# Abstract book

# SCIENTIFIC MEETING

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

Part 1:

ABSTRACTS

# *A PRIORI* MODEL-INFORMED PRECISION DOSING OF TAMOXIFEN: A PROSPECTIVE IMPLEMENTATION STUDY

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**Background:** Tamoxifen is an estrogen-receptor antagonist, used in the adjuvant treatment of estrogen-sensitive breast cancer. It is converted by CYP2D6 enzymes to endoxifen, its most active metabolite. Research indicates that patients with endoxifen plasma concentrations <16 nM are at higher risk of cancer recurrence. Performing therapeutic drug monitoring (TDM) avoids undertreatment in this population. Development of population-pharmacokinetic (POP-PK) models allow *a priori* model-informed precision dosing (MIPD), leading to faster attainment of target concentrations, less required TDM samples, and potentially better patient outcomes.

**Methods**: This prospective MIPD intervention study enrolled patients who started adjuvant tamoxifen treatment. An individualized tamoxifen dose was predicted, and prescribed at baseline using a validated POP-PK model developed at the Erasmus MC Cancer Institute. The starting dosages were based on a continuous CYP2D6 activity score, body mass index (BMI), age, and height. After 4-6 weeks and after three months (in which steady-state is reached) of therapy, endoxifen levels were measured. The primary endpoint of the study was the percentage of patients with an endoxifen level ≥16 nM after three months of tamoxifen therapy which was compared to a historical cohort receiving the standard dose of 20 mg (77.9% ≥16 nM). Sample size calculations showed that 106 patients were needed to detect a difference between the two groups.

**Results:** A total of 106 evaluable patients were included. Patients received a predicted dose (65.1% 20 mg, 16.0%, 30 mg, 4.7% 40 mg, and 14.2% potential switch to aromatase inhibitor (AI), although these received 40 mg). After three months of therapy, 84.0% of patients reached endoxifen levels ≥16 nM. This was improved compared to the standard dosing cohort, although not significantly (p=0.17). The proportion of patients who were predicted to reach inadequate endoxifen levels using the maximum dose of 40 was 14.2% in the intervention cohort and 7.7% in the historical cohort. The median endoxifen level in this cohort when treated with the maximum dose was 15.1 nM (range: 8.9 – 20.4 nM). If all patients for whom the model recommended a switch toward an AI were excluded, 91.5% of patients would have attained endoxifen levels ≥16 nM. The model showed the most improvement in the groups with a predicted dose of 30mg (44.1% to 23.5% <16 nM) and 40 mg (87.5% to 0% <16 nM). Early endoxifen samples reduced the mean absolute prediction error from 21.2% to 15.0%. Moreover, no relevant changes in side effects from start of treatment compared to steady-state were observed in any dosing group.

**Discussion/Conclusion:** In this first MIPD implementation study in oncology, MIPD did lead to a larger proportion of patients achieving endoxifen levels ≥16 nM compared to the one-dose-fits-all strategy, although not significant. This may be explained by a larger proportion of patients who were recommended to switch to an AI in the intervention cohort compared to the control cohort. The model correctly identified patients *a priori* requiring higher starting doses and those who did not. MIPD seems beneficial compared to one-size-fits-all-dosing, but TDM still remains an important addition.

# CLINICAL IMPLICATIONS OF NINTEDANIB PHARMACOKINETICS IN PATIENTS WITH PULMONARY FIBROSIS

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**Background:** Nintedanib is used to treat both idiopathic and progressive pulmonary fibrosis (IPF/PPF) in which it slows the progression of fibrosis and thereby the loss of forced vital capacity (FVC). In a combined analysis of phase II and III trials, an exposure-response relation for FVC decline was found. Additionally, diarrhea and nausea, the two most common side effects of nintedanib occurred more often in patients receiving higher dosages in a phase II trial. This suggests potential value of therapeutic drug monitoring. We aimed to elucidate the exposure-response and toxicity relationships in real-world patients with IPF and PPF that were treated with nintedanib.

**Methods**: Data from both a prospective cohort study and an pharmacokinetic (PK) interaction study with green tea capsules were pooled for this analysis. To quantify exposure to nintedanib, a population-pharmacokinetic (PK) model was developed. Associations between PK and decline in FVC and diffusing capacity ($DL_{co}$) were performed using linear-mixed-effect models (LMEM). The mean nintedanib exposure was compared when treated with the standard starting dose of 150 mg BID between patients that did and did not experience dose-limiting toxicities (DLTs) in the first twelve months of treatment using a t-test. Additionally, a time-to-DLT analysis was performed using a Cox proportional hazard analysis.

**Results:** In total, 911 PK samples and 517 spirometries from 99 patients were included in the analysis. Median follow-up was 620 days for toxicities and spirometries. Inter-individual variability (IIV) of nintedanib clearance was 41.7% and partially explained by both concomitant green tea capsule use (19% increase) and sex (58% higher clearance in men). The model incorporated a relatively high additive error of 0.512 on a logarithmic scale which was mostly apparent in the absorption phase of nintedanib. The LMEM with random slopes and intercepts showed that patients in this cohort lost an average of 79.8 mL of FVC per year. Although nintedanib exposure did not significantly affect the rate of FVC decline, the administered nintedanib dose was a predictor of FVC decline (p=0.002). Per 50 mg decrease of daily dosage, from the median dose of 250 mg per day, the rate of FVC decline increased by an additional 53.5 mL/year. Nintedanib exposure nor dose significantly affected the rate of $DL_{co}$ decline. In total, 64 out of the 99 patients experienced DLTs. No significant difference in nintedanib exposure when treated with the standard starting dose was found between patients with and without toxicity (234 vs. 223 ng*mL/hour). Moreover, in the time-to-DLT analysis nintedanib exposure or dose was not significantly associated with the occurrence of a DLT.

**Conclusion:** Nintedanib dose was significantly associated with a decreased rate of FVC loss. However, no significant relationship between nintedanib exposure occurrence of DLTs was found in this real-world population. The findings in this study indicate that performing therapeutic drug monitoring is unsuitable for nintedanib.

# PHARMACOVIGILANCE OF NEPHROTOXIC DRUGS IN NEONATES: THE POTTEL METHOD FOR ACUTE KIDNEY INJURY DETECTION IN ELBW NEONATES

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## Background

Extreme Low Birth Weight (ELBW) neonates (birth weight ≤1000 grams) are at high-risk to develop drug-induced acute kidney injury (AKI). However, we lack a pragmatic detection tool to capture their time-dependent (patho)physiologic serum creatinine (Scr) patterns. Pottel et al. suggested rescaling Scr by dividing Scr with the mean Scr-value of the age and sex specific reference population. We explored if this Pottel method can detect drug-related nephrotoxicity in ELBW neonates.

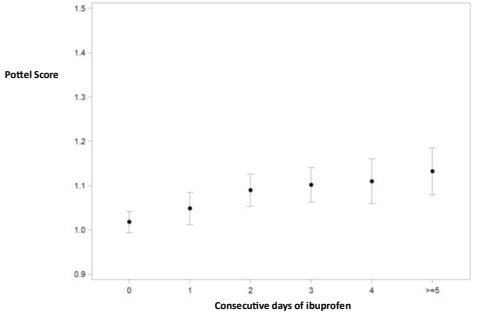
## Methods

A previously reported dataset on Scr changes in ELBW neonates exposed to ibuprofen, amikacin or vancomycin was updated to calculate Pottel scores for every available Scr value in the first 28 postnatal days.

We hereby used previously published postnatal age specific 50$^{th}$ centile values in an ELBW population. Linear mixed models were applied, analyzing Pottel scores as response variable and continuous time (day), drug exposure, and interaction thereof in the explanatory model.

## Results

Serum creatinine (n = 3 231) observations in 201 ELBW neonates were collected. A statistically significant rise of Pottel scores was observed with ibuprofen starting from postnatal day 4. A cumulative effect of treatment with mean Pottel scores on day 0 of 1.020 and on day 3 during treatment of 1.106 (95% CI 1.068-1.145, $p<0.001$) was observed, corrected for effect of antibiotics (Figure). Antibiotic administrations showed a small but statistical significant difference up to postnatal day 5.




## Discussion

As rescaled Scr biomarker, the Pottel method showed a clear association with ibuprofen-exposed ELBW neonates, suggesting its applicability as pragmatic bedside alternative tool to assess nephrotoxicity.

# EFFECT OF PLASMAPHERESIS ON DRUG PHARMACOKINETICS IN CHILDREN: A STRUCTURED REVIEW OF THE CURRENT LITERATURE.

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**Background**
Plasmapheresis is a technique used to separate plasma from whole blood designed for the removal of substances from blood plasma. Throughout childhood, several physiological changes alter the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug. Currently, information on the effect of plasmapheresis on the PK of drugs in children is mainly extrapolated from adult studies. However, drug PK fluctuates throughout childhood because of both physiological and non-physiological changes. As a result, plasmapheresis-related PK data, such as drug removal (%), cannot be extrapolated from adults to children. In this structured review, we present an overview on the effect of plasmapheresis on PK of drugs in children.

**Methods**
A literature search assessing drug PK/exposure in children undergoing plasmapheresis was conducted in EMBASE and PubMed Studies were labelled as relevant when data on PK/exposure after plasmapheresis in patients younger than 18 years-old was reported.

**Results**
Twenty-six studies were identified, 96% being case reports. PK/exposure data for eighteen drugs in children undergoing plasmapheresis were included, 69% of the studies reported significant change in drug exposure after plasmapheresis. In 38% of the studies plasmapheresis was used in combination with drugs at therapeutic dosages, with no significant drug removal. No evidence-based drug dosing regimen for drugs at therapeutic dosages in children undergoing plasmapheresis have been developed. However, the usefulness of plasmapheresis in drug poisoning has been demonstrated, in case reports, for 31% of the overdosages.

**Discussion/Conclusion**
This structured review shows that a limited number of studies have been performed on the PK/exposure of drugs in children undergoing plasmapheresis. Furthermore, it became clear that predicting the risk of drug removal by plasmapheresis based on theoretical (drug properties or PK) criteria for adults is not always applicable to children (Table 1).

| Drug | Drug removal by plasmapheresis in adults (%) | Drug removal by plasmapheresis in children (%) | Drug Removed by plasmapheresis in adults? |
|---|---|---|---|
| **Amitriptyline** | 63.0 | 91.4; 99.6; 59.5 | Yes |
| **Amlodipine** | 73.3 | 48.9 | Yes |
| **Carbamazepine** | 6.0 | 88.4; 73.0; 89.5 | Possibly |
| **Cisplatin** | 88.9 | 98.3 | Yes |
| **Cyclosporine** | 0.2-0.3 | 37.9; 71 | No |
| **Digoxin** | 0.1-1.85 | 13.8 | No |
| **Methotrexate** | NR | 99.6; 99.9 | Possibly |
| **Phenytoin** | 10.0 | 3.6 | Possibly |
| **Propranolol** | 74.4 (decrease of elimination half-life) | 79.2 | Possibly |
| **Tacrolimus** | 0 | 0 | No |
| **Thyroxine** | 6.0-12.0 | 7.0 | No |
| **Vancomycin** | 11.0 | 6.6 | Possibly |
| **Verapamil** | 58.0 | 58.4; 60.0 | Yes |
| **Vincristine** | 70.4 | 57.7; 71.5; 7.6 | Possibly |

Table 1: Comparison of drug removal by plasmapheresis between children and adults

# THE EFFECT OF DEXAMETHASONE USE ON THE INCIDENCE OF DELIRIUM IN OLDER PATIENTS HOSPITALIZED BECAUSE OF COVID-19 INFECTION. A RETROSPECTIVE COHORT STUDY,

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**Background**
Delirium has been suggested as a complication from corticosteroid use [1,2]. Therefore the association between dexamethasone and delirium in COVID-19 patients was studied.

