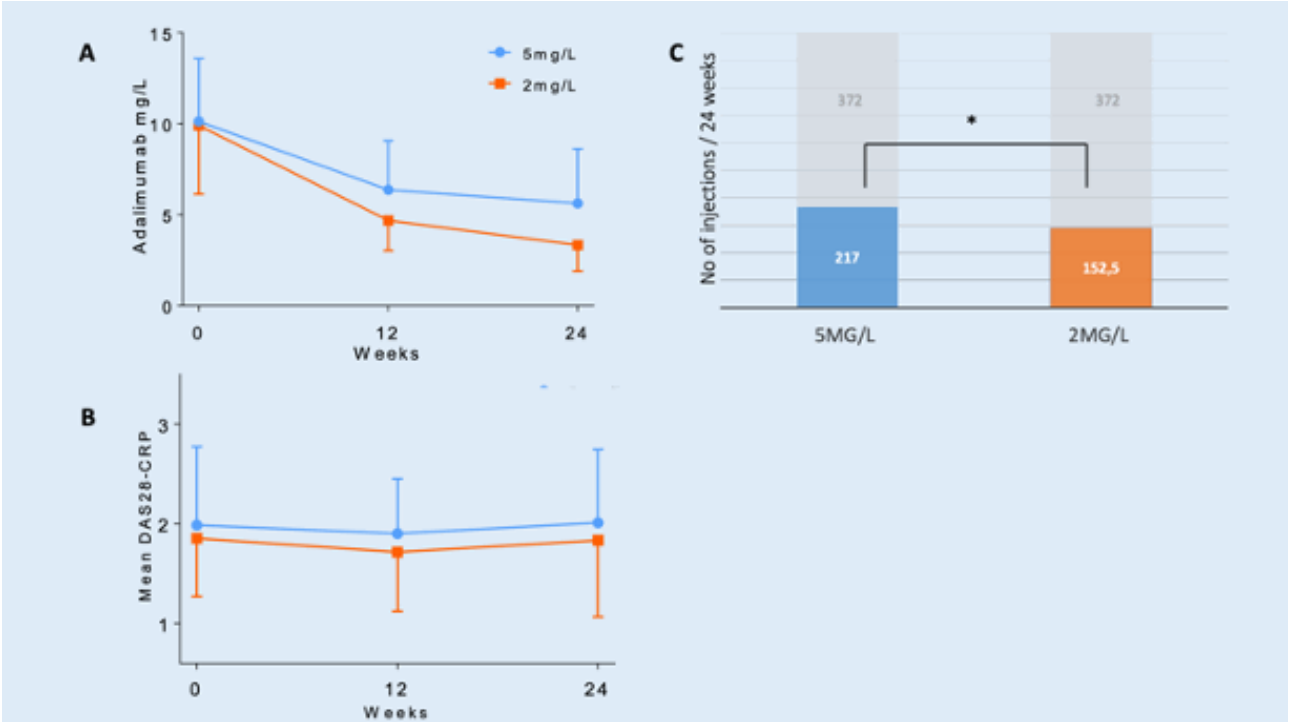
Table 1. Dosing interval algorithm

Drug level at baseline Dosing interval (weeks)	<3 mg/L	≥3 <5 mg/L	≥5 <6 mg/L	≥6 <8 mg/L	≥8<10 mg/L	≥10 mg/L
Target [5 mg/L]	2	2	3	3	4	4
Target [2 mg/L]	2	4	4	5	5	6

Table 2. Baseline characteristics. SD standard deviation; no number; IQR interquartile range; MTX methotrexate

	5mg/L(n=31)	2mg/L(n=31)
Demographics		
Age mean (SD)	60.1 (12.3)	55.0 (15.6)
Female No (%)	22.0 (71.0)	18.0 (58.1)
BMI median (IQR)	26.1 (23.5-28.1)	26.0 (23.5-28.7)
Alcohol no (%)	15.0 (68.2)	18.0 (75.0)
Smoking (ever & current) no (%)	12.0 (47.0)	12 (47.0)
Disease status		
Disease duration years median (IQR)	9.0 (3.0-14.0)	5.0 (2.0-14.0)
IgM rheumatoid factor positive (%)	23.0 (76.7)	19.0 (61.3)
Anti-CCP positive (%)	22.0 (73.3)	20.0 (64.5)
Erosion (%)	12.0 (54.5)	11.0 (42.3)
DAS28-score mean (SD)	2.0 (0.8)	1.9 (0.6)
CRP median (IQR)	2.0 (1.0-3.0)	1.6 (1.0-2.6)
ESR median (IQR)	7.5 (2.8-18.3)	7.0 (5.0-16.0)
Medication		
MTX use no (%)	22.0 (71.0)	23 (74.2)
Biological naive no (%)	27 (87.1)	29 (93.5)
Adalimumab serum concentration mean (SD)	10.1 (3.5)	9.8 (3.8)

Figure 1. (A) mean (SD) ADA concentration and (B) mean (SD) disease activity score of 28 joints (DAS28-CRP) both at baseline (prior to tapering), 12 weeks and 24 weeks thereafter (C) total number of ADA injections used during 24 weeks in blue for 5mg/L group and in orange for 2mg/L group. The gray areas represent the number of ADA injections needed for 24 weeks if standard



PHARMACOGENETICS IN PSYCHIATRY: A CASE SERIES DEMONSTRATING THE CLINICAL IMPACT OF GENETIC-GUIDED TREATMENT

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Introduction Pharmacogenetics (PGx) is an emerging field that studies the impact of heritable genetic variations on drug metabolisation and can enhance treatment outcome by reducing side effects or enhancing desired clinical effect [1]. Although there have been significant advancements in pharmacogenetic testing, and dosing guidelines are available for many psychopharmaceutic, grand scale uptake in clinical psychiatric care remains limited even though treatment efficacy is not optimal [1]. At the Outpatient Clinic Pharmacogenetics (www.PSY-PGx.nl) a personalised medication advise is provided based on psychiatric evaluation (diagnosis), treatment history, comorbidity, comedication, and pharmacogenetics profile for psychiatric patients This case series aims to describe the clinical relevance of PGx-guided pharmacotherapy psychiatric patients with inefficacy or multiple or severe side effects of psychopharmaceutic.

Methods Two psychiatric patients, diagnosed with treatment-resistant mood disorder, were evaluated. Each patient underwent pharmacogenetic testing to determine their metaboliser status for the key cytochrome P450 enzymes. Based on their genetic profile, personalised medication advice was formulated, focusing on drug selection and dose adjustments according to the guidelines proposed by the Dutch Pharmacogenetics Working Group (DPWG) [2;3]**.** A follow-up consultation assessed treatment response and patient-reported outcomes and found enhanced treatment effectiveness and reduction of side effects.

Results Both patients showed variations in their genetic metabolism requiring medication adjustments. Case 1, an ultrarapid metaboliser for CYP2C19 and a poor metaboliser for CYP2D6 benefited from dose alterations and switching to antidepressants that were not metabolised by CYP2D6 [3]. This led to improved symptom management and fewer side effects. Case 2, a poor metaboliser for CYP2C19 and CYP2B6 and having one inactive allele for CYP2D6 experienced enhanced treatment effectiveness and symptom relief following dose alterations. This also included reduction in the use of CYP2C19-dependent and CYP2D6-dependent psychotropic medication [2;3]. Patient-reported outcomes as measured with the RAS (Recovery Assessment Scale) at follow-up indicated increased satisfaction with treatment and significant reduction of side effects.

Conclusions This case series highlights the potential of PGx-guided pharmacotherapy in psychiatric practice. By personalising medication partly based on pharmacogenetics, the chance of side effects can be minimised thereby enhancing treatment efficacy. Large-scale studies are underway aiming to establish standardised clinical use of PGx guidelines in psychiatric care (www.PSY-PGx.org). Enhanced awareness and accessibility of pharmacogenetic testing worldwide could facilitate its broader implementation, improving clinical outcomes for many psychiatric patients.

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THE EFFECTS OF CONTINOUS INFUSION OF NOREPINEPHRINE ON BLOOD PRESSURE IN AWAKE AND ANAESTHETISED HEALTHY VOLUNTEERS

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Introduction

Norepinephrine (NE) is frequently administered during general anaesthesia (GA) to treat hypotension [1], yet little is known of its quantitative effect on arterial blood pressure (ABP) [2]. We aimed to describe the concentration- and dose-response relationship between NE and ABP in awake and anaesthetised healthy volunteers.

Methods

36 volunteers (18–70 years) participated. NE infusion began at 0.04 µg kg^{-1} min^{-1} and increased in four equal increments to 0.20 µg kg^{-1} min^{-1}, each lasting 15 minutes. After a washout, GA (propofol/remifentanil) was induced and maintained. Steady state invasive ABP measurements and assays of plasma NE concentrations were performed. Concentration- and dose-response relationship were assessed by linear mixed-effects modelling.

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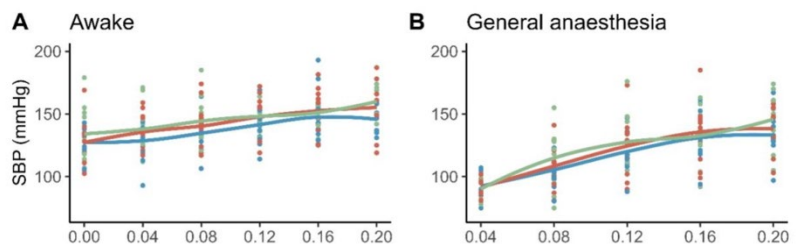

Figure 1 –Raw dataplot of the Relationship between Systolic Blood pressure (mmHg) and NE infusion rate (mcg kg^{-1}).

Results

The relationship of SBP (systolic blood pressure) and infusion rate is plotted in figure 1. An increase of 0.01 mcg kg-1 min-1 in NE infusion rate resulted in a 1.20 mmHg [95%CI: 0.52 to 1.87] increase of SBP during the awake phase, and this effect increased with an additional 1.69 mmHg [95%CI: 1.47 to 1.92] under GA. Similar results were found for diastolic blood pressure (0.50 mmHg [95%CI: 0.35 to 0.62] and 0.75 mmHg [95%CI: 0.65 to 0.85]) and mean arterial pressure (1.02 mmHg [95%CI: 0.79 to 1.25] and 1.23 mmHg [95%CI 1.22 to 1.23]). Age further increased the effect on SBP with 0.04 mmHg [95%CI: 0.01 to 0.06] per year (p=0.007). Female gender independently decreased SBP (-8.32 mmHg [95%CI: -14.74 to -1.91],p=0.022).

Conclusions

GA enhances the dose-response relationship between NE and ABP, so that a larger increase in ABP is attained during GA, than attained in an awake state. Increasing age was associated with a steeper dose-response curve for SBP.

A POOLED POPULATION PK ANALYSIS INVESTIGATING ONCE-DAILY STANDARD DOSING OF DOLUTEGRAVIR IN HIV/TB CO-INFECTED CHILDREN WEIGHING 3 KG OR MORE.

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Introduction

Dolutegravir is a key HIV treatment, but rifampicin co-treatment increases its clearance via UGT1A1 and CYP3A4 induction. This is typically managed by doubling the dose for twice-daily (BID) administration, but BID dosing can hinder adherence in children. Griesel et al. (2021) showed that once-daily (OD) dolutegravir with rifampicin achieves similar virological suppression in adults. To explore this in children, we developed a population pharmacokinetic model using data from three large paediatric trials, aiming to assess whether OD dosing with rifampicin reaches sufficient dolutegravir concentrations (C_{trough}).

Methods

We developed a paediatric population pharmacokinetic model of dolutegravir in NONMEM (v7.5) using data from three trials: ODYSSEY, CHAPAS-4, and EMPIRICAL. Volume and flow parameters were scaled to 70 kg, and maturation of dolutegravir clearance was assessed. Covariates were tested using dOFV and VPC. Simulations were performed with a virtual population of 7000 children (3-<40 kg). The main endpoint was the proportion of children reaching dolutegravir trough levels above the PA-IC90 (0.064 mg/L).

Results

The model, developed using 1942 dolutegravir concentrations from 235 children (3 months to 18 years), was a two-compartment model with first-order elimination and Erlang-type absorption. Rifampicin coadministration increased dolutegravir clearance by 61%. A broken-stick model described the maturation of clearance, reaching adult levels by 2.67 years. We observed that film-coated tablets (FCT) with food had 22% higher bioavailability, while dispersible tablets (DT) with food had 47% lower bioavailability. Simulations based on the optimal scenario (DT without food, FCT with food) showed that 94.5% of children reached dolutegravir trough levels above the PA-IC90 with once-daily dolutegravir co-administered with rifampicin.

Conclusions

Simulations showed that 94.5% of children in the virtual population achieved dolutegravir trough levels above the PA-IC90 with OD dosing co-administered with rifampicin, which compares favourably to the 78% observed in adults (Griesel et al.). These results suggest that OD standard dosing dolutegravir could be an effective treatment option for children with HIV-TB coinfection.

THE IMPACT OF CO-MEDICATION USE ON IRINOTECAN-RELATED TOXICITY

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Introduction: Emerging evidence suggests that commonly prescribed drugs, such as antibiotics (AB), proton-pump-inhibitors (PPI) and metformin, can affect the efficacy and toxicity of chemotherapy through their influence on the gut microbiome [1,2]. Specifically, interference with the gut bacterial enzyme β-glucuronidase may affect irinotecan-related gastrointestinal toxicity [3]. This study aims to determine the extent to which antibiotics, PPI and metformin influence the incidence of irinotecan-related diarrhea.

Methods: This retrospective multicenter observational cohort study used electronic records of patients aged ≥ 18 years, who were initiated treatment with systemic irinotecan-based chemotherapy between August 2017 and April 2024. The primary outcome was the incidences of severe diarrhea in AB-users versus non-AB users. Secondary outcomes were the incidence of severe diarrhea in PPI and metformin users. AB use was defined as a prescription of any type of oral antibiotics within the period of 30 days before the start of irinotecan-based treatment. PPI and metformin use was defined as consecutive use of ≥ 14 days prior to the start of irinotecan treatment. Severe diarrhea was defined as grade 3 or higher following the Common Terminology Criteria for Adverse Events (CTCAE).

Results: 502 patients were included. AB use was known for 360 patients, of whom 28 (7.8%) used AB, of which the most commonly applied AB were doxycycline and amoxicillin/clavulanic acid. PPI use was documented for 483 patients of whom 184 (38%) used a PPI. Metformin use was available for 489 patients of whom 54 (11%) were using metformin at the start of irinotecan treatment. The incidence of severe diarrhea was lower in patients who used AB compared to non-AB users, although not significantly (0% vs 12.3%, p = 0.057). The incidence of severe diarrhea appeared to be higher in PPI users compared to non-users (20% vs. 13%, p = 0.029). In contrast, the incidence of severe diarrhea did not differ significantly in metformin users compared to non-users (13% vs. 16%, p = 0.552).

Conclusions: A possible lower incidence of severe diarrhea in patients who used AB prior to the start of irinotecan treatment suggests that antibiotics might reduce the burden of irinotecan-related diarrhea, potentially improving the tolerability of irinotecan treatment. In contrast, PPI use might exacerbate severe irinotecan-related diarrhea and could be considered a potential risk factor. No significant effect of metformin use on the incidence of diarrhea was observed

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A RANDOMIZED, DOUBLE-BLIND, MULTIPLE ASCENDING DOSE STUDY OF ENX-102, A NOVEL GABA-A-R α2/3/5 SUBTYPE-SELECTIVE POSITIVE ALLOSTERIC MODULATOR, IN HEALTHY VOLUNTEERS

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Introduction
ENX-102 is a gamma-aminobutyric acid type A receptor (GABA-AR) positive allosteric modulator (PAM) that enhances inhibitory neurotransmission. By agonizing GABA-AR containing alpha-2,3,5 subunits and blocking alpha1 subunits, ENX-102 may be anxiolytic while avoiding sedative, psychomotor, and cognitive effects associated with non-selective GABA-A-PAMs such as benzodiazepines (BZDs).

Methods
ENX-102 was evaluated in a randomized, double-blind, placebo-controlled, multiple ascending dose study in healthy volunteers (N=40). Doses ranging from 0.5 mg to 5.0 mg, administered once daily for 12 days, were evaluated.
Safety and pharmacokinetic (PK) data were collected.
Pharmacodynamic measures included qEEG, biomarkers assessing neurophysiological and neuropsychological function (NeuroCart test battery), and declarative memory (Visual Verbal Learning Test) analyzed by mixed model analyses of covariance.
The NeuroCart test battery assessed saccadic peak velocity, subjective alertness as measured by a visual analogue scale, sustained attention as measured by an adaptive tracking task, and psychomotor function as measured by body sway.
Sedation was measured by the clinician-administered Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale.

Results
The majority (85%) of adverse events (AEs) were mild, and all were transient. The most frequent AEs were somnolence and fatigue, with no difference from placebo in clinician-observed sedation. Furthermore, no clinically meaningful changes in safety measures were observed. Pharmacokinetics indicated dose-proportional exposure and a half-life up to 66 hours. Of particular interest, a dose of 2 mg ENX-102 was associated with a mean Cmax value of 49.82 ng/mL and AUC of 873.17 h*ng/mL. Tmax was reached at 3 hours postdose. Mean t1/2 was 61 hours.
ENX-102 significantly decreased qEEG alpha- (p=0.0271) and theta-power (p=0.0001), and saccadic peak velocity (p=0.0002), all of which were sustained with repeated administration. No consistent effects were observed on body sway (p=0.1917), adaptive tracking (p=0.3936), alertness (p=0.3419), or immediate- (p=0.3069) and delayed word recall (p=0.3445). No sedation was observed on the MOAA/S.

Conclusions
ENX-102 was safe and well tolerated and exhibited a favorable PK profile with a long half-life consistent with once daily oral administration. Central target engagement was evidenced by qEEG changes consistent with sustained reduced arousal, with no evidence of reduced alertness, impaired psychomotor function, or impaired memory, a pattern distinct from GABA-AR non-selective BZDs in humans.

TREATMENT OF LEPTOMENINGEAL DISEASE WITH INTRATHECAL AND INTRAVENOUS NIVOLUMAB: PHARMACOKINETIC AND PHARMACODYNAMIC RESULTS IN TWO CASES

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Introduction
Recent phase 1/1b trial data showed promising efficacy and safety of adding intrathecal (IT) to intravenous (IV) nivolumab in melanoma patients with leptomeningeal disease (LMD). In this trial, patients received IT nivolumab 50 mg every two weeks, combined with IV nivolumab 240 mg from the second cycle onwards. Clinical evaluation was performed based on MRI, without assessing pharmacokinetic (PK) and pharmacodynamic (PD) data. Here, we determined the PK and PD of nivolumab in cerebrospinal fluid (CSF) and blood in two melanoma patients with LMD with the same treatment regimen to investigate the added value of IT nivolumab in combination with IV nivolumab treatment.

Methods
Two melanoma patients with LMD, for whom no regular treatment options were available, were treated with concurrent IT and IV nivolumab. During the treatment period, pre-dose CSF and blood were taken to assess nivolumab levels and PD-1 receptor occupancy (RO). Nivolumab plasma levels were measured with LC-MS/MS and ELISA, and PD-1 RO was determined with a multiparameter flow cytometry assay.

Results
Patient 1 had received systemic treatment with nivolumab four weeks before the first IT treatment. CSF examination before IT treatment showed detectable nivolumab levels in both CSF and plasma, with a PD-1 RO of 96.6% and 98.8% on CD4+ T-cells and 95.7% and 95.2% on CD8+ T-cells in CSF and blood, respectively. Concurrent IT and IV treatment did not increase the PD-1 RO at trough level. Patient 2 showed detectable nivolumab levels and a maximal RO of 100% at trough level for both CD4+ T-cells and CD8+ T-cells in CSF and blood after the first treatment cycle with IT nivolumab only. PD-1 RO did not change after the addition of IV nivolumab in subsequent cycles.

Conclusions
Based on our PK and PD analyses in two patients, we found no pharmacological evidence supporting concurrent IT and IV administration of nivolumab in melanoma patients with LMD. The distribution of nivolumab is bidirectional (CSF-plasma), with a nearly maximal PD-1 RO in CSF after IV nivolumab, indicating that the route of administration (IT or IV) does not play a significant role. Other beneficial PD effects than the measured PD-1 RO, however, cannot be ruled out.

INCIDENCE OF RASH AND ACUTE KIDNEY FAILURE IN A REAL-LIFE COHORT OF HOSPITALIZED PATIENTS WITH LEGIONELLA PNEUMONIA TREATED WITH LEVOFLOXACIN OR CIPROFLOXACIN

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Introduction

Legionella pneumonia is a serious condition as Dutch surveillance data show a death rate of 5-10% of the reported cases. Directed antibiotic therapy consist of a fluorquinolone or macrolide. Since the introduction of levofloxacin instead of ciprofloxacin as first-line agent in 2020 health care providers in our hospital seem to observe more rash and acute kidney injury (AKI). In the literature these both side-effects are described for both fluorquinolones with a incidence of 1-3%, but no difference in incidence has been described. This study therefore aimed to objectively test this hypothesis with a retrospective database study in our hospital.

Methods

In this single centre retrospective cohort study patients who were hospitalized with a positive legionella urine antigen and/or PCR test and treated with levofloxacin and/or ciprofloxacin between June 2015 and December 2024 were included using CTcue v4.13.1. The primary endpoint was a descriptive comparison of the incidence of rash and AKI for ciprofloxacin versus levofloxacin. Rash was based on the description of rash (or a synonym) as assessed by the treating health care provider in the patient file. AKI was defined as an increase in serum creatinine since start medication conform the KDIGO 2012 criteria.

Results

A total of 192 patients with a median (min-max) age of 69 (29-96) years met the inclusion criteria. Treatment consisted of ciprofloxacine alone (45%), levofloxacine alone (26%) or both (switch therapy; 30%). Rash had occurred in 12 (6%) of the patients, and AKI occurred in 27 (14%). Table 1 shows the proportion of patients with an adverse event per treatment group. Of the patients with a rash or AKI, respectively 50% and 67% were admitted to the intensive case.

Table 1. proportion with rash or AKI in each treatment group

	Rash, n (%)	AKI, n (%)
- Ciprofloxacin therapy	- 6/86 (7%)	13/86 (15%)
- Levofloxacin therapy	- 2/49 (4%)	7/49 (14%)
- Switch therapy	- 4/57 (9%)*	7/57 (12%) **

* 2 patients levofloxacin –> ciprofloxacin; 2 patients ciprofloxacin –> levofloxacin
** 7 patients ciprofloxacin -> levofloxacin

Conclusions / Discussion

Rash and AKI are common adverse events for hospitalized patients with legionella pneumonia, and we observed a higher incidence of these adverse events as described in the literature. The levofloxacin therapy group did not experience more rash or AKI as the ciprofloxacin therapy group. These results give no reason to change the first line policy, and reflect that switching to other fluorquinolone upon a rash or AKI does not seem sensible.

PHARMACODYNAMIC EFFECTS OF LEVETIRACETAM ON TRANSCRANIAL MAGNETIC STIMULATION IN GENERALIZED EPILEPSY PATIENTS: A BIOMARKER FOR EARLY-PHASE DRUG DEVELOPMENT

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Introduction

Transcranial magnetic stimulation-electromyography (TMS-EMG) is a non-invasive method to study corticospinal excitability. Epilepsy's pathophysiology relates to cortical hyperexcitability [1] and different anti-epileptic drugs (AEDs) affect TMS measures [2]. A TMS-EMG validation study demonstrated effects of levetiracetam, valproic acid and lorazepam several parameters of cortical excitability in healthy volunteers [3]. This follow-up study evaluated levetiracetam's effects on cortical excitability in generalized epilepsy patients, with the goal to validate TMS as a translational biomarker for early-phase drug development of new AEDs.

Methods

This randomized, double-blind, two-way cross-over study included two groups of patients with generalized epilepsy: 13 patients on levetiracetam (2dd 500mg) and 10 valproic acid (max 1000mg/day) monotherapy (levetiracetam-naïve). During two visits (washout 7 days), patients received a single dose of levetiracetam 2000mg or placebo after refraining from their morning AED dose. Single- and paired-pulse TMS-EMG was performed pre-dose, 1.5h and 3h post-dose. TMS-EMG endpoints were analyzed with a mixed effects model analysis of variance with baseline as covariate.

Results

The single pulse MEP amplitude (estimated difference (ED): -382 μV, 95%CI: -580;-184, p=0.0009) and long intracortical inhibition at interstimulus interval (ISI) 50 msec ($LICI_{50}$)(ED: -98%, 95%CI: -154;-44, p=0.0015) were significantly reduced in the combined group, with the greatest effect in the levetiracetam-naïve patients. The LICI at ISI 100 msec ($LICI_{100}$) was significantly reduced in patients on levetiracetam monotherapy (ED: -7%, 95-CI: -12;-2, p=0.0155). The resting motor threshold, short intracortical inhibition at ISI of 2 msec ($SICI_2$) and LICI at ISI of 300 msec ($LICI_{300}$) were not affected.

Conclusions

In summary, levetiracetam reduced the cortical excitability in patients with generalized epilepsy, with a greater effect size in the levetiracetam-naïve patients. These effects are in line with findings in healthy volunteers and confirm the value of TMS-EMG as a translational biomarker for AED effects in epilepsy patients.

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RETROSPECTIVE VALIDATION OF CLINICAL DECISION SUPPORT TOOLS FOR PREDICTING EFFECTIVENESS OUTCOMES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE TREATED WITH VEDOLIZUMAB

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Introduction

Two clinical decision support tools (CDSTs) have been developed to predict treatment effectiveness of vedolizumab in Crohn's disease (CD) and ulcerative colitis (UC)[1,2]. This study aimed to validate the CDSTs with real world data from a Dutch teaching hospital.

Methods

Patients with CD or UC treated with vedolizumab or ustekinumab (for testing specificity of the CDSTs) between October 2014 and July 2023 were included. The primary outcomes were rates of clinical remission (CREM), biochemical remission (BioREM), composite (both clinical and biochemical) remission (CompREM) and corticosteroid-free clinical remission (CSF-CREM) at week 14, 30 and 54. Secondary outcomes included drug survival, rates of bowel resection and the incidence of a reduced dosing interval at week 54. Both correlation between drug survival and baseline serum albumin and sensitivity and specificity were assessed.

Results

A total of 101 patients were included, of which 32 CD patients treated with vedolizumab, 28 CD patients treated with ustekinumab and 41 UC patients treated with vedolizumab. Among UC patients treated with vedolizumab, the high probability group had statistically significant higher rates of CREM, CompREM and CSF-CREM at week 54 than the low+intermediate probability group. At week 14 and week 30 there was also an increasing trend for all outcomes, but not statistically significant. Differences in rates of drug survival differed significantly between low+intermediate and high probability groups for patients with UC. The CDST of UC discriminated drug survival with an AUC of 0.843 (95%CI 0.718-0.968). For both CD patients treated with vedolizumab and CD patients treated with ustekinumab, no statistically significant differences were found between low+intermediate and high probability groups for all outcomes.

Conclusions

The CDST for UC is able to predict various effectiveness outcomes for treatment with vedolizumab and can therefore help in the selection of optimal treatment for patients with UC. For CD patients treated with vedolizumab, the CDST could not predict effectiveness outcomes in our cohort.

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SWITCHING FROM INTRAVENOUS TO SUBCUTANEOUS INFLIXIMAB IN ADULT PATIENTS WITH INFLAMMATORY BOWEL DISEASE: EVALUATION OF EXPOSURE PARAMETERS (SHUFFLE STUDY)

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Introduction: Infliximab (IFX), an anti-TNF-alpha monoclonal antibody, is approved for Inflammatory Bowel Disease (IBD) treatment. Recently, the subcutaneous (SC) IFX biosimilar CT-P13 was introduced, offering higher pharmaco-kinetic exposure compared to intravenous (IV) administration. The main objective of this study is to evaluate the IFX exposure before and after switching from IV IFX to fixed dose (120 mg) Q2W SC IFX in IBD patients in remission on maintenance IV monotherapy. IFX trough levels and treatment duration were evaluated in an additional analysis.

Methods: In this single-centre study, 18 adult IBD patients in remission on stable IV IFX were switched to SC CT-P13 and followed for 24 weeks. Preliminary analysis included nine patients. The primary endpoint was the area under the concentration-time curve (AUC) at steady state before and after switching to SC CT-P13. AUCs were calculated using MwPharm++ (Mediware, version 2.40; Hanzel et al., 2021).

Preliminary Results: By January 2025, nine patients had completed the study; six of whom had Crohn's disease, 3 had Ulcerative Colitis. The mean ± SD dose and interval of IV IFX administration were 5.0 ± 0.78 mg/kg every 7.1 ± 1.0 weeks. AUCs for IV and SC were comparable, with a mean ± SD $AUC_{6\text{-}8\ weeks}$ of 29,633 ± 7,457 mg·h/L for IV therapy and 28,450 ± 9,369 mg·h/L (p=0.591) for SC therapy. Mean ± SD trough level at 4 months post-switch was 17.6 ± 6.7 mg/L with a mean $AUC_{2\ weeks}$ of 7,872 ± 2614 mg·h/L. Treatment-related time expenditure was reduced from mean ± SD 554 ± 126 minutes to 18 ± 18 minutes per six months (p<0.001).

Conclusion: SC IFX may provide equivalent pharmaco-kinetic exposure to IV IFX while reducing treatment-related time expenditure as suggested by these preliminary results. Full data is expected by the end of February.

THE IMPACT OF PREGNANCY ON THE PHARMACOKINETIC PARAMETERS OF ADALIMUMAB AND INFLIXIMAB USING POPULATION PHARMACOKINETIC MODELLING

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Introduction
It is known that pregnancy influences the pharmacokinetics (PK) of drugs in pregnant women. However, there limited evidence investigating the effect of pregnancy on large molecules, like adalimumab and infliximab, in pregnant women with inflammatory bowel disease (IBD). For this study two population (pop) PK models on non-pregnant adults from literature were optimised to investigate the effect of pregnant on the PK of ADA and IFX.