**Methods**
In this single centre retrospective cohort study 435 patients who were hospitalised because of COVID-19 between March 2020 and January 2021 were included. Delirium was diagnosed with the delirium observation screening scale. The association between the use of dexamethasone six mg daily and delirium was measured with a multivariate logistic regression analysis, corrected for possible confounders.

**Results**
The incidence of delirium was 10.7 % in the group treated with dexamethasone and 9.3% in the group without dexamethasone (RR 1.16, CI 0.65-2.09, p=0.604). When corrected for age, comorbidity score and use of remdesivir the Odds ratio for developing a delirium with the use of dexamethasone was 0.93 (CI 0.45-1.91, p=0.844).

**Discussion/Conclusion**
In contrast to other studies we did not find an effect of dexamethasone use on the incidence of delirium. The contradiction might be explained by the studied population and, more importantly, the dose of dexamethasone which was above ten mg daily in the other studies [1,2].
Possibly, the effect of dexamethasone on the incidence of delirium was underestimated in our study. We excluded patients having a delirium before and within two nights after admission, since we felt this could not have been caused by dexamethasone. The low incidence of delirium of 10.1% can also be explained by this [3].
The use of dexamethasone six mg daily in hospitalised COVID-19 patients did not affect the incidence of delirium.

**References**

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2. Wu Z, Li H, Liao K, Wang Y. Association between Dexamethasone and Delirium in Critically Ill Patients: A Retrospective Cohort Study of a Large Clinical Database. J Surg Res 2021;263:89
3. Shao SC et al. Prevalence, incidence and mortality of delirium in patients with COVID-19: a systematic review and meta-analysis. Age and Ageing. 2021; 50:1445

# PHARMACOKINETICS OF PEGASPARAGINASE IN INFANTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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**Background**

Pegylated (PEG-) *E.coli* asparaginase (asp) is known to be a critical drug for treating pediatric acute lymphoblastic leukemia (ALL), however, there is insufficient evidence to determine the optimal dose for infants who are less than one year of age at diagnosis. This international study was conducted to identify the pharmacokinetics of PEGasp in infants with newly diagnosed ALL and gather insight into the clearance and dosing of this population.

**Methods**

68 Infants with ALL, from Australia, Belgium, Denmark, Italy Netherlands, Poland, and USA, receiving PEGasp were included in our population pharmacokinetic (popPK) assessment employing non-linear mixed effects modelling (NONMEM).

**Results**

A total of 388 samples were collected. PEGasp doses ranged from 400 to 3,663 IU/m$^2$ and were administered either intravenously or intramuscularly. A one-compartment model with time-dependent clearance, modeled using a transit model, provided the best fit to the data. Body weight was significantly correlated with clearance and volume of distribution. The final model estimated a half-life of 11.7 days just after administration, which decreased to 1.8 days 14 days after administration (table 1). We found that clearance was 19.5% lower during the post-induction treatment phase compared to induction. The final model predictions were precise as the visual prediction curve (figure 1) showed no indications of either over- or under-prediction.

**Discussion/Conclusion**

To the best of our knowledge, this is the first popPK model that simultaneously describes PEGasp data from different dosing schedules in an infant population. We observed that the half-life of PEGasp in infants with ALL is comparable to that of older children (aged 1-18 yrs). The time-dependent clearance of PEGasp increases overtime by loss of PEG as the native asp clearance rate is higher. Clearance was lower during the post-induction treatment phase compared to induction.

We recommend a PEGasp dosing at 1,500 IU/m$^2$ for infants without dose adaptations according to age, and implementing therapeutic drug monitoring as standard practice.

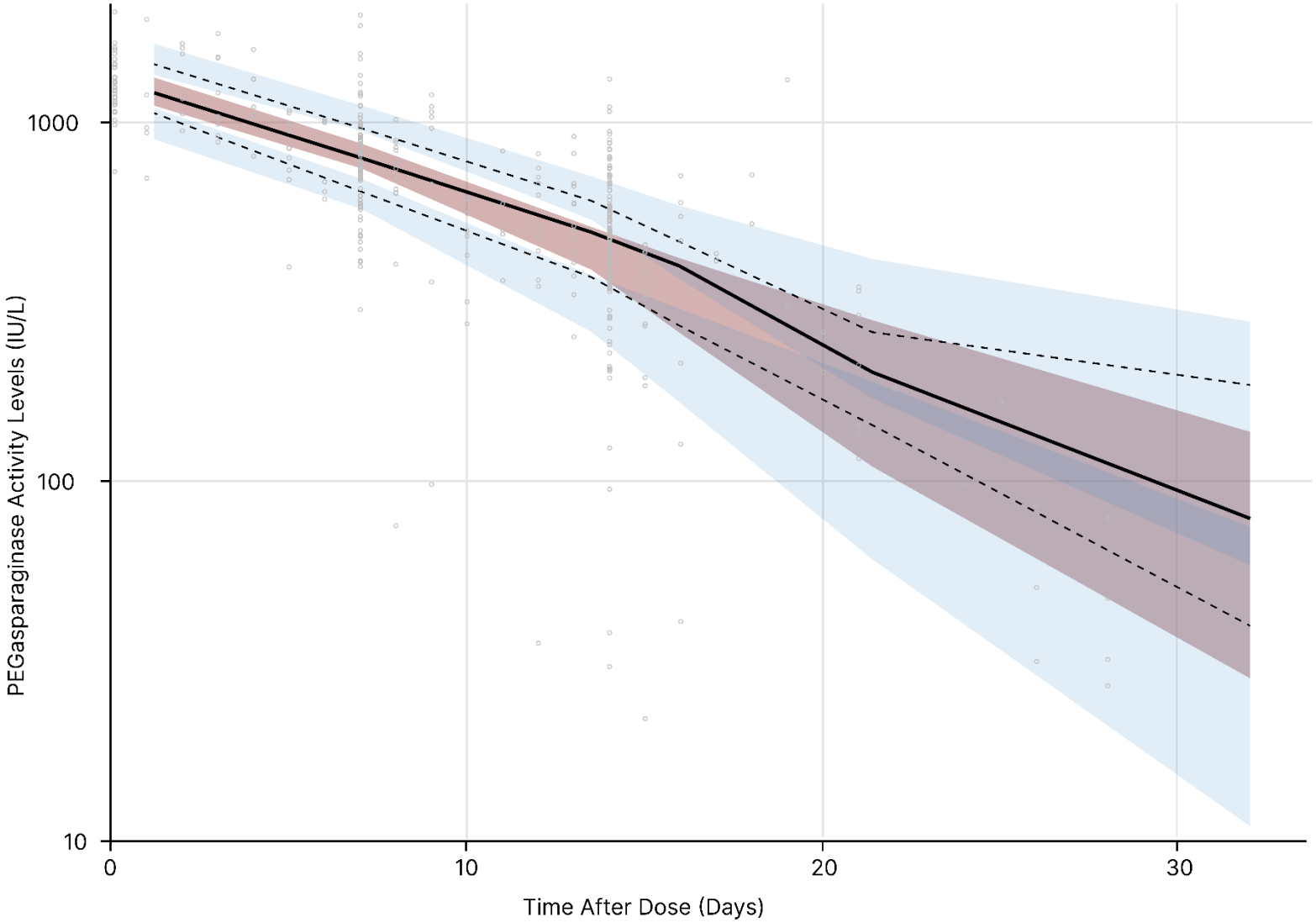


**Figure 1** Visual Predictive Check**.** Black lines are the observed mean (solid) and 5$^{th}$ and 95$^{th}$ percentile (dashed) concentrations. Red and blue areas represent 90% prediction intervals of the simulated mean and the 5$^{th}$ and 95$^{th}$ percentiles, respectively.

**Table 1** Estimates of final model

| **PK Parameter** | | | **Estimate** | **95% CI** |
|---|---|---|---|---|
| $CL_{initial}$ (L/day, 8.8 kg*) | | | 0.0318 | 0.0275-0.0362 |
| | $t_{1/2}$ (days) [#] | | 11.7 | |
| | IIV (%) | | 46.6 | 39.0-56.8 |
| | Allometric scaling exponent | | 0.5 | 0.5-0.5 |
| | *Phase* | | | |
| | | Induction (ref) | 1 (fixed) | - |
| | | Post-induction | 0.8 | 0.7-0.9 |
| | $CL_{induced}$ (L/day, 8.8 kg*) | | 0.212 | 0.135-0.341 |
| | | $t_{1/2}$ (days) [#] | 1.8 | |
| $Vd$ (L, 8.8 kg*) | | | 0.538 | 0.504-0.575 |
| | IIV (%) | | 12.2 | 7.0-16.3 |
| | Allometric scaling exponent | | 0.7 | 0.5-0.9 |
| $K_{tr}$ ($day^{-1}$) | | | 0.386 | 0.0353-0.421 |
| IIV (%) | | | | |
| | *Correlation between CL and Vd* | | 12.4 | 7.7-19.3 |
| Residual proportional variability (%) | | | 27.5 | 25.7-29.9 |

$CL_{initial}$ , initial population clearance rate; $CL_{induced}$, induced population clearance rate; $IIV$, inter-individual variability; $K_{tr}$, loss of PEG; T, transit compartments; $Vd$, volume of distribution. $t_{1/2}$, half-life calculated for the typical value $CL_{initial}$ and $CL_{induced}$ reflecting the half-life at the start and end of elimination, respectively.

*parameters correspond to a patient of 8.8 kg.

[#]The half-life represents the initial half-life just after administration and was not estimated, but we calculated half-life with the following formula: $\ln 2/\left(\frac{CL}{Vd}\right)$.

Allometric scaling exponents were estimated to account for differences in size among subjects and predict how these pharmacokinetic parameters change with size. Allometric scaling was based on median body weight during treatment (8.8 kg). The 95% confidence interval (CI) was obtained through the sampling importance resampling (SIR) analysis and demonstrates the parameter precision.

# THE DEVELOPMENT OF AN ELISA ASSAY TO DETERMINE PEMBROLIZUMAB IN A HEEL PRICK

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## Background

Immune-checkpoint inhibitors (ICI) have improved the outcomes of patients with advanced stage melanoma drastically. When ICIs are administered during pregnancy, these may transfer the placenta. The highest transplacental transfer of PD-1 antibodies occurs during the third trimester, with fetal IgG concentrations exceeding maternal concentrations at term. Therefore, it is hypothesized that fetal or infantile immune related adverse effects are more likely to occur when maternal treatment takes place from the late second trimester onwards. A bioanalysis method was developed to determine pembrolizumab in a heel prick to link possible *in utero* exposure of this agent to a described case of immune-related gastroenterocolitis in an infant of 2.5 months old.

## Methods

A sample preparation procedure and ELISA method were developed to extract and measure pembrolizumab from the heel prick. Calibration standards were prepared by spiking diluted pembrolizumab stock solutions to control whole blood (haematocrit 50%) and plasma in concentrations of 3,500, 1500, 750, 300, 150, 75, 30 and 0 ng/mL. 40 µL of the whole blood and 22 µL of the plasma standards were applied to blanc heel prick cards. After drying, 4 mm punches of the standards and heel prick were made. Pembrolizumab was extracted by the addition of 85.5 µL PBSTF10% during overnight incubation for 12 hours at 4°C. Other extraction buffers and extraction conditions were tested to improve extraction. 50 µL of every sample was applied to a Nunc MaxiSorpTM white 96-well flat-bottom plate coated with 50 µL of 2 µg/mL human PD-1; calibration standards in duplicate, QCs in triplicate. The heel prick was analysed in two independent assays (n=3 total). After incubation with anti-human $IgG_4$-HRP in PBSTF1% and addition of Pierce standard ECL, pembrolizumab concentrations were measured with a plate reader.

## Results

The recovery of the sample preparation was 33 ± 4.3%. Different extraction buffers and conditions did not improve the pembrolizumab extraction from the heel prick. Haematocrit did not affect pembrolizumab determination by ELISA. The mean concentration of pembrolizumab in the heel prick determined in two independent assays, was 38.6 µg/mL (range 34.7-41.5 µg/mL).

## Discussion/Conclusion

We successfully developed an ELISA assay to determine pembrolizumab in a heel prick. From analysis of the heel prick sample of the newborn, we can confirm *in utero* exposure to pembrolizumab.

Method was applied *in Baarslag MA, Heimovaara JH, Borgers JSW, van Aerde KJ, Koenen HJPM, Smeets RL, Buitelaar PLM et al. Severe Immune-Related Enteritis after In Utero Exposure to Pembrolizumab. NEJM. 2023 Nov 9;389(19):1790-1796. doi: 10.1056/NEJMoa2308135.*

# DEVELOPMENT AND EVALUATION OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR IVACAFTOR, TEZACAFTOR AND ELEXACAFTOR (KAFTRIO®) IN HEALTHY MALE ADULTS

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**Background**

Since the triple combination of cystic fibrosis transmembrane conductance regulator (CFTR) modulators consisting of ivacaftor, tezacaftor, and elexacaftor (Kaftrio®) is widely used to treat cystic fibrosis (CF). There is a lack of understanding of the drug-drug interaction (DDI) potential of Kaftrio® and its pharmacokinetics (PK) in special populations. Therefore, insights regarding the PK of CFTR modulators are crucial in order to optimize therapeutic efficacy while avoiding toxicity. This study aims to provide whole-body physiologically-based pharmacokinetic (PBPK) models for ivacaftor, tezacaftor, and elexacaftor, investigating healthy adults before scaling PK of Kaftrio® to special populations.

**Methods**

PBPK models were independently developed for ivacaftor, tezacaftor and elexacaftor, using physicochemical parameters from literature and observed data from twenty-one clinical studies. Their predictive performance was assessed by comparing predicted pharmacokinetic profiles with in vivo PK to develop, evaluate, and verify the models. Furthermore, $C_{max}$ and AUC ratios were calculated to compare predicted and observed ratios. An acceptance range of 0.75-1.25 was used.

**Results**

92.2% of the predicted peak plasma concentration ($C_{max}$) ratios and 87.8% of the predicted area under the plasma concentration-time curve (AUC) for the ivacaftor model are within the 0.75-1.25 acceptance range of the observed values. For the tezacaftor model, 77.8% of the predicted $C_{max}$ ratios and 88.9% of the AUC ratios are within the acceptance range. Lastly, for the elexacaftor model, 83.3% of the predicted $C_{max}$ ratios and 86.7% of the AUC ratios are within the acceptance range.

**Discussion/Conclusion**

The PBPK models are successfully developed by incorporating all open-access data and current knowledge on the PK processes of these drugs. This study lays the foundation for the PBPK models of ivacaftor, tezacaftor, and elexacaftor and their use in clinical practice to describe PK and subsequently develop evidence-based dosing regimens in special populations (e.g. liver cirrhosis, children, pregnant women).

# OPIOID PRESCRIBING IN THE NETHERLANDS DURING THE COVID-19 PANDEMIC: A NATIONAL REGISTER-BASED STUDY.

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**Background**
The COVID-19 pandemic and related lockdown measures disrupted global healthcare provision, including opioid-prescribing. In North-America, opioid sales declined while opioid-related deaths increased. In Europe, the effect of the pandemic on prescribing is not yet known. Given the ongoing increase in opioid-related harm, and mortality, it is crucial to analyze the impact of the COVID-19 crisis and lockdown measures on opioid prescribing. Therefore, the objective of this study was to characterize opioid prescribing in the Netherlands during the COVID-19 pandemic.

**Methods**
We conducted a nationwide register-based study characterizing opioid prescribing for the whole Dutch population using aggregated insurance reimbursement data. We analyzed data from one year pre-COVID-19 and the first two years of the COVID-19 pandemic. We compared the number of opioid prescriptions during the pandemic with a pre-pandemic period using a risk ratio, with separate analysis on prescription type (first-time or repeat prescription), patient sex, age, and socio-economic status. We also explored lockdown effects.

**Results**
During the first lockdown, the total number of new opioid prescriptions and prescriptions to young patients (briefly) decreased, but the overall number of opioid prescriptions remained stable throughout the pandemic. (+0.8% in the first year of the COVID-19 pandemic compared to pre-pandemic and -0.1% in the second year compared to pre-pandemic). Women, older patients, and patients living in lower socio-economic areas received more opioids per capita, but the pandemic did not amplify these differences.

**Discussion/Conclusion**
The pandemic appears to have had a limited impact on opioid prescribing in the Netherlands. Yet, chronic use of opioids remains an important public health issue.

# PHARMACOKINETICS OF IVACAFTOR, TEZACAFTOR, ELEXACAFTOR, AND LUMACAFTOR IN PATIENTS WITH CYSTIC FIBROSIS: A SYSTEMATIC REVIEW

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**Background:** Cystic fibrosis (CF) is a progressive autosomal disease caused by a mutation in cystic fibrosis conductance regulator (CFTR) gene. With the development of CFTR modulating therapy as ivacaftor, lumacaftor, tezacaftor, and elexacaftor the prognosis for patients with CF has been improved. Nevertheless, CF affects multiple organ systems and is associated with changes in drug disposition thereby altering the pharmacokinetic (PK) profile of CFTR modulators. This systematic review describes the PK of CFTR modulators in different patient populations, including adults, pancreatic insufficient patients, patients with moderate hepatic impairment and children.

**Methods:** A systematic literature search was carried out in PubMed and Embase. In addition, supportive studies conducted by the EMA and FDA were utilized. Articles were labelled as relevant when information on PK of CFTR modulating therapy was available. Extracted parameters included bioavailability (*F*), volume of distribution (*Vd*), trough and peak concentrations ($C_{min}$, $C_{max}$), time of maximum concentration ($T_{max}$), area under the curve (*AUC*), clearance (*CL*), and half-life ($t_{1/2}$).

**Results:** Nineteen relevant articles were included, seven of which discussed triple combination elexacaftor-tezacaftor-ivacaftor (Kaftrio®), five ivacaftor monotherapy (Kalydeco®), four lumacaftor-ivacaftor (Orkambi®), three tezacaftor-ivacaftor (Symkevi®), and one lumacaftor monotherapy. Seventeen out of nineteen articles provided information on *AUC*, ten $C_{max}$, five $t_{1/2}$, two *Vd/F*, and two *CL/F*. Overall, the *AUC* of all CFTR modulators exhibit variations in adults and children. For ivacaftor and tezacaftor, both $C_{max}$ and *AUC* were higher in patients with moderate hepatic impairment compared to healthy subjects. However, pancreatic insufficiency reduces the reached *AUC* and $C_{max}$ of ivacaftor in CF patients.

**Discussion/Conclusion:** This systematic review aims to offer an overview of the PK of CFTR modulating drugs in diverse patient populations. These new insights provide the first steps towards evidence-based dosing regimens for optimal therapeutic efficacy while avoiding toxicity.

# VARIABILITY IN PERFUSION CONDITIONS AND SET-UP PARAMETERS USED IN EX VIVO HUMAN PLACENTA MODELS: A LITERATURE REVIEW

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**Background:** The *ex vivo* human placenta perfusion model has proven to be clinically relevant to study transfer- and fetal exposure of various drugs. Although the method has existed for a long period, the setup of the perfusion model has not been generalized yet. This review aims to summarize the setups of *ex vivo* placental perfusion models used to examine drug transfer across the placenta to identify generalized properties and differences across setups.

**Methods:** A literature search was carried out in PubMed September 26, 2022. Studies were labelled as relevant when information was reported, between 2000 and 2022, on the setups of ex vivo placental perfusion models used to study drug transfer across the placenta. The placenta perfusion process, and the data extraction, was divided into phases of preparation, control, drug, and experimental reflecting the chronological timeline of the different phases during the entire placental perfusion process.

**Results:** 135 studies describing an *ex vivo* human placental perfusion experiment were included. Among included studies, the majority (78.5%) analysed drug perfusion in maternal to fetal direction, 18% evaluated bi-directional drug perfusion, 3% under equilibrium conditions, and one study investigated drug perfusion in fetal to maternal direction.

**Discussion/Conclusion:** This literature review facilitates the comparison of studies that employ similar placenta perfusion protocols for drug transfer studies and reveals significant disparities in the setup of these *ex vivo* placental perfusion models. Due to interlaboratory variability, perfusion studies are not readily comparable or interchangeable. Therefore, a stepwise protocol with multiple checkpoints for validating placental perfusion is needed.

# PHARMACOKINETICS OF MONOCLONAL ANTIBODIES THROUGHOUT PREGNANCY: A SYSTEMIC LITERATURE REVIEW

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**Background**
Although little information is available on the pharmacokinetics (PK) of monoclonal antibodies (mAbs) during pregnancy, multiple mAbs are being used during pregnancy for various indications. The aim of this systematic literature review was to characterize the PK of mAbs throughout pregnancy.

**Methods**
A systematic literature search was carried out in PubMed and Embase on April 21, 2023. Articles were included when information on PK or exposure parameters of mAbs in pregnant women was available.