Methods
Patient, disease characteristics, laboratory values and drug information of pregnant women with IBD were collected from three Dutch hospitals (UMCG, Erasmus MC and the Radboud UMC). A literature search was conducted to verify the most optimal popPK model for non-pregnant adults treated with ADA or IFX. Two models were selected, one to describe the women using ADA and one for IFX treatment. The models were optimised to describe the study population of interest using non-linear mixed effects modelling. Validation methods were used to determine the predictive performance of the models. The effect of pregnancy was tested on both models and a power analysis was done to determine the likelihood of detecting a true effect of pregnancy on the PK parameters clearance and volume of distribution.

Results
Two popPK models were selected from literature. For ADA the model of Vande Casteele et al. was selected as a base model and for IFX the model of Fasanmade et al. 2011. In total 76 ADA and 69 IFX concentrations from pregnant women were available for analysis. The available pregnant data was used to optimise the two popPK models from literature. The optimized popPK models effectively described the pregnant population, as demonstrated by goodness-of-fit plots, bootstrap analysis, and a prediction-corrected visual predictive check. Pregnancy did not show a statistically significant effect when it was added as a covariate to both popPK models. The power analysis confirmed that pregnancy had no impact on ADA and IFX levels in the population and ruled out sparse datasets as a contributing factor.

Conclusions
Two popPK models were established to investigate the behaviour of ADA and IFX in pregnant women with IBD. The addition of pregnancy as a covariate to both popPK models demonstrated no significant effect.

EFFECT OF ANTITHROMBOTIC STEWARDSHIP ON THE EFFICACY AND SAFETY OF ANTITHROMBOTIC THERAPY DURING AND AFTER HOSPITALIZATION

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Introduction

Although the benefits of antithrombotic drugs are indisputable to reduce thrombotic events, they carry a high risk of compromising patient safety [1,2]. No previous studies investigated the implementation and (cost-) effectiveness of a hospital-based multidisciplinary antithrombotic team on bleeding and thrombotic outcomes. The primary aim of this study was to compare the proportion of patients with a composite endpoint consisting of one or more bleeding episodes or one or more thrombotic event from hospitalization until three months after hospitalization.

Methods

A prospective, multicenter before-after intervention study was conducted in two Dutch hospitals. Adult patients hospitalized between October 2015 and December 2017 treated with anticoagulant therapy were included. The intervention was the implementation of a multidisciplinary antithrombotic team focusing on education, medication reviews by pharmacists, implementing of local anticoagulant therapy guidelines based on national guidelines, patient counselling and medication reconciliation at admission and discharge. The primary endpoint was analysed using segmented linear regression.

Results

We obtained data for 1,886 patients: 941 patients were included in the usual care period and 945 patients in the intervention period. The S-team study showed that implementation of a multidisciplinary antithrombotic team over time significantly reduced the composite end point consisting of one or more bleeding episodes or one or more thrombotic event from hospitalization until three months after hospitalization in patients using anticoagulant drugs (-1.83% (-2.58% to -1.08%) per 2 month period).

Conclusions

This study shows that implementation of a multidisciplinary antithrombotic team over time significantly reduces the composite end point consisting of one or more bleeding episodes or one or more thrombotic event from hospitalization until three months after hospitalization in patients using anticoagulant drugs.

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TARGET ENGAGEMENT AND IMMUNOGENICITY OF AN ACTIVE IMMUNOTHERAPEUTIC TARGETING PATHOLOGICAL A-SYNUCLEIN: A PHASE 1 PLACEBO-CONTROLLED TRIAL

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Introduction
Investigational therapeutics that target toxic species of α-synuclein (αSyn) aim to slow down or halt disease progression in patients with Parkinson's disease (PD). Here this 44-week, randomized, placebo-controlled, double-blind, single-center phase 1 study investigated safety, tolerability and immunogenicity of UB-312, an active immunotherapeutic targeting pathological αSyn, in patients with PD.

Methods
The primary outcome measures were adverse event frequency and change in anti-αSyn antibody titers in blood and cerebrospinal fluid (CSF). Exploratory outcomes were changes in clinical scales and biomarker-based target engagement as measured by seed amplification assay.

Results

Twenty patients were randomized 7:3 (UB-312:placebo) into 300/100/100 µg or 300/300/300 µg (weeks 1, 5 and 13) intramuscular prime-boost dose groups. Safety was similar across groups; adverse events were mostly mild and transient. Two patients experienced three serious adverse events in total, one possibly treatment related; all resolved without sequalae. Anti-αSyn antibodies in serum from 12/13 and CSF from 5/13 patients who received three UB-312 doses confirmed immunogenicity. Mean serum titers (in log-dilution factor) increased from baseline by 1.398 and 1.354, and peaked at week 29 at 2.520 and 2.133, for 300/100/100 µg and 300/300/300 µg, respectively. CSF titers were 0 at baseline and were 0.182 and 0.032 at week 21, respectively. Exploratory analyses showed no statistical differences in clinical scales but a significant reduction of αSyn seeds in CSF of a subset of UB-312-treated patients.

Conclusions

These data support further UB-312 development.

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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF IVACAFTOR/TEZACAFTOR/ELEXACAFTOR IN CHILDREN WITH CYSTIC FIBROSIS: EVALUATING HEPATIC AND INTESTINAL CYP3A4 ONTOGENY

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Introduction: In pediatric physiologically-based pharmacokinetic (PBPK) modeling, uncertainty persists regarding the ontogeny functions of hepatic and intestinal cytochrome-P-450 (CYP3A4) enzymes. This study evaluates the predictive accuracy of available CYP3A4 ontogeny functions using cystic fibrosis transmembrane conductance regulator (CFTR) modulators ivacaftor, tezacaftor, and elexacaftor, which are widely used in cystic fibrosis (CF) treatment.

Methods: An adult PBPK model was developed using the physicochemical properties and absorption, distribution, metabolism and excretion characteristics of CFTR modulators, with virtual populations from Open Systems Pharmacology. The PBPK model was calibrated using single-dose data from healthy adults and validated by predicting pharmacokinetic (PK) data following multiple doses in adults with CF. The PBPK model was scaled to children aged 0 to 6 years, accounting for developmental changes in anatomy and physiology, including various CYP3A4 ontogeny functions. The pediatric PBPK model was assessed against observed data for ivacaftor in children aged 4 to 6 years, and for tezacaftor and elexacaftor in children aged 2 to 6 years. Simulations were performed using each of the five available hepatic (Edginton, Upreti, Salem, Anderson, and Björkman) and two intestinal (Johnson, and Flat) CYP3A4 ontogeny functions to evaluate the predictive performance of these functions. Additional simulations were performed to optimize dosing recommendations of CFTR modulators in children aged 0 to 6 years.

Results: Overall, the PK predictions generated using Edginton's ontogeny function demonstrated the highest accuracy for hepatic CYP3A4 ontogeny, whereas the Flat ontogeny function provided the most precise predictions for intestinal CYP3A4 ontogeny.

Conclusions: PBPK modeling of ivacaftor, tezacaftor, and elexacaftor demonstrated that dosing recommendations by the Food and Drug Administration are appropriate for children with CF. Additionally, identifying accurate hepatic and intestinal CYP3A4 ontogeny functions is crucial for application of PBPK modeling in pediatric drug development, especially in the youngest pediatric populations.

ASSESSMENT OF THIOPURINE-INDUCED TOXICITY IN HUMAN FULL-TERM PLACENTA USING THE PLACENTAL PRECISION CUT SLICING METHOD

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Introduction: Thiopurines, including 6-mercaptopurine (6-MP) and 6-thioguanine (6TG), are widely used immunosuppressive agents for the treatment of autoimmune disease and leukemia. Despite their clinical benefits, concerns remain regarding their safety during pregnancy. Understanding the potential toxicity of thiopurines is crucial to ensure maternal and fatal safety. This study aimed to assess thiopurine-induced toxicity in human full-term placental tissue using the placental precision cut slicing method and complementary assays to evaluate cellular viability, cytotoxicity, and metabolic activity.

Methods: Full-term human placentas from uncomplicated pregnancies were obtained. The maternal and fetal sides of each placenta were carefully separated. Tissue sections were prepared into uniform slices using a Krumdieck tissue slicer and incubated for up to 48 hours in Williams' E culture medium supplemented with GlutaMAX, gentamicin, and glucose. The placental slices were exposed to both therapeutic and toxic concentrations of 6-MP and 6-TG. Tissue viability was assessed using the Presto Blue assay for metabolic activity, ATP determination for energy metabolism, and LDH release assay for cytotoxicity. Thiopurine concentrations in maternal and fetal slices were quantified using LC-MS/MS to evaluate drug accumulation. Additionally, hormone levels, including human placental lactogen (hPL), human chorionic gonadotropin (hCG) and estrogen were measured to assess endocrine function. Morphological changes were analysed to evaluate structural integrity and tissue preservation.

Results: Presto Blue assay results demonstrated stable metabolic activity across all tested concentrations of 6-MP and 6-TG, with no significant reduction in viability observed in either maternal or fetal placental slices. Similarly, LDH release assays showed no indication of increased cytotoxicity, with consistent levels of LDH release across all treatment groups, further confirming the absence of toxic effects. Other analyses, including ATP determination, LC-MS/MS for thiopurine concentrations, hormone measurements, and morphological assessments, are still ongoing.

Conclusions: The results from the Presto Blue and LDH assays indicate that exposure to both therapeutic and toxic concentrations of 6-MP and 6-TG does not negatively affect placental tissue viability or cause cytotoxicity in maternal or fatal slices. These findings suggest that thiopurines may be safe for placental tissue under the tested conditions. However, ongoing analyses, including ATP determination, hormone measurements, and morphological assessments, are required to provide a more comprehensive understanding of their effects on placental function and structure.

SAFETY OF SGLT2 INHIBITORS IN OLDER ADULTS: A RETROSPECTIVE COHORT STUDY

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Introduction: Sodium–glucose cotransporter-2 (SGLT2) inhibitors have demonstrated effectiveness in managing type 2 diabetes mellitus and heart failure, providing both glycemic control and cardio-renal benefits [1]. However, their safety profile in older adults remains insufficiently characterized, largely because individuals with age-related physiological changes and comorbidities have been underrepresented or excluded from many clinical trials. Therefore, this study aims to evaluate the differences in adverse events (AEs) between age groups among users of SGLT2 inhibitors.

Methods: A retrospective cohort study was conducted at Haga Teaching Hospital (2016–2024), including 3,417 patients who initiated SGLT2 inhibitors. Patients were stratified by baseline age (<60, 60–70, >70 years), with <60 years as the reference group. Data were extracted from electronic medical records (EMRs) using CTcue software, covering structured fields (diagnosis codes, lab values) and unstructured fields (clinical notes). Adverse events (AEs) were identified within the hospital system, potentially missing those outside care. Key AEs included infections (diagnoses, positive urine cultures, antibiotic use), acute kidney injury, hypotension (systolic BP <110 mmHg), syncope/falls, and metabolic abnormalities. Logistic regression estimated adjusted odds ratios (ORs) for AEs in older groups relative to <60 years, controlling for sex and comorbidities (heart failure, kidney disease, diabetes).

Results: The median age of the cohort was 70 years (IQR: 61–76), with 66.8% men. Compared to those <60 years, patients >70 years had significantly higher odds of acute kidney injury (OR 1.65, p=0.021), urinary tract infection (OR 1.93, p=0.003), positive urine cultures (OR 1.52, p=0.031), antibiotic use (OR 1.25, p=0.007), and syncope/falls (OR 2.44, p<0.001). Hypotension was most prevalent in the 60–70 age group (OR 3.92, p<0.001). The overall incidence of genital fungal infections (0.9–1.3%) and diabetic ketoacidosis (0.5%) was low, without significant differences across age groups.

Conclusions: with an increased risk of specific adverse events, including acute kidney injury (AKI), urinary tract infections (UTIs), and syncope/falls. Hypotension was most prevalent in the 60–70 age group. These findings align with existing literature, which reports higher incidences of adverse events, particularly in the oldest adult populations [2,3]. Interestingly, while real-world data highlight a high incidence of genitourinary infections, especially in the oldest age group [2], our study observed a notably low incidence of these infections. The low incidence of genital infections in our cohort may be influenced by the underrepresentation of women (33.2%). These findings highlight the importance of close monitoring and individualized prescribing when initiating SGLT2 inhibitors in older populations.

References are available upon request.

IMPACT OF STATIN USE ON MUSCLE HEALTH IN OLDER ADULTS

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Introduction: Statins are a cornerstone in the management of cardiovascular diseases [1], yet their potential effects on muscle health, particularly among older adults, remain contentious. By inhibiting the mevalonate pathway, statins may disrupt mitochondrial function, promote muscle degradation, and lead to adverse muscle-related events [2]. Given the importance of muscle health for maintaining independence and overall well-being in older adults, this study investigates the association between statin use and muscle strength, muscle mass, and physical performance in individuals aged 75 and older over a one-year period.

Methods: This prospective observational study was conducted among outpatients aged 75 years and older. Patients using statins were compared to non-users. Muscle strength, muscle mass, and function were assessed using handgrip strength, Skeletal Muscle Index (SMI), and the Short Physical Performance Battery (SPPB). Changes in these measurements were evaluated over a one-year period. The results were stratified by sex, and adjustments were made for potential confounders, including polypharmacy, age, BMI, and the total CIRS score.

Results: A total of 301 participants were included, of whom 154 were statin users and 147 were non-users. Statin users had a higher prevalence of polypharmacy (83.7% vs. 58.4% in non-users) and a higher cumulative illness burden, with a CIRS-G total score of 14.1 (95% CI: 13.4-14.9) compared to 11.6 (95% CI: 10.9-12.4) in non-users ($p < 0.001$). At baseline, no significant differences were found in muscle health parameters between statin users and non-users for both men and women. Longitudinal analysis revealed a significant difference in the change in SMI between male statin users and non-users. Male statin users showed a smaller decline in SMI (0.8%, 95% CI: -0.7% to 2.3%) compared to non-users, who had a decrease of -2.1% (95% CI: -3.8% to -0.4%, $p = 0.02$). Other muscle health parameters in both men and women remained unchanged.

Conclusion: This study found no overall effect of statin use on muscle strength, skeletal muscle index (SMI), or physical performance in older adults over one year. However, a significant difference in SMI was observed in men, with non-statin users experiencing a greater decline in muscle mass compared to statin users, for which we have no clear explanation other than the potential influence of uncontrolled factors (e.g., physical activity, therapy adherence). What distinguishes this study from other literature is its exclusive focus on individuals aged 75 and older, as well as the use of validated muscle health assessments [3]. Despite its strengths, limitations include its observational design and limited generalizability to frail populations.

References are available upon request

IN THE REAL-WORLD, A PHARMACOKINETICALLY GUIDED FOOD INTERVENTION ALSO OPTIMIZES ABIRATERONE TREATMENT IN MCRPC

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Introduction Abiraterone therapy is an effective and well-tolerated treatment for metastatic castration-resistant prostate cancer (mCRPC), improving survival by 4 to 15 months. Optimal therapeutic exposure, defined by trough plasma concentrations (Cmin) >8.4 ug/L, correlates with extended progression-free survival (PFS) (12 vs. 7 months) [1]. Standard fasting-state administration resulted in subtherapeutic levels in 65% of patients in a tertiary care academic hospital. Hereby, a food intervention improved abiraterone absorption, enhancing therapeutic levels in 87.5% of patients [1]. We performed a prospective study to evaluate the feasibility of using a food-based intervention to achieve therapeutic exposure in a real-world multicentre hospital setting.

Methods mCRPC patients from two non-academic hospitals were monitored for abiraterone trough levels during routine three-monthly blood evaluations. Abiraterone plasma concentrations were measured using LC-MS/MS. Patients with Cmin < 8.4 ug/L, were instructed to take their 1000 mg abiraterone concomitantly with a light meal, instead of in the fasting state.

Results In total, 47 patients were included in the study with a median of 2 samples per patient. Overall, 14 patients (30%) had Cmin < 8,4 ug/L (median 6.1 ug/L; range 2.0–8.3 ug/L). In 9 patients with follow-up abiraterone levels post food intervention, Cmin increased significantly from a median of 6.3 ug/L (range 2.0–8.3 ug/L) to 9.8 ug/L (range 3.9–688 ug/L; p = 0.021), without evidence of additional toxicity. Adequate exposure (Cmin >8.4 ug/L) was achieved in 7 of these 9 patients (78%; p = 0.016). Among patients with Cmin >8,4 ug/L during the entire study period were 11 patients with Cmin >100 ug/L, which suggests non-trough sampling.

Conclusions In this community hospital setting, 30% of patients exhibited subtherapeutic abiraterone levels. The food intervention resulted in adequate exposure in 78% of these patients. As in the tertiary care academic setting, our patients achieved therapeutic levels following the food intervention to a similar extend. The lower proportion of our patients with subtherapeutic abiraterone levels, may be attributed to more non-trough measurements in our population. This study demonstrates that the pharmacokinetically guided food intervention significantly improves the proportion of patients reaching therapeutic abiraterone levels, supporting its integration into routine clinical practice for mCRPC management.

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DOSE-RESPONSE RELATIONSHIPS OF MARALIXIBAT AND ODEVIXIBAT IN PATIENTS WITH ALAGILLE SYNDROME

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Introduction

Alagille syndrome (ALGS) is a multisystem autosomal dominant disorder that is associated with cholestasis from a young age. For the treatment of cholestatic pruritus in patients with ALGS two selective reversible Ileal Bile Acid Transporter inhibitors (IBATi), maralixibat and odevixibat, are approved. IBATi interrupt the enterohepatic circulation of bile acids by inhibition of IBAT in the terminal ileum. The optimal doses for these IBATi have not been determined, and the current literature reports a wide range of doses, with varying responses. This study aims to determine relationships between dose and efficacy in reducing pruritus and serum bile acid (sBA) based on available publications.

Methods

A systematic literature search was carried out in Pubmed, EMBASE, Web of Science, and Cochrane Library of articles, oral presentations, poster presentations and abstracts. The impact of different doses on treatment outcomes was evaluated for maralixibat and odevixibat. Extracted parameters are sBA and pruritus scores from clinical studies, their long-term extensions and retrospective studies.

Results

There are 25 reports included in this systematic review. Maralixibat maintenance dose ranges from 66.5 to 760 µg/kg/day, with variable results on dose-response. In studies with maralixibat, higher dosages, showed no increase change in mean pruritus score or sBA. The median decrease in pruritus at 8-10 weeks in two separate studies was less with 266 µg/kg/day maralixibat than with 133 µg/kg/day maralixibat (-0.4;-2.2 and -0.8;-2.5, respectively). For sBA the median decrease was less pronounced after 8-10 weeks on maralixibat dose of 266 µg/kg/day than on 66.5 µg/kg/day (-27 µmol/L vs -177 µmol/L). In contrast, in another study, sBA and pruritus decrease were smaller at a lower than at higher dose (380 and 760 µg/kg/day, respectively).

For odevixibat, doses ranged from 10 to 200 µg/kg/day. However, individual outcomes have only been reported for odevixibat 120 µg/kg/day, with similar results on pruritus and sBA across different studies.

Conclusions

Present reports on IBATi for patients with ALGS do not establish reliable dose-response relationships for pruritus or sBA. We advocate to address optimization of dose regimens to enhance clinical efficacy at the lowest possible doses.

EXPLORING HYPERKALEMIA RISK IN FRAIL OLDER PATIENTS USING RAAS INHIBITORS

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Introduction
Renin–angiotensin–aldosterone system inhibitors (RAASi) are widely used in treatment of cardiovascular and renal disease. While effective, they pose a risk of hyperkalemia. In the general population, risk factors for hyperkalemia include chronic kidney disease, congestive heart failure, and use of medication affecting potassium balance. These risk factors are highly prevalent in frail older patients. Therefore, this study aims to explore the prevalence and risk factors for hyperkalemia associated with RAASi use in this vulnerable population.

Methods
This single-center, cross-sectional study included RAASi users aged ≥ 70 years who presented at the emergency department. Clinical Frailty Scale (CFS) according to Rockwood was calculated retrospectively from information in clinical files. All patients with CFS ≥ 5 were considered frail. Hyperkalemia was defined as serum potassium ≥ 5.5 mmol/L at time of presentation at the emergency department. Potential risk factors for hyperkalemia in older patients were identified using logistic regression models.

Results
Of the 2023 participants, 86 (4.3%) were hyperkalemic, with no significant difference between frail and non-frail patients (4.7% versus 3.3%, p-value 0.157) and between different individual frailty scales (figure 1). Hyperkalemic patients were slightly younger than non-hyperkalemic patients (median age 83 versus 84 years, p-value 0.023), and females were slightly overrepresented in both groups (52.6% and 53.5%, p = 0.867). Risk factors associated with hyperkalemia in older RAASi users included younger age (odds ratio (OR) 0.95, 95% confidence intervals (CI) 0.92–0.99, p = 0.010), diabetes mellitus (OR 1.67, 95% CI 1.05–2.65, p = 0.030), moderate to severe kidney failure (OR 9.87, 95% CI 6.01–16.21, p < 0.001), and use of potassium-sparing diuretics and potassium-binding agents.

Conclusions
Contrary to expectations, this study found no association between frailty and hyperkalemia in older RAASi users visiting the emergency department. These results suggest that frail older patients without additional risk factors can be treated with RAASi when indicated, similar to the general population. The main risk factors for hyperkalemia in this population remain consistent with those in the general population, emphasizing the importance of monitoring kidney function and medication use.

IMPACT OF AZOLE CO-ADMINISTRATION ON TACROLIMUS PHARMACOKINETICS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction

Tacrolimus is a key immunosuppressant to prevent graft-versus-host-disease (GvHD) after allogeneic hematopoietic stem cell transplantation (HSCT). Pharmacokinetics (PK) of tacrolimus is characterised by large variability and a narrow therapeutic window, making therapeutic drug monitoring (TDM) essential, specifically in the first weeks after HSCT. Tacrolimus therapy is further complicated by drug-drug interactions with co-medication such as azoles, commonly used for antifungal prophylaxis or treatment. This study aimed to evaluate the impact of azole co-administration on tacrolimus PK during the first four weeks post-transplant.

Methods

This retrospective study included allogeneic HSCT patients (>18 years) who received IV or PO tacrolimus as GvHD prophylaxis between January 2016 and November 2024 in Amsterdam UMC. Data on patient characteristics, tacrolimus dosing (weight-based), tacrolimus whole blood concentrations, clinical laboratory results, and co-administration of azoles were collected from the first four weeks after HSCT. Population pharmacokinetic modelling was used for data-

Results

A total of 170 patients (791 whole blood samples) were included. The patient population was predominately male (n =110) with an age of 55 ± 15.7 years (mean ± standard deviation), bodyweight of 78 ± 14.3 kg and an eGFR) of 83 ± 29 mL/min. Tacrolimus PK was described by expanding on a previously published PK-model of Dutch renal transplant patients. Interindividual variability in clearance was 129% (CV% 1.9). Co-administration of azoles was associated with a 16.8% reduction in tacrolimus clearance (CV% 1.3, P=0.37), accounting for only 5% of the inter-individual variability in clearance.

Conclusions

Tacrolimus PK is highly variable in allogeneic HSCT patients within the first four weeks post-transplant. Concomitant azole therapy did not significantly decrease tacrolimus clearance. These results suggest that upfront dose reduction is not warranted and may lead to underexposure early after transplantation and hereby increase GvHD risk. Further optimization of the predictive performance of our models by additional covariate analyses (eg including the effect of letermovir and haematocrit on whole blood PK) may help guide dosing in these patients.

PHARMACOKINETICS OF 5-FLUOROURACIL IN PATIENTS TREATED WITH CAPECITABINE CARRYING THE C.1236G>A *DPYD* VARIANT ALLELE

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Introduction

DPYD-guided fluoropyrimidine dosing is a feasible method for limiting severe toxicity while maintaining clinical efficacy. Nonetheless, recent data noted that patients carrying the c.1236G>A variant, who started on a 25% reduced dose, had shorter progression-free survival than wild-type patients on a full dose. Although their overall survival was not affected, this still highlights the need for further investigation [1]. Hence, we compared 5-fluorouracil (5-FU) exposure in c.1236G>A carriers to that of *DPYD* wild-type patients.

Methods

For this analysis, pharmacokinetic data from nine historical trials involving patients with cancer receiving capecitabine treatment was pooled. Patients were sampled before and after administration of capecitabine to assess systemic capecitabine and 5-FU levels. Dose reductions for c.1236G>A variant carriers were performed in accordance with the clinical guidelines at the time, meaning either no reduction, or a 25 or 50% reduction of the total capecitabine dose. Pharmacokinetic exposure, expressed as area under the curve ($AUC_{0-\infty}$) in ng*h/mL, was determined using non-compartmental analysis and dose-normalized to 850 mg/m^2.

Results

In total, 36 c.1236G>A heterozygous *DPYD* variant carriers and 68 *DPYD* wild-type patients were included. Dose-normalized geometric mean 5-FU exposure was 37% lower in c.1236G>A carriers compared to *DPYD* wild-type patients: 365 ng*h/mL (coefficient of variation (CV) = 58%) vs. 582 ng*h/mL (CV = 48%), ($P < 0.001$). Notably, all c.1236G>A carriers who received a 50% dose reduction had exposure values below the range observed in the wild-type group. Among c.1236G>A carriers who received a 25% dose reduction, 6 out of 16 patients exhibited exposure values below this range.

Conclusions

An upfront 50% dose reduction for capecitabine in c.1236G>A carriers results in low 5-FU exposure. We stress the need of performing upward dose titration in carriers of this variant if treatment is well tolerated.

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DOSE-INDIVIDUALISATION OF FLUOROPYRIMIDINES BASED ON PRE-TREATMENT SERUM URACIL LEVELS

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Introduction

Although *DPYD*-based dosing has markedly improved the safety of fluoropyrimidine (FP) treatment, ~23% of wild-type *DPYD* ($DPYD_{wt}$) patients still continue to experience severe toxicity. Previous studies linked pre-treatment uracil (U) levels to severe FP-related toxicity. In this prospective multicentre trial (NCT04194957), we investigated if U-based dose-individualisation for FP treatment can further improve safety in $DPYD_{wt}$ patients.

Methods

Genotyping (*DPYD**2A, *13, c.2846A>T and c.1236G>A), U levels (in fasted state between 8-10h AM) and DPD enzyme activity in peripheral blood mononuclear cells were measured prior to FP treatment. $DPYD_{wt}$ patients with U levels > 16 ng/mL (U_{high}) received a 50% dose-reduction, as per French RNPGx guidelines. The incidence of CTCAE grade ≥ 3 FP-related toxicity in $DPYD_{wt}/U_{high}$ patients was compared to $DPYD_{wt}$ patients with U ≤ 16 ng/mL (U_{normal}) from this study and to a historical cohort of $DPYD_{wt}/U_{high}$ patients (n = 14), both treated at full dose. Pharmacokinetic (PK) data was compared to a historical cohort (n=20).

Results

Twenty-two of 612 evaluable patients were $DPYD_{wt}/U_{high}$. Dose-reduced $DPYD_{wt}/U_{high}$ patients had a significantly lower incidence of severe toxicity compared to historical full-dose $DPYD_{wt}/U_{high}$ patients (20% vs. 43%, p=.03). Moreover, the incidence of severe toxicity during the first 2 treatment cycles was comparable to $DPYD_{wt}/U_{normal}$ patients from this study (10% vs. 11%). PK analysis of 19 $DPYD_{wt}/U_{high}$ patients treated at a 50% dose showed a substantially lower 5-fluorouracil (5-FU) exposure compared to historical $DPYD_{wt}$ patients (177 vs. 381 ng*h/mL). Hereafter, five $DPYD_{wt}$ /U_{high} patients were treated at full dose and showed comparable 5-FU exposure to the historical cohort (456 vs 381 ng*h/mL). No correlation (R=.006, p=.98) between U levels and DPD enzyme activity was observed.

Conclusions

While U-based dosing of FPs in $DPYD_{wt}$ patients decreased severe toxicity, it also resulted in clinically relevant underexposure to 5-FU. This renders it unsuitable for dose-individualisation in $DPYD_{wt}$ patients. Hence, we advise to reconsider the position of U-based dosing within the EMA guideline.

DRUG REPURPOSING FOR EWING SARCOMA: QUANTITATIVE LC-MS/MS ANALYSIS OF DISULFIRAM, METABOLITES AND THE DIETHYLDITHIOCARBAMATE-COPPER COMPLEX

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Introduction

Ewing sarcoma is a high-risk bone or soft-tissue cancer mainly affecting children and adolescents. Drug repurposing can help overcome ineffective therapies in aggressive cancers such as Ewing sarcoma. Disulfiram (an anti-alcohol abuse drug) is converted into multiple metabolites including diethyldithiocarbamate (DDTC), which can chelate with copper to form the diethyldithiocarbamate-copper complex (CuET). CuET has demonstrated anticancer effects by disrupting essential tumor cell processes.[1-3] However, the hydrophobicity of CuET complicates its systemic distribution, which may be improved using nanoparticles. To assess its therapeutic potential, it is crucial to quantify the amount of CuET reaching the tumor site. This study aimed to develop a quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to accurately measure CuET, disulfiram, and the metabolites diethyldithiocarbamate (DDTC), methyl-diethyldithiocarbamate (Me-DDTC) and methyl-diethyl thiocarbamate sulfoxide (Me-DETC-SO) (Figure 1).

Methods

CuET was measured in isocratic mode using a mobile phase of 99.9% acetone, 0.1% water and 0.03% formic acid. CuET was extracted from plasma samples using acetone. Disulfiram, Me-DDTC and Me-DETC-SO were analysed using a mobile phase of ammonium bicarbonate pH 7 and acetonitrile. Since DDTC is highly reactive and quickly degrades or complexes with copper it is not directly detectable and, therefore, derivatization of DDTC using N-ethylmaleimide is currently being optimized. CuET-d_{20} was synthesized for internal standard correction.