**Results**
A total of 40 relevant articles were included, of which eight discussed adalimumab (ADL), three certolizumab pegol (CZP), five eculizumab (ECU), one golimumab (GOL), twelve infliximab (IFX), two natalizumab (NAT), five tocilizumab (TCZ), eight ustekinumab (UST) and six vedolizumab (VDZ). One of the 40 studies reported information on clearance and volume of distribution of IFX; all other studies only reported on serum concentrations in pre-pregnancy state, different trimesters and in the post-partum period. For all of the assessed mAbs except IFX, serum concentrations were similar to concentrations in the pre-pregnancy state or modestly decreased. In contrast, IFX trough concentrations trended toward increases in the second and third trimesters in comparison to the non-pregnant state.

**Discussion/Conclusion**
Available information suggests that the anatomical and physiologic changes throughout pregnancy may have meaningful effects on the PK of mAbs. For most mAbs (and not for IFX), modestly higher dosing (mg) may be needed during pregnancy to sustain a similar serum exposure compared to pre-pregnancy.

# POPULATION PHARMACOKINETIC ANALYSIS OF GUANABENZ IN CHILDREN WITH VANISHING WHITE MATTER: EVALUATING SCALING ADULT DRUG SAFETY AND PHARMACOKINETICS

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**Background:** Vanishing White Matter (VWM) is a leukodystrophy characterized by degeneration of central nervous system white matter, resulting in decline of cognitive and motor functions and premature death. There is currently no treatment. Preclinical studies using VWM mouse models have shown that guanabenz, a central $\alpha_2$-adrenergic receptor agonist, has positive effects on the disease's progression by inhibiting the integrated stress response (ISR). The aims of the current study were to validate the translational pharmacokinetics (PK) and pharmacodynamic (PD) model of oral guanabenz in pediatric VWM patients in an ongoing phase 1 study. In this context, we also aimed to assess the feasibility of using guanabenz's effect on blood pressure as a possible non-invasive biomarker, and to perform an explorative analysis of the relation between Guanabenz blood level and side effects.

**Methods:** Translational modelling was performed using adult guanabenz PK parameters in blood and rodent PK in blood and brain tissue, toxicity and efficacy data upon multiple dose levels. The translational model was used to determine the safe and potentially effective Guanabenz doses. In a following phase 1/2 clinical with paediatric VWM patients, drug titration was carried out until the individual maximum tolerated dose. Extensive blood plasma PK-sampling was performed during the initial dose and consecutive annual visits during MRI and capillary sampling in between. Blood pressure and heart rate were measured 4 times per day during this phase and more intermittently thereafter. Adverse events were registered continuously during the trial. Population PK modelling and simulations were performed on samples in NONMEM®.

**Results:** Translational modelling, based on brain AUC, resulted in an estimated minimum effective dose of 1 mg/kg/day and an optimal dose of 2 mg/kg/day. Venous and capillary samples highly correlated. In the clinical trial population PK modelling was performed with 25 participants, aged 2.4 – 12.9 years. The model that was developed by extrapolating adult PK parameters showed an overall under-prediction with an prediction error of 50.4%. Re-estimation of all PK-parameters improved the model (PE 6.0%). Hallucinations were an unexpected sides-effect. Time-to-event modelling and Kaplan-Meier analysis showed no significant covariates or concentration-response relationship for hallucinations, although the data suggested a predisposition in younger patients for the first few weeks. Population pharmacodynamic modelling showed that tolerance develops to guanabenz's effect on blood pressure, impeding, although not ruling out, its use as a biomarker for efficacy and tolerability.

**Discussion/Conclusion:** Extrapolation of adult guanabenz PK was able to effectively predict safe dosages for a pediatric population, Guanabenz was tolerated in terms of physiological and α2 side effects, although unexpected hallucinations did occur during the trial.

# CATCHING THE CULPRIT: BENZYLPENICILLIN NEUROTOXICITY CONFIRMED BY THERAPEUTIC DRUG MONITORING IN A PATIENT WITH CVVH

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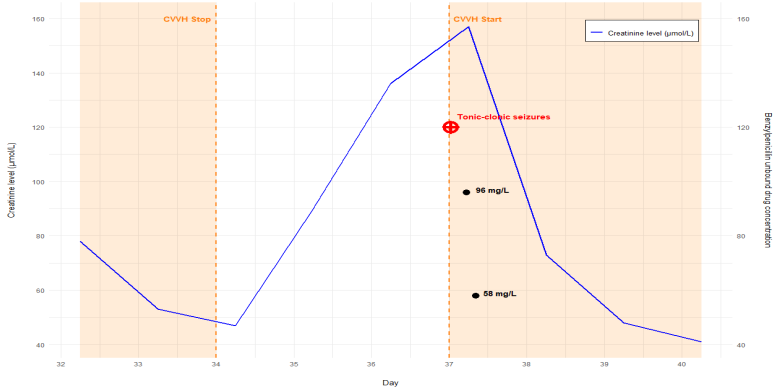
## Background
Neurotoxicity is a known complication of high doses of benzylpenicillin, especially in critically ill patients with predisposing factors like renal insufficiency. Therapeutic drug monitoring (TDM) of β-lactam antibiotics like benzylpenicillin has been proposed as a strategy to prevent excessively high exposure and resultant neurotoxicity. However, threshold unbound benzylpenicillin concentrations above which neurotoxicity events should be anticipated are unknown.

## Methods (Case presentation)
A 65-year-old Caucasian woman was admitted to the intensive care unit with severe invasive pneumococcal pneumonia, complicated by pleural empyema, purulent pericarditis and a myocardial abscess. She developed acute renal failure, necessitating continuous venovenous hemofiltration (CVVH). She was treated with 7200 mg (12 million units) of benzylpenicillin per day, administered intravenously as a continuous infusion for 3,5 weeks. One day after cessation of CVVH, she experienced multiple tonic-clonic seizures.

## Results
TDM revealed significantly elevated unbound benzylpenicillin serum concentrations (96 mg/L). See Figure 1 for the creatinine level over time, CVVH, the tonic-clonic seizures and the serum concentrations. These findings suggested a direct link between the high serum levels of benzylpenicillin and the neurotoxicity symptoms. The antibiotic therapy was switched, and neurological signs recovered rapidly. At a post-ICU follow-up, she showed no cognitive impairments.




## Discussion/Conclusion
This case highlights the challenges in dosing benzylpenicillin in patients with severe infections and renal impairment. TDM has a critical role to prevent (neuro)toxicity, though a therapeutic range is unknown. This case provides the first documented neurotoxic level of unbound serum benzylpenicillin concentration providing a reference for clinicians to adjust benzylpenicillin dosages to minimize the risk of neurotoxic effects.

# THE EFFECT OF THE IMPLEMENTATION OF A CLINICAL DECISION SUPPORT (CDS) TOOL ON THE PERCENTAGE OF ADEQUATELY PRESCRIBED LMWH-THROMBOPROPHYLAXIS(: AN INTERRUPTED TIME SERIES)

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**Background**

Nonsurgical patients have an increased risk of developing a venous thromboembolism (VTE) during hospital admission. This risk can be reduced by thromboprophylaxis with low molecular weight heparins (LMWHs) [1]. In thromboprophylaxis guidelines the Padua Prediction Score (PPS) has been suggested as the best available model to assess the risk of VTE in these patients, but adherence to these guidelines is known to be low. Objective of this study was to increase the percentage of adequately prescribed thromboprophylaxis with a LMWH in patients with a high VTE risk by implementation of an intervention consisting of a clinical decision support (CDS) tool in the electronic health record. Secondary specificity and sensitivity of the CDS tool were determined.

**Methods**

Data on the PPS and thromboprophylaxis in non-surgical patients (≥ 18 years) were prospectively collected for a total of ten time points before and after the implementation of the intervention. Patients who recently (<1 week) underwent a percutaneous coronary intervention, and palliative patients were excluded. A time series analysis was performed using an autoregressive integrated moving average (ARIMA) model to examine the effect of the intervention on the percentage of adequately prescribed LMWH-thromboprophylaxis.

**Results**

400 patients were included, 200 patients pre- and 200 patients postintervention. Assuming a direct effect of the intervention on the percentage of adequately prescribed LMWH-thromboprophylaxis a level effect of 7,5% was calculated, this level effect was not significant ([CI] -4,4-19,5; P=0,19) as can be seen in figure 1. The CDS tool had a sensitivity of 92% and a specificity of 68%.

**Discussion/Conclusion**

The implementation of a CDS tool had no significant effect on the percentage of patients adequately treated with thromboprophylaxis in an interrupted time series analysis. The CDS tool was able to successfully identify patients with a high risk of developing a VTE.

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# IMPACT OF DISCONTINUATION OF FALL RISK INCREASING DRUGS IN HOSPITALIZED MULTIMORBID OLDER PATIENTS WITH POLYPHARMACY

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**Background:** Falls are a major concern in the older population. Recently, a STOPFALL screening tool was presented to support clinicians in the management of fall risk increasing drugs (FRIDs). However, not much is known if discontinuation of FRIDs leads to a reduction of falls. Therefore, the aim of this study was to assess the association between discontinuation of FRID on the occurrence of falls in older patients with polypharmacy.
**Methods:** A prospective cohort study was conducted using data of the OPERAM trial [1], a cluster randomized controlled trial that enrolled adults ≥70 years with multimorbidity and polypharmacy to assess hospital pharmacotherapy optimization. Participants who were alive 2 months after inclusion and provided data on the occurrence of falls were included. FRID discontinuation was defined as the discontinuation of ≥1 drug(s) within 2 months after inclusion, including the following groups: antidepressants, antiepileptics, antihistamines, antipsychotics, benzodiazepines, diuretics, opioids, and alpha-blockers. Falls were self-reported and were defined as one or more falls in the follow-up period of 2 to 12 months after inclusion. Multivariable cox regression analysis was performed to assess the association between discontinuation of FRIDs and the occurrence of falls.
**Results:** Our analysis included 1,546 participants, with a median age of 79 years (IQR 10) and 44% were female. FRID were discontinued in 878 (57%) participants. During follow-up, 211 (24%) participants whose FRIDs were discontinued experienced a fall compared to 167 (25%) in the group were FRIDs were continued. No association was found between the discontinuation of FRIDs and the incidence of falls (adjusted HR 0.88, 95% CI 0.72-1.09).
**Discussion/Conclusion:** In multimorbid older patients using FRID, incident falls are highly prevalent. No association was found between the discontinuation of FRID and the risk of falls.

**References:**

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# CONVERSION TO BELATACEPT IN KIDNEY TRANSPLANTATION: THE ROTTERDAM EXPERIENCE

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**Background**

After kidney transplantation, belatacept, a costimulation blocker, is an alternative immunosuppressive agent for tacrolimus, a calcineurin inhibitor (CNI). It has a favourable cardiovascular profile, but a higher acute rejection rate early after transplantation compared to a CNI-based regimen. We share our experience of converting kidney transplant patients from a CNI-based to a belatacept-based immunosuppressive regimen, and give recommendations for general clinical practice.

**Methods**

All kidney transplant patients who were or had been on belatacept in our center after 2012 were included for analysis. Patient and transplant characteristics; reason for and method of conversion were studied; as well as rejection rate after conversion from CNI to belatacept. Finally, trends in estimated glomerular filtration rates (eGFR) before and after conversion to belatacept, were analyzed.

**Results**

Patients converted to belatacept (n=19) had a low immunological risk for rejection: 63% living donors, median calculated panel reactive antibody (cPRA) was 0%, and 75% received their first kidney. Median time between transplantation and conversion was almost 3 years. Most prevalent reasons for conversion were renal in nature (n=12), i.e. interstitial fibrosis and tubular atrophy (IFTA: 26%); thrombotic microangiopathy (TMA: 26%); and acute tubular necrosis (ATN: 11%). The majority of these patients showed a decline in eGFR before conversion to belatacept (67%). However, after conversion, most of these patients (89%) had a stable or improving eGFR in the long term. This positive effect of belatacept was not seen in patients who were converted for non-renal causes (n=7). In total, 7 of 19 patients had a biopsy proven acute rejection (BPAR) after transplantation, of which 4 were after conversion to belatacept. Of these 4 patients, 3 were converted within the first 3 months after transplantation, one of them was converted for renal cause. Moreover, in these patients, CNI was stopped immediately after start belatacept, without tapering down the dosage. The 4 patients with a BPAR after conversion, all showed a decline in eGFR in the long term.

**Discussion/Conclusion**

Conversion of a CNI-based to a belatacept-based regimen can be beneficial and safe in kidney transplant patients experiencing CNI-toxicity. The risk for acute rejection is smaller when conversion is not shortly after transplantation and when a period of overlap of CNI and belatacept is applied.

# THE INFLUENCE OF METFORMIN AND CIMETIDINE ON THE EXPOSURE OF TRIFLURIDINE

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**Background**

Trifluridine/tipiracil (T/T), registered for the treatment of patients with metastatic gastric and colorectal cancer, is a substrate and an inhibitor for the organic cation transporter 2 (OCT2) and the multidrug and toxin extrusion protein 1 (MATE1). Therefore, we prospectively examined the effect of an OCT2/MATE1 inhibitor (cimetidine) and substrate (metformin) on the pharmacokinetics of trifluridine.

**Methods**

This was a pharmacokinetic (PK), three-phase, cross-over trial. Patients for whom T/T was indicated according to label were eligible if they did not have diabetes mellitus and did not use any OCT2/MATE1-affecting drug/supplement. Patients were hospitalized for 12h dense PK blood sampling after 5 days of T/T monotherapy (phase A) and 5 days of T/T concomitantly with metformin (phase B) during the first cycle, and 5 days of concomitant use with cimetidine (phase C) the next course after. The primary endpoint was the relative difference (RD) in $AUC_{0\text{-}INF}$ trifluridine between phase A and B, and A and C, calculated using non-compartmental models (Phoenix WinNonlin, version 8.3) and analysed on log-transformed observations by means of paired t-tests against $\alpha$=0.025. A >30% change in exposure was considered clinically relevant.

**Results**

Eighteen patients were included in this study. PK parameters of all phases are shown in Table 1. Metformin did not significantly alter the exposure to trifluridine (RD: -12.6%; 97.5% CI: -25.0 to 1.8%; p = 0.045). Cimetidine did statistically significantly alter the exposure to trifluridine (RD: +18.0%; 97.5% CI: 4.5 to 33.3%; p = 0.004), but this increase did not meet our threshold for clinical relevance. The trough concentrations of metformin in combination with T/T (0.24 mg/L) did not significantly differ from metformin monotherapy (0.15 mg/L, p = 0.192).

Table 1: PK parameters of trifluridine. Data are represented as geometric mean (CV%).

| | T/T (phase A) | T/T + metformin (phase B) | T/T + cimetidine (phase C) |
|---|---|---|---|
| $AUC_{0\text{-}INF}$ (mg*h/L) | 22.5 (43%) | 19.8 (57%) | 26.7 (52%) |
| Cmax (mg/L) | 6.79 (36%) | 5.66 (50%) | 6.41 (35%) |

**Discussion/Conclusion**

Concomitant use of either metformin or cimetidine during treatment with trifluridine/tipiracil does not result in a clinically relevant change in trifluridine/tipiracil exposure in patients. These results suggest that in daily practice, combining trifluridine/tipiracil with any of these drugs can be done safely.

# DEVELOPMENT AND VALIDATION OF AN EX VIVO HUMAN PLACENTA PERFUSION MODEL

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**Background**
There is limited data on fetal drug exposure during pregnancy resulting from the inability to sample in the uterus. An ex vivo placenta perfusion model can be used to determine fetal drug exposure. Nonetheless, this model still lacks a standardized protocol. This study is to create an ex vivo human placenta perfusion model that can be used in future placental drug transfer studies, providing valuable insights into fetal drug exposure.

**Methods**
This model was developed based on relevant literature found in PubMed. Human placentas used for the model were obtained from planned C-sections at term pregnancy.

**Results**
The developed ex vivo human placenta perfusion model involves two parts: blood removal on the fetal side using a 37 °C flush, followed by a room temperature flush with a flow rate of 3-9 mL/min and a pressure <70 mmHg. Secondly, the 37 °C placental perfusion with a maternal flow rate of 12 mL/min and a fetal flow rate of 6 mL/min with a pressure of 50-70 mmHg (2.5 hours)(figure 1).
The preparation period, from birth until the start of the perfusion, was 2 hours. Validation with FITC-dextran (40 kDa, negative control) and antipyrine (positive control) confirmed circuit functionality.
A significant perfusate decrease during perfusion indicated placental leakage. This led to improvements in the flush and the finalization of the development of the ex vivo placenta perfusion model.

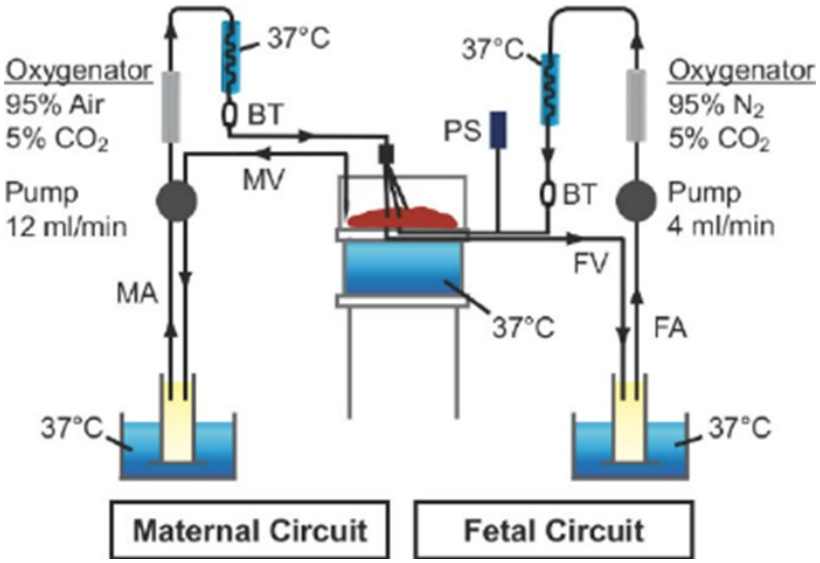

Figure 1. Schematic of the ex vivo placenta perfusion model

**Discussion/Conclusion**
This study developed an ex vivo human placenta perfusion model, overcoming challenges and establishing a framework for future placental drug transfer studies.

# CLINICAL FACTORS ASSOCIATED WITH ERYTHROCYTE METHOTREXATE-POLYGLUTAMATE CONCENTRATIONS IN DIFFERENT DISEASES AFTER THREE MONTHS OF THERAPY

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**Background:** Erythrocyte methotrexate-polyglutamates (MTX-$PG_n$) concentrations vary among patients in several immune-mediated inflammatory diseases (IMID) and are associated with the treatment response. We aimed to investigate clinical and biochemical determinants of the erythrocyte MTX-$PG_n$ concentrations in patients across multiple diseases.

**Methods:** This study included rheumatoid arthritis, juvenile idiopathic arthritis, Crohn's disease, sarcoidosis and pediatric acute lymphoblastic leukemia (ALL) patients. MTX-$PG_n$ concentrations were measured in nmol/L packed erythrocytes after three months of therapy or after the fourth cycle of IV-MTX for ALL. To evaluate possible determinants multivariate linear regression was conducted in R (v4.2.1) including age, gender, BMI, smoking status, MTX dose, eGFR, route of administration, DMARDs (mesalazine, hydroxychloroquine and sulfalazine), prednisone and folic acid/leucovorin use.

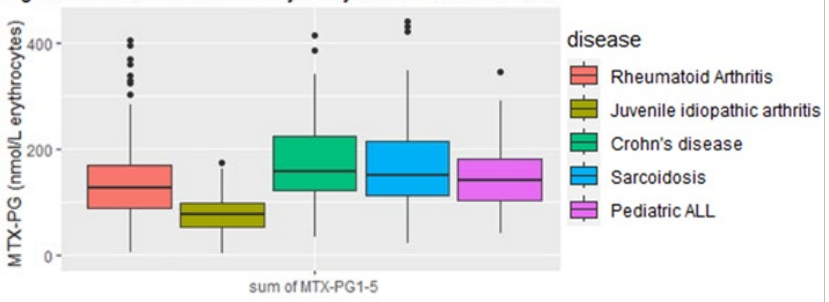
Table 1. Multivariate analysis of MTX-$PG_n$ determinants

| | Age | BMI | Dose | S.C. | IV. | DMARD | FA |
|---|---|---|---|---|---|---|---|
| PG1 | 1.01* | | -0.97* | | | | |
| PG2 | 0.15* | -0.34* | | -4.52* | | -4.63* | |
| PG3 | 0.38* | -0.59* | | 12.35* | | -4.77* | 6.72* |
| PG4 | 1.01* | -0.98* | | 2.40* | 15.42* | | 1.46* |
| PG5 | 1.02* | 2.67* | -1.00* | 2.67* | 57.65* | -0.80* | 1.44* |

Abbreviations: dose: in mg/m²/week, s.c.: subcutaneous, IV: intravenous administration, FA:>5mg/week.

**Results:** 580 patients were included. Figure 1 shows similar concentration MTX-$PG_{1-5}$ for IMIDs treated with low-dose and ALL treated with high-dose MTX. In the combined IMIDs and ALL model, age, subcutaneous and IV route associated positively with MTX-$PG_n$, whereas MTX-dose negatively associated with MTX-$PG_1$ and MTX-$PG_5$. BMI and DMARD use associated negatively with MTX-$PG_n$ (Table 1). Multivariate analysis of IMIDs only, showed a positive association with MTX-dose and subcutaneous route for MTX-$PG_4$ (stβ 1.07,p <0.001) and MTX-$PG_5$ (stβ 1.06, p= 0.002) and a negative association for eGFR for MTX-$PG_4$ (stβ -1.01 ,p= 0.005) and MTX-$PG_5$ (stβ -1.01, p= 0.016).