Results

Two methods were developed as the LC method for CuET cannot contain any water. The synthesized internal standard CuET-d_{20} cannot be used for quantitation due to chemical instability. A combined compound (CuET-d_{10}) between analyte and IS was detected. Disulfiram response was observed in samples containing only CuET. CuET response appeared at the retention time of disulfiram, indicating copper adduct formation in the source. No DDTC response was detected.

Conclusions

The developed methods enable the analysis of CuET, disulfiram, Me-DDTC, and Me-DETC-SO. DDTC was measured indirectly via derivatization. These methods will be used to assess the therapeutic potential of CuET and will pave the way for subsequent studies and clinical trials in treating Ewing sarcoma.

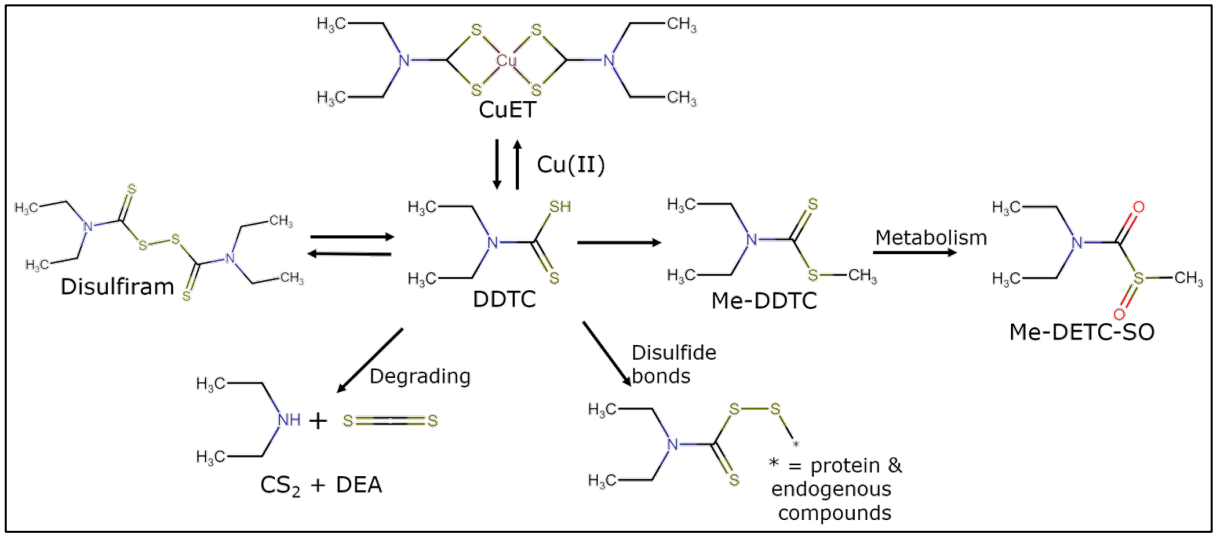

Figure 1: Overview of compounds in this method and how they are related. [Created in RSCB.org and Biorender].

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OPTIMIZING PEDIATRIC OPIOID CONVERSION AND METHADONE TAPERING: PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING IN CRITICALLY ILL CHILDREN

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Introduction: Long-term opioid use is common in critically ill children and can cause withdrawal symptoms at discontinuation. To avoid withdrawal, short-acting opioids are usually converted into a long-acting drug, such as methadone, which is subsequently tapered carefully. However, literature is inconclusive when it comes to the best way of converting to and subsequent tapering of methadone. In this study, we used physiologically-based pharmacokinetic (PBPK) modelling to explore the conversion from intravenous fentanyl and morphine into oral methadone and methadone tapering.
Methods: We extracted PBPK models for fentanyl, morphine, and methadone from literature, rebuilt the models, and verified them with pharmacokinetic (PK) data from published clinical PK studies. After model approval, we simulated the opioid infusion, the conversion to methadone according to several conversion ratios found in literature, followed by different methadone tapering schedules (Figure 1)[1-3]. Plasma concentrations were compared to the minimal analgesic concentration reported in literature (i.e., 1 ng/mL for fentanyl, 4 ng/mL for morphine, and 60 ng/mL for methadone). An expert panel was consulted to make final recommendations on conversion ratio and tapering schedule.

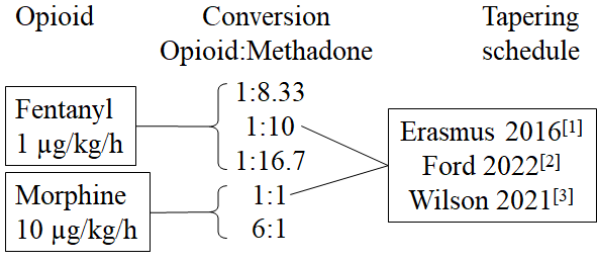

Figure 1. Overview of PBPK simulations. For fentanyl, three ratios were simulated, for morphine two. The ratio leading to the most optimal methadone level was used for simulations of three different tapering schedules.

Results: Model verification for all models proved adequate. Simulations showed that the opioid doses resulted in mean plasma concentrations just above the minimal analgesic concentration. Conversion ratios of 1:10 for fentanyl and 1:1 for morphine led to a mean methadone plasma concentration of 60 ng/mL. Simulations of three tapering schedules showed similar maximum plasma concentrations (C_{max}) and T_{max}, only the duration of total tapering (varying from 3.5 to 26 days) and the classification of risk categories differed. The expert panel agreed on the conversion ratios supported by the modelling, and based on clinical experience and the clear dosing directions, it was concluded that the tapering schedule by Ford 2022[2] was most appropriate.
Conclusions: This study showed that PBPK modelling can support dosing decisions when literature is inconclusive. Conversion and dosing recommendations are implemented in the Dutch Pediatric Formulary to guide pediatric opioid tapering.

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CHALLENGES IN OPTIMIZING TACROLIMUS THERAPY IN PATIENTS TREATED WITH RIFAMPIN: A CASE SERIES

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Introduction
Tacrolimus, a cornerstone immunosuppressant in solid organ transplantation, presents substantial management challenges when co-administered with rifampin, a potent CYP3A4 and P-glycoprotein inducer. Rifampin significantly reduces tacrolimus bioavailability, necessitating intensive therapeutic drug monitoring (TDM) and frequent dose adjustments to maintain therapeutic concentrations. This study presents two cases illustrating the impact of rifampin on tacrolimus dosing and the need for a multidisciplinary approach.

Methods
Two transplant recipients receiving rifampin and tacrolimus were analyzed. Tacrolimus dosing, trough concentrations, and concentration-to-dose ratios (CDR) were assessed during rifampin co-administration and following its discontinuation. TDM data were evaluated to determine the extent of rifampin-induced changes in tacrolimus pharmacokinetics.

Results
In Case 1, tacrolimus dose increased from 6 mg/day to 120 mg/day, while the CDR dropped from 0.66 to 0.13 µg/L/mg over five days. In Case 2, an initial 14 mg/day tacrolimus dose resulted in 3.3 µg/L, necessitating an increase to 20 mg/day, with a CDR of 0.17 µg/L/mg. After rifampin discontinuation, tacrolimus exposure rose sharply. In Case 1, the dose was reduced from 76 mg/day to 13 mg/day as the CDR increased to 0.57 µg/L/mg, with plasma concentrations stabilizing around 5.2–8.7 µg/L. In Case 2, concentrations surged from 15.7 µg/L to 33.4 µg/L, requiring a dose reduction from 16 mg/day to 4 mg/day as the CDR increased to 2.5 µg/L/mg. Rifampin's inductive effects waned over 7–15 days

Conclusions
Rifampin initiation, continuation and cessation have major effects on tacrolimus bioavailability, metabolism and elimination, leading to significant changes in blood concentrations, necessitating frequent TDM. These two cases highlight the need for individualized dosing strategies, guided by a multidisciplinary team, involving transplant specialists and clinical pharmacologists. Whenever possible, this combination should be avoided to simplify management and minimize the risk of underexposure or toxicity.

EFFECTIVENESS OF PHARMACOGENETICS IN THE TREATMENT OF MAJOR DEPRESSIVE DISORDER WITH ANTIDEPRESSANTS

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B.

Introduction

Due to the chronic relapsing nature of mental disorders and increased life expectancy, the societal burden of these non-communicable diseases will increase even further. Treatments for mental disorders, such as depression, are available, but their effect is limited due to patients' (genetic) heterogeneity, low treatment compliance and frequent side effects. In general, only one-third of the patients respond to treatment. Today, medication selection in psychiatry relies on a trial-and-error approach based mainly on physicians' experience. Pharmacogenetic (PGx) testing can help in this process by determining the person-specific genetic factors that may predict clinical response and side effects associated with genetic variants that impact drug-metabolizing enzymes, drug transporters or drug targets.

Methods

A literature study was performed to evaluate prospective pharmacogenetic clinical trials that included patients with MDD who received treatment with an antidepressant and were compared with a control group who received treatment as usual. The outcome measures we were interested in were reduction of symptoms, response and remission (measured with a standardized questionnaire, such as HAMD-17). Medline (via OVID), Embase (via Embase.com), the Cochrane Library (via Wiley), Web of Science, PsychINFO (via OVID) and Google Scholar were searched for prespecified search terms.

Results

In total we could include 17 articles: 11 randomized controlled trials (RCT's) and 6 open label studies.

Most studies showed a significant, but moderate effect on symptom reduction, response and remission in the group that received an antidepressant based on PGx. There were limited studies performed that looked on the amount of side effects.

Conclusions

The 17 studies we found in our literature search showed a modest but significant effect in symptom reduction, response, and remission. A lot of these studies had methodological limitations (for example: single blind design, a treatment algoritm that was unclear, limited n-numbers, commercial sponsorship). Large randomized clinical trials in psychiatric patients across the world will deliver more results on how to best implement pharmacogenetics in psychiatric care.

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Medication Extravasation Management and Outcomes in Patients Who Experienced an Extravasation of Medication During Hospital Admission

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Introduction
Intravenous (IV) medication administration is a common procedure for hospitalized patients but carries the risk of complications, such as extravasations. An extravasation occurs when a vesicant drug unintentionally leaks into surrounding tissue, potentially causing tissue damage. Despite its clinical significance, existing literature on the prevalence, management, and patient outcomes associated with extravasations is limited. This study aims to determine the prevalence, nature, and management strategies of extravasations in hospitalized patients and to assess patient-reported outcomes post-discharge.

Methods
A single-centre, retrospective, mixed-method study was conducted. Patients who experienced extravasations between 01-01-2022 and 01-01-2024 were included. Eligible patients were invited to complete a questionnaire. The primary outcome was the prevalence and nature of extravasations, along with management strategies used. Secondary outcomes involved patients' post-discharge experiences and outcomes. Data were extracted from the Electronic Health Record system, and questionnaire responses were analysed using descriptive statistics.

Results
A total of 200 patients with 205 extravasations were included, yielding a prevalence of 0.014% of all IV administrations. Anti-infectives were the most common drugs (32.7%). Peripheral parenteral nutrition (4.2%) and acyclovir (0.5%) were the most frequently extravasated drugs. Extravasations occurred mainly in the arm (61.5%) and were predominantly of moderate severity. Symptoms, including swelling (70.8%), redness (40.9%), and pain (38.5%), were most common on day 1 and resolved within 2-3 days. Standard management strategies were used in 96.6% of cases, with cold compresses and hot compresses with hyaluronidase being most frequent. Patient questionnaires (n=41) revealed that 70.7% recalled experiencing swelling, pain, and redness, with 46.3% reporting a burning sensation. Post-discharge, 34.1% of patients continued to experience symptoms.

Conclusions
This study is the first to provide comprehensive data on the prevalence, nature, management strategies, and patient outcomes related to medication extravasation. The findings highlight areas for improvement in both management practices and post-discharge follow-up.

DUTCH PHARMACOGENETICS WORKING GROUP (DPWG) GUIDELINE FOR THE GENE-DRUG INTERACTION BETWEEN *CYP2D6* AND *CYP2C19* AND TRICYCLIC ANTIDEPRESSANTS.

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Introduction: The study of effects of heritable genetic variation on drug response is referred to as pharmacogenetics (PGx) and contributes to personalized pharmacotherapy.
The Dutch Pharmacogenetic Working Group (DPWG) aims to develop evidence-based guidelines to optimize pharmacotherapy based on a patient's genotype.
Methods: The current guideline describes the gene-drug interactions between *CYP2D6* and *CYP2C19* and the pharmacokinetics of various tricyclic antidepressants (TCAs). A detailed description of literature collection, assessment and preparation of the gene-drug monograph methods has previously been published. In brief, a systematic review of literature was performed, relevant articles were summarized, and therapeutic recommendations were formulated. Additionally, the DPWG also developed the Clinical Implication Score and uses this to develop guidelines to determine whether genotyping prior to treatment is beneficial. This Clinical Impact Score is based on four criteria: the clinical effect associated with the gene-drug interaction, the level of evidence supporting the associated clinical effect grade ≥ 3, the number needed to genotype in the Dutch population to prevent one clinical effect grade ≥ 3, and the PGx information in the Summary of Product Characteristics (SmPC).
Results: For *CYP2D6* poor metabolisers (PM), dose reductions are advised for amitriptyline (reduction to 60% of the normal dose), clomipramine (reduction to 50% of the normal dose for the indication depression or in case of side effects at the normal dose for other indications), doxepin (reduction to 40%), imipramine (reduction to 30%), and nortriptyline (reduction to 40%). For *CYP2D6* intermediate metabolisers (IM), reduced dose is also recommended for amitriptyline (to 75%), clomipramine (to 70%), doxepin (to 80%), imipramine (to 70%), and nortriptyline (to 60%). Also, *CYP2D6* ultra-rapid metabolisers (UM) require tailored dose adjustments: amitriptyline (1.6 times the normal dose), clomipramine (1.5 times), doxepin (2 times), imipramine (1.7 times), and nortriptyline (1.7 times). Additionally, alternative drugs may be needed for *CYP2D6* UM due to potential safety concerns. For *CYP2C19* PM, a reduction to 30% of the normal dose is advised for imipramine. For *CYP2C19* IM no action is required for TCAs. For *CYP2C19* UM alternative medication is recommended for clomipramine prescribed for anxiety and obsessive-compulsive disorder (OCD).
The DPWG scored *CYP2D6* genotyping for all TCAs and *CYP2C19* genotyping for clomipramine (in anxiety/OCD) and imipramine "potentially beneficial". Testing may be considered on individual basis.
Conclusions: For *CYP2D6* gene variants, dose adjustments are advised for TCAs. For *CYP2C19* gene variants, adjustment of therapy might be advised. In these cases, testing may be considered on individual basis if the genotype of the patient is not known.

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INFLAMMATION-DRIVEN VARIABILITY IN DRUG METABOLISM: INSIGHTS FROM VORICONAZOLE TREATMENT

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Introduction

Voriconazole is widely used to prevent fungal infections in patients receiving a hematopoietic stem cell transplantation (HSCT). The drug shows highly variable pharmacokinetics, and therapeutic drug monitoring (TDM) is used to guide dosing (1). Voriconazole is extensively metabolized by CYP2C19 (2). In recent years both genetic variation and inflammation have been shown to influence CYP2C19 activity. Together these factors can lead to phenoconversion (3). However, both factors are currently disregarded and not incorporated into hematologic treatment guidelines. Here, we investigated the effect of *CYP2C19* drug metabolizer phenotypes in HSCT patients receiving voriconazole in absence and presence of inflammation. Moreover, the influence of inflammation on target attainment and the association between inflammation and CYP2C19 activity was explored.

Methods

We analyzed *CYP2C19* metabolizer phenotypes of 126 HSCT patients from the *Biobank Hematologische Ziekten* (LUMC) receiving voriconazole in the absence (CRP < 10 mg/L) and presence (CRP > 10 mg/L) of inflammation.

Results

Higher (dose-corrected) voriconazole concentrations were observed during inflammation compared to the non-inflamed state (LMM p=<2e-16, effect size of phenotype β=-5.99e-02 mg/L per mg voriconazole, SE=2.75e-02, p=0.0315; CRP β=1.54e-03 mg/L, SE=2.79E-04, p=5.49e-08). During inflammation, an increase in the frequency of supra-therapeutic levels of voriconazole was observed, while the percentage of sub-therapeutic levels decreased (χ^2 test p=0.0080). This effect was most pronounced for IMs (3% supra-therapeutic without inflammation versus 33% in presence, p=0.02). Finally, a subgroup analysis of 25 patients with longitudinal follow-up including data prior-, during, and post-inflammation, showed that voriconazole levels changed in parallel to inflammatory status.

Conclusions

Our data confirm the combined effect of inflammation and *CYP2C19* genotype on voriconazole metabolism, and suggest TDM to prevent supra-therapeutic voriconazole levels during inflammation is most beneficial in CYP2C19 IMs and RMs.

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OPTIMIZING HIGH DOSE METHOTREXATE DOSING FOR THE TREATMENT OF INFANT ALL

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Introduction

The pharmacokinetics (PK) of HD-MTX in infants with acute lymphoblastic leukemia (ALL) is complex due to rapid physiological development in the first year of life. Current dosing guidelines, based on body surface area and extrapolated from pediatric or adult standards, may not adequately account for the unique physiological characteristics of infants. The PK of HD-MTX is characterized by large inter-individual variability, highlighting the need for a more precise dosing strategy. Currently, there is no PK-based dose recommendation for HD-MTX in infants. We aim to develop a whole-body physiology-based (PB)PK model describing HD-MTX in infants with ALL, targeting an optimal end-of-infusion concentration (C_{max}) for efficacy (56 – 75 µM) [1] without exceeding the time-above-threshold concentration for toxicity (<0.2 µM at 48 hours after start of infusion). With this PBPK model, we ultimately aim to individualize HD-MTX dosing for each infant with ALL. To build the PBPK model for HD-MTX in infants, we first developed the model for an adult population as this group has the most extensive PK data available to evaluate model performance.

Methods

The adult PBPK model for HD-MTX was built in Simcyp (v23). Model performance was evaluated iteratively by comparing its predictions to clinical data. Concentration-time profiles for the evaluation of the adult PBPK model were obtained from a previously published clinical study [2]. Model performance was evaluated visually by assessing if the observed concentration-time profiles fell within the 5th and 95th percentiles of the model predicted concentration-time profiles. Additionally, the ratios of predicted to observed values for the C_{max}, area under the curve (AUC) and the half-life ($t_{1/2}$) were calculated.

Results

For the adult model, the concentration-time profiles fall within the 95% confidence interval, and the ratio of predicted to observed values ranged from 0.9 to 2.0 indicating the PBPK model fits the observed data well (see figure 1). However, $t_{1/2}$ between 24 and 48 hours is slightly overpredicted.

Conclusions

The developed PBPK model describes the adult population well, but slightly overpredicts $t_{1/2}$ between 24 and 48 hours. Since our endpoint for toxicity is a time-above-threshold concentration after 48 hours, we are currently optimizing the characterization of the elimination phase of the concentration-time curve by adding a fluid compartment where MTX is known to distribute. Building on this, we will scale the model to first a pediatric and then an infant population by aligning the physiological parameterization with these populations, followed by further evaluation against clinical data (figure 2). Ultimately, we will evaluate the current dosing schedule for infants to determine if it reaches the C_{max} endpoint for efficacy and the time-above-threshold endpoint for toxicity. Additionally, we will propose strategies to optimize the dosing schedule to more precisely achieve these endpoints.

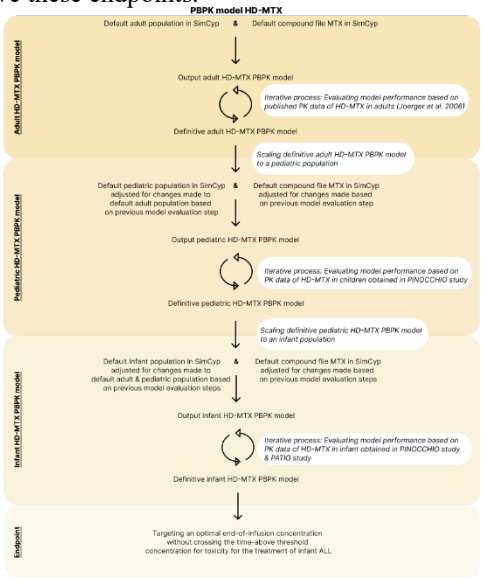

Figure 2. Overview of the workflow for development and evaluation of the PBPK model.

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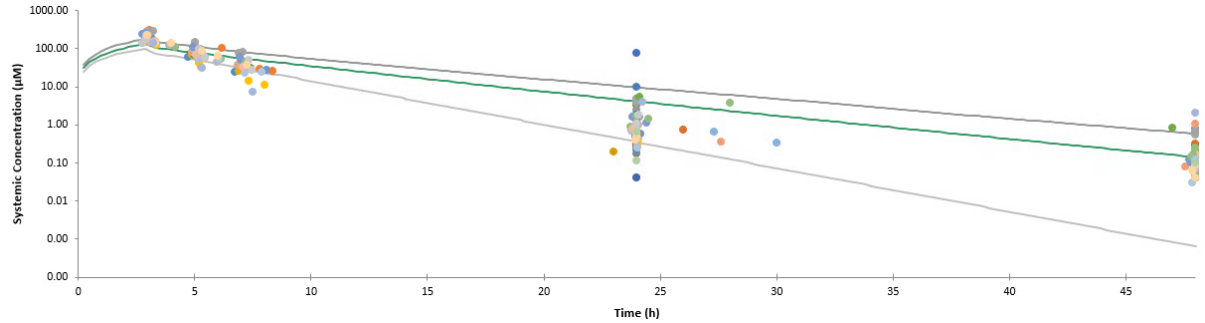
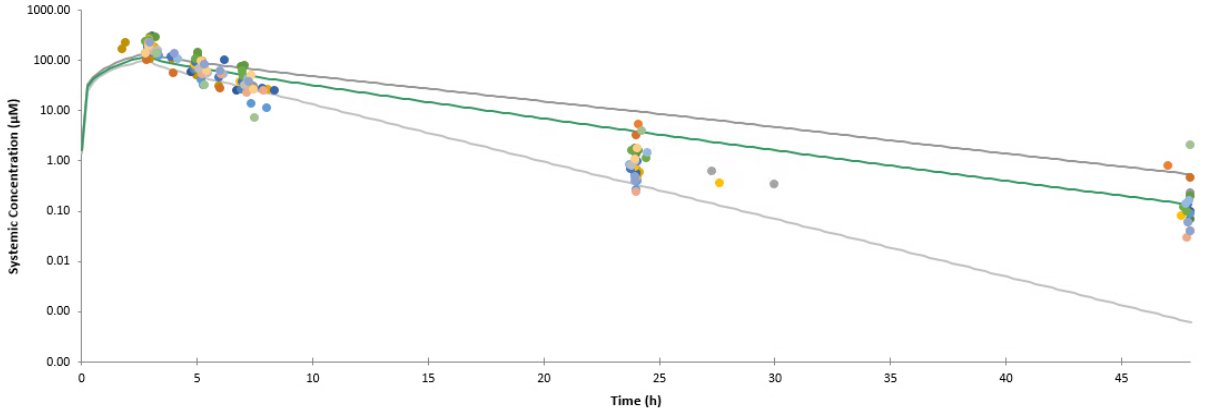
A



Median AUC_{obs} (µM*h)	95% CI	Median AUC_{pred} (µM*h)	95% CI	Ratio
861.5	701.6 – 1451.6	953.8	552.4 – 1341.8	0.9
Median $C_{max, obs}$ (µM)	95% CI	Median $C_{max, pred}$ (µM)	95% CI	Ratio
145.0	82.9 – 158.8	134.1	101.7 – 182.6	1.1
Median $T_{1/2, obs}$ (h)	95% CI	Median $T_{1/2, pred}$ (h)	95% CI	Ratio
8.4	3.3 – 13.0	4.3	2.7 – 5.8	2.0
Median AUC_{obs}/ Median $C_{max, obs}$		5.9		
Median AUC_{pred}/ Median $C_{max, pred}$		7.1		

B



Median AUC_{obs} (µM*h)	95% CI	Median AUC_{pred} (µM*h)	95% CI	Ratio
1734.9	1324.4 – 2028.6	885.4	527.3 – 1182.4	2.0
Median $C_{max, obs}$ (µM)	95% CI	Median $C_{max, pred}$ (µM)	95% CI	Ratio
219.2	176.1 – 224.5	125.9	103.4 – 146.3	1.7
Median $T_{1/2, obs}$ (h)	95% CI	Median $T_{1/2, pred}$ (h)	95% CI	Ratio
5.8	3.3 – 14.3	4.3	2.7 – 5.8	1.3
Median AUC_{obs}/ Median $C_{max, obs}$		7.9		
Median AUC_{pred}/ Median $C_{max, pred}$		7.0		

Figure 1. Simulation of the adult PBPK model (green line) with two subsets of the adult data published by Joerger et al. (dots, A = 3 g i.v. in 3 hours, B = 1.5 g/m^2 i.v. in 3 hours). The grey lines indicate the 95% confidence interval of the simulated concentration-time curve.

DEVELOPMENT OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS FOR DASATINIB AND ALECTINIB IN PREGNANT WOMEN

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Introduction
Limited information exist regarding pharmacokinetics (PK) of tyrosine kinase inhibitors such as dasatinib and alectinib throughout pregnancy. The increased usage of tyrosine kinase inhibitors in non pregnant users due to their improved safety and efficacy necessitates the understanding of their PK in special patient populations such as pregnant women. The aim of this study was to develop Physiologically Based Pharmacokinetic (PBPK) models of dasatinib and alectinib to predict their PK throughout pregnancy and to compile evidence-based dosing for the population of pregnant women.

Methods
PBPK models of dasatinib and alectinib were developed for non-pregnant healthy adults, cancer patients and pregnant women. Both models were validated against observed in vivo PK data obtained from clinical trials and from real-world cancer patients. Pregnancy-related changes in the anatomy, physiology, and activity of enzymes and transporters were taken into account for the development of pregnant PBPK models. The pregnant PBPK models of dasatinib and alectinib were validated with PK data from second and third trimester respectively, as only PK data from those trimesters was available. Most relevant PK parameters (maximum concentration, trough concentration and area under the curve) were compared between the non-pregnant and pregnant populations to evaluate whether dose adjustments are needed during pregnancy.

Results
Both PBPK models accurately predicted PK of dasatinib (47% of C_{max} and 65% of AUC ratio's within 1.30 fold CI) and alectinib (67% of C_{max} and 60% of AUC ratio's within 1.30 fold CI) in non-pregnant healthy adults, cancer patients and pregnant women. There was no significant difference in the C_{max} and C_{trough} concentration of dasatinib between the non-pregnant and pregnant population. The AUC of dasatinib in the pregnant population was significantly lower than in the non-pregnant population. This geometric mean AUC was 24%, 27% and 29% lower in the first, second and third trimester respectively. With regard to alectinib no significant difference was found in the C_{max}, C_{trough} and AUC between pregnant and non-pregnant population.

Conclusions
PBPK models of dasatinib and alectinib were successfully developed and validated for non-pregnant healthy adults, cancer patients and pregnant women in the second (dasatinib) and third trimester (alectinib). The comparison of PK parameters in the non-pregnant and pregnant population showed that there is no need for dose adjustment for both drugs in the pregnant population.

EFFECT OF CYP3A4 METHYLATION ON TACROLIMUS PHARMACOKINETICS

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Introduction
Common genetic variants in CYP3A4 together only explain ~10% of variability in tacrolimus clearance. This cross-sectional study aims to explore how much of the PK variability can be explained by methylation of the CYP3A4 gene.

Methods
The study was approved by the relevant ethical committee. Residual tissue material from liver biopsies routinely collected 6 months after transplantation were used. Transplant recipients had to use tacrolimus once daily (Advagraf™) in steady state (i.e., no dose change in the last 3 days), had to have an assessment of tacrolimus PK within 3 weeks of the biopsy and be without a documented rejection episode for at least 3 months prior. Patients and liver tissue were genotyped. Only patients where both the patient and donor had a CYP3A5 non-expressor genotype were included in this study. The liver biopsy tissue material was then analyzed using an Illumina Infinium MethylationEPIC array.

Results
From the 28 patients who fit the inclusion criteria, 23 passed the quality control check required for methylation analysis. Increased methylation measurement of one of the ten methylation probes within the CYP3A4 gene region (cg19046783) was positively correlated (Spearman correlation of +0.52) with the dose-normalized $AUC_{0\text{-}24h}$ ($P = 0.01$). When quantified using univariate linear regression, this probe explains 18% of the variation in dose-normalized $AUC_{0\text{-}24h}$. Interestingly, cg19046783 also showed the lowest mean methylation and highest biological variation.

Conclusions
Increased methylation of one of the ten methylation probes within the CYP3A4 gene region (cg19046783) was positively correlated with increased dose-normalized $AUC_{0\text{-}24h}$ which can explain 18% of the variation in dose-normalized $AUC_{0\text{-}24h}$ using univariate linear regression.

ANTI-XA LEVELS ARE NOT CORRELATED WITH RENAL FUNCTION IN PATIENTS WITH KIDNEY FAILURE WHO ARE TREATED WITH DALTEPARIN

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Introduction
Low molecular weight heparins (LMWHs) are commonly used anticoagulants, with anti-Xa (aXa) monitoring recommended in patients with renal impairment due to concerns about drug accumulation and bleeding risk. However, guidelines provide conflicting recommendations regarding dose adjustments and aXa monitoring in this population. This study investigates the correlation between the first measured peak aXa level and renal function in therapeutic twice daily dalteparin. Additionally, the association between aXa levels, bleeding, thrombosis, and mortality is explored.