Figure 1 sum of MTX-PGn in erythrocytes after three months




**Conclusion/Discussion:** This is the first study investigating determinants influencing MTX-$PG_n$ concentration across both multiple IMIDS and pediatric ALL. Consistent with previous studies in smaller cohorts, age, MTX dose and route of administration associated positively with the MTX-$PG_n$ concentrations, while BMI and DMARD co-medication are the newly found determinants. This study enhances our understanding of factors affecting MTX-$PG_n$ accumulation, which could facilitate therapeutic drug monitoring-based dosing of MTX for personalized treatment.

# INFLIXIMAB IN PEDIATRIC INFLAMMATORY BOWEL DISEASE: EXTERNAL EVALUATION OF POPULATION PHARMACOKINETIC MODELS

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**Background**

The use of infliximab (IFX) has significantly improved outcomes for children with inflammatory bowel disease (IBD). However, variability in response among children led to the increasing need for drug monitoring. Population pharmacokinetic (PopPK) modelling is a promising approach for therapy optimization in children but with the increasing number of models in the literature, model evaluation is essential. This study aims to externally evaluate the validity of existing PopPK models for IFX in children with IBD.

**Methods**

A detailed search on PubMed was conducted to identify IFX PopPK models in children with IBD and data was collected using electronic healthcare data collected at the University Medical Center Groningen from 2017 to 2023. Selected PopPK models were reconstructed and analysed using R mapbayr and mrgsolve packages. Model performance was assessed using goodness-of-fit plots, residuals against time, eta-distributions, prediction error, and prediction-corrected visual predictive checks (pcVPC).

**Results**

Nine PopPK models were chosen, and the evaluation included 73 children. Individual predicted (IPRED) values exhibited bias ranging from -9.29% to 8.01%, while population predicted (PRED) values displayed bias within the range of -82.86% to 118.22%. Models 6 and 8, developed by Vande Casteele et al [1] and Passot et al [2], respectively, demonstrated superior performance (IPRED bias of 2.13 and -0.24, PRED bias of -6.11 and -13.409).

**Discussion/Conclusion**

Model 6 demonstrated the highest performance and is considered the most suitable for clinical applications. This model can be further used in practice for predicting IFX levels in patients, enabling early detection of primary and secondary non-response.

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# THE EFFECT OF COLCHICINE ON COAGULATION IN PATIENTS USING VITAMIN K ANTAGONISTS WITH CHRONIC CORONARY DISEASE

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**Background**
Low-dose (0.5mg/day) colchicine improves cardiovascular outcomes in patients with coronary disease. Around 12% of these patients simultaneously use anticoagulant therapy. In vitro studies and case reports describe a possible drug interaction between colchicine and Vitamin-K antagonists (VKAs).

The aim of this study was to investigate if there is a clinically relevant drug interaction between colchicine and VKAs in patients with chronic coronary disease.

**Methods**
This study was a sub-analysis of the randomized LoDoCo2 trial in which colchicine 0.5mg once daily was compared with placebo in patients with chronic coronary disease. For the current study we included a sample of patients who used a VKA. All patients were subject to a one month open-label run-in phase in which they received 0,5mg colchicine once daily. The primary outcome was the within-patient difference in International Normalized Ratio (INR) after starting or stopping colchicine as compared to one month before. Secondary outcomes were difference in mean daily dosage of VKAs and Time in Therapeutic Range (TTR).

**Results**
In total, 73 patients were included (35 colchicine- and 38 placebo group). No difference in mean INR was found after the starting colchicine (+0.07; 95% confidence interval [CI]: -0.13–0.26; p=0.50) or when stopping (+0.11; 95%CI: -0.12–0.33; p=0.34). The change in mean VKA daily dosage was -0.01mg (95%CI: -0.033–0.012; p=0.35) when starting colchicine and -0.01mg (95%CI: -0.029–0.012; p=0.41) when stopping colchicine. The change in TTR one year prior to the study compared to one year after randomization to colchicine was +7.56% (95% CI: -0.14–15.3; p=0.054).

**Conclusion**
We found no clinically relevant difference in mean INR in patients using VKAs after starting, using or stopping colchicine. These results suggest that low-dose colchicine can be used safely in patients treated with VKAs, without the need for additional INR monitoring other than the standard of care.

# ASSESSING THE IMPORTANCE OF SEX AND AGE-RELATED INFLUENCES OF CYP ENZYME ACTIVITY; THE NEXT STEP TOWARDS PERSONALISED MEDICINE

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## Background

Between 40% and 70% of patients experience variability in their drug response, encountering either adverse drug reactions (ADRs) or ineffectiveness. The human CYP450 system, influencing drug metabolism, plays a crucial role. Psychotropic drugs, metabolized by CYP450 enzymes, are susceptible to variations in enzyme activity, paving the way for personalized medication selection based on genotype [1]. Genetic polymorphisms may explain ADRs and inefficiencies in approximately 15-30% of patients. Notably, sex and age differences in CYP450 enzyme activity have been observed, potentially influencing drug metabolism and toxicity risk [2,3].

## Methods

A systematic search across Medline, Embase, PsycINFO, clinicaltrials.gov, and The Cochrane Library via the Ovid app identified peer-reviewed articles focusing on the activities of five major CYP enzymes. Papers meeting the criteria underwent screening for data on metabolism rates, CYP enzyme activity, age, sex, and pharmacogenetics. The analysis will undergo descriptive statistics and visual aids, with heterogeneity addressed through appropriate statistical tests.

## Results

An initial search yielded nearly n=2,200 articles, which were subsequently narrowed down to n=583 articles after refining search terms. Title and abstract screening resulted in n=116 potentially useful articles based on set criteria. Two researchers independently and blindly assessed articles for inclusion or exclusion. A final screening involved a thorough review of the full-text articles, leading to the selection of n=30 articles for further detailed study and data extraction.

## Discussion/Conclusion

In some studies assessing P450 activity subjects were not genotyped, introducing a potential confounding effect to their results. Preliminary findings indicate a marginal difference in CYP2C19 and CYP1A2 activity between sexes, with other CYPs appearing unaffected. Age influences CYP3A4 and CYP2C19 activity, while CYP2D6 seems largely unaffected. Integrating CYP genotyping with data on sex and age differences in drug metabolism promises a more efficient guided pharmacological treatment, empowering clinicians to make informed decisions. This integration represents a significant step towards personalized medicine in psychiatry.

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# VARIABILITY IN MAXIMUM BINDING CAPACITY OF VINCRISTINE TO BETA-TUBULIN NOT EXPLAINED BY DIAGNOSIS OF ACUTE LYMPHOBLASTIC LEUKEMIA

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## Background

Previously, we investigated the rationale behind reducing vincristine doses in infants compared to older children using a pharmacokinetic (PK) model[1]. This PK model contained a saturable compartment resembling the binding of vincristine to beta-tubulin in blood. In this model, the maximal binding capacity (Bmax) to beta-tubulin was estimated and found to be strongly influenced by weight and age. This PK model suggested that young children tolerate higher doses of vincristine and, therefore, challenges the common practice that infants should receive a lower dose than older children. The data on which this model is based included patients across all types of cancer. As tubulin expression in the blood might be influenced by underlying disease, we hypothesize that tubulin blood expression might be different for haematological malignancies versus solid tumours. Therefore, we tested if diagnosis could explain the variability seen in Bmax in the published PK model and should be considered when determining the optimal vincristine dose.

## Methods

Diagnosis was added to the original dataset (n = 206) and tested as a dichotomous covariate (acute lymphoblastic leukaemia (ALL) vs. other cancer types) in the published PK model for vincristine to test if this would improve its fit to this original dataset. Improvement in fit was determined based on the change in objective function value (OFV), goodness of fit (GOF)-plots and a visual predictive check (VPC). In addition, we checked for correlations between thrombocyte count and Bmax.

## Results

Adding diagnosis as a dichotomous covariate on Bmax improved the model fit (ΔOFV = -43). Yet, the inclusion of this covariate suggested that Bmax is only 4.8% lower in patients treated for ALL compared to other cancer types. No correlations were found between absolute thrombocyte count and Bmax (see figure 1). Interestingly, higher values for Bmax were found at lower thrombocyte counts for patients diagnosed with ALL.

## Discussion/Conclusion

We hypothesized that an ALL diagnosis could influence Bmax in multiple ways. For example, not only might the thrombocyte count reach lower levels in this population, but there might also be more fluctuations in these levels or even less tubulin available within the thrombocytes to bind to. The amount of tubulin available within a thrombocyte might even vary over the course of treatment, depending for example on the number of dividing cells. This last hypothesis is supported by the lack of correlation between Bmax and thrombocyte count shown in figure 1. Adding diagnosis as a covariate on Bmax improved the description of vincristine disposition slightly, yet does not relevantly explain the large variability in this parameter.

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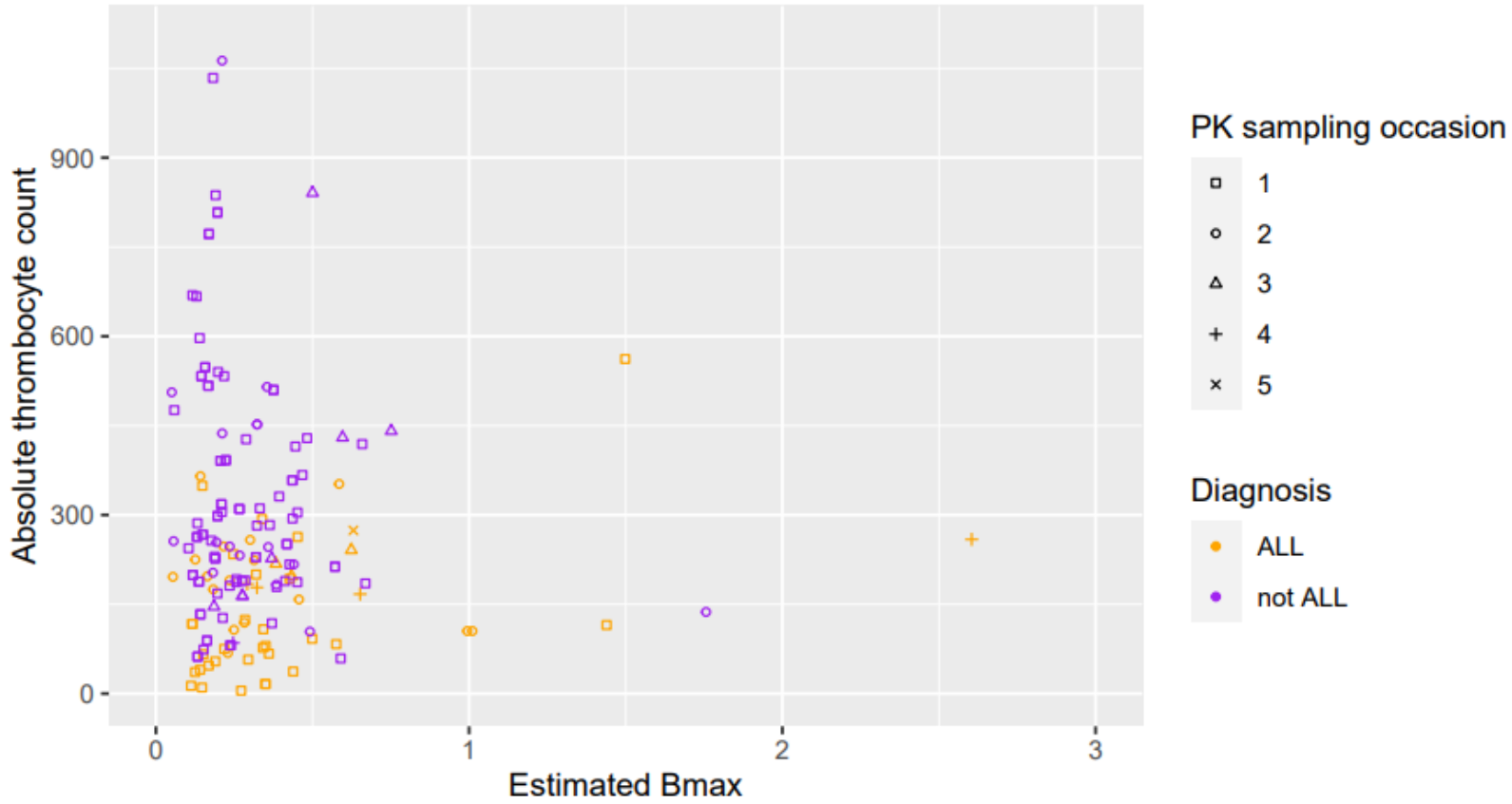