Methods
A retrospective cohort study was conducted across two Dutch hospitals between dec-2017 and jun-2024. Adult patients with an estimated glomerular filtration rate (eGFR) ≤60 mL/min/1.73m² who received therapeutic dalteparin and had an aXa level measurement 3–5 hours after administration were included. All patients started with a full first dose, which was 100% continued in patients with eGFR between 30 and 60, 75% if the eGFR was between 10 and 30 and 50% if the eGFR was < 10 ml/min/1,73m^2. Anti-Xa levels < 0,5 IU/mL were considered subtherapeutic. Patients on dialysis or pregnant patients were excluded. Statistical analyses included Spearman's rho, linear regression, and Mann-Whitney U tests.

Results
157 patients were included. No significant correlation was found between aXa levels and renal function. 55% of patients had subtherapeutic aXa levels. Bleeding occurred in 24 patients (15.3%), thrombosis in 2 (1.3%), and 39 (24.8%) patients deceased within one month of dalteparin discontinuation. Neither bleeding, thrombosis or mortality was associated with increased or reduced aXa levels respectively, or dalteparin dose.

Conclusions
This study shows that renal function was not correlated with aXa levels during therapeutic twice daily dalteparin use. Furthermore, bleeding, thrombosis, and mortality were not associated with sub- or supratherapeutic aXa levels. Given that a large proportion of patients had subtherapeutic aXa levels dose reduction strategies in renal impairment may need reconsideration.

TWO FATAL CASES OF METHANOL POISONING AS A RESULT OF PROLONGED INHALATION DURING PRODUCTION OF ILLEGAL DRUGS

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Introduction: Methanol poisoning is classically described following ingesting of homemade alcohol ('moonshine') or antifreeze. It is used as a solvent in the production of illegal drugs, like amphetamines and MDMA. When exposed to the fumes of methanol, accidental intoxication can occur due to inhalational exposure. Initial symptoms include gastro-intestinal complaints, neurological effects similar to alcohol and visual disturbances. Methanol is metabolized into toxic metabolites (e.g. formic acid), subsequently leading to a high anion gap metabolic acidosis. In case of severe poisoning blindness, haemodynamic instability, confusion, seizures, cerebral oedema and haemorrhages, and even death has been described.

Methods: We describe the separate cases of two young healthy males who presented at the Emergency Department after 'a night out'.

Results: The first patient was confused at presentation and eventually lost consciousness; the second patient was admitted unresponsive with fixed dilated pupils. Cerebral CAT scans did not show any abnormalities.
Laboratory results showed severe metabolic acidosis (pH 6.95 resp. 6.8); lactate (3.7 resp. 9.1); high anion gap (29 resp. 37); and osmol gap (48 resp. 95).
Drug screening in urine revealed high concentrations of amphetamines and cocaine; ketones in urine were also positive in both cases.
Toxicological analysis revealed extremely high methanol serum concentrations of 728 resp. ~2500 mg/L, which appeared to be the result of prolonged exposure during illegal drug production.
After recognition of severe methanol poisoning, both cases received maximal treatment with sodium bicarbonate and tromethamine for correcting metabolic acidosis, ethanol and folinic acid to promote breakdown of formic acid.
Additionally, dialysis was used to remove methanol and formic acid. Both intermittent hemodialysis (IHD) and continuous veno-venous hemodialysis (CVVHD) were effective to clear methanol to a concentration below 200 mg/L.
However, despite maximal treatment efforts, both patients succumbed to cerebral herniation within 4 resp. 3 days after ICU admission.

Conclusions: Prolonged inhalation of methanol can be potential fatal. Not only IHD, but also CVVHD is effective, especially in haemodynamically instable patients.
Clinicians should be aware of the increasing incidence of inhalational methanol exposure due to elicit drug production. Although intentionally inadequate history can delay the diagnosis, prompt recognition of the possibility of methanol intoxication and initiating timely treatment is essential for these patients' survival.

Assessing the analytical validity of Agena MassARRAY for CYP2D6 copy number variation analysis

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Introduction

Copy number variation (CNV) in the *CYP2D6* gene is a significant factor influencing individual drug metabolism and response. However, detection of CNVs can be difficult. We investigated the ability to asses CNVs with the VeriDose CYP2D6 CNV panel from Agena and compared results to long-range PCR.

Methods

Between May 2023 and May 2024, diagnostic samples were analyzed both with the Agena MassARRAY system and with long-range PCR to detect CNVs. MassARRAY calls were evaluated on accuracy compared to long-range PCR. The MassARRAY systems assigns a continuous CNV score which is subsequently categorized into deletion, duplication or no CNV. For discrepancies in CNV calls the continuous CNV score was used to assess its potential as an indicator of uncertainty regarding the call.

Results

A total of 456 samples were analyzed. Of these, 54 were a tandem CYP2D6-CYP2D7 hybrid arrangement without clinical relevance. Four were *13 type hybrids which were confirmed by PCR based tests. After exclusion of the hybrids, 398 calls remain with high agreement (N= 393, kappa =0.97 $p<0.001$). Discrepancies were caused by MassARRAY assigning 2N to samples with both a deletion and duplication (N=2) and by inaccurately called duplications (N=3). Using the continuous CNV would help to detect the inaccurately called duplications (mean CNV score of 2.54 for the inaccurate duplications and 2.84 for true duplications).

Conclusion

These results show that analysis with the VeriDose CYP2D6 CNV panel from Agena is well suited to assess CYP2D6 duplication and deletion. However, for CNV calls within the range of insecurity a second analysis should be used to confirm. Moreover, the MassARRAY panel is well suited to detect clinically relevant *13 hybrid alleles.

THERAPEUTIC DRUG MONITORING OF 5-FU AS 24-HOUR INFUSION IN PATIENTS WITH GASTRIC CANCER TREATED WITH FLOT (THOMAS FU FLOT)

Authors

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Introduction

Gastric cancer is treated with perioperative FLOT, consisting of 5-fluorouracil (5-FU), oxaliplatin and docetaxel. The International Association of Therapeutic Drug Monitoring and Clinical Toxicology recommends therapeutic drug monitoring (TDM) of 5-FU for treatment regimens in gastrointestinal cancer. In these regimens with 46-hour continuous infusion, therapeutic exposure was defined by an area under the curve (AUC) of 20-30 mg*h/l. The therapeutic and population AUC of 5-FU as 24-hour infusion in FLOT is still unknown. The primary aim was to establish population AUC of 5-FU as 24-hour infusion in patients treated with FLOT. Secondary objectives were to establish intra-patient AUC variation, correlation of plasma sample with dried blood spot concentrations, and correlation of AUC with toxicity.

Methods

This was a prospective, multicentre pharmacokinetic study in 19 patients treated with FLOT. 5-FU administered as 24-hour continuous infusion of 2600 mg/m^2. Pharmacokinetic sampling was performed at 1, 2 and 22 hours after start of 5-FU infusion in cycles 1, 2, 5 and 6. AUC was calculated using NONMEM. Dried blood spots were drawn at the same time points as plasma samples. Toxicity was assessed for each FLOT cycle.

Results

Mean±SD AUC in the first FLOT cycle was 25.4±4.1 mg*h/l (n=10; interim analysis) with interpatient variability of 20%. Intra-patient variability between cycles 1 and 2 was 10.5%. 5-FU related toxicity grade ≥2 was more frequent in patients with AUC >30 mg*h/L. 6 out of 10 patients needed 5-FU dose reduction or treatment delay due to 5-FU related toxicity. Mean±SD AUC in the next cycles was 22.6±4.3 mg*h/l. The Pearson's correlation test demonstrated acceptable correlation (r^2=0.674) between plasma sample and dried blood spot concentrations (p=0.006; n=16).

Conclusions

Population AUC of 5-FU was comparable to the 20-30 mg*h/l range in patients treated with FLOT. 5-FU related toxicity was frequently present and could be reduced by TDM of 5-FU.

EVALUATION OF LOW DOSE DEXAMETHASON AS PREMEDICATION FOR DOCETAXEL INFUSION IN PATIENTS WITH PROSTATE AND BREAST CANCER

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Introduction

Docetaxel is a commonly used chemotherapeutic agent for the treatment of prostate and breast cancer. Prior to its administration, relatively high doses of dexamethasone are recommended as premedication to prevent hypersensitivity reactions (HSR) and to reduce the risk of fluid retention syndrome (FRS). However, this high-dose regimen is associated with adverse effects and supporting evidence is limited. A recent phase 1 trial shows the feasibility of a low-dose dexamethason regimen [1]. Therefore, we modified our dexamethasone regimen from three doses of 8 mg (24 mg total) to a single 4 mg dose and evaluated incidence of HSR and FRS.

Methods

Patients treated with docetaxel within the 21 months preceding and following the implementation of the new dexamethason regimen for prostate and breast cancer were included. Patients in the transitional period who received both the low and high dose dexamethasone regimen were excluded. Patients were identified using the validated search program CTcue. We reviewed records for signs and symptoms of HSR or FRS, which were graded according to the Common Terminology Criteria for Adverse Events (CTCAE). We used descriptive statistics to evaluate the results.

Results

We included 172 patients: 82 in the high-dose and 90 in the low-dose dexamethason group. HSR occurred in 3 patients (3.3%) in the low-dose group and 1 patient (1.4%) in the high-dose group (table 1). No patient had to discontinue docetaxel due to HSR. These HSR rates are comparable to 2-3% reported in literature for high dose dexamethasone regimens [2]. FRS was observed in 6 patients (6.7%) in the low-dose group and 7 (8.5%) in the high-dose group.

	High dose (3 x 8mg)	Low dose (4mg once)
HSR (any)	1 (1,4%)	3 (3,3%)
grade 1	1	0
grade 2	1	1
FRS (any)	7 (8,5%)	6 (6,7%)
grade 1	6	5
grade 2	1	1

Conclusions

Our findings suggest that for docetaxel infusions a low dose dexamethasone premedication regimen is as safe and effective as a high dose regimen in preventing both HSR and FRS.

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THE KEYHOLE LIMPET HEMOCYANIN (KLH) CHALLENGE MODEL TO ESTABLISH EARLY CLINICAL PROOF-OF-MECHANISM OF INVESTIGATIONAL DRUGS FOR AUTO-IMMUNE DISEASES.

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Introduction

Keyhole limpet hemocyanin (KLH) is a suitable neo-antigen for studying adaptive immune responses, which can be utilized to investigate the pharmacological activity of novel immuno-modulatory drugs on T and B cell mediated immunity. The use of KLH has been widely adapted in pre-clinical studies, though healthy volunteers (HV) studies could also benefit from the use of KLH to overcome the limitation of frequently lacking suitable biomarkers in this population. By eliciting a controlled immune response, KLH could support the pathophysiological understanding of autoimmune diseases and the early clinical efficacy evaluation of novel immune-targeted treatments.
We present here applications and outcome measures of the human KLH challenge model used in clinical studies at the Centre for Human Drug Research.

Methods

The KLH challenge model consists of 1 to 3 intramuscular KLH administrations, evoking a cellular immune response which can be studied *in vivo* by circulating KLH-specific antibodies or by *ex vivo* analyses of KLH-driven cellular responses. The subsequent intradermal challenge elicits a local, cellular recall response, which can be quantified by cutaneous blood flow and erythema. Minimally invasive assessments include cellular and molecular markers in skin biopsies and blister exudates.

Results

A randomized, double-blind, placebo-controlled study in HV showed that anti-KLH antibody titers increased incrementally after KLH immunization. KLH-specific IFN-γ and IL-13 responses were demonstrated by ELISpot analyses. Maximal increase in erythema of 50% (95% CI 35 – 68%, $p < 0.0001$) and increased perfusion of 173% (95% CI 134 – 219%, $p < 0.0001$) compared to baseline was observed 24h after the skin challenge. Blister fluid analysis showed cell influx (T cells; B cells; monocytes; dendritic cells), as confirmed by immuno-fluorescent staining of skin biopsies. Furthermore, a randomized, double-blind intervention study with an anti-OX40L monoclonal antibody (KY1005, currently amlitelimab) showed dose-dependent decrease of KLH-driven cutaneous blood perfusion and erythema at doses from 0.45 mg/kg, compared to placebo. Based on these results, the phase 2 dose was selected, which was demonstrated to be effective in atopic dermatitis patients. The translation of the KLH model from HV to patients will be explored further by investigating the KLH response in treatment naïve rheumatoid arthritis patients that are starting methotrexate therapy.

Conclusions

Our studies demonstrate that KLH drives an antigen-specific immune response in man *in vivo*, displaying Th1, Th2 and Th17 characteristics. The KLH model allows evaluation of systemic responses but also in peripheral tissue. As demonstrated by the amlitelimab data, the KLH model can be used to establish proof-of-mechanism in early phase drug development with novel compounds targeting the adaptive immune system and may facilitate effective dose selection for future phase 2 studies.

WHOLE GENOME SEQUENCING-BASED PHARMACOGENETIC PROFILING OF *DPYD* TO PREVENT FLUOROPYRIMIDINE-INDUCED TOXICITY

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Introduction

Whole genome sequencing (WGS) is increasingly used for tumour characterization and is expected to play a greater role earlier in cancer care. WGS also enables pharmacogenetic profiling, including germline analysis of the full *DPYD* gene, encoding for the enzyme dihydropyrimidine dehydrogenase (DPD). The enzyme DPD metabolizes fluoropyrimidines into inactive metabolites. Reduced DPD activity can cause severe toxicity due to an increase in active metabolites. To identify patients with DPD deficiency who require upfront fluoropyrimidine dose reduction to prevent severe toxicity, *DPYD* is currently genotyped for four single nucleotide polymorphisms (SNPs) using a RealTime-Polymerase Chain Reaction (RT-PCR)-test [1,2]. Although WGS is expected to produce similar results to the RT-PCR test, its clinical applicability remains unvalidated. This study aims to cross-validate WGS against RT-PCR for detecting these four *DPYD* SNPs.

Methods

Patients genotyped for *DPYD* with RT-PCR between 01-11-2014 and 01-05-2023, with available WGS data, were included. WGS was carried out as part of the WIDE-study or routine care by the Hartwig Medical Foundation [3]. Pharmacogenetic results on the following four *DPYD* SNPs were compared between RT-PCR and WGS: IVS14+1G>A, 2846A>T, 1236G>A, 1679T>G.

Results

Of 602 patients, 496 were included. Exclusions were due to failed WGS (N=46), missing WGS number (N=46), withdrawn consent for WGS (N=13), or unavailable WGS data (N=1). All SNPs identified with RT-PCR were also detected with WGS (Table 1).

Table 1: Comparison of pharmacogenetic profiling *DPYD* SNPs between RT-PCR and WGS (N = 496)

	RT-PCR	**WGS**
Heterozygote 1679T>G	2	2
Heterozygote IVS14+1G>A	15	15
Heterozygote 1236G>A	20	20
Homozygote 1236G>A	1	1
Heterozygote 2846A>T	7	7
No genotype call for one of the four SNPs	451	451

Conclusions

This cross-validation confirms that WGS is clinically applicable for pharmacogenetic profiling of these four *DPYD* SNPs.

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BASELINE RISK AND LONGITUDINAL CHANGES IN KIDNEYINTELX.DKD AND ITS ASSOCIATION WITH KIDNEY OUTCOMES IN THE CANVAS AND CREDENCE TRIALS

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Introduction
KidneyintelX.dkd is a composite risk score incorporating biomarkers and clinical variables at baseline for diabetic kidney disease (DKD) progression. We sought to determine the clinical relevance of kidneyintelX.dkd and corresponding thresholds in a large cohort of patients with type 2 diabetes (T2D) and a broad range of chronic kidney disease (CKD).

Methods
We measured tumor necrosis factor receptor (TNFR)-1), TNFR-2, and kidney injury molecule (KIM-1) on banked plasma samples from CANVAS and CREDENCE participants, and executed kidneyintelX.dkd at baseline and year 1. Subsequently, we assessed the association of baseline and changes in kidneyintelX.dkd with a composite kidney outcome of 40% decline in eGFR or kidney failure. Hazard ratios were estimated using multivariate Cox regression.

Results
There were 4672 participants (mean eGFR 69.4 mL/min/1.73m2; median UACR 77.0 mg/g) with available plasma samples. At baseline, kidneyintelX.dkd scored 867 (18.6%) participants as high, 1520 (32.5%) as moderate, and 2285 (48.9%) as low risk. The adjusted HR per doubling in kidneyintelX.dkd was 2.26 (95% CI 1.80-2.84; Figure 1A). At year 1, the median change in kidneyintelX.dkd was 0.0%, with >10% reduction in 25.7% of canagliflozin vs. 17.0% of placebo-treated patients. The adjusted HR for the change in kidneyintelX.dkd from baseline to year 1 was 2.07 (95% CI 1.51-2.83; Figure 1B). Canagliflozin led to more patients shifting to lower risk at year 1 (32.1% vs. 16.2% moderate to low; 38.9% vs 19.7% high to moderate or low). Low risk at year 1 was associated with very low kidney outcome risk of 0.3 events per 100 person-years, while staying at moderate had 1.4, moving from high to moderate had 2.2, and high risk at year 1 had 8.9 (Figure 1C).

Conclusions
Baseline and year 1 kidneyintelX.dkd risk assessments in patients with T2D and CKD robustly stratified patients for DKD progression independently of classic predictors and should be considered for enriching clinical trials and assessing treatment response.

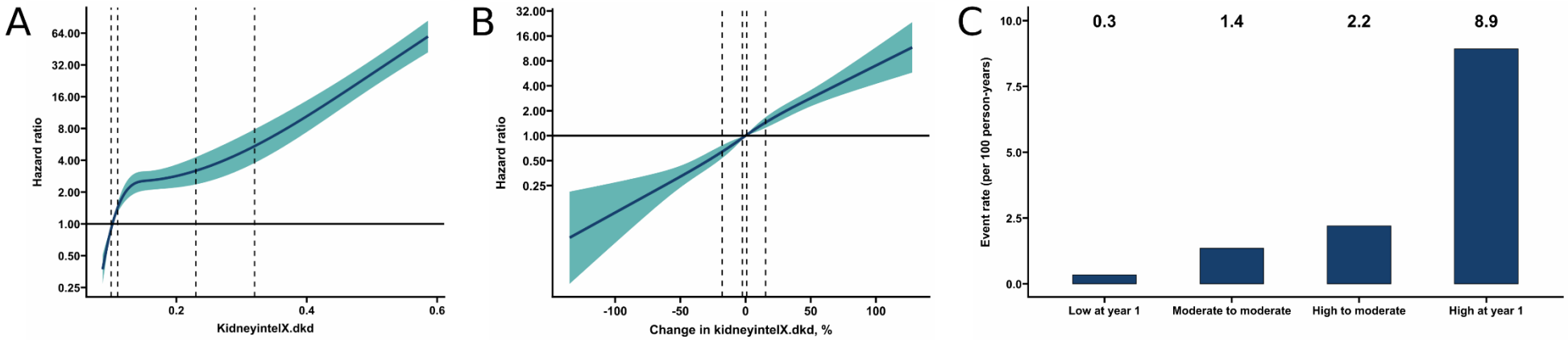

Figure 1: Association between baseline and early change in kidneyintelX.dkd with kidney outcomes. A. Association between baseline kidneyintelX.dkd and kidney outcome analyzed on a continuous scale. B. Association between change in kidneyintelX.dkd and kidney outcome analyzed on a continuous scale. C. Change in kidneyintelX.dkd risk level from baseline to 1 year and kidney event rates.

REDUCING THE BURDEN OF A DRUG-FOOD INTERACTION STUDY IN CANCER PATIENTS USING A STABLE ISOTOPICALLY LABELLED MICROTRACER: A PROOF-OF-CONCEPT STUDY WITH ALECTINIB

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Introduction

Traditional drug-food interaction studies of oral anticancer agents have a high patient burden. These interactions need to be evaluated at a steady state, requiring relatively long periods of standardized meal intake and long hospitalizations especially for drugs with long half-lives. An alternative way of studying this interaction could be the use of stable isotopically labelled microtracers, where results are gathered while allowing normal dosing of oral anticancer agents to continue. We present a pilot study using the microtracer alectinib-d6 to investigate the food effect on alectinib pharmacokinetics (PK).

Methods

A prospective, single-centre, open-label, cross-over, food effect study with a microtracer alectinib-d6 (100 µg) was conducted in patients with ALK-positive non-small cell lung cancer currently on treatment with 600 mg alectinib twice daily. Patients were hospitalised for only 12 hours at two occasions with a minimum wash-out period of seven days in between. On the first study day (fed state), patients received 100 µg alectinib-d6 in addition to their normal dose of 600 mg alectinib and a standardized Dutch breakfast (320 – 392 kcal and 7.5 – 7.8 grams fat). On the second study day (fasted state), patients received alectinib-d6 and alectinib under fasting conditions. During the wash-out period, patients did not have to adhere to specific food dietaries. For both study days, PK samples were taken up to 8 hours after intake of alectinib-d6. A population PK model was developed to investigate the food effect on relative bioavailability (F) and mean transit time (MTT). Simulations (taking into account uncertainty in final model parameters) were performed to assess area under the curve (AUC) and maximum concentration (C_{max}) differences between fed and fasted states.

Results

Ten patients were included with a total of 140 PK samples of alectinib-d6, of which 21 samples were below the lower limit of quantification (LLOQ).

F and MTT were 34.7% (relative standard error (RSE): 6.5%) and 28% (RSE: 20.5%), respectively higher in the fed state than in the fasted state. The C_{max} and AUC were 1.52x (coefficient of variation (CV): 13.2%) and 1.60x (CV: 12.5%), respectively higher in the fed state than in the fasted state. This means that intake with a Dutch breakfast leads to a delay in absorption, but higher plasma samples.

Conclusions

This proof-of-concept study demonstrated the feasibility of a microtracer food effect study using a study design with a relatively low patient burden with only two food controlled drug administrations and a short period of sample collection.

TOWARDS 2- ARACHIDONOYLGLYCEROL (2-AG) AS A RELIABLE PHARMACODYNAMIC BIOMARKER IN EARLY PHASE DRUG DEVELOPMENT: A VALIDATION STUDY IN HEALTHY VOLUNTEERS

Authors

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Introduction

The endocannabinoid (eCB) system is a highly intricate and evolutionarily conserved biological system ubiquitously expressed in the CNS and peripheral nervous system. The involvement of eCB in functional processes such as emotion, motivation, and cognition offer interesting novel targets for the pharmacological treatment of mood, anxiety, and trauma-related disorders. Among these, selective inhibition of the eCB-degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) is under investigation. Since 2-arachidonoylglycerol (2-AG) is a major substrate for MAGL, it may serve as a pharmacodynamic biomarker for investigative compounds that selectively inhibit MAGL (MAGL inhibitors). However, the reliability of 2-AG as a pharmacological biomarker is currently uncertain due to i) the unknown correlation of central with peripheral 2-AG concentrations and ii) the potential impact of circadian rhythm on the interpretation of changes in 2-AG levels. Systematic data addressing these issues is currently lacking and needs to be elucidated before applying 2-AG as a biomarker in early clinical drug development. This clinical study characterized the time-concentration profiles of 2-AG concurrently in plasma and cerebrospinal fluid (CSF) and aimed to establish whether 2-AG demonstrates a circadian rhythm in these compartments.

Method

A non-interventional biomarker sampling study in 12 healthy (female/male) volunteers between 25 and 75 years old. Plasma and CSF 2-AG were sampled hourly for 24 and 16 hours, respectively. Spinal catheter insertion for CSF sampling was carried out by certified anaesthesiologists. Plasma and CSF concentrations of 2-AG were determined in triplicate using LC-MS/MS at Keystone Bioanalytical, Inc, North Wales, United States of America.

Results

In total, six males and six females with a mean age of 52.4 years (range 26 – 75 years) with a mean BMI of 24.0 kg/m2 (range 18.9 -29.0 kg/m2) were included. 2-AG showed a coefficient of variation (CV) < 20% in 98% and 92% of the triplicate measurements for plasma and a CSF, respectively. Average levels (CV%) of 2-AG demonstrated a nadir of 486.8 pg/mL (45.7%) and 27.2 pg/mL (36.3%) in plasma (4 AM) and CSF (9 AM), respectively. The concentration of 2-AG peaked around 2 PM in both plasma and CSF at 724.5 pg/mL (39.2%) and 40.0 pg/mL (78.5%), respectively. Preliminary data suggest no apparent circadian rhythm for 2-AG in CSF but potentially a rhythm in plasma.

Conclusions

The endocannabinoid 2-AG demonstrated a nadir in the early morning (4 AM-9 AM), while peak concentrations occurred in the early afternoon (2 PM) in both plasma and CSF of healthy volunteers. The concentrations of 2-AG in plasma and CSF showed high inter-subject variability across the 12 subjects. Plasma 2-AG concentrations showed comparable circadian rhythmicity as in the literature. These findings provide important guidance for the potential use of 2-AG as a pharmacodynamic marker in future clinical studies with therapeutics targeting the eCB system.

THE INCIDENCE OF DIURETIC ASSOCIATED SEQUENTIAL PRESCRIBING CASCADES: A FEASIBILITY STUDY

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Introduction

Prescribing cascades occur when adverse drug reactions of a medication (index) are inappropriately treated with additional medications (marker). They are associated with negative outcomes for patients and health care in general. While individual prescribing cascades involving a single index and marker medication are well-documented, little is known about the occurrence of sequential prescribing cascades. We therefore aimed to determine the incidence of sequential prescribing cascades in a large prescription database.

Methods

This feasibility study used the Clinical Practice Research Datalink (CPRD) to identify patients aged ≥65 years who were newly prescribed diuretics as a primary marker within 12 months of initiating a calcium channel blocker or gabapentinoid (index) between 2018-2022. The incidence of secondary markers prescribed within 12 months after diuretic prescription was determined, including antigout medications, urinary frequency and incontinence medications, and potassium salts and potassium-sparing agents.

Results

608,225 new index users were identified, of which 28,240 patients were newly prescribed a diuretic. Of these, 1,877 (6.6%) were prescribed a secondary marker within 12 months. Antigout medications were prescribed for 311 patients (1.1%), urinary frequency and incontinence medications for 518 patients (1.8%), and potassium salts or potassium-sparing agents for 1,090 (3.7%) patients.

Conclusions

These findings suggest that a substantial amount of patients experience sequential prescribing cascades in clinical practice, highlighting the need for strategies to improve recognition, prevention and management of prescribing cascades.

DEVELOPMENT OF AN ONLINE TOOL FOR THE MANAGEMENT OF DRUG-DRUG INTERACTIONS WITH THE OLD DRUG METAMIZOLE

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Introduction

Metamizole (dipyrone) is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and spasmolytic properties. It is commonly used intramurally in the Netherlands for management of postoperative pain for durations of less than 24 hours. However, it is increasingly being prescribed for longer periods as an alternative to other NSAIDs. In some other countries (Germany, Switzerland) metamizole is a preferred NSAID both intra- and extramurally. In addition to the rare adverse effect of agranulocytosis, metamizole can also interfere with several cytochrome P450 (CYP) enzymes, including CYP3A4. Despite being on the market for decades, little is known about its interaction potential.

Methods

We reviewed the FDA and EMA Product Information documents and data published in scientific literature to establish the Drug-Drug Interaction (DDI) profile for metamizole. We used the DDI studies to extrapolate to unstudied drug combinations with metamizole and constructed a recommendation for the DDI management of each drug combination. We used a "traffic light" system to classify the DDIs in "no or minimally (clinically relevant) interaction expected; no action" (green); "clinically relevant interaction expected; action needed" (orange); or "severe interaction expected, do not co-administer" (red).

Results

Metamizole is a substrate for CYP3A4, CYP2B6, CYP2C8 and CYP2C9. Furthermore, metamizole is a moderate inducer of CYP3A4, CYP2B6 and CYP2C19, a weak CYP2C9 inducer and a weak CYP1A2 inhibitor. On January 28, 2025, metamizole was reviewed with 983 comedications for potential DDI interactions. We found that 59.9% (589), 21.8% (214) and 18.3% (180) of the comedications can be classified for green, orange and red interactions, respectively. Metamizole use up to 24 hours is not considered clinically relevant, due to CYP induction not being at maximum effect after a maximum of three gifts and pragmatical reasons[1].

Conclusions

Metamizole has an high DDI potential and can be involved in many DDIs. However, these DDIs are only considered clinically relevant when metamizole is used for more than 24 hours. An online tool can guide clinicians in managing DDIs with long-term use of metamizole and relevant comedications. All DDI pairs will be included in an online tool (www.DDIManagers.com).

1. Breithaupt, M.H., Krohmer, E., Taylor, L., et al. Time course of CYP3A4 activity during and after metamizole (dipyrone) in healthy volunteers. *Br J Clin Pharmacol*. 2023;89(8):2458-2464. doi: 10.1111/bcp.15720

IMPLEMENTATION FIDELITY OF A MULTI-COMPONENT PHARMACIST INTERVENTION TO IMPROVE HYPERPHOSPHATEMIA IN DUTCH HEMODIALYSIS PATIENTS

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Introduction
A multi-component pharmacist intervention (PIDO-P, Pharmacist Intervention and Dose Optimization of Phosphate-binding Medication) was designed to improve hyperphosphatemia in dialysis patients with a high phosphate-binding medication (PBM) pill burden. Although the PIDO-P intervention improved PBM adherence, phosphate levels remained high. To determine if the intervention was delivered as intended, we studied its implementation fidelity (IF).

Methods
This mixed methods study was performed in the FGV, a large teaching hospital with a hemodialysis facility for 250 patients. The PIDO-P intervention consisted of 3 pharmacist-patient consultations in 3 months, in which barriers to adherence to PBM were addressed and PBM dose was reduced. The IF of the PIDO-P intervention was studied with Carroll's IF framework, evaluating adherence to the intervention (coverage, content, frequency, and duration); and moderating factors (intervention complexity, facilitation strategies, quality of delivery, and participant responsiveness). Six key intervention components were identified. Feasibility in clinical practice was also explored. Qualitative data from evaluations and semi-structured interviews with patients and healthcare professionals were thematically analysed according to Braun with Atlas.ti, quantitative data using descriptive statistics in SPSS.