**Figure 1.** Absolute thrombocyte count over estimated maximal binding capacity of vincristine to thrombocytes.

# OPIOID USE AND OPIOID OVERDOSE IN THE NETHERLANDS – A 10 YEARS RETROSPECTIVE STUDY OF NALOXONE ADMINISTRATION AT DUTCH EMERGENCY DEPARTMENTS.

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**Background**

The number of opioid users in the Netherlands has doubled in the last two decades. To assess if the rise in use also means a rise in severe opioid intoxications, we studied the number opioid intoxications necessitating naloxone administration in Dutch emergency departments during the last 10 years.

**Method**

A multi-center retrospective cohort study that uses information of Dutch emergency department visits between 2011 and 2020 was conducted. Information on type of opioid, reason for use, and duration of use was collected. A severe opioid intoxication was defined as a patient with an opioid toxidrome in whom a rapid improvement of symptoms occurred after administration of naloxone.

**Results**

Five emergency departments participated in this study. The number of episodes of naloxone administrations was 311 in 292 unique patients. 187 cases were considered a severe opioid intoxication. Mean annual incidence was 19.5 cases with a maximum annual incidence of 28 cases. The opioid mostly involved was oxycodone in 69 cases (37%); followed by methadone in 41 cases (22%) and fentanyl in 26 cases (14%). 127 cases (68%) were using opioids since more than 3 months. 176 cases (94%) were admitted of whom 96 to the intensive care unit. Twenty three cases died within 30 days (12%).

**Discussion/Conclusion**

The increase in naloxone administrations as a proxy for opioid intoxications in Dutch emergency departments between 2011 and 2020 is not comparable to the increase in opioid users. The increment in incidence over time was largely attributable to one of the participating hospitals.

# EFFECT OF THERAPEUTIC DRUG MONITORING ON THE TOXICITY OF IMATINIB IN PATIENTS WITH GASTROINTESTINAL STROMAL TUMOURS

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**Background** Imatinib trough concentrations (Cmin) ≥ 1100 ng/mL are associated with treatment response in patients with gastrointestinal stromal tumours (GISTs). To prevent underexposure, therapeutic drug monitoring (TDM) is used. With this tool, the imatinib dose is increased based on the measured blood concentration. We evaluated whether this strategy increases the incidence and severity of imatinib-related adverse events (AEs) and we aimed to elucidate predictive concentration thresholds for toxicity.

**Methods** This study included GIST patients starting TDM-guided treatment with 400 mg imatinib at the Netherlands Cancer Institute. AEs during the treatment period were retrospectively collected from the medical files and graded using the Common Terminology Criteria for Adverse Events (CTCAE) system. The incidence and severity of AEs was compared between different dose groups in our TDM cohort. Predictive Cmin thresholds for the occurrence of AEs were determined using receiver operating characteristics (ROC) analysis. The incidence and severity of toxicity in the TDM cohort was compared with two historical cohorts receiving non-TDM guided imatinib treatment, using data from the Dutch GIST Registry (DGR) and the imatinib-only arm of the ALT-GIST study.

**Results** A total of 74 patients were included. The incidence of AEs did not significantly increase in the 47 patients with a dose increase from 400 mg to 600 mg (89.4% to 93.6%, $p$ = 0.48). In the 16 patients with an additional dose increase to 800 mg, the incidence of grade 2 toxicity increased significantly (400 mg: 25%, 600 mg: 31% and 800 mg: 63%, $p$ = 0.006). We found that a Cmin of ≥ 1248 ng/mL could predict the occurrence of severe (grade 3 and higher) toxicity ($p$ = 0.004). Most patients (93%) from the DGR and all patients from the ALT-GIST study a received a dose of 400 mg imatinib daily. Patients with a dose increase in our TDM-cohort more often experienced dose-limiting toxicity compared to patients in the DGR (61.2% and 16.3%, respectively; $p$ < 0.001). We found no difference in severity of AEs between patients in the TDM-cohort and patients from the ALT-GIST study (all toxicity grades $p$ > 0.05).

**Discussion/Conclusion** This study showed that implementing routine TDM for imatinib led to an (acceptable) increase in toxicity. Information on high imatinib exposure may have resulted in physicians being more inclined to perform dose reductions in case of toxicity, which can explain the high proportion of patients with a DLT in our TDM cohort. Maintaining a Cmin < 1248 ng/mL could prevent severe toxicity, however, the implementation of this threshold in daily practice will likely be challenging given the high intrapatient variability in exposure and the available dosage strengths.

# IMPACT OF ANTITHROMBOTIC THERAPY ON THROMBOTIC AND BLEEDING COMPLICATIONS AFTER ELECTIVE ENDOVASCULAR ANEURYSM REPAIR

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**Background**
The aim of this study was to investigate the influence of various antithrombotic therapies on the occurrence of thrombotic and bleeding complications after endovascular aneurysm repair (EVAR).

**Methods**
We conducted a retrospective single centre cohort study of patients undergoing elective EVAR for abdominal aortic aneurysm from 2010 to 2020. Patients were categorized into antithrombotic groups: single antiplatelet therapy (SAPT), anticoagulants, or dual antiplatelet therapy (DAPT). Primary outcome measures were the incidence of major adverse cardiovascular events (MACE), prosthetic limb occlusions, and bleeding complications during follow-up.

**Results**
Among 616 patients (SAPT: n=450, anticoagulants: n=84, and DAPT: n=82), Kaplan-Meier (KM) time-to-event analysis showed no significant difference (log-rank p=0.37) in incidence of MACE between patients receiving SAPT (20.9%), anticoagulants (25.0%), and DAPT (14.6%) during a mean follow-up of 4 years and 9 months. In multivariable Cox regression analysis only age (HR=1.03; 95% CI 1.01-1.06, p=0.01) and ASA classification (HR=1.46; 95% CI 1.12-1.91; p=0.01) were significant predictors for MACE. Prosthetic Limb occlusion was observed in 38 patients, incidence between patients receiving SAPT (5.8%), anticoagulants (10.7%), and DAPT (3.7%) was not significantly different (log-rank p=0.08). Age (HR=0.96; 95% CI 0.92-1.00; p=0.03) and use of anticoagulants (HR=3.79, 95% CI 1.46-9.83; p<0.01) were significant predictors for prosthetic limb occlusion in multivariable Cox regression analysis. Bleeding complications occurred in 73 patients, without significant difference (log rank p=0.06) in incidence between patients receiving SAPT (10.7%), anticoagulants (19.0%), and DAPT (11.0%). In multivariable Cox regression analysis ASA classification (HR=1.74; 95% CI 1.23-2.46; p<0.01) was a significant predictor for bleeding complications.

**Discussion/Conclusion**
Use of anticoagulants after EVAR is associated with a higher risk of prosthetic limb occlusion compared to SAPT or DAPT.

# CLINICAL VALIDATION AND FEASIBILITY OF VOLUMETRIC ABSORPTIVE MICROSAMPLING FOR THERAPEUTIC DRUG MONITORING OF ORAL ANTICANCER DRUGS

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**Background** Therapeutic Drug Monitoring (TDM) is applied for oral anti-cancer drugs (OADs) to optimise treatment outcomes by measuring trough levels in plasma. Volumetric Absorptive Microsampling (VAMS) could be used to collect a small amount of capillary blood (10µL) from the finger. This sampling technique offers the advantages of being less invasive and less painful, and outlines possibilities for home-sampling. However, since VAMS collects whole blood, development of conversion methods is necessary to determine plasma concentrations. Therefore, this study aimed to establish a conversion method for six frequently used OADs in a clinical validation setting: abiraterone, alectinib, cabozantinib, imatinib, olaparib and sunitinib, and metabolites. Furthermore, feasibility of home-sampling was examined.

**Methods** VAMS samples were analyzed using a validated LC-MS/MS method. For each patient, three different samples in duplicate were collected: plasma, VAMS dipped in a tube of whole blood collected by venepuncture (VAMS tube) and VAMS collected via finger prick (VAMS finger). VAMS tube and VAMS finger were compared to determine if a difference between capillary and venous blood existed, which was defined as a difference within ± 20% for at least 67% of the patients. Two conversion methods were tested, Passing-Bablok regression (PB-reg) and conversion factor (CF), using the plasma and VAMS finger samples. For both methods, estimated plasma concentrations were derived, and the measured plasma concentrations and the estimated plasma concentrations were required to fall within ± 20% for ≥67% of the pairs for every OAD. The feasibility of home-sampling involved a visual inspection of the received VAMS samples and a questionnaire.

**Results** Capillary and venous whole blood concentrations were in accordance with each other, except for D4A (65.5% within ± 20%). At higher concentrations (> 1.5 ng/mL) D4A capillary and venous whole blood were similar (84% within ± 20%). For cabozantinib, imatinib and metabolite, olaparib, sunitinib and metabolite, both conversion methods met the acceptance criteria. Only the CF method fulfilled the requirements for D4A. For abiraterone and alectinib, both conversion methods did not met the requirements (respectively 64.5% and 60% within ± 20%). In 94% of the patients who participated in home-sampling, at least 1 VAMS sample could be used. Sampling time was correct in 63% of the patients. Patients were positive towards home-sampling.

**Discussion/Conclusion** The study successfully established the conversion methods for several OADS using VAMS. The identified discrepancies, particularly regarding the conversion of abiraterone and alectinib, emphasise the necessity for additional research. Furthermore, the feasibility of home-sampling gives promising results.