Results
Coverage was high: the selection procedure was performed as planned and a high percentage of invited patients participated. 5 of 6 key components were delivered to a high degree (content). IF was also high for frequency and duration: 72 of 75 patients received all 3 consultations, total duration of the consultations was 61 minutes. The pharmacists considered the intervention not complex with easy-to-use intervention materials. All respondents perceived the facilitation strategies as helpful. The quality of delivery and participant responsiveness were generally good. To optimize the effectiveness of the intervention on phosphate and to improve feasibility in clinical practice, pharmacists and prescribers thought the intervention should focus on patients with higher phosphate levels.

Conclusions
The IF of the PIDO-P intervention was high. Therefore, its lack of effect on phosphate concentrations cannot be explained by a low IF of the intervention. However, during the intervention, PBM dose was reduced to 5.8 sevelamer tablet equivalents. This dose might have been too low to reduce phosphate in patients with high dietary phosphate content. Further research is needed to improve the effectiveness of the intervention, focusing on patients with higher phosphate levels.

12 MONTHS FOLLOW-UP OF A PHARMACIST INTERVENTION WITH DOSE OPTIMIZATION OF PHOSPHATE-BINDING MEDICATION (PIDO-P) TO REDUCE HYPERPHOSPHATEMIA IN HEMODIALYSIS PATIENTS

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Introduction
Suboptimal adherence to phosphate-binding medication (PBM) is common in the nearly 50% of hemodialysis patients with uncontrolled hyperphosphatemia. Important barriers to PBM adherence are forgetfulness, complex treatment regimens, and a high pill burden. We hypothesized that a reduction in PBM pill burden would improve hyperphosphatemia by improving adherence. To test this hypothesis, we investigated the effects of the PIDO-P intervention on phosphate levels, PBM pill burden, and PBM adherence during 12 months.

Methods
This was a prospective, pre-post intervention study in 75 hemodialysis patients with hyperphosphatemia (>1.50 mmol/l) and a high PBM pill burden (≥6 sevelamer tablet equivalents) in the FGV in Rotterdam. The PIDO-P intervention consisted of 3 pharmacist-patient consultations within 3 months on the hemodialysis ward in which barriers to adherence were addressed, and PBM pill burden was reduced. Self-reported adherence to PBM (MARS-5) was investigated at baseline (BL) and after 3 and 12 months. After 3 months the patient returned to usual care by the nephrologist alone. The primary outcome parameter was the average phosphate concentration in the 3 months (3M) after versus 3M before the intervention. Secondary outcome parameters were phosphate levels, percentage of patients within target range for phosphate, PBM pill burden, and self-reported adherence after 3, 6 and 12 months. Data were analyzed with SPSS version 29.0.2.

Results
The phosphate concentration was 1.99±0.34 mmol/L in the 3M before versus 2.03±0.37 mmol/L in the 3M after start of the intervention (p=0.268). Phosphate remained stable for the first 4M, whereafter it decreased gradually (from 2.04±0.45 mmol/L at BL to 1.86±0.57 mmol/L at 12M, p=0.025). The percentage of patients within target range increased from 0 (at BL) to 16.4, 24.2, and 26.4% after 3, 6, and 12M (p<0.001 vs BL for all time points). PBM pill burden decreased the first 3M (from 8.8±3.1 at BL to 5.8±2.7 at 3M, p<0.001), whereafter it increased gradually to approach BL after 12M (8.4±3.8, p=0.261 vs BL). Self-reported adherence increased the first 3M and remained higher during total follow-up (median 24, IQR 22-25 at BL vs median 25, IQR 23-25 after 12M, p=0.048).

Discussion/Conclusion
Contrary to our hypothesis, the intervention did not reduce hyperphosphatemia after 3 months. However, the percentage of patients within target range increased. The long-term improvement in PBM adherence combined with an increase in PBM pill burden over time probably contributed to lower phosphate levels at 12 months. The PIDO-P intervention seems promising in improving PBM adherence, but focusing on patients with higher phosphate levels may improve its effectiveness on phosphate levels, as post-hoc analyses show.

THE INCIDENCE OF BLEEDING AND THROMBOTIC COMPLICATIONS AFTER BARIATRIC SURGERY AMONG VITAMIN K ANTAGONIST USERS AND DIRECT ORAL ANTICOAGULANT USERS.

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Introduction
Bariatric surgery is a popular weight loss method, but alters gastrointestinal (GI) anatomy, leading to challenges in medication administration. [1] This study aims to identify the incidence of bleeding and thrombotic complications after bariatric surgery among DOAC and VKA users. Also, the incidence in DOAC subtypes and in different bariatric surgical techniques is examined.

Methods
A retrospective study was conducted at Frisius Medical Centre Leeuwarden, including patients who used a DOAC (apixaban, dabigatran, edoxaban or rivaroxaban) or a VKA (acenocoumarol or phenprocoumon) after bariatric surgery (sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB) and anastomosis gastric bypass (OAGB)). Patients who underwent surgery before 2016 or who had a gastric or intestinal tube were excluded. The postoperative period was defined as a minimum of 30 days after bariatric surgery. Bleeding events were classified using the Bleeding Academic Research Consortium (BARC) score. The primary outcome was the incidence of bleeding and thrombotic complications among DOAC and VKA. Data were analysed using SPSS Statistics 28, including chi-square test, Fisher's exact test or Fischer Halton test and survival analysis using Kaplan-Meier curves, log-rank test and cox regression.

Results
This study included 310 patients (164 VKA, 146 DOAC users). Postoperative bleeding occurred in 14 patients (4.5%), significantly higher in DOAC users (7.5%) than VKA users (1.8%) ($p = 0.025$). No bleeding was observed with apixaban or dabigatran; one case (7.1%) occurred with edoxaban. Rivaroxaban had the highest bleeding rate (13.7%). There was a significant difference in the incidence of bleeding among the DOAC subgroups ($p = 0.027$). Thrombotic events were rare, with only one event (0.3%) reported in the DOAC group. Furthermore, no significant difference was found between surgical techniques (SG versus bypass techniques, $p = 0.995$). Additionally, survival analysis using Kaplan-Meier curves showed that DOAC users had a significantly higher bleeding risk compared to VKA users, with a hazard ratio of 3.95 (95% CI 1.1 -14.16).

Conclusions
This study highlights the need for caution when prescribing DOACs, especially rivaroxaban, after bariatric surgery. The significant difference in bleeding risk between the DOAC and VKA users indicates that VKA might be a safer choice following bariatric surgery. Our results suggest that follow-up and monitoring are crucial and examination for bleeding is necessary. Future research should focus on larger-scale studies to compare the risk of thrombotic complications and to compare bleeding and thrombotic complications for dabigatran and edoxaban

[1] Kingma, J.S., et al., Oral drug dosing following bariatric surgery: General concepts and specific dosing advice. Br J Clin Pharmacol, 2021. 87(12): p. 4560-4576.

QUANTIFICATION OF METABOLITE CONTRIBUTION TO ESKETAMINE SUBJECTIVE EFFECTS AFTER ORAL AND INTRAVENOUS ADMINISTRATION IN HEALTHY VOLUNTEERS

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Introduction

Esketamine is an antidepressant that has potential to be administrated orally. However, through this route, esketamine undergoes extensive first pass metabolism, resulting in low esketamine exposure and high exposure of pharmacologically active metabolites, including esnorketamine. To what extent the parent and metabolites each contribute to the antidepressant effects remains to be determined. Therefore, this study aims to quantify the pharmacokinetic/pharmacodynamic (PK/PD) relationship of esketamine as measured on the visual analogue scale (VAS) 'Feeling High' in healthy volunteers receiving both oral and intravenous esketamine, and to investigate the potential contribution to the effect of esnorketamine.

Methods

Data of 17 healthy volunteers receiving oral (0.20 mg/kg and 0.45 mg/kg) and intravenous (0.4 mg/kg over 40 min) esketamine in a double-blind double-dummy placebo controlled clinical trial was available. A semi-physiologic PK model was developed based on measured esketamine and metabolite ensorketamine levels over 24h. VAS 'Feeling High'(range: 0-100 mm) was measured at 8 different timepoints post-dose, with the first timepoint at 31 min after IV infusion started and 16 min after oral dosing. PK parameters were fixed to the individual estimates for determination of the PK/PD relationship. The esketamine model was established first, after which the addition of an esnorketamine model was tested. A bounded integer modeling approach was used to account for the discrete nature and boundaries of the VAS scale, by linking the VAS scores to the probability of observing that score dependent on the underlying latent variable. The latent variable is then correlated to the esketamine and esnorketamine concentration through a linear, power, exponential, or Emax relationship. Data transformation and visualisation was done in R(V4.4.1), modeling was done in NONMEM(V7.5).

Results

A linear relationship was found to best describe the VAS scores in relation to esketamine concentration. The addition of a linear esnorketamine effect was significant ($p<0.01$), resulting in a baseline latent variable of -4.69 [relative standard error: 7%] with 14% (CV) inter-individual variability [71%], and slopes of 0.037 [8%] and 0.018 $(ng/ml)^{-1}$ [28%] for esketamine and esnorketamine, respectively. However, estimated variance in the data was high (SD: 1.26 [11%]), resulting in overpredicted variability when using the model for simulations.

Conclusions

Esketamine was found to be the main driver of the effects measured on VAS 'Feeling High', while esnorketamine was found to significantly contribute but be about half as efficacious. Although these findings were associated with large variability, they may inform future clinical studies on alternative esketamine formulations, thereby improving accessibility to adequate antidepressant treatment for patients.

USING PATIENT VIDEOS IN PHARMACOLOGY EDUCATION WITHIN MEDICINE AND PHARMACY CURRICULA

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Introduction

Safe and effective pharmacotherapy not only requires biomedical and pharmacological knowledge, but also insight into the patient's perspective. Although factors such as personal beliefs, acceptance of side effects or medicine costs influence pharmacotherapy, these non-clinical factors are not extensively discussed within the health professions education (HPE) curricula. Incorporating patient-perspective into pharmacology could therefore help minimize drug-related problems in patients. As videos provide a holistic depiction of the patient's life, using patient videos, instead of commonly used, paper-based case studies, could be suitable to reach this objective. Here we aim to study effectiveness of patient videos as a tool for teaching HPE students on the value of patient's perspective in pharmacology and pharmacotherapy.

Methods

The study was conducted in two academic years. First-year bachelor students from two different programs (medicine and pharmacy) following a course on pharmacokinetics were invited to participate in the study. The educational intervention was an interactive lecture which included watching a recorded interview with a patient with epilepsy, followed by focused exercises and plenary discussions on various pharmacological and patient-perspective related topics. The lecture concluded with students filling up a questionnaire with both open-ended questions and Likert-scale based statements. Thematic analysis was conducted for analyzing open-ended questions.

Results

The video was generally rated as more useful by medicine students, especially for understanding epilepsy, its treatment, and the clinical application of pharmacokinetics. Besides learning about pharmacological principles (e.g. clinical relevance of pharmacokinetics), students additionally learnt about other prescribing-related topics (e.g. therapy failure, shared decision making) and were able to identify skills required of healthcare professionals, beyond those connected directly to pharmacotherapy (e.g. empathy, listening).

Conclusions

The current study shows that educational intervention with a short patient video followed by exercises and discussions is an effective tool to teach students about multiple topics, necessary for effective pharmacotherapy and patient care in general. Besides learning about the basic pharmacological principles students were able to identify multiple latent, implicit messages the video depicted). Valuable lessons like understanding patient's perspective and empathy are drawn through this educational intervention.

INTRAVENOUS LIPOPOLYSACCHARIDE CHALLENGE INDUCED EXPRESSION OF POTENTIALLY DRUGGABLE ONCOLOGICAL TARGETS IN PERIPHERAL BLOOD AND BONE MARROW OF HEALTHY VOLUNTEERS: AN INNOVATIVE APPROACH FOR EARLY PHARMACOLOGY TRIALS

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Introduction
Human challenge models accelerate clinical drug development by providing critical insights during early-phase trials. The intravenous (i.v.) lipopolysaccharide (LPS) challenge, commonly used in autoimmune and inflammatory research, offers a novel model in oncology due to the pro-inflammatory nature of tumors and their microenvironment (TME). We hypothesize that, by mimicking pro-inflammatory conditions in healthy volunteers, i.v. LPS induces the expression of druggable targets in various immune cells and tissues typically involved in TME. Furthermore, assessing bone marrow could reveal a unique set of targets otherwise inaccessible in peripheral blood. This study aims to deeply characterize LPS-induced responses in peripheral blood and bone marrow to demonstrate the potential of the LPS challenge model in identifying and assessing druggable targets for early oncology trials.

Methods
An open-label study was conducted in healthy male subjects (aged 18-35 years). Ten participants were administered either 1 ng/kg (n=5) or 2 ng/kg (n=5) of i.v. LPS. Bone marrow samples were taken at baseline and 4 hours post-dose, while blood samples were collected at multiple timepoints. Immunophenotyping, cytokine profiling, and bulk RNA sequencing were used to evaluate LPS responses in both blood and bone marrow. Differential gene expression analysis, gene set enrichment analysis (GSEA), and gene set variation analysis (GSVA) were performed on the transcriptomic data. The Open Targets database helped identify druggable targets.

Results
LPS triggered a dose-dependent inflammatory response. Transcriptome analysis revealed activation of TNF, IL-1, RANKL, IFN-alpha/gamma, IL-6-JAK-STAT3, and complement pathways in blood, alongside upregulated druggable targets including DHRS13, PIM3, ITGAM, HCAR3, S100A12, PFKFB3, and GRN. These targets influence tumor metabolism, immune evasion, and survival, offering promising candidates for antagonists or agonists depending on their role in tumor progression. In bone marrow, LPS additionally activated E2F transcription factors, critical for cell cycle regulation, and the G2/M checkpoint pathway. Bone marrow-specific differentially expressed druggable targets included CD163, CXCR4, HSPA8, and CD36. This compartmentalization suggests that certain oncological targets can only be exclusively studied within the bone marrow microenvironment.

Conclusions
This study identifies LPS-induced druggable oncological targets in bone marrow and blood, supporting the use of the LPS challenge model in early oncology trials. This model could serve as a valuable tool to assess target engagement for novel cancer therapies by replicating tumor-like inflammatory responses in healthy volunteers.

AZITHROMYCIN EXPOSURE AND THE EFFECT OF CO-ADMINISTRATION OF RIFAMPICIN IN PATIENTS WITH NON-TUBERCULOUS MYCOBACTERIAL INFECTIONS

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Introduction
Treatment of non-tuberculous mycobacterial (NTM) disease requires multi-drug antibiotic regimens, which are still associated with high failure rates. The treatment regimen typically contains the macrolide antibiotic azithromycin, considered the most potent drug against NTM. Azithromycin is often combined with rifampicin, a strong inducer of metabolic enzymes which leads to decreased azithromycin exposures. There are no clinically validated plasma concentration targets for azithromycin. Therefore, average population measures of exposure are used as targets for dose adjustments.
We aimed to (1) describe azithromycin exposure measures in a Dutch population, (2) assess the effect of co-administration of rifampicin on azithromycin exposure, and (3) identify predictors of azithromycin exposure.
Methods
Plasma azithromycin area-under-the-curve from 0 to 6 hours after administration in mg/L*hours (AUC0-6), peak (Cmax) in mg/L and trough (Cmin) concentrations in mg/L were collected at Radboucumc from 2021 and 2024. Geometric means and ranges were assessed to describe the azithromycin exposure. The effect of rifampicin on azithromycin exposure was assessed using a between-group t-test on log-transformed measures in patients with and without rifampicin co-administration. Additionally, a bio-equivalence approach was applied to patients who had azithromycin concentrations measured both with and without rifampicin. Predictors of exposure were identified using multiple regression analyses for age, sex, rifampicin use and dose per kg.
Results
A total of 124 patients were included (45% male; median age 64 years; median weight 63 kg). The geometric mean dose of azithromycin was 6.7 mg/kg (range 2.14 – 17.86).
In patients receiving rifampicin, the geometric mean for azithromycin AUC0-6 was 0.95 mg/L*hours, compared to 1.78 mg/L*hours for patients without rifampicin (53%). Similarly, Cmax was 0.22 mg/L vs 0.43 mg/L (52%), and Cmin 0.047 mg/L vs 0.12 mg/L (39%) ($p<0.001$ for all comparisons).
Among the subset of patients (n=14) who had azithromycin concentrations measured both with and without concurrent rifampicin use, exposure measures were significantly lower with rifampicin: geometric means of AUC0-6, Cmax, and Cmin (and 90%-confidence interval) were 36% (25 – 52%), 41% (28 – 60%) and 31% (21 – 45%) of those without rifampicin (all $p\leq0.001$).
In multiple regression analyses, azithromycin dose (mg/kg) and rifampicin co-administration were predictors of all exposure measures, whereas age and sex were not.
Conclusions
This study provides average population measures of azithromycin exposure in a Dutch population, offering more appropriate clinical target values in the Netherlands. Rifampicin co-administration reduces azithromycin exposure by half, underscoring the need for upfront azithromycin dose adjustment or consideration of alternative drugs, such as clofazimine, in the combination regimen.

Assessing Safety of the Transition to Generic Imatinib in GIST Treatment

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Introduction
In 2021, after expiration of Glivec patent, all Dutch patients with Gastrointestinal Stromal Tumour (GIST) switched from branded *Glivec* imatinib to generic forms to reduce costs. Following this switch, an increase of adverse events was reported by many patients, which underlined the need for further investigation. We therefore conducted this retrospective cohort study to assess safety of generic imatinib among GIST patients in the Netherlands.

Methods
GIST patients from four hospitals that switched to different forms of generic imatinib (Amarox, Accord and Sandoz) were included. Using a self-controlled case series design a control group of patients who remained on *Glivec* was created. Adverse events (AEs) after the switch were retrospectively assessed in the control and generic groups. In order to identify potential causes of different AE rates, excipients were reviewed and plasma trough levels from 1 year prior to 1 year after the switch were analysed.

Results
In total, 81 patients switched to Amarox, 107 to Accord and 13 to Sandoz. Of these patients, a total of 150 were already on Glivec a year prior and was thus included in the reference group In the reference Glivec group, 20.7% experience new AEs, compared to 29.9%-32.1% in the different generic groups. Relative to the reference group, we observed a significant odds ratio of 2.3 after switching to Amarox, whereas the rates for Accord and Sandoz were not significant. No differences in plasma trough levels were observed among the generic formulations. Excipients were similar, except for the presence of titaniumdioxide and the absence of several binding agents in Amarox. The latter is thought to have led to unpleasant taste, potentially influencing AE rates indirectly.

Conclusion
The difference in AE rates between generics and the reference group was not due to differences in pharmacokinetics and can only partially be attributed to differences in excipients. Therefore, we hypothesize that the nocebo effect, which describes worsened symptoms caused by negative expectations about the treatment, likely played a large role in observed event rates. Concluding, the switch to generic imatinib among GIST patients in the Netherlands was safe.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING OF URIDINE 5'DIPHOSPHOGLUCOROSULTRANSFERASE (UGT) SUBSTRATE DRUGS IN PREGNANT WOMEN

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Introduction
While research on the effects of pregnancy on phase I enzyme expression/activity has reached a critical mass, there is a paucity of information regarding how the expression/activity of phase II enzymes may be affected. This study aimed to test the hypothesis that pregnancy-related changes in the pharmacokinetics (PK) of sensitive substrates of uridine 5'-diphosphoglucuronosyltransferase (UGT) enzymes (UGT1A1, UGT1A4, UGT1A9, and UGT2B7) could be attributed to changes in the expression/activity of these enzymes, using physiologically based pharmacokinetic (PBPK) modelling of pregnant populations.

Methods
PBPK models for UGT substrates (UGT1A1, UGT1A4, UGT1A9, and UGT2B7) were developed or obtained from literature and scaled to pregnancy by incorporating anatomical, physiological, and UGT-specific enzymatic changes. UGT1A1, UGT1A4, and UGT1A9 induction were modelled using literature-based equations, while UGT1A3 and UGT2B7 expression/activity remained unchanged during pregnancy. The equations are based on temporal changes in UGT activity during pregnancy, accounting for hormonal influences like 17β-estradiol and progesterone. The validity of these enzymatic changes was assessed by comparing predicted pharmacokinetics and pregnant-to-nonpregnant ratios to observed clinical data.

Results
All pregnant-to-non-pregnant ratios fall within the acceptance criteria for all UGT compounds, except for paracetamol in the first trimester. Results on additional UGT2B7 substrates will be added soon.

Conclusions
Changes in UGT enzyme expression/activity during pregnancy, based on pregnancy related hormones, were successfully incorporated into PBPK modelling to predict pharmacokinetic changes in pregnant population. This enhances our understanding of phase II metabolism during pregnancy and may support dose adjustments.

CORTICOSTEROID USE AND LONG-TERM CHANGES IN WEIGHT AND WAIST CIRCUMFERENCE: THE LIFELINES COHORT STUDY

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Introduction
The use of corticosteroids (CS) has been associated with higher body mass index (BMI) and waist circumference in cross-sectional studies. However, longitudinal data are scarce, particularly for locally administered forms.

Methods
We analyzed weight and waist circumference changes in 81,361 Lifelines Cohort Study participants (mean age 46.3 years, mean BMI 26.0 kg/m^2, 41% male, mean follow-up 3.9 years) via linear regression. Sensitivity analyses included stratification by sex and BMI. Short-term weight changes post-start were assessed in a subset using linear mixed effect models.

Results
We found 23.8% CS users during the study period. Individuals reporting any new use of CS gained significantly more weight compared to non-users at follow-up (β 0.034 kg/year, p=0.021), particularly among those initiating local CS use (β 0.037 kg/year, p=0.017). Use of new systemic CS was associated with increased waist circumference (β 0.200 cm/year, p<0.001), Discontinuation of CS led to decreased waist circumference (β -0.078 cm/year, p=0.028). These effects were particularly observed in females and individuals with BMI ≥25 kg/m^2 but not in males and participants with BMI< 25kg/m^2. Short-term weight-inducing effects of CSs were not observed in the weeks after initiation of CS use.

Conclusions
This study demonstrates that corticosteroid use, including locally administered forms, is associated with long-term increases in weight and waist circumference, notably in females and individuals with overweight or obesity. Discontinuing corticosteroids was linked to reductions in waist circumference. These findings underscore the need to carefully assess chronic systemic and local corticosteroid use, as discontinuation could benefit obesity-related outcomes in certain patients.

BRIGATINIB MONOTHERAPY IN CHILDREN WITH R/R ALK+ ALCL, IMT, OR OTHER SOLID TUMORS: DOSE-FINDING AND PHARMACOKINETIC ANALYSIS FROM THE BRIGAPED PHASE 1 STUDY

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Introduction
Brigatinib is a potent anaplastic lymphoma kinase (ALK) inhibitor (ALKi) with good central nervous system penetration, approved for the treatment of adult ALK+ non-small cell lung cancer (7 day lead in: 90 mg/day, followed by 180 mg/day). This report presents the dose-finding and pharmacokinetic (PK) analysis of a pediatric phase 1 study (NCT04925609, ITCC-098) in patients with ALK+ malignancies.

Methods
This multicenter phase 1 study enrolled pediatric patients aged ≥1-<18 years and >10 kg with relapsed/refractory ALK+ malignancies. Brigatinib was administered as tablets once daily in 28-day cycles. Doses were escalated according to the rolling-six design to a maximum of three dose levels (DL). Dose limiting toxicities (DLTs) were evaluated during lead-in and cycle 1 to determine the recommended phase 2 dose (RP2D). PK sampling was scheduled at first dose and at steady state. Brigatinib concentrations were measured centrally using LC-MS/MS. PK parameters were estimated with non-compartmental analysis (NCA). Target exposure was defined as the geometric mean (CV%) steady state AUC_{tau} +/- 20% observed in adults dosed 180 mg/day (20.3 h*µg/mL (61.6%); +/- 20% range: 16.2-24.3).

Results
Ten patients with a median age of 9 years (range: 6-17) were enrolled: 9 anaplastic large cell lymphoma (ALCL) and 1 sarcoma patient. At database cut-off (21-June-2024), a median of 18 cycles (range 10-23) were administered. No DLTs were observed on DL1 (n=4), and 1 on DL2 (n=6, grade (G)3 neutropenia > 7 days). Following dose reduction, this patient could resume brigatinib successfully. Common treatment-related adverse events (AEs) were (n any grade (G); n ≥G3): asymptomatic CPK increase (8;2), nausea/vomiting (7;0), and abdominal pain (6;0). The geometric mean (CV%) brigatinib exposure was 12.0 h*µg/mL (69.7%) on DL1 (n=3 evaluable) and 19.2 h*µg/mL (29.0%) on DL2 (n=6). The objective response rate was 100%, including CR or CR unconfirmed (CRu) in all evaluable ALCL patients (n=8) and PR in the sarcoma patient.

Conclusions
Brigatinib monotherapy is well tolerated and showed promising preliminary activity. Exposure at DL2 was within the predefined target range. The RP2D was established at DL2 (18-40 kg: 150 mg/day, >40 kg: 240 mg/day), which is higher than the adult approved dose. A pediatric population PK model for brigatinib is currently in development.

MEASUREMENT OF NON-INVASIVE TRACES OF PARACETAMOL

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Introduction: Personalized monitoring of medications has become increasingly important for individualizing dosage regimens and preventing disease progression. There is significant interest in developing a cost-effective, simple, and robust measure to evaluate medication levels in the body and medication adherence. This study aimed to evaluate a headspace solid-phase microextraction (HS-SPME) assisted gas chromatography-mass spectrometry (GC-MS) method for measuring in-vitro volatile organic compound (VOC) emissions (chemical fingerprints) from medications, specifically paracetamol.

Methods: In this study, HS-SPME was combined with GC-MS analysis to capture chemical fingerprints in glass vials containing 0.5 gram paracetamol tablets. Statistical analysis involved unsupervised (Principal Component Analysis (PCA)) and supervised (sparse partial least square discriminant analysis (sPLS-DA)) analyses to distinguish between blanks and samples based on their chemical fingerprints followed by identifying the discriminatory VOCs. Additionally, the influence of excipients was assessed using vials containing the active pharmaceutical ingredient (API) of paracetamol. Subsequently, the Mann– Whitney U test was employed to compare signal intensities between 0.5 g and 1 g paracetamol samples.

Results: A total of 41 samples and 27 blanks were prepared for the paracetamol experiments, 19 samples and 17 blanks were analysed for the 0.5 g condition, while 22 samples and 10 blanks were analysed for the 1 g condition. Although no statistically significant differences in signal intensities were detected based on quantities, the median intensity was higher for 1 g paracetamol compared to 0.5 g paracetamol and therefore, this condition was chosen for further exploration. PCA effectively distinguished between blanks and samples. The sPLS-DA model identified four discriminatory VOCs—hexanal, 6-methyl-2-heptanone, nonanal, and 4,8-dimethyl-1-nonanol—that effectively distinguished between blanks and samples with AUC of 1.00. After exploring the impact of excipients, 6-methyl-2-heptanone, and 4,8-dimethyl-1-nonanol remained as the key VOCs distinguishing blanks from samples.

Conclusions: This study provided a foundation for the use of VOCs (chemical fingerprints) in medication monitoring, suggesting their potential as markers for medication presence. Future research focusing on exhaled breath samples is essential to validate these findings and advance toward a point-of-care test for estimating paracetamol intake in emergency departments.

COST ANALYSIS OF *ADRB2* GENOTYPE-GUIDED TREATMENT FOR CHILDHOOD ASTHMA: RESULTS OF THE PUFFIN AND PACT TRIAL

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Introduction
Long-acting β2-agonists (LABA) are commonly used to treat asthma, however, some children do not respond to LABA. This might be due to the Arg16Gly polymorphism in the *ADRB2* gene encoding the β2 receptor, in particular the rs1042713 A allele (Arg16 amino acid). Several trials have shown that Arg16Gly *ADRB2* genotype-guided treatment before start of LABA in children with uncontrolled asthma despite inhaled corticosteroids (ICS) can improve clinical outcomes. We investigated whether *ADRB2* genotype-guided treatment decreases asthma-related healthcare costs.

Methods
Total semiannual healthcare costs for children with and without exacerbations were calculated using data of the PUFFIN trial. 102 Dutch and Swiss children were randomised over a genotype-guided treatment arm (adding LABA (Gly16Gly) or a double dose of ICS (Arg16Arg/Arg16Gly)) and control arm where the children were randomised to one of the two treatment regimens. Thereafter, we used half-yearly exacerbation rates of two trials (PUFFIN and PACT) to calculate asthma-related healthcare costs per arm based on the Dutch guideline for economic evaluations in healthcare. The PACT trial consisted of 91 children from Scotland and England with uncontrolled asthma randomised to a genotype-guided treatment arm (adding LABA (Gly16Gly) or montelukast (Arg16Arg/Arg16Gly)) and control arm.

Results
Total mean costs excluding genotyping costs per child were €421.10 (336.88 – 505.32) in the genotype-guided treatment arm (23 of the 90 children experienced exacerbations) and €514.81 (411.85 – 627.77) in the control arm (40 of the 103 children experienced exacerbations). Genotyping costs are currently €83 (range €66-99) per patient in the Netherlands.

Conclusions
To conclude, *ADRB2* genotype-guided treatment reduces asthma exacerbations, increases quality of life, leads to less school absence and is cost saving, outweighing the screening costs. *ADRB2* genotyping should therefore become standard of care in treating children with uncontrolled asthma that require step-up treatment with LABA.

APPLICABILITY OF LIPID-LOWERING RANDOMIZED CLINICAL TRIALS TO CONTEMPORARY REAL-WORLD PATIENTS WITH ESTABLISHED ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

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Introduction
Lipid-lowering randomized trials guide recommendations on lipid-lowering therapy (LLT) for atherosclerotic cardiovascular disease (ASCVD) prevention, but strict eligibility criteria may limit their applicability to real-world ASCVD patients. This study compared clinical characteristics and outcomes between trial-eligible and ineligible real-world patients with coronary artery disease (CAD), cerebrovascular disease (CeVD), and peripheral artery disease (PAD).

Methods
Eligibility criteria from eight lipid-lowering randomized clinical outcome trials were applied to patients with established CAD, CeVD, and PAD enrolled in the Utrecht Cardiovascular Cohort – Second Manifestations of ARTerial disease (UCC-SMART) study from 2000 to 2023. Eligibility proportions were calculated for each trial, and differences in clinical characteristics and long-term risks of cardiovascular (CV) events and all-cause mortality were analyzed, accounting for competing risks.

Results
The study included 8,537 patients with manifest ASCVD, of whom 5,673 had CAD, 2,493 had CeVD, and 1,302 had PAD. The median follow-up was 8.8 years (interquartile range: 4.2–13.9). Eligibility proportions for lipid-lowering trials ranged from 9-85% for CAD, 7-42% for CeVD, and 8-78% for PAD. Although trial-eligible and ineligible patients exhibited differences in clinical characteristics, they had largely comparable cumulative incidences of CV events and all-cause mortality.

Conclusions
Eligibility for lipid-lowering trials varied substantially by ASCVD subtype and trial. Despite differences in clinical characteristics between trial-eligible and ineligible patients, their risks of CV events and mortality were similar. These findings support the applicability of lipid-lowering trials to contemporary real-world CAD, CeVD, and PAD populations in clinical practice.

DEVELOPMENT OF A PREDICTION RULE FOR BENEFIT AND HARM OF THE ENDOTHELIN RECEPTOR ANTAGONIST ATRASENTAN IN PATIENTS WITH TYPE 2 DIABETES AND CHRONIC KIDNEY DISEASE

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Introduction

Endothelin receptor antagonist (ERAs) slow the progression of chronic kidney disease (CKD), but are associated with an increased risk of fluid retention. We aimed to develop a personalized benefit-harm score to guide effective and safe use of the ERA atrasentan in patients with type 2 diabetes and CKD.

Methods

Two Cox proportional hazards models were developed to predict the risk of the composite kidney outcome and hospitalization for heart failure (HHF) among patients with type 2 diabetes and CKD (urine albumin-creatinine ratio [UACR] ≥300 to 5000 mg/g, and estimated glomerular filtration rate [eGFR] of ≥25 to < 75 mL/min/1.73m^2]) participating in the SONAR trial. The composite kidney outcome consisted of doubling in serum creatinine or end-stage kidney disease (ESKD). HHF was defined as a hospitalization ≥24 hours in duration with acute heart failure. The difference between predicted risk with atrasentan (0.75 mg/day) and placebo was used to estimate the absolute risk change for both the kidney and HHF outcome. The benefit-harm score was calculated as the difference between these two estimates. Using linear regression, the study population was dichotomized into "benefit" and "harm"groups. For both outcomes, we compared the treatment effect on the absolute risk as well as the hazard ratios between the benefit and harm groups.

Results

A total of 3300 participants were included in the analysis. The composite kidney outcome occurred in 319 (9.7%) participants and 111 (3.4%) participants were hospitalized for heart failure. The Cox regression models for the kidney and heart failure outcome had a c-statistic of 0.86 and 0.82 respectively. Variables included in the benefit-risk score included age, sex, race, systolic- and diastolic blood pressure, eGFR, UACR, serum albumin, hemoglobin, B-type natriuretic peptide, beta-blocker use, diabetes duration, BMI, edema, and smoking. In the benefit group, atrasentan reduced the risk for the kidney outcome (absolute risk reduction [ARR] -4.5%, 95% CI -8.1, -0.9) without increased heart failure risk (ARR 0.4%, 95%CI -1.4, 2.3). The harm group had minimal kidney benefit (ARR -0.4%, 95% CI -1.8, 1.0) with an increased heart failure risk (ARR 1.7%, 95% CI 0.2, 3.2).

Conclusions

The benefit-harm score enabled identification of patients with type 2 diabetes and CKD who are likely to experience a net benefit or harm from atrasentan treatment. This personalized approach may facilitate informed decision-making, especially in populations at higher risk of heart failure.

ASSOCIATION BETWEEN GENETIC VARIANTS AND ESOPHAGITIS IN PATIENTS WITH NSCLC TREATED WITH FIRST-LINE PLATINUM BASED (RADIO)THERAPY: A PROSPECTIVE OBSERVATIONAL STUDY

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Introduction
Lung cancer is prevalent in the Netherlands, with NSCLC accounting for 70% of all patients. Treatment typically involves chemo-, radio- and/or immunotherapy. Toxicity, such as acute esophagitis, is common and often necessitates adjustments in the treatment regimen.
The primary objective was to explore genetic risk factors for esophagitis in NSCLC patients undergoing platinum-based (radio)therapy. Secondary objectives include determining the incidence and severity of esophagitis and assessing whether patient and treatment regimens characteristics influence the risk of developing esophagitis.

Methods
A candidate gene approach was applied based on the PGxLUNG study, a multicenter prospective study that included 320 NSCLC patients (stage II-IV) treated with first-line platinum-based chemotherapy [1]. Esophagitis was assessed regularly during follow-up using CTCAE v4.03.The association between 28 SNPs (selection based on literature) and esophagitis was analyzed by multivariate logistic regression.

Results
Of the 320 patients included in the analysis, 55% were treated with chemotherapy (sometimes combined with immunotherapy) and 45% with chemoradiotherapy. The incidence of severe esophagitis (CTCAE grade 2 or higher) was higher in patients undergoing chemoradiotherapy (24.5%) compared to those not receiving radiotherapy (0.6%). Moreover, it was 2.5 times more frequent in patients undergoing concurrent chemoradiotherapy compared to those receiving sequential chemoradiotherapy (37.9% vs 15.3% respectively). Significant associations with severe esophagitis were found for *OGG1* (rs1052133), *RPS6KB2* (rs10274), and *ERCC2* (rs13181). Patients with the GG-genotype (n=6) for *OGG*1 showed a 7.6-fold higher risk (95%CI: 1.2-50.9), the AA-genotype (n=20) for *RPS6KB2* a 3.2-fold higher risk (95%CI: 1.1-9.4), and the TT-genotype (n=51) for *ERCC2* a 2.5-fold higher risk (95%CI: 1.11-5.0). Numbers needed to genotype (NNG) were respectively 45, 20 and 17.

Conclusions
Genetic variants in *OGG1*, *RPS6KB2*, and *ERCC2* are associated with the risk of radiotherapy-induced esophagitis in NSCLC patients. Genotype guided selection for a less burdensome radiotherapy regimen may be beneficial for those with risk-associated genotypes.

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Onset and progression of atherosclerosis in patients with melanoma treated with immune checkpoint inhibitors

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Introduction
Immune checkpoint inhibitors (ICIs) are effective anti-cancer agents but significantly increase cardiovascular risk. This could be due to its potential to induce or worsen atherosclerosis. We evaluated the onset and progression of atherosclerosis during ICI treatment in patients with melanoma and investigated risk factors for substantial (>10%/year) atherosclerotic plaque growth in five segments of the arterial tree.

Methods
Onset and yearly progression of atherosclerosis were assessed in the aortic arch, descending thoracic aorta, abdominal aorta, left and right iliac arteries via CT scans performed prior to and one year (+/-three months) after ICI therapy initiation in patients with melanoma in the adjuvant (resected melanoma) and advanced disease (irresectable stage III and stage IV) setting. The primary outcome was defined as yearly progression of maximal plaque thickness in each arterial segment. Secondary outcomes were changes in the number of plaques and factors associated with substantial plaque growth in the descending thoracic aorta.

Results
In total, 244 patients were included. Plaque thickness increased significantly in all aortic segments, ranging from 3.0-8.0% per year. In 75% of included patients, substantial plaque growth in ≥1 segment occurred. Number of plaques remained identical in 64-86% of arterial segments. ICI combination therapy demonstrated a trend towards increased risk of substantial plaque growth compared to monotherapy (OR 2.10 [0.95-4.66; P = 0.068]), whereas antihypertensive drug usage associated with a lower risk (OR 0.53 [0.29-0.99; P = 0.048]).

Conclusions
The majority of melanoma patients experience substantial atherosclerotic plaque growth during ICI therapy. The number of plaques remained relatively stable, suggesting that ICIs particularly affect preexisting plaques.

METHYLPREDNISOLONE AND ECULIZUMAB FOR COMPLEMENT MEDIATED CEREBRITIS

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Introduction

Complement factor I (CFI) is a complement regulatory glycoprotein that, in the presence of cofactors, specific binding proteins and/or complement receptor 1, can inhibit all pathways of the complement system. Loss of function of CFI can lead to ongoing complement activation by C3 and a decrease in circulating C3 and factor B by consumption and absent alternative complement pathway. Partial CFI deficiency has also been described in association with immune-complex mediated diseases, like atypical Hemolytic Uremic Syndrome (aHUS). Eculizumab (humanized monoclonal antibody against C5) is a registered treatment for aHUS.

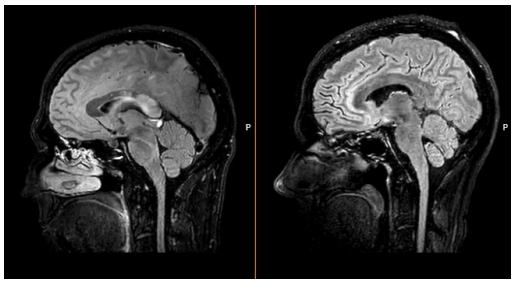
Methods (case presentation)

A 32-year-old Caucasian male presented with progressive headache, neck pain and vomiting and since a few hours increasing mental alteration. The medical history yielded a congenital CFI deficiency. Because a meningitis was suspected, high dose antibiotics, antivirals and corticosteroids were started. Initial CT scan showed diffuse elapse of gyri and sulci patterns, consistent with oedema. Results of lumbar puncture came back negative for infection. The patient deteriorated quickly requiring Intensive Care Unit (ICU) admission and intubation. MRI scan showed diffuse T2 hyperintensity consistent with fulminant CNS inflammation.

Results

Intracranial pressures increased upon which methylprednisolone was started (5 days 1000mg, followed by 80mg/day) and the treatment was expanded with off-label Eculizumab (900mg once a week, for 4 weeks). The hypothesized working mechanism of this treatment regimen is shown in Figure 1.
For the persistent high intracranial pressures, barbiturates were added to the sedative regimen and even a decompressive hemicraniectomy was performed. Seven days post-ICU admission sedation was weaned and the methylprednisolone was tapered, with complete discontinuation by week 6. Over de following days the patient kept improving neurologically, achieving near-complete clinical recovery. Eculizumab was continued in a maintenance dosage (900mg, once a month). A follow-up MRI, 46 days after hospital admission, and respectively 43 and 42 days after starting methylprednisolone and Eculizumab, showed near complete normalisation of the inflammatory changes in the brain (figure 2).

Figure 2. MRI scan pre- and post treatment.



Conclusion

This case describes a patient with known complete CFI deficiency with a presentation of fulminant CNS inflammation, effectively treated with methylprednisolone and Eculizumab.

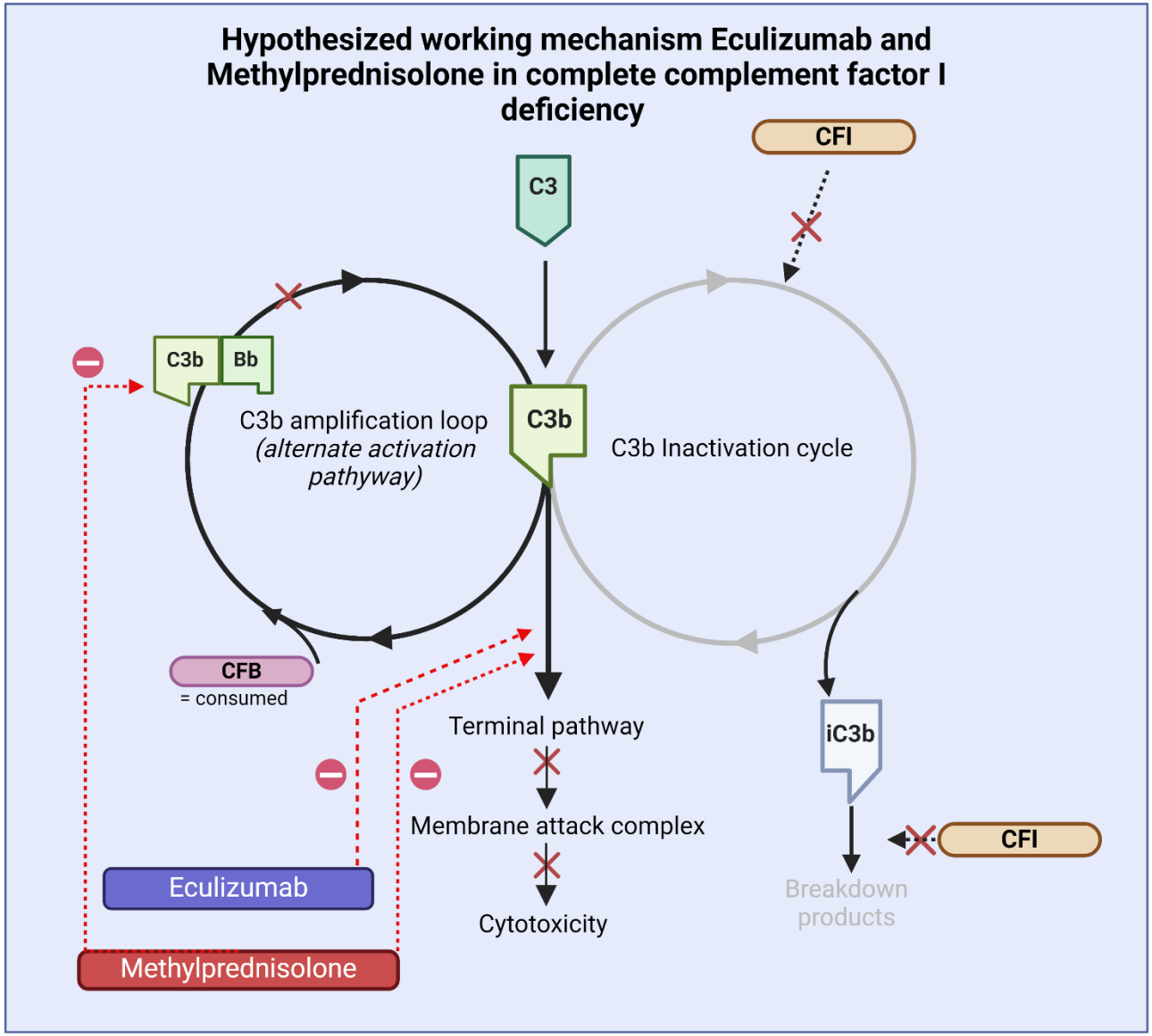


Figure 1. Hypothesized working mechanism Eculizumab and Methylprednisolone in complete complement factor I deficiency. Simplified representation of complement amplification loop. Loss of function of complement factor I (CFI) leads to ongoing complement activation by C3 by a lack of C3b inactivation causing uncontrolled activation of C3b, consumption of C3 and cofactor B (CFB) and activation of downstream terminal pathway. Eculizumab targets C5 (not shown in this figure), inhibiting activation of the terminal pathway and Methylprednisolone limits the activation of the amplification loop by inhibiting formation of C3bBb (C3 convertase) and also inhibiting the function of the terminal part of the complement system. Created in https://BioRender.com.

PREDICTIVE VALUE OF GASTRIN FOR THE ABSORPTION OF PH-DEPENDANT SMALL-MOLECULE INHIBITORS IN PATIENTS WITH NON-SMALL CELL LUNG CANCER; DOES ACHLORHYDRIA MATTER?

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Introduction

Achlorhydria (AH) is the pathophysiological state of chronic increased gastric pH, due to destruction of the gastric acid producing parietal cells. Gastrin stimulates gastric acid production and is elevated in high gastric pH. It is known that when the gastric pH is iatrogenically elevated by proton pump inhibitors (PPIs), the absorption of multiple small-molecule inhibitors (SMIs) in patients with metastatic lung cancer (LC) can be vastly decreased. Subsequent lower drug exposure can cause treatment failure. Solubility and absorption of the SMIs erlotinib and sotorasib are pH dependent. Hence, we hypothesized that gastrin as marker of AH could function as predictor of drug absorption and exposure in patients with LC.

Methods

We measured gastrin levels in prospectively collected samples from two cross-over drug-interaction studies with erlotinib [1,2] in which dense blood sampling was performed during 24h. Also, consecutive single-day erlotinib and sotorasib samples from a prospective cohort study (NCT05221372) were used to quantify SMI and gastrin levels. For erlotinib, Area Under the Curve (AUC) and maximum (C_{max}) and trough concentrations (C_{min} 24h) were calculated. For sotorasib, only C_{min} 24h was calculatable. Spearman's correlation test used gastrin as a continuous variable, where the Mann-Whitney U test used gastrin ≥150 pg/ml to distinguish patients with AH for dichotomous comparison of the data.

Results

In total, SMI and gastrin levels were quantified from 52 patients who used erlotinib and 54 who used sotorasib. Gastrin as continuous variable showed a significant inverse correlation with erlotinib $AUC_{0\text{-}12h}$ ($R = -0.277$; $p = 0.047$) and C_{max} ($R = -0.423$; $p = 0.002$). In 11 patients with AH, C_{max} was 35% lower compared to 41 patients with physiological levels of gastrin (i.e. <150 pg/mL), c.q. 1200 vs 1890 pg/mL; $p = 0.024$. $AUC_{0\text{-}12h}$ was 29% lower in patients with AH, but this was not statistically significant (10500 vs 14800; $p = 0.087$) due to limited sample size. For sotorasib C_{min}, no correlation was found with gastrin ($R = 0.158$; $p = 0.255$) in the total cohort.

Conclusions

Gastrin functions as a good marker for the absorption and exposure of the pH-dependant SMI erlotinib. This was not the case for sotorasib C_{min}. Patients with AH are hence more at risk to have diminished erlotinib drug exposure and treatment failure. Future studies should thus focus on the pre-emptive assessment of gastrin, in order to identify patients with AH, and on interventions that might prevent treatment failure, *e.g.* a higher SMI erlotinib starting dose or therapeutic drug monitoring during treatment.

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Investigating the Effects of Paracetamol on Human Placenta Using the Placental Slice Model

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Introduction

Paracetamol (PCM) is widely used during pregnancy, yet its potential impact on placental function remains unclear. This study investigates the effects of PCM on human placental viability, function, and morphology using the placental slice model.

Methods

Placental slices from both maternal and fetal sides were treated with PCM at 0.01 mM, 0.1 mM, 1 mM and 10 mM, and incubated for 24 hours. Viability was assessed using ATP content and PrestoBlue assays, function was evaluated by hormone concentration, and morphology was examined via hematoxylin and eosin (H&E) staining.

Results

ATP content measurements indicated no significant differences in viability across all groups. However, PrestoBlue assay revealed reduced viability in maternal slices exposed to the highest PCM concentration (100 mmol). Other functional and morphological analyses are ongoing.

Conclusions

Our findings suggest that high PCM concentrations may affect maternal placental slice viability without altering ATP levels. Further analyses will clarify the functional and structural implications of PCM exposure.

DORAVIRINE IN BREASTMILK OF HEALTHY, LACTATING, HIV-NEGATIVE WOMEN AFTER A SINGLE DOSE OF 100MG

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Introduction
Breastfeeding in context of HIV-1 infection has long been avoided in high and middle income countries out of fear of vertical transmission. In low income countries, this risk is also recognized, but is weighed out by the risk of pneumonia and diarrheal disease associated with unsafe drinking water used for formula preparation. Due to effective antiretroviral therapy, vertical transmission risk has been proven to be very low. Question remains, however, if exposure to antiretroviral drugs through breastmilk can be hazardous to the infant. The aim of this study is to describe breastmilk transfer of doravirine, a first line antiretroviral agent.

Methods
In this non-randomized, open-label, single center clinical trial healthy, lactating women without HIV were administered a single dose of 100mg doravirine, after which blood- and breastmilk samples were obtained during a 24 hour period. The participants were instructed to provide alternative feeding (formula or previously pumped breastmilk) 4 days after ingestion of study drug, in order to prevent exposure to the study drug through breastmilk of their infants.
Doravirine concentrations were measured with the use of LC-MS/MS. Pharmacokinetic parameters in blood were determined with the use of non-compartmental analysis. Milk: plasma ratio was calculated using the area under the curves during 24 hours (AUC0-24h) (Breastmilk AUC0-24h / maternal plasma AUC0-24h). Daily infant dose (mg/day) was calculated using Σ (total drug concentration in each milk collection multiplied by the expressed milk volume in each milk collection) and the relative infant dose (RID) by dividing the infant dosage (mg/kg/day) by the maternal dosage (mg/kg/day) and multiplied by 100. As cumulative expression of breastmilk may vary across participants, a RID will be calculated assuming breastmilk intake of 150mL/kg/day per infant ((AUC/24)*150). A RID <10% was considered safe, in accordance with EMA/FDA recommendations.

Results
8 healthy, lactating women without HIV were included. The geometric mean (CV%) AUC0-24h(h*mg/L) in plasma was 14.47 (26.1) and 3.86 (25.2) in breastmilk, resulting in a median (IQR) breastmilk : plasma ratio of 0.29 (0.27-0.31). Not all participants were exclusively breastfeeding, resulting in a wide range of expressed milk volumina. So did the daily - and relative infant dosage (%), with a median (IQR) of 0.07 (0.01-0.11) mg/day and 0.86 (0.08-1.42)% respectively. When assuming a daily milk intake of 150mL/kg the median (IQR) daily - and relative infant dosages were 0.18 (0.15-026) mg/day and 1.90 (1.50-2.44)% respectively.

Conclusions
Doravirine does transfer from maternal plasma into breastmilk, however, the measured concentrations and subsequent daily- and relative infant dosages do not exceed the safety threshold. Even though no clear relationship between exposure and toxicity has been established for doravirine, a relative infant dose of <10% is reassuring.

Tacrolimus Dosing and Therapeutic Drug Monitoring in Steroid-Refractory Immune-Related Adverse Events

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Introduction

Immune therapy is crucial in oncology but often leads to immune-related adverse events (irAEs). In patients receiving CTLA-4 and PD(L)-1 inhibitors, 30-50% develop grade 3-4 irAEs, typically treated with prednisolone.[1] However, some cases are steroid-refractory requiring other immunosuppressive therapy such as tacrolimus. Despite its clinical use beyond ESMO guidelines, optimal tacrolimus dosing and therapeutic drug monitoring (TDM) remain unclear.[2] This study evaluates dosing strategies, serum trough levels, and TDM policies in steroid-refractory irAEs.

Methods

A retrospective analysis included patients treated with tacrolimus for irAEs post-immune checkpoint inhibitors (ICIs) between January 2018 and October 2024 in the Antoni van Leeuwenhoek hospital. Patients with incomplete tacrolimus dosing or serum trough data were excluded. Two tacrolimus dosing protocols were compared:

1. **Bodyweight-based dosing** (0.15 mg/kg/day, target: 5–15 ng/mL, non-standardized TDM).
2. **Fixed dosing** (5 mg twice daily for 60–100 kg, target: 10–15 ng/mL initially, 5–10 ng/mL maintenance, standardized TDM).

Additionally, we investigated whether the patient characteristics age, BMI, sex, bodyweight, gender, length, body surface area and eGFR influenced tacrolimus serum trough levels.

Results

Among 58 patients (Group 1: n=39; Group 2: n=19), bodyweight-based dosing led to more: (1) frequent high-dose prescriptions (≥6 mg), (2) toxic serum through levels (≥20 ng/mL, 1 vs 6 patients) and (3) variability in serum trough levels, with mean tacrolimus levels of 12.4 ± 9.1 ng/mL compared to 9.7 ± 5.5 ng/mL. Strict TDM guidance resulted in significantly lower variability in second serum trough levels (mean: 11.4 ± 4.9 ng/mL; p=0.0049), and less discontinuations due to toxicity (0 vs. 6 patients). No significant correlations were found with patients' factors and serum trough levels (BMI, age, sex, bodyweight, gender, length, body surface area and eGFR).

Conclusion

Fixed tacrolimus dosing minimizes toxicity and optimizes therapeutic outcomes in steroid-refractory irAEs. A regimen of 5 mg twice daily (>60 kg) with an initial target of 10–15 ng/mL, followed by 5–10 ng/mL maintenance, under TDM, is feasible and safe. Further studies are needed to refine dosing for patients <60 kg and to assess the efficacy of different trough targets

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POPULATION MODELING TO EXPLOIT PHASE I DOSE-FINDING TRIALS DATA IN PEDIATRIC ONCOLOGY EXEMPLIFIED BY THE BRIGAPED (ITCC-098) PHASE I STUDY

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Introduction
In pediatric oncology phase I trials, determining the recommended phase II dose (RP2D) often involves aligning with target adult pharmacokinetics (PK) parameters. While non-compartmental analysis (NCA) is commonly used, its accuracy struggles with sparse sampling. Incorporating prior adult PK knowledge in the pediatric population PK (popPK) modeling process via the $PRIOR subroutine in the model software (NONMEM) offers an alternative to NCA to characterize PK at an early trial stage. We examined the feasibility of this modeling approach in the completed phase I portion of an ongoing pediatric trial and, in simulated trials, compared its performance in exposure estimation to NCA.

Methods
Pediatric PK data were obtained from the dose-finding part of a Brigatinib (Briga) trial, ITCC-098. The pediatric popPK model was informed by pediatric data and the selective use of prior results from the published Briga adult popPK model. Full PK profiles were simulated to integrate the true exposure, and to generate 1000 simulated trials. In each trial (n=12, 6 per dose level) exposure was estimated by NCA and by a re-developed model. The capacity to generate evaluable estimates and the accuracy (power: the percentage of evaluable estimates within ±20% of the true value) of both methods were assessed.

Results
95 Briga PK samples from ten patients were analyzed. The pediatric PK model was a three-compartment model with a two transit compartments input and fixed allometric scaling. The pediatric apparent clearance (CL/F) was 14.0 L/h (RSE: 14.6%) for a 70-kg patient, 32.1% higher than the adult value of 10.6 L/h. Parameters without estimation issues were estimated with uninformative priors ensuring that the estimates were driven by pediatric data. Informative priors were used for the other parameters. In simulated trials, the modeling approach achieved 100% (n=12000) evaluable estimates vs. 81.6% (n=9788) with NCA. Power of the modeling approach among evaluable estimates was higher: 84.0% vs. 66.5%.

Conclusions
This study highlights the feasibility of the modeling approach as an alternative to NCA to leverage existing knowledge and limited phase I trial data in pediatric populations. It can also be applied to other vulnerable populations with sparse sampling. Higher pediatric CL relative to adults supports the preliminary observations in the Briga trial that a higher dose was required in children to reach adult exposure. In realistic scenarios with sparse data, compared to NCA the higher number of evaluable estimates and higher accuracy suggest the modeling approach may support reduced sampling per patient in future trial designs.

Physiologically Based Pharmacokinetic Modeling of Ponatinib in Pregnancy: Predicting Maternal Drug Exposure

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Introduction

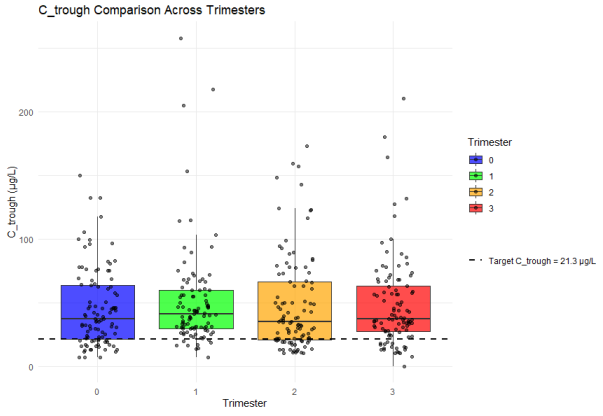
Tyrosine kinase inhibitors (TKIs) play a crucial role in cancer treatment, but their use during pregnancy remains a challenge due to limited pharmacokinetic (PK) data. Physiological changes in pregnancy can significantly alter drug disposition, yet evidence-based dosing guidelines for these agents are lacking. While some data exist for earlier-generation TKIs, little is known about third-generation TKIs, particularly ponatinib. To address this gap, this study aimed to develop physiologically based pharmacokinetic (PBPK) models for ponatinib, to characterize their PK throughout pregnancy and support informed dosing recommendations for this special population.

Methods

A PBPK model for ponatinib was developed using data from a monkey intravenous study, as well as single and once daily administrations in healthy and diseased individuals. The models were validated against observed in vivo PK data from clinical trials and real world-cancer patient data from the UMCG. Model verification included drug-drug interaction studies with the CYP3A4 inducer rifampin and inhibitor keto-conazole. Pregnancy-related changes in anatomy, physiology, and the activity of enzymes and transporters were incorporated into the development of the pregnant PBPK model. For dose evaluation, PK parameters, including peak concentration (Cmax), concentration prior to next dose (Ctrough), and area under the curve (AUC), were compared between non-pregnant and pregnant populations."

Results

The PBPK model accurately predicted ponatinib PK in non-pregnant healthy adults and in cancer patients. There was no significant difference in the Cmax, Ctrough AUC of ponatinib between the non-pregnant and pregnant population across the three trimesters.



Conclusions

PBPK models for ponatinib were developed and validated for non-pregnant healthy adults, cancer patients, and pregnant women. No dose adjustment is needed for ponatinib in the pregnant population.

A SYSTEMATIC EVALUATION TO REFINE DOSING REGIMENS FOR APPROVED TARGETED THERAPY IN METASTATIC RENAL CELL CARCINOMA FROM A PROJECT OPTIMUS PERSPECTIVE

Authors

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Introduction

Targeted therapies have recently been dominant in the treatment of metastatic renal cell carcinoma (mRCC), leading to notable improvements in patient survival. However, the high incidence of adverse events often leads to treatment interruptions and discontinuation[1]. The FDA's recent initiative, Project OPTIMUS[2], emphasizes the importance of optimizing dosing regimens during oncology clinical development, moving beyond the conventional maximum tolerated dose approach. In this study, we aimed to review and optimize the approved dosing strategies for targeted therapies in mRCC.

Methods

First, we conducted a comprehensive review of the dose-finding strategies by analyzing FDA clinical pharmacology reviews of pazopanib, axitinib, cabozantinib, sunitnib, everolimus and nivolumab. Second, we summarized routine clinical practice studies on dose reduction and alternative dosing regimens concerning the actual tolerated and effective doses observed in clinical practice. Finally, published pharmacokinetic models were assessed and selected for model-informed simulations to evaluate labelled dosing or alternative regimens from the perspective of optimal target achievement in the post-marketing context for these drugs.

Results

Across all targeted therapies except nivolumab, the observed actual tolerated dose in routine clinical practice ranged from 46.1% to 86% of the approved recommended dosage, with up to 75% of patients requiring dose reductions or treatment interruptions due to intolerance. Evidence from clinical practice and model-informed simulations suggests that for most investigated drugs, a lower dose (14-50% reduction) could provide comparable efficacy with improved tolerability as summarized in Table 1. For nivolumab, model-informed simulations confirmed that approved flat-dose regimens provide adequate drug exposure without an increase of adverse effects.

Conclusions

We identified optimized dosing regimens that could improve drug tolerability while maintaining efficacy for the approved targeted therapies in mRCC. We suggest that these optimized dosing regimens should be considered for updating drug labels for existing therapies and that the optimal exposure range should be included in drug labels to support pharmacokinetically guided dose individualization in clinical practice.

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Table 1. Recommended starting dose based on labelled dose, routine clinical practice studies and model-informed results

Drug	Labelled dose	Dose in routine clinical practice*	Efficacy target concentration [REF]	Toxicity target concentration [REF]	Model-informed dose$	Conclusions and recommended starting dose&
Pazopanib	800 mg QD	600 mg QD	$C_{min,ss} \geq 20.5$ mg/L[1, 2]	$C_{min,ss} < 46$ or 50 mg/L[1, 2]	600 mg QD	600 mg QD
Axitinib	5 mg BID	4 mg BID	$C_{min,ss} > 1.76$ ng/mL[2, 3]	$C_{max,ss} > 40.2$ ng/mL[2, 3]	5 mg BID	5 mg BID
Cabozantinib	60 mg QD	40 mg QD	$C_{min,ss} > 336$ ng/mL[4]	$C_{min,ss} < 750$ ng/mL[4]	60 mg QD * 2 days + 1 day skip 40 mg QD	60 mg QD * 2 days + 1 day skip 40 mg QD
Sunitinib#	50 mg QD 4/2	37.5 mg QD	$C_{min,ss} \geq 50$ ng/mL[2]	$C_{min,ss} < 100$ ng/mL[2]	50 mg QD 2/1	50 mg QD 2/1 37.5 mg QD
Everolimus	10 mg QD	-	$C_{min,ss} > 10$ ng/mL[2]	$C_{min,ss} \leq 26.3$ ng/mL[2]	5 mg QD	5 mg QD
Nivolumab	3 mg/kg 240 mg Q2W 480 mg Q4W	-	$C_{min,ss} > 2.5$ mg/L[5]	/	3 mg/kg 240 mg Q2W 480 mg Q4W 360 mg Q3W	3 mg/kg 240 mg Q2W 480 mg Q4W 360 mg Q3W

QD: once daily; BID: twice daily; Q2W: once every 2 weeks; Q3W: once every 3 weeks; Q4W: once every 4 weeks; 4/2: 4 weeks on and 2 weeks off treatment; 2/1: 2 weeks on and 1 week off treatment; $C_{min,ss}$: trough concentration at steady state; $C_{max,ss}$: maximum concentration at steady state

*: The information of this column was taken from published studies and rounded to the nearest tablet/pill/vial strength

$: Derived from the model-informed simulations, using the selected PK models and exposure targets

&: The conclusions and recommended starting dose regimens are based on the 2nd, 3rd, 5th columns and expert opinion if there is discrepancy between dose in routine clinical practice and model-informed dose

-: No solid evidence from routine clinical practice studies

#: Sunitinib exposure metric is the composite $C_{min,ss}$ of both parent drug and main metabolite (sunitinib and SU12662)

/: No solid relationship available of nivolumab exposure with toxicities

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POPULATION PHARMACOKINETICS OF NEO-ADJUVANT IPILIMUMAB AND NIVOLUMAB IN PATIENTS WITH LOCOREGIONALLY ADVANCED UROTHELIAL CARCINOMA

Authors
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Background
Patients with locoregionally advanced urothelial carcinoma (UC) have a high recurrence rate after surgical resection. Immune checkpoint inhibitors have proven to be very effective in the neo-adjuvant metastatic setting. Therefore, in the NABUCCO trial[1,2], the effect of three neo-adjuvant cycles of anti-CTLA-4 IgG1 antibody ipilimumab (IPI) and anti-PD-1 IgG4 antibody nivolumab (NIV) was tested in three different dosing combinations: A) 3 (cycle 1+2) mg/kg IPI + 1 (cycle 2) or 3 (cycle 3) mg/kg NIV, B) 3 (cycle 1+2) mg/kg IPI + 1 (cycle 1+2) or 3 (cycle 3) mg/kg NIV, and C) 1 (cycle 1+2) mg/kg IPI + 3 (cycle 1+2+3) mg/kg NIV. Overall trial results showed convincing clinical outcomes. However, the pathological complete response (pCR) rate for patients in arm C was relatively low. The aim of the current study is to determine variability in IPI and NIV pharmacokinetics (PK) to explain differences in pCR rate among treatment arms.

Methods
Before (and in a few cases after) each treatment cycle and surgery, serum samples were taken from participating patients. IPI and NIV levels were measured using a validated UPLC-MS/MS method[3]. Previously published population PK models (popPK)[4,5] for IPI and NIV were implemented in the NONMEM (v7.5) PRIOR subroutine to develop popPK models for both drugs for this population.

Results
IPI and NIV serum levels were measured in 139 samples from 54 patients. Using the NONMEM PRIOR subroutine, the essential PK parameters (CL, V1, Q, V2) could be estimated for this population. For IPI, typical CL was estimated as 0.28 L/day (RSE of 5.5%). For NIV, CL was also estimated as 0.28 L/day (RSE of 7.1%). Individual CL estimates of both drugs were associated. Patients with a (relatively) low CL of both drugs were more likely to have a pCR after treatment. In arm C, the amount of such patients was relatively lower.

Discussion/Conclusion
The PK parameters of IPI and NIV have been studied for the first time in a neo-adjuvant locoregionally advanced UC population. The differences in clinical outcome among treatment arms in the NABUCCO trial might be explained by PK differences. Relatively low IPI and NIV CL during neo-adjuvant therapy was associated with a higher pCR rate in locoregionally advanced UC patients.

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Abstract book

SCIENTIFIC MEETING

Dutch Society for Clinical Pharmacology and Biopharmacy (NVKFB) &
Dutch Society for Pharmacology (NVF)

Part 2:

ABSTRACTS

Development of a Parent-Metabolite Model for Tramadol and Its Metabolite M1 in Rat Plasma for Mechanistic Investigation of Their CNS Distribution

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Organisations

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Introduction

In Europe, 38% of chronic pain patients report inadequate symptom relief and significant side effects with conventional treatments. To address this, a combination drug therapy incorporating the opioid tramadol is of interest. Tramadol and its metabolite M1 target opioid and monoaminergic pathways in the central nervous system (CNS).

Aim: To develop a rat plasma population PK (PopPK) model in order to mechanistically describe their CNS distribution using a physiology-based pharmacokinetic (PBPK) model.

Methods

Twelve male Sprague-Dawley rats received a single I.V. bolus dose of tramadol (15 or 25mg/kg). Tramadol and M1 concentrations were measured in plasma, striatum, and lateral ventricles through microdialysis. The PopPK model was developed in Monolix and evaluated using diagnostic plots, Objective Function Value (OFV), and the corrected Bayesian Information Criterion (BICc). Covariate and covariance models were tested using stepwise inclusion and deletion.

Results

A parent-metabolite model with two compartments for tramadol and one for M1 best described their plasma kinetics. Tramadol elimination involved saturable transformation to M1 (V_{max}: 10506nmol/h [7.01 %RSE]; K_m: 3167nM [11.4 %RSE]) and linear clearance (0.29L/h [38.9 %RSE], IIV: 95.58%, [33.0 %RSE]). The clearance of M1 was also linear (48.46L/h [7.19 %RSE], IIV: 17.08% [25.4 %RSE]). Analysis of the microdialysis data indicates passive as well as active influx of tramadol and M1 to the brain extracellular fluid.

Conclusions

The final model adequately described the plasma PK of tramadol and M1 in rats. It quantified saturable transformation of tramadol, serving as a starting point for model-based assessment of the microdialysis results and mechanistic modelling of the CNS distribution of tramadol and M1.

HIGH-THROUGHPUT SCREENING OF THE EXPRESS-PICK LIBRARY IDENTIFIES A NEW INHIBITOR OF SULFUR BIOCHEMISTRY IN COLORECTAL CANCER CELLS

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, with Slovakia among the countries with the highest incidence. Cystathionine-β-synthase (CBS) plays a key role in sulfur metabolism and contributes to CRC progression by promoting tumor growth. Targeting CBS with selective inhibitors could disrupt cancer cell metabolism and offer a novel therapeutic approach.

Methods

High-throughput screening of the Express-Pick Library (SelleckChem) containing 3010 structurally diverse, drug-like compounds was performed using a fluorescence-based CBS activity assay. Hit CBS inhibitors were confirmed using two absorbance-based assays. The most effective inhibitor WAY-230765 was characterized for potency, direct CBS binding, cofactor binding, selectivity in enzyme counter-screen, and effects in HCT116 human colorectal cancer cells.

Results

WAY-230765 had a lower potency (IC50 = 18.20 μM) than the reference inhibitor AOAA (IC50 = 5.62 μM), but did not act via the CBS cofactor PLP (pyridoxal-5'-phosphate). WAY-230765 also did not inhibit other PLP-dependent enzymes. Gradual thermal denaturation of CBS suggested target engagement by WAY-230765. Unlike AOAA, WAY-230765 inhibited hydrogen sulfide production by HCT116 cells.

Conclusion

We identified WAY-230765 as a novel CBS inhibitor with a unique cofactor-independent mechanism of action. Being a drug-like compound capable of inhibiting sulfur biochemistry in colorectal cancer cells, WAY-230765 represents a starting point for development of new CRC treatment.

Funding

This work was supported by APVV-23-0178; VEGA 1/0619/24.

VIRAL GPCR US28 AS A DRIVER OF ONCOGENIC EXTRACELLULAR VESICLE SECRETION IN BRAIN CANCER

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Introduction

US28 is a vial G protein-coupled receptor (vGPCR) encoded by the human cytomegalovirus. An infection is often asymptomatic, however, in immune-compromised individuals such as cancer patients it can give rise to complications. In glioblastoma, a fast-growing and aggressive form of brain cancer, it has been demonstrated that US28 expression enhances oncogenic signaling pathways. US28 has also been linked to the emerging field of extracellular vesicles (EVs). These are nanosized membrane enclosed vesicles that contain heterogenous bioactive cargo which were shown to display multi-faceted cancer-promoting functions. We propose that US28 modulates EV secretion and/or composition via pathways that require elucidation to further understand the GPCR-mediated oncomodulation.

Methods

To determine whether US28 itself and its presence in glioma cells changes EV secretion or composition, Western blotting looking at various EV markers was employed. In addition, tunable resistive pulse sensing (TRPS) was used to determine the number of secreted particles. For localization studies of US28 and EV markers of interest, immunofluorescence imaging was used. EVs were isolated from conditioned media using ultrafiltration and size exclusion chromatography. Further, luminescence-based assays were performed to determine the molecular determinants of US28 that are responsible for EV secretion and/or cargo selection.

Results

Our results showed the presence of US28 in EVs derived from glioma cells. The latter seems partially dependent on its constitutive signaling and intracellular cycling activity. Further, a comprehensive EV marker panel and TRPS indicated a US28-dependent change in EV particle amount upon its expression in glioma cells.

Conclusion

Our findings identify US28 as a regulator of EV secretion and/or cargo selection, changes that may explain its reported oncomodulatory role.

DIFFERENTIATION OF LRRK2-G2019S IPSC-DERIVED MICROGLIA TO STUDY ALTERATIONS IN MICROGLIA FUNCTION IN THE CONTEXT OF PARKINSON'S DISEASE

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Introduction

Parkinson's Disease (PD) is a neurodegenerative disorder characterised by the progressive loss of dopaminergic neurons in the substantia nigra, leading to motor impairments. The Leucine-Rich Repeat Kinase 2 (LRRK2) G2019S mutation is the most common genetic risk factor for PD and is associated with neuroinflammation. Microglia, the resident immune cells of the central nervous system, are key regulators of the neuroinflammation. They play a crucial role maintaining homeostasis in the brain, thus microglial dysfunction may contribute to the onset of PD pathology and the characteristic degeneration of dopaminergic neurons. To perform research on CNS brain cells such as microglia, certain limitations have to be overcome since human samples can only be obtained post-mortem, and murine models don't fully recapitulate the human PD pathology. Patient-derived induced pluripotent stem cells (iPSCs) provide a valuable model to obtain human brain cells for in vitro research.

Methods

We established a protocol that allows robust differentiation of patient-derived iPSCs carrying the LRRK2 G2019S mutation into microglia. We subsequently verified LRRK2 function via downstream phosphorylation of Rab10 and analysed different aspects of microglia function including phagocytosis, metabolism, ROS production and calcium homeostasis after different inflammatory stimuli like LPS, IFNy or Diesel Exhaust Particles (DEP) to study the role of the LRRK2-G2019S mutation in microglia function under inflammatory conditions.

Results

Our data shows impaired phagocytic activity and metabolic reprogramming in the G2019S microglia, and increased ROS production after different inflammatory stimuli.

Conclusions

Altogether this shows the relevance of iPSC-derived LRRK2-G2019S microglia to study cellular function in the context of PD.

TARGETING METHYLGLYOXAL: A NOVEL APPROACH TO IMPROVE VASCULAR FUNCTION IN DIABETES

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Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare neurological disease characterized by chronic white matter oedema, leading to motor problems, epilepsy and cognitive decline. Two distinct heterozygous amino acid duplications (I176dup and A177dup) have been found in transmembrane region 4 (TM4) of GPRC5B of three unrelated MLC patients. GPRC5B is an orphan class C G-protein coupled receptor (GPCR). Class C GPCRs typically form obligate dimers, suggesting that GPRC5B also dimerizes. Recently, dimerization through a TM4-TM4 interface has been identified in GPRC5D, another member of the GPRC5 family. Based on sequence conservation among the GPRC5 family members, I175, I176, and E179 have been proposed as critical residues in the dimerization interface. We characterize the changes induced by MLC-related mutations, which are located inside the dimerization interface, in dimerization of GPRC5B.

Methods

Structural and interaction predictions were generated using AlphaFold3 supplemented by PyMOL for structural analysis.

Results

AlphaFold3 predicts that wildtype GPRC5B dimerizes through TM4, while I176dup and A177dup shift the dimerization interface to TM6. PyMOL analysis revealed structural changes in GPRC5B TM4 with MLC related mutations, causing residues I175, I176 and E179 to shift upward in the transmembrane helix.

Conclusion

Literature and AF3 predictions suggest that wildtype GPRC5B dimerizes through TM4-TM4 interface. Moreover, MLC patient mutations I176dup and A177dup may disrupt this interface, potentially interfering with dimerization. These findings highlight AI as a valuable tool to explore the effect of patient mutations on GPRC5B homodimerization, and generate hypotheses which can be validated through experimental work in living cells.

EXPLORING CXCL12 - GLYCOSAMINOGLYCANS INTERACTIONS

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Organisations

Introduction

CXCL12 (stromal cell-derived factor-1, SDF-1) belongs to the family of chemokines, small, secreted proteins of molecular weight ranging from 8-12 kDa. CXCL12 exerts its biological effect by interacting with two chemokine receptors, namely the CXC chemokine receptor (CXCR4) and an atypical chemokine receptor 3 (ACKR3). Chemokines play critical roles in regulating immune responses, angiogenesis, haematopoiesis, and embryogenesis. However, they are also implicated in the development and progression of various pathological conditions, such as cancer metastasis. To achieve their specific effects, extracellular distribution of chemokines is essential and regulated by binding to glycosaminoglycans (GAGs), which are prominently expressed on cell surfaces. Glycosaminoglycans are involved in extracellular distribution and gradient formation, receptor binding and signal modulation. Therefore, we aim to understand CXCL12-GAG interactions which appear crucial in understanding the regulation of cell signalling and migration and could be a relevant interface to probe with novel therapeutics.

Methods

We established a framework of biochemical techniques to explore CXCL12 – GAGs interactions. Initially, we determined how GAGs modulate CXCL12 binding and signalling via its cognate receptors using Nano-Bioluminescence Energy Resonance Transfer (Nano BRET). Moreover, we developed a solid-phase binding assay to study its binding affinities for different GAGs. As endothelial cells are known to contain various CXCL12 binding molecules (CXCR4, ACKR3 and heparan sulfate) we are characterizing endothelial cells, derived from human-induced pluripotent stem cells, as a model that closely resemble the physiological glycocalyx composition.

Results and conclusions

Using the established methodological framework, we are now exploring CXCL12 specificity for various GAGs, together with functional effects in hiPSC-ECs.

TARGETING ANGIOTENSIN II-INDUCED ENDOTHELIAL DYSFUNCTION IN PORCINE CORONARY ARTERIES: METFORMIN AND COLCHICINE AS THERAPEUTIC AGENTS

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Introduction

Endothelial dysfunction is a hallmark of heart failure with preserved ejection fraction (HFpEF) and coronary artery disease (CAD), driven by inflammation and impaired nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) signaling. Targeting these mechanisms may offer novel therapeutic strategies.

Objective: This study aimed to establish an ex vivo porcine coronary artery model to investigate endothelial dysfunction induced by (Ang II) and evaluate the protective effects of colchicine and metformin.

Methods

Coronary arteries from slaughterhouse pigs were incubated for 24 hours with angiotensin II (Ang II,100 nM), doxorubicin (50 nM), or exposed to UV irradiation (40J) to induce endothelial dysfunction and DNA damage. Wire myography assessed endothelium-dependent and -independent vasorelaxation, while specific inhibitors were used to evaluate the involvement of NO-cGMP, endothelial-dependent hyperpolarization (EDH), and cyclooxygenase (COX) pathways. Baseline cGMP levels were quantified via ELISA.

Results

Among tested exposures, Ang II significantly impaired endothelial function ($p<0.05$). Colchicine restored endothelial function primarily via the COX and NO-cGMP pathways, as indicated by partial inhibition following indomethacin and L-NAME treatment. Metformin improved endothelial function via the EDH pathway, independent of cGMP bioavailability, but through AMPK activation, as evidenced by the diminished protective effect after AMPK inhibition.

Conclusions

Colchicine and metformin exhibit distinct protective mechanisms against Ang II-induced endothelial dysfunction, supporting their potential as therapeutic agents for vascular disease management.

TGFB INDUCES CHANGES INDICATIVE OF NEUROPLASTICITY IN NOVEL MODEL OF HPSC-DERIVED SENSORY NEURONS

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Introduction

Neuroplasticity is a pathological feature of asthma, characterized by a lowered firing threshold, increased neuron density and neuron length, resulting in hyperresponsiveness. We recently reported an increased presence of mast cells in the vicinity of airway neurons in patients with fatal asthma. Unfortunately, no adequate model exists to study human sensory neuron plasticity. To address this, we have developed an in vitro human model of sensory neurons and exposed these to TGFβ, a cytokine produced by mast cells and other lung cells and important for remodeling processes.

Methods

We developed and validated a 40-day protocol to differentiate hPSCs into mature sensory neurons. Subsequently, we exposed these sensory neurons for 5 days to TGFβ and assessed changes in the neurons.

Results

Immunofluorescence, flow cytometry, and RNA analysis confirmed successful generation of β3-tubulin+ neurons, with ~35% being sensory neurons (TRPV1+/NAV1.8+) in both standard culture and our microfluidic axon-guidance model. TGFβ treatments altered network structure, resulting in more branching and longer segments. This was supported by RNAseq and GSEA, which showed that TGFβ exposure upregulates gene sets associated with peripheral neuron development (e.g. ASCL1, POU4F1, CRLF1, NTRK1), protein synthesis (e.g. RPL, RPS) and immune cell recruitment (e.g. PLAUR, IL11, CCL19).

Conclusions

We present a novel in vitro model of hPSC-derived sensory neurons that replicates neuroplasticity, offering a valuable tool to study its pathological mechanisms. Our findings show that TGFβ can induce neuroplasticity-like changes within 5 days of exposure, providing a new route to study the onset of neuroplasticity in asthma.

SIGNAL TERMINATION OF THE CHEMOKINE RECEPTOR CCR9 IS GOVERNED BY AN ARRESTIN-INDEPENDENT PHOSPHORYLATION MECHANISM

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Introduction

The chemokine receptor CCR9 coordinates immune cell migration from the thymus to the small intestine along gradients of CCL25. Receptor dysregulation is associated with a variety of inflammatory bowel diseases such as Crohn's and ulcerative colitis, while aberrant CCR9 overexpression correlates with tumor metastasis. Despite being an attractive therapeutic target, attempts to clinically antagonize CCR9 have been unsuccessful. This highlights the need for a deeper understanding of its specific regulatory mechanisms and signaling pathways

Methods

Results and conclusion

CCR9 is a G protein-coupled receptor (GPCR) and activates Gi and Gq pathways. Unexpectedly, live-cell BRET assays reveal only limited G protein activation and signaling is rapidly terminated. Truncating the receptor C-terminus significantly enhanced G protein coupling, highlighting the regulatory role of this domain. Signal suppression was not due to canonical arrestin-coordinated desensitization. Rather, removal of GPCR kinase (GRK) phosphorylation led to sustained and robust G protein activation by CCR9. Using site-directed mutagenesis, we identified specific phosphorylation patterns that attenuate G protein coupling. Receptor internalization does not correlate with G protein activation capabilities. Instead, CCR9 phosphorylation appeared to directly destabilize the interaction of G protein heterotrimers with the receptor. This interference could lead to rapid loss of productive coupling and downstream signaling as phosphorylation would effectively render the receptor incapable of G protein coupling. An arrestin-independent, phosphorylation-driven deactivation mechanism could complement arrestin-dependent regulation of other GPCRs and have consequences for therapeutically targeting these receptors.

EFFECTS OF BUDESONIDE ON AIRWAY NEUROPLASTICITY IN ASTHMA

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Introduction

Asthma is a chronic inflammatory disease for which inhaled corticosteroids are the first-line option. Airway remodeling, including neuroplasticity of airway nerves, has recently been recognized as a pathological feature of asthma [1], even though the effect of corticosteroids on it remain poorly investigated. Our study unravels the impact of the corticosteroid Budesonide on neuronal remodeling.

Reference: [1] Dragunas G, Koster CS, de Souza Xavier Costa N, et al. Neuroplasticity and neuroimmune interactions in fatal asthma. Allergy. 2024; 00: 1-12. doi:10.1111/all.16373.

Methods

For the in-vivo study, a murine chronic asthma model sensitized to Ovalbumin (OVA) at days 1, 14 and 21 (IP, 10 µg) was used. Two experimental groups were formed: Preventive: Budesonide (0.1 mM, inhaler concentration) 24h before OVA challenge (1% inhaler concentration); Therapeutic: Budesonide (0.1 mM, inhaler concentration) during the last 3 weeks of OVA challenge. An in-vitro model was performed with the cell line SH-SY5Y: Preventive: Budesonide (10 nM) 24h prior to BDNF challenge (50 ng/mL) for 5 days; Therapeutic: Budesonide (10 nM) for the last 24h of BDNF challenge. Neuroplasticity was assessed through immunofluorescence analysis (Tubulin-III+ areas) and the Angiogenesis Analyzer Plugin in Fiji.

Results

In mouse lungs, preventive budesonide decreased Tubulin-III+ neuronal area (~60%) compared to the OVA-induced Tubulin-III+ area. Conversely, therapeutic Budesonide did not reverse OVA-induced neuroplasticity. Airway eosinophilia was reduced in both treatment groups.

In the in-vitro model, preliminary results with a Network Analysis (n=3) showed that BDNF induced neuroplasticity in SH-SY5Y cells, partially reverted both in Preventive and Therapeutic Budesonide.

Conclusions

Our study demonstrates that in a mouse model of chronic asthma airway neuroplasticity can be prevented but not reversed by the corticosteroid Budesonide. In vitro preliminary results show a non-significant decrease in network density in Budesonide-treated neurons.

PERSISTENT BENEFICIAL EFFECTS OF ANGIOTENSINOGEN SMALL INTERFERING RNA (SIRNA) AND VALSARTAN IN SPONTANEOUSLY HYPERTENSIVE RATS AFTER DRUG REMOVAL

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Introduction

Dual blockade of the renin-angiotensin system (RAS) with siRNA targeting hepatic angiotensinogen (AGT) plus the angiotensin receptor blocker valsartan synergistically lowers blood pressure and diminishes cardiac hypertrophy in spontaneously hypertensive rats (SHR). Since the effects of one siRNA injection may last for up to six months, here we evaluated whether the REVERSIR (reverse siRNA silencing, RVR) technology and/or stopping valsartan might acutely reverse the effects of dual RAS blockade in SHR.

Methods

Ten-week old SHR were subjected to a 3-week treatment with vehicle or AGT-siRNA (10 mg/kg) + valsartan (4 mg/kg per day), followed by administration of vehicle or AGT-RVR at doses of 1, 10 and 20 mg/kg, both with and without continuation of valsartan, for 1 week. Mean arterial blood pressure (MAP) was monitored using radiotelemetry, and circulating AGT and renin were measured by enzyme-kinetic assay.

Results

Baseline MAP was 143±2 mm Hg. Dual treatment lowered MAP by 70 mm Hg, cardiac hypertrophy (heart weight/tibia length) by ~30%, and circulating AGT by >99%, while renin increased >100-fold versus vehicle. Discontinuing valsartan or applying AGT-RVR (1, 10 or 20 mg/kg) increased MAP similarly (by ~20 mm Hg), while discontinuing valsartan + AGT-RVR (10 mg/kg) increased MAP by ~40 mm Hg. Changes in cardiac hypertrophy mimicked those in MAP. Only valsartan + AGT-RVR fully restored circulating AGT and renin to normal. When discontinuing valsartan only, AGT remained suppressed by >95%, and renin was up 10-fold, while with AGT-RVR 1, 10 and 20 mg/kg only, AGT suppression was 25, 50 and 50%, while renin was up 25-, 15- and 15-fold, respectively.

Conclusions

Discontinuing valsartan or applying AGT-RVR in SHR treated with valsartan + AGT siRNA partially restored MAP, AGT, and renin, likely reflecting the fact that this approach replaces dual RAS blockade by single RAS blockade. However, although discontinuing valsartan plus AGT-RVR fully restored RAS activity, MAP and cardiac hypertrophy remained suppressed under this condition. This agrees with observations that early RAS inhibition resets genetic pathways and networks, allowing persistent blood pressure normalization even when treatment has stopped.

A Machine-Learning Approach to Finding Gene Target Treatment Options for Long COVID

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Introduction: Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection (PASC), involves a range of symptoms that persist for weeks or months after the acute phase of COVID-19. These symptoms affect multiple organ systems, including respiratory, cardiovascular, neurological, and gastrointestinal systems, significantly impacting the quality of life. The study aims to identify gene targets for treating Long COVID using machine learning.

Methods: The study used Recursive Ensemble Feature Selection (REFS) to identify key genes associated with Long COVID. REFS was applied to the discovery dataset GSE275334 and validated with test datasets GSE270045 and GSE157103 using a 10-fold cross-validation scheme. The identified genes were further analyzed with Open Targets and DrugBank for potential treatments, and clinical trials were reviewed to validate these targets.

Target Gene	Known Drug	Clinical Trials in COVID-19/Long COVID (Max 5)
PPP2CB		
SOCS3	Resveratrol	NCT05601180, NCT04400890, NCT04799743, NCT04542993, NCT04666753
ARG1	Bitolterol, albuterol	NCT04681079
FZD2		
IL6R	Tocilizumab	NCT04317092, NCT04363736, NCT04924829, NCT04479358, NCT04331795,
TRGV3/5		
ECHS1	Clopidogrel+Aspirin	NCT04333407, NCT04368377, NCT04518735,

Connected to COVID-19 in recent studies.

Gene Target drugs in clinical trials for COVID-19

Table 1: Clinical trials, with drugs that target the found genes.

Results: The application of REFS reduced the number of genes from 635 to 7 key genes. The identified genes were validated using the MLP classifier, achieving high diagnostic accuracy in test datasets. Five of the seven genes were connected to COVID-19, with four of them being part of ongoing clinical trials.

Conclusions: The study demonstrates the effectiveness of using machine learning, specifically REFS, to identify key gene targets associated with Long COVID. The identified genes, including PPP2CB, SOCS3, ARG1, IL6R, and ECHS1, show significant therapeutic potential. Ongoing clinical trials and pharmacological interventions are being explored to manage Long COVID symptoms. The findings underscore the potential of machine learning in advancing personalized treatment strategies for Long COVID patients, offering a promising avenue for developing targeted and effective therapies.

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CONSEQUENCES OF INFLAMMATION-DRIVEN CHANGES IN ALPHA-1 ACID GLYCOPROTEIN AND ALBUMIN ON THE PROTEIN BINDING AND PLASMA CLEARANCE OF CYP450 PHENOTYPING PROBES

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Introduction

Inflammation is increasingly linked to altered pharmacokinetics and drug exposure, presumably due to impaired CYP enzyme activity(1). However, changes in fraction unbound (fu) due to altered protein binding by albumin and alpha-1acid glycoprotein (AAG), may also affect plasma clearance(2). How inflammation-driven changes in these plasma proteins affect drug-binding and plasma clearance remains incompletely understood and was therefore investigated for the CYP450 phenotyping probes midazolam and diclofenac.

Methods

In-vitro studies (n=4) were performed with midazolam and diclofenac using different concentrations of albumin (21–32.5–42.5g/l) and AAG (0.75–1.8–2.35g/l), representative for healthy, arthritis, and sepsis conditions. Equilibrium dialysis with LC-MS was used to determine the fu of the probes. Fu values for the inflammatory conditions were used to calculate change in plasma clearance using a hepatic dispersion model(2).

Results

For diclofenac, fu changed from 0.7±0.2% to 1.6±0.7%, with a relative increased fu of +59.5% in arthritis and +117.2% in sepsis. Fu of midazolam changed from 1.7±0.2% to 2.6±0.3%, with a relative increase in fu of +9.8% in arthritis and +51.7% in sepsis. Importantly, changes in fu for midazolam were smaller than anticipated, due to increased AAG binding.

These fu changes were predicted to increase plasma clearances of diclofenac and midazolam, respectively, in arthritis (+61.8% and +6.2%) and sepsis (119.7% and +30.1%) conditions.

Conclusion

Inflammation-associated changes in albumin and AAG increase fu and plasma clearance of midazolam and diclofenac. This study demonstrates that changes in protein binding may counteract and underestimate the impact of impaired CYP activity for CYP450 probe drugs in patients with inflammation.

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CYCLOALKYLAMINE ANALOGUES AS NOVEL INHIBITORS FOR THE NOREPINEPHRINE TRANSPORTER

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Introduction

Depression is one of the most common mental disorders that is caused by dysregulation of neurotransmitter release and uptake by monoamine transporters. Current treatment strategies for depression include inhibitors targeting the norepinephrine transporter (NET, SLC6A2), the serotonin transporter (SERT, SLC6A4) or dual NET and SERT inhibitors (SNRIs). Disadvantageously, these inhibitors have a slow onset of action and show a lack of efficacy in over 50% of the patients1. Recent in-house virtual screening efforts utilizing a proteochemometric model discovered GIFT1147, as a potent inhibitor for NET2. Here we further optimize the cycloalkylamine scaffold of GIFT1147 to establish a structure-activity relationship on NET.

Methods

Twenty cycloalkylamine analogues were designed and purchased based on the scaffold of GIFT1147. These analogues were screened for their ability to inhibit NET utilizing an impedance-based screening assay3. Hit compounds were further characterized to determine their NET inhibitory potency.

Results and conclusion

Ten out of twenty GIFT1147 analogues displayed over 70% inhibition of NET. Alterations at the R3-position decreased or even abrogated NET inhibition, indicating dichloro-substitution of the phenyl ring is optimal for NET inhibition. Interestingly, rigidification of the R2-position resulted in hit compound GIFT1215 with a potency (pIC50 8.3 ± 0.1). Pharmacological characterization of all eight stereoisomers of GIFT1215 revealed varying inhibitory potencies, favoring a trans-orientation of the N,2-substituted cyclopentyl moiety. Molecular docking highlighted key interactions and the impact of a hydrophilic region in the binding pocket. This study presents a novel set of moderate to highly potent NET inhibitors and elucidates the influence of molecular orientation within the NET binding pocket. These insights may contribute to drug discovery efforts for novel monoamine transporter-related treatments.

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INVESTIGATION OF THE ANTIARRHYTHMIC EFFECTS OF SZV-2649, A NEW AMIODARONE-LIKE MEXILETINE ANALOG COMPOUND

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Introduction

Cardiovascular diseases, particularly heart rhythm disorders such as ventricular fibrillation, remain a leading cause of mortality in developed countries. Consequently, cardiac arrhythmias represent a critical focus in cardiovascular research. Pharmacological treatment remains the cornerstone for managing ventricular and supraventricular arrhythmias. The development of novel antiarrhythmic agents with enhanced efficacy and reduced proarrhythmic risk requires a thorough understanding of their mechanisms at the organ, tissue, cellular, and subcellular levels. One promising avenue involves designing amiodarone-like antiarrhythmics that combine repolarization-prolonging effects (class III) with additional advantageous pharmacological properties, such as combined class III+I/B, II, and/or class IV activities.

Methods

In this study, we investigated the antiarrhythmic and cardiac electrophysiological effects of a novel amiodarone-like compound, SZV-2649, which lacks the benzofuran chemical structure of amiodarone. Experiments were conducted using rat and dog cardiac preparations. SZV-2649 demonstrated antiarrhythmic effects in a rat model of ventricular fibrillation induced by coronary artery occlusion-reperfusion and in a dog model of atrial fibrillation induced by acetylcholine and burst stimulation.

Results

SZV-2649 inhibited hERG/Ikr and GIRK/IK_ACh currents in HEK293 cell lines expressing the relevant channels and in vitro canine atrial muscle preparations. These findings suggest that SZV-2649 exerts antiarrhythmic effects similar to amiodarone, mediated through multi-ion channel blocking properties.

Conclusions

Due to its distinct chemical structure, SZV-2649 is anticipated to have a more favorable side-effect profile compared to amiodarone. These promising results highlight SZV-2649 as a potential candidate for further development as a novel antiarrhythmic agent.

NEW HOMOCYSTEINE CONSUMPTION ASSSAY FOR HIGH-THROUGHPUT SCREENING OF HUMAN CYSTATHIONINE-B-SYNTHASE

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Introduction

Homocysteine, an established risk factor in cardiovascular disease, is metabolized by Cystathionine-β-synthase (CBS), making CBS an attractive target for new therapies. Currently, there are no compounds enhancing CBS activity aside from the endogenous activator S-adenosyl-L-methionine (SAM) which has limited membrane transport. Existing assays to identify CBS activators in high-throughput screening (HTS) are complicated by autofluorescence of screening compounds. Therefore, we developed a new HTS-capable homocysteine consumption assay for CBS (HconCBS).

Methods

Human CBS was produced in E. coli. The assay was based on the CBS-catalysed reaction between homocysteine and serine, and remaining homocysteine was detected by Ellman's reagent. We optimised the reaction time, activating concentration of SAM, assay parameters (signal/background (S/B), uniformity (CV), Z prime (Z'), readout stability), and automated the assay for HTS. Finally, we carried out HTS of the Selleck Express-Pick Library, containing 3010 drug-like compounds using HconCBS and fluorescence-based detection of hydrogen sulphide using 7-azido-4-methylcoumarin (AzMC).

Results

The HconCBS assay had an optimal reaction time of 30 min, was spatially uniform, had 10-hour readout stability, and far exceeded the Royal Society of Chemistry criteria for HTS assay parameters. HconCBS identified fewer interfering compounds (101 compounds with autoabsorbance (HconCBS) vs. 383 autofluorescence (AzMC)) and had a lower hit rate (0.33% HconCBS vs. 4.7% AzMC).

Conclusion

We present a new activity assay for isolated human CBS with parameters optimised for automated HTS that is simple, cost-efficient, sensitive, and robust. The assay should facilitate the discovery of homocysteine-lowering compounds.

Funding

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NEW TOOL COMPOUND FOR TRANSSULFURATION PHARMACOLOGY: ENHANCING FLUORESCENCE SENSING OF HYDROGEN SULFIDE IN LIVING SYSTEMS

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Introduction

Hydrogen sulfide (H2S) is a cytoprotective molecule abundant in colorectal cancer, where it contributes to tumor development and immune evasion. H_2S is produced by the transsulfuration enzymes cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). Detecting H_2S in living systems remains challenging, as probes like 7-azido-4-methylcoumarin (AzMC) have sensitivity limitations. We introduce WAY-310829 as a tool compound that enhances the sensitivity and imaging capabilities of AzMC to help advance transsulfuration pharmacology.

Methods

High-throughput screening of a library containing 3010 compounds was performed with an assay in which recombinant human CBS produced H2S that was measured using AzMC. Direct interaction of WAY-310829 with CBS and H2S was excluded. Selectivity of signal enhancement for H2S was verified against several reducing agents. Effect of WAY-310829 was confirmed by re-purchase and inactive analogs were identified. H2S produced by human colorectal cancer cells (HCT116) was imaged using fluorescence microscopy.

Results

Enhancement of AzMC signal by WAY-310829 was selective for H2S and pH-independent. Methylated derivatives of WAY-310829 did not enhance AzMC signal. Fluorescence imaging of HCT116 cells showed intracellular H2S signal when WAY-310829 was used together with AzMC, but no H2S signal when AzMC (10µM) was used alone. The H2S signal enhanced by WAY-310829 corresponded with pharmacological inhibition of H2S-producing enzymes and activation of CBS.

Conclusions

Taken together, we characterized WAY-310829 as a tool compound that selectively enhanced H2S sensing by the fluorescent probe AzMC and revealed pharmacological modulation of transsulfuration in human colorectal cancer cells.

Funding

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TRAPPING THE EFFECTS OF SMOKING: ELUCIDATING THE FUNCTION OF ACP5 EXPRESSION IN LUNG TISSUE

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Introduction

Tartrate-resistant acid phosphatase (TRAP, gene Acp5) is highly expressed in alveolar macrophages with proposed roles in lung inflammation and lung fibrosis development. We previously showed that its expression and activity are higher in lung macrophages of smokers and COPD patients, suggesting involvement in smoke-induced lung damage. In this study we explored the function of TRAP and regulation of its different mRNA transcripts (Acp5 201-206) in lung tissue exposed to cigarette smoke to elucidate its function in lung tissue.

Methods

Results

In mice exposed to cigarette smoke or air for 4-6 weeks, higher Acp5 mRNA expression in lung tissue after smoking was mainly driven by transcript Acp5-202, which originates from macrophages. Expression of Acp5-202 correlated with transcription factors previously found to drive proliferation of macrophages. We found that ACP5-deficient alveolar macrophages and macrophages treated with a TRAP inhibitor proliferated less than control macrophages. Mechanistically this lack of proliferation after TRAP inhibition was associated with higher presence of phosphorylated β-catenin compared to nontreated controls. Phosphorylation of β-catenin is known to mark it for ubiquitination and degradation by the proteasome, preventing its activity in promoting cell proliferation.

Conclusions

In conclusion, TRAP stimulates alveolar macrophage proliferation via dephosphorylation of β-catenin. The smoke-induced increase of TRAP expression in macrophages may be a compensatory mechanism for increased cell loss due to uptake of toxic smoke particles by alveolar macrophages. By promoting proliferation, TRAP may help sustain the macrophage population after smoke exposure.

FAMILY C ORPHAN GPRC5B IN MEGALENCEPHALIC LEUKOENCEPHALOPATHY: FROM GENETICS TO LIGAND DISCOVERY

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Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a leukodystrophy with onset in early childhood, characterized by chronic brain white matter oedema. In most patients, mutations in the MLC1 or GLIALCAM gene were found, but recently we have identified two dominant mutations in GPRC5B in three unrelated patients. These mutations result in amino acid duplications in transmembrane helix 4 and increased expression levels of GPRC5B in patient-derived cells. GPRC5B is an orphan class C GPCR with no identified endogenous ligand so far. Three GPRC5B splice variants have been reported: the canonical sequence, a longer isoform transcribed from an upstream start codon, and a brain specific variant with a distinct C-tail.

Methods

Results

We characterized expression of GPRC5B isoforms and MLC-associated mutants at the HEK293T cell membrane after transfection, and find that the short and brain isoform, and corresponding MLC-associated mutants, are readily expressed at the membrane. The expression of the long isoform is hampered. We also show specific interaction of the short and brain isoforms with MLC1 as assessed by saturation BRET, and the propensity of this interaction was not affected by MLC-associated mutations in GPRC5B. GPRC5B has previously been shown to constitutively increase NFκB signalling through its interaction with the tyrosine kinase Fyn, which could be used as a possible screening method for ligand-induced GPRC5B activation. A constitutive increase of NFκB signalling in HEK293T cells by GPRC5B can only be replicated in our experiments when overexpressing MLC1, suggesting a role for MLC1 in the activation of NFκB signalling by GPRC5B.

Conclusions

MODEL-BASED DOSE OPTIMIZATION FOR TEICOPLANIN IN PATIENTS RECEIVING CHRONIC HEMODIALYSIS

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Introduction

Patients undergoing chronic hemodialysis (HD) exhibit altered pharmacokinetics (PK), leading to variable drug exposure with associated risks of treatment failure or toxicity. Teicoplanin, a renally cleared antibiotic, is commonly used for treatment of Gram-positive infections[1], including those associated with the vascular access in HD. Due to variability in residual renal function, determining the appropriate dose for patients undergoing HD is challenging. This study aims to develop a population PK model for teicoplanin in patients undergoing intermittent HD and propose an optimized dosing regimen.

Methods

Teicoplanin population PK model was developed on NONMEM (version 7.4.3) from total and unbound plasma concentrations sampled from 31 patients undergoing thrice weekly HD. Drug transfer between the central compartment and the dialyzer were semi-mechanistically represented in the model. Monte Carlo simulations with the final model were used to evaluate the standard thrice weekly dosing regimen and explore alternative dosing strategies. Loading and maintenance doses were optimized to maximize population attainment of the therapeutic C_{trough} range (20 to 50 mg/L). The impact of C_{trough}-based therapeutic drug monitoring on target attainment was also evaluated.

Results

A two-compartment model best described the plasma PK of teicoplanin. None of the tested covariates significantly influenced teicoplanin PK. The standard 12 mg/kg (840 mg for a 70 kg individual) thrice weekly regimen[2] was found to attain the C_{trough} target range in 6.8% and 47.2 % of the patients after the first and subsequent doses, respectively. We propose a fixed 1600 mg loading dose and intersession period-adjusted 800 mg maintenance dose as an optimized dosing regimen. The proposed regimen improved target attainment to 67.4% and 51.8% after the first and subsequent doses, respectively. Further improvement was limited by the clearance variability observed within the studied patient population. C_{trough}-based therapeutic drug monitoring can be employed to further improve target attainment to up to 87.5%.

Conclusions

Patients are at risk of subtherapeutic teicoplanin exposure when using the standard dosing regimen. We propose an optimized dosing regimen with fixed loading and maintenance doses, as well as C_{trough}-based therapeutic drug monitoring. An external model validation is the next step before implementing this dosing regimen in routine practice.

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SIMULATION-BASED EXPLORATION OF THE P-GLYCOPROTEIN EXPRESSION-ACTIVITY RELATIONSHIP SHOWS A DRUG AND SYSTEM DEPENDENCY

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Introduction

For mechanistic prediction of transporter-mediated pharmacokinetics (like P-glycoprotein, P-gp) at the blood-brain-border (BBB) it is commonly assumed that *in vitro* transporter activity can be scaled to *in vivo* using differences in protein expression. This assumes a drug-independent, directly proportional relationship between protein expression and activity (E-Ar). Though this approach has shown promise, there is conflicting experimental information on the relationship itself. Additionally, some studies rely on empirical scaling factors to match observed data. In this simulation study, we aimed to theoretically explore the assumption of drug-independent linearity, and to investigate the factors governing the P-gp E-Ar.

Methods

A P-gp binding kinetic model, derived from literature, was used to simulate membrane permeation of seven P-gp substrates. For each substrate, the activity of P-gp was determined at a baseline condition (100% P-gp expression). Then, P-gp expression was varied (2%-300%), and P-gp activity at different P-gp concentrations was compared to baseline activity for each drug, providing E-Ar. Further simulations of virtual drugs with wide ranges in dissociation (k_{off}) and efflux rate constant (k_e), as well as different drug doses and baseline P-gp concentrations, were performed.

Results

Drugs with different binding kinetic properties show distinct E-Ar. Four of the seven drugs showed non-linear, whereas two showed linear E-Ar. P-gp E-Ar shifted towards non-linearity for drugs with a low k_{off}/k_e ratio and/or high baseline P-gp/drug concentration ratio.

Conclusion

The P-gp E-Ar is drug and system dependent. The assumption that P-gp activity scales linearly with P-gp expression might therefore work for some but not all drugs.

TARGETING METHYLGLYOXAL: A NOVEL APPROACH TO IMPROVE VASCULAR FUNCTION IN DIABETES

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Introduction

Diabetes is characterized by insulin resistance and chronic hyperglycemia, increasing the risk of cardiovascular complications. Elevated levels of methylglyoxal (MGO) contribute to advanced glycation end product formation, which exacerbates vascular dysfunction. Therefore, this study aimed to investigate whether reducing endogenous MGO improves vascular and cardiac function in diabetic mice. It was hypothesized that reducing MGO would mitigate endothelial dysfunction and improve cardiovascular health.

Methods

8-week-old C57BL/6 mice were injected intraperitoneally with streptozotocin (50mg/kg) or citrate buffer (vehicle) for five consecutive days to induce diabetes (n=12–14/group). Diabetes was confirmed at 10 weeks (blood glucose>14 mmol/L, WTdiabetic: 23.3±1.8), after which mice received an MGO-lowering cocktail (2g/L pyridoxamine, 0.1g/L hesperidin, 0.1g/L resveratrol)/vehicle in drinking water for 8 weeks. Cardiac function was assessed via echocardiography, while vascular function was evaluated in isolated thoracic aortas at 20 weeks.

Results

Diabetic mice exhibited significantly impaired acetylcholine (ACh)-induced vasorelaxation compared to non-diabetic controls (maximal response (mean±SEM): -37.0%±4.7% vs. -69.6%±3.6%, $p<0.05$, general linear model). Treatment with the MGO-lowering cocktail significantly improved endothelial function (diabetic+cocktail: -50.5%±7.1%, $p<0.05$). No significant differences were observed in heart rate ($p=0.59$) or pulse-wave-velocity ($p=0.14$) between groups. Inhibition of endothelial nitric oxide synthase (eNOS) and endothelium-dependent hyperpolarization (EDH) indicated that nitric oxide was the predominant mediator of ACh-induced relaxation, with a minor contribution from EDH.

Conclusion

MGO-lowering treatment improved endothelium-dependent vasorelaxation in diabetic mice, underscoring the role of MGO in vascular dysfunction. These findings suggest that targeting MGO could be a promising strategy to restore endothelial function and mitigate diabetes-associated vascular complications.

APPLICATIONS OF QUANTITATIVE SYSTEMS PHARMACOLOGY MODELLING FOR VACCINE DEVELOPMENT

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Introduction

Mathematical Modelling and Simulation is widely used in many areas of drug development. Quantitative Systems Pharmacology (QSP) has been gaining recognition as a decision-making tool with a growing number of FDA submissions supported by QSP models. Using Certara's QSP Platform Vaccine Model, we demonstrate the value of QSP modelling to support dose predictions for SARS-CoV-2 vaccine (Tozinameran) administration in paediatric age groups.

Methods

Certara's Vaccine Model integrates a QSP model of liquid nanoparticle mRNA administration to an immune response model. The model is validated for SARS-CoV-2 neutralising titers in an adult population. Predictions for SARS-CoV-2 neutralising titers in paediatric age groups were simulated compared to clinical trials [1,2]. We simulate a dose selection trial, to find the optimal two-dose regimen in 2–4-year-old, reaching the immunobridging threshold.

Results

The model reproduces clinical results in different age groups within a 95% CI. We predict a dose of 5 µg to be sufficient to pass the immunobridging threshold in the 2-4 y.o. population after two doses.

Conclusions

Prospective use of our QSP model in paediatric COVID-19 vaccine development could have prevented a failed trial and resulted in a more convenient dosing regimen.

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STUDENTS' PERCEPTION OF A NEWLY DEVELOPED INTERACTIVE PHARMACOLOGY E-LEARNING PRACTICAL IN A LIFE SCIENCES CURRICULUM

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Introduction

In higher education, practicals of scientific experiments play an essential role in aiding students to understand relations between phenomena that are observable in the real world and the often abstract, scientific, principles that are used to explain these phenomena. Digital forms of these scientific experiments are particularly useful in supporting students' grasping difficult and abstract concepts and scientific inquiry self-efficacy, i.e. the students' confidence in their ability to reach study targets. In our pharmacology education in a life sciences programme at the VU University in Amsterdam, The Netherlands, we designed an interactive digital practical and accompanying assessment to actively engage our students with certain core concepts of pharmacology.

Methods

To gain insight into students' perspective on their experience on the implementation of this novel interactive e-learning practical and its assignment in our course, we used a dedicated questionnaire, that includes a Likert scale and free text elements, and subsequent (statistical) analysis of its outcome.

Results and Conclusions

We observed that students found the design and implementation of our interactive digital practical of asset value to our course, and especially appreciated both the interactive nature and quality of the e-learning as well as the form of execution of the assignment was setup.

LIPOPOLYSACCHARIDE PRESENCE PROMOTES, BUT IS NOT REQUIRED FOR, HEN'S EGG ALLERGEN OVALBUMIN INDUCED TYPE-2 MUCOSAL IMMUNE RESPONSE IN VITRO

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Introduction

Although food allergens share some structural and physicochemical properties contributing to their intrinsic allergenicity, concurrent mucosal immune activation, via e.g. LPS binding Toll-like receptor 4 (TLR4), has been linked to promoting allergic sensitization. We aimed to investigate the contribution of LPS in ovalbumin (OVA), the major allergen in hen's egg, to inducing mucosal type-2 immune activation in vitro.

Methods

HT29 human intestinal epithelial cells (IEC) were exposed to OVA, which contained 96ng/mL (OVA+), or 3,6ng/mL (OVA+/-) LPS or OVA without LPS (OVA-)- for 24h. Alternatively, IEC and/or monocyte-derived dendritic cells (moDCs) were exposed to the LPS-containing (+)/(+/-)(-) OVAs for 48h. Primed moDCs were cocultured for 5 days with allogenic naïve Th cells. Cytokine secretion was determined by ELISA, phenotype of moDCs was assessed by flow cytometry.

Results

MoDCs directly exposed to OVA+ or OVA+/- secreted enhanced levels of IL8 and IL6. However, IL6, IL12p70 and IL10 decreased in a coculture of IEC/moDC exposed to OVA+, while the percentage of CD80 expressing moDCs increased. Subsequent coculture of OVA exposed moDCs with allogenic naïve Th cells resulted in increased Th2 IL13 secretion by Th cells, reaching significance for OVA+. Alternatively, reduced Th1 IFNγ secretion was measured upon OVA+ exposure via IEC, but also in absence of LPS OVA reduced Th1 IFNγ and IL-10 secretion.

Conclusions

Although simultaneous exposure to LPS and ovalbumin in vitro yields a more prominent IEC and moDC response, LPS is not required to steer towards a type-2 T cell cytokine response, the first step in initiating allergic sensitization.

ILLUMINATING THE LIGAND-INDUCED RECEPTOR FATE OF CC CHEMOKINE RECEPTOR (CCR2)

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Introduction

CC Chemokine receptor 2 (CCR2) is a G protein-coupled receptor (GPCR) with key roles in inflammation and immunity, making it a relevant target in drug discovery. CCR2 can be activated by several chemokines, including CCL2, CCL7, CCL8 and CCL13. Previous studies have reported differences in CCR2 signaling after stimulation with different chemokines; however, it is not clear if these chemokines also lead to distinct intracellular trafficking profiles (i.e. internalization, recycling, and degradation). Thus, we aimed to explore the fate of CCR2 after activation by several chemokines.

Methods

We first used a label free assay (xCELLigence) to characterize the functional effect of the different chemokines in CCR2. Next, we performed bioluminescence resonance energy transfer (BRET) assays using a HiBiT-tagged CCR2 and Venus-tagged biosensors to investigate CCR2 trafficking and interaction with G protein-coupled receptor kinases (GRKs) upon chemokine stimulation.

Results

In the xCELLigence assay, all chemokines induced a CCR2 functional response with varying potencies and efficacies. BRET assays showed different trafficking profiles after chemokine stimulation. While none of the chemokines appeared to induce a Rab11-dependent recycling mechanism, stimulation by CCL2 and CCL7 induced CCR2 trafficking to both early and late endosomes. CCL2 also induced interaction of CCR2 with GRK2 and GRK3, while this was not observed with other chemokines.

Conclusions

Our results indicate that activation of CCR2 by different chemokines elicits unique trafficking dynamics, in addition to their differential effects in receptor signaling. Overall, these findings suggest an extra layer of complexity when targeting this receptor.

BIOCATALYSIS MEETS RATIONAL DESIGN: ENHANCING SULFUR METABOLISM

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Introduction

Cystathionine-β-synthase (CBS) is a regulator of sulfur amino acid metabolism, serving as an entry point for homocysteine, a toxic intermediate, into the transsulfuration pathway. CBS produces hydrogen sulfide and facilitates downstream production of glutathione (GSH), both of which are important antioxidants. The activity of CBS is enhanced by endogenous S-adenosyl methionine (SAM). However, SAM has poor membrane transport and is a universal substrate in methylation reactions. The CBS/H2S/GSH axis is impaired in diabetic nephropathy. As novel compounds aimed to protect the kidney via CBS activation, we developed analogs of SAM using rational design and biocatalysis.

Methods

In principle, endogenous SAM is made by SAM synthetase from methionine and ATP. We made analogs of SAM from analogs of methionine and of ATP. We designed 60 compounds, which we filtered based on molecular docking to CBS and the availability of substrates for synthesis. We attempted the synthesis of 15 compounds and quantified their yields from phosphate released from ATP. Activation of CBS was measured in terms of hydrogen sulfide production and homocysteine consumption. Methyl transfer from SAM analogs to Lambda DNA was measured using EcoRI methyltransferase/endonuclease.

Results

We successfully synthesized 12 analogs, out of which 3 activated CBS, and 1 activated CBS more than unmodified SAM. The active compounds remained methylation substrates.

Conclusions

We demonstrate that rational design combined with biocatalysis presents a promising avenue to enhance homocysteine metabolism via the targeting of CBS. The active compounds provide a starting point for further development into redox-enhancing, organ-protective agents.

EXPLORING CXCL12 - GLYCOSAMINOGLYCANS INTERACTIONS

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Introduction

Particulate matter can enter the human brain and accelerate the development of neurodegenerative diseases. Ferroptosis is an emerging form of cell death that can cause large accumulation of lipid peroxidation and reactive oxygen species (ROS) in cells [1]. Increased intracellular calcium release has been shown to lead to ferroptosis [2]. Therefore, we investigated whether regulating mitochondrial calcium can be prevented ferroptosis induced by particulate matter.

Methods and results

We used NIST DEP as a standardized diesel exhaust particle sample and treated HT22 mouse hippocampal neuron cell line as a cell model. MTT assays showed that the viability of HT22 cells was not altered after exposure to NIST DEP, while co-exposure with the ferroptosis inducer RSL-3 increased ferroptosis cell death. Based on fluorescence image analysis, co-treatment with NIST DEP and RSL-3 caused an increase in HT22 calcium levels and mitochondrial calcium levels, increased ER-mitochondrial contact points, aggravated mitochondrial fragmentation, and increased lipid peroxide levels and mitochondrial ROS. However, the addition of mitochondrial calcium uniporter (MCU) inhibitor MCUi4 and SK channel activator CyPPA attenuated the negative effects caused by RSL-3 and NIST DEP.

Conclusions

NIST DEP exposure further exacerbated RSL-3-induced ferroptosis and caused calcium homeostasis disorders and mitochondrial damage. Calcium modulators prevented the increased HT22 cell death and calcium level increase induced by combined NIST DEP and RSL-3 exposure.

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MODELING NONLINEAR BRAIN PHARMACOKINETICS OF FLUVOXAMINE USING THE LEICNS-PK MODEL: ROLE OF ACTIVE INFLUX AND INTRACEREBRAL METABOLISM

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Introduction

Fluvoxamine, a widely prescribed selective serotonin reuptake inhibitor for major depressive disorder [1], obsessive-compulsive disorder, and social anxiety disorder [2], exhibits complex CNS pharmacokinetics. While previous studies attribute the fact that its brain distribution does not increase proportionally with administration dosages, so-called nonlinear distribution, to possible efflux transporters or binding to the serotonin reuptake transporter [3-5], we propose an alternative mechanism. This study tests the hypothesis that active influx transport ($Kp_{,uu,brain}$ >1 [6, 7]) primarily drives fluvoxamine brain accumulation, while intracerebral metabolism contributes to its dose-dependent nonlinear kinetics, particularly during elimination.

Methods

We developed the LeiCNS-PK model via a bottom-up approach, extending rat CNS-PBPK frameworks [8-10], and added two important innovations. First, a sequential approach quantified active influx clearance (CL_{in}) through a nonlinear mixed-effects model, integrating a fixed plasma PK model [5] with brain extracellular fluid (brainECF) microdialysis data [3]. Second, Michaelis-Menten kinetics was incorporated for brain metabolism using in-vitro Km and Vmax values from rat liver microsomes to explain dose-dependent nonlinearity with 1, 3.7, and 7.3 mg/kg doses.

Results

Without metabolism, the model incorporating only CL_{in} failed to capture elimination-phase brainECF concentrations, especially at 1 mg/kg. Adding metabolism significantly improved predictions, supporting its role in nonlinearity.

Conclusions

The LeiCNS-PK model accurately characterizes fluvoxamine brain kinetics, improving dose-response predictions and facilitating human translation. This refined PK model also provides insights into pharmacokinetic variations due to drug interaction, environmental factors, or genetic polymorphisms.