# PREDICTIONS OF IMATINIB PHARMACOKINETICS IN PREGNANT WOMEN BY PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING

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**Background**
When a pregnant woman is diagnosed with, or develops, cancer, healthcare providers are confronted with the dilemma of selecting an appropriate therapeutic approach. Yearly approximately 30 women are diagnosed with (or develop) cancer during pregnancy in the Netherlands. The overarching goal for pregnant women with cancer is to administer standard treatments to the greatest extend possible, with the primary aim to increase their chances of survival. Numerous conventional chemotherapy regimens have been established as safe for use during pregnancy. However, there is a notable shift in non-pregnant women towards incorporating targeted therapies such as tyrosine kinase inhibitors into standard treatment protocols, and may therefore regard also pregnant women. These novel treatments are frequently more effective than chemotherapy. Currently, the effective and safe utilization of those tyrosine kinase inhibitors in pregnant women, while minimizing fetal exposure, remains a critical knowledge gap. One of those tyrosine kinase inhibitors of which little is known is imatinib pharmacokinetics throughout pregnancy. The aim of this study was to develop a Physiologically Based Pharmacokinetic (PBPK) model to predict imatinib PK for second and third trimester pregnant women.

**Methods**
PBPK models were developed for non-pregnant patients and second and third trimester pregnant women. First trimester pregnant women were not invested due to an absolute contra-indication within this period. Physiological and enzymatic changes in pregnant women expected to impact imatinib PK were taken into account. Models were evaluated by comparing predictedPK profiles with in vivo PK data, both from literature as well as real world data from the UMCG. Simulations were performed to illustrate the average minimal concentration ($C_{min}$), maximum concentration ($C_{max}$) and area-under the curve (AUC) values, used as an indicator for efficacy, of imatinib achieved following administration of 400mg/day.

**Results**
PBPK models successfully predicted the PK of imatinib in non-pregnant and second and third trimester pregnant women. Predictions resulted in the lowest $C_{min,}$ $C_{max}$ and AUC in third trimester pregnant women in the third trimester namely < 1000 ng/mL), 1243 [interquartile range: 958-1545] ng/mL and 20196 [18756-35495] ng*h/mL in third-trimester pregnant populations, respectively. Those values were <1000 ng/mL, 1848 ng/mL and 23295 ng*h/mL for non-pregnant populations.

**Conclusion/discussion**
Imatinib exposure is lower in second and third trimester pregnant women than in non-pregnant women. Our next step is to develop evidence-based dosing regimens for imatinib in pregnant women and fetus, by also taking data from ex vivo placenta perfusion experiments.

# DESCRIPTION OF TACROLIMUS PHARMACOKINETICS IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS DURING THE FIRST MONTH AFTER TRANSPLANTATION

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**Background**
Tacrolimus is the cornerstone within immunosuppressive treatment protocol in most pediatric transplant centers. Regardless of dosing protocols and tight therapeutic drug monitoring, the number of children after transplantation reaching levels within the therapeutic range present high variability. The aim of this study was to describe tacrolimus whole-blood pharmacokinetics and to identify physical, clinical and laboratory parameters which are associated with whole-blood tacrolimus trough concentrations and can explain interpatient variability in children during the first month (31 days) after liver transplantation.

**Methods:** In this single-center retrospective cohort study whole blood tacrolimus trough concentrations (corrected for the dose; C/D ratios) were described in pediatric liver transplant recipients aged 0-2 years, of which a liver transplantation occurred between 2018 and 2021. Descriptive statistics and linear mixed models were used to characterize changes in tacrolimus C/D ratios in the first month after liver transplantation in children.

**Results**
The total study population included 36 pediatric liver transplant recipients (535 C/D tacrolimus trough concentrations). Albumin, bilirubin, aspartate transaminase, alanine aminotransferase and the co administration of fentanyl, fluconazole and prednisolone can explain interpatient variability ($P<0.05$).

**Discussion/ conclusion**
Tacrolimus whole-blood pharmacokinetics showed great interpatient variability in pediatric liver transplant recipients during the first month after transplantation. In general, interpatient variability can be largely explained by liver function laboratory parameters and certain concomitant medications.

# 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 could be affected. The aim of this study was to test the hypothesis that pregnancy-related changes in the PK of sensitive substrates of UGT1A1, UGT1A4 and UGT2B7 could be attributed to hormone-dependent changes in the expression/activity of the enzymes using physiologically based pharmacokinetic (PBPK) modelling of pregnant individuals (p-PBPK).

**Methods:** Estrogen- and progesterone-dependent profiles of UGT expression/activity were extracted from literature for UGT1A1, UGT1A4, and UGT2B7. PBPK models for nine sensitive substrates of UGT in healthy adults were developed or obtained from the scientific literature. The models were scaled to pregnant women accounting for anatomical, physiological, and other enzymatic changes, plus the hypothesized hormone-dependent induction of UGT expression/activity. The hypothesis was accepted or rejected based on the accuracy of the p-PBPK models for predicting PK data in pregnant women from literature, and the observed pregnant:non-pregnant ratio of PK parameters.

**Results:** The PK of sensitive substrates of UGT2B7 (namely zidovudine and indomethacin) in pregnant women was adequately predicted by implementing no hormone-dependent changes in expression/activity. For UGT1A1 and UGT1A4, while overall trends were captured, the hypothesized hormone-dependent functions over-estimated the magnitude of pregnancy-related changes in the PK of sensitive substrate drugs.

**Conclusion:** Quantitative translation of in vitro findings to understand the magnitude of induction of UGT expression/activity in pregnancy by evaluating the PK of sensitive substrates is challenging, but early patterns are emerging to support a mechanistic link with hormone-dependence.

# EVALUATION OF FLUCLOXACILLIN PHARMACOKINETICS IN PEDIATRIC AND ADULT INTENSIVE CARE PATIENTS
TITLE IN CAPITAL LETTERS

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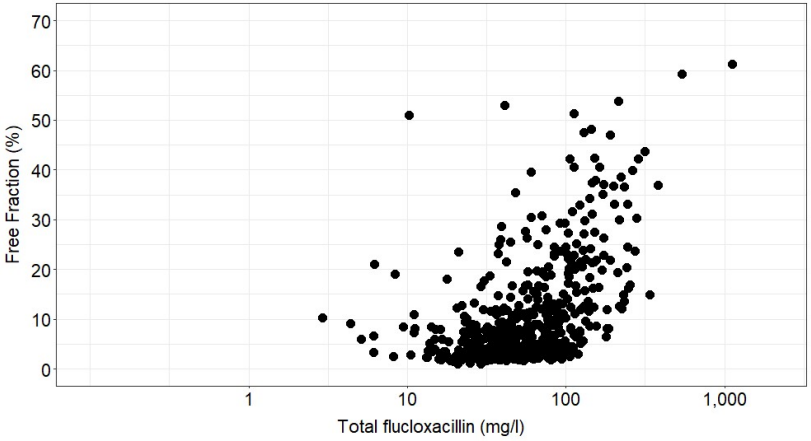
## Background
Flucloxacillin is a widely used antibiotic in critically ill patients on the Intensive Care Unit (ICU). The pharmacokinetics of flucloxacillin is complicated with multiple processes influencing the behaviour of flucloxacillin such as protein binding, renal clearance, and metabolism. These processes may change in critically illness. Understanding the magnitude of the influence these processes have on flucloxacillin pharmacokinetics is important to optimize dosing in patients. The aim of this study is to gain knowledge on the flucloxacillin pharmacokinetics in critically ill pediatric and adult patients by evaluating the behaviour of the drug.

## Methods
A retrospective study evaluating the impact of different covariates on the flucloxacillin pharmacokinetics was performed. Both non-ICU and ICU-admitted paediatric and adult patients were selected for inclusion. The significance of multiple covariates, such as weight, albumin, and creatinine concentration, to the total concentration, free concentration, and free fraction was assessed through linear mixed effects models.

## Results
In total 166 patients, consisting of 548 cycles, were included. The overall trend in the observed population can be seen in the figure. When a certain total concentration is reached, the free fraction increases considerable suggesting saturation. In adults, the creatinine concentration seems to have a significant effect on the total concentration, free concentration, and free fraction ($p < 0.05$). In pediatric patients, no specific covariate stood out to have the most impact on the flucloxacillin concentration.




## Discussion/Conclusion

Due to the limited dataset not all trends reached significance. To gain a good understanding of the impact of all covariates, including a healthy patient group is needed as a reference dataset. By broadening the inclusion of patients, the significance of the covariates can be determined with more certainty which can help with future model development for flucloxacillin.

# INVESTIGATING THE IMPACT OF EBV INFECTION ON THE PHARMACOKINETICS OF TACROLIMUS IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS

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## Background

Epstein-Barr virus (EBV) is a severe viral infection among pediatric liver transplant (LTx) recipients. EBV infection is suggested to affect tacrolimus concentrations by increasing cytokines regulating the function of cytochrome-P-450 enzymes, involved in tacrolimus metabolism [1-3]. Hence, the aim of this study is to examine the effect of EBV, expressed as both EBV-serostatus and viral load, on tacrolimus whole-blood trough concentrations corrected for the dose (concentration-to-dose [C/D] ratios).

## Methods

The study includes pediatric LTx recipients aged 0-18 years old and transplanted between January 2008 and September 2021 in the University Medical Center Groningen (UMCG). The study was conducted using two cohorts. The first cohort had a cross-sectional study design looking at the effect of EBV serostatus (EBV- vs EBV+ on tacrolimus C/D ratios). The second cohort had a longitudinal study design looking at the effect of both EBV serostatus and EBV viral load on tacrolimus C/D ratios between and within pediatric LTx recipients. Descriptive statistics and linear mixed models were used to characterize changes in tacrolimus C/D ratios with EBV-serostatus and EBV viral load.

## Results

The total study population included 89 pediatric LTx recipients (42 in the first cohort, 47 in the second cohort). No statistically significant effect of EBV infection for both serostatus and viral load on the tacrolimus C/D ratio ($P>0.005$) was observed.

## Discussion/Conclusion

The findings of this study suggest that there are no differences in the C/D ratio of tacrolimus between EBV- and EBV+ states within and between pediatric LTx recipients, as well as among different EBV viral loads. For clinical practice this implies that there would be no need to adapt dosages when there is A EBV infection.

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# REPLACING A RADIOACTIVE WITH A NON-RADIOACTIVE MEASUREMENT METHOD TO RELIABLY MEASURE KIDNEY FUNCTION

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## Background

Accurate assessment of kidney function by measuring glomerular filtration rate (mGFR) is crucial for diagnosing chronic kidney disease, determining kidney donation eligibility, adjusting drug doses, and evaluating the renal hemodynamic profile of drugs. The current method ("warm method") of determining the mGFR and effective renal plasma flow (ERPF) is unfavorable due to the radioactive burden it places on patients [1, 2]. In this study we tried to determine whether mGFR can be accurately assessed by measuring non-radioactive iothalamate and hippuran ("cold method").

## Methods

The $^{125}$I- and $^{131}$I- radioactivity levels were measured in serum, urine, and infusion solution, of 166 patients, using a gamma counter. These samples were re-analyzed for iothalamate and hippuran concentration using a validated LC-MS/MS method. The mGFR and ERPF, determined using both methods, were compared for each patient. Blant-Altman plots and Passing-Bablok regression were used to determine bias (unbiased when 95% CI included 0 and 1 for intercept and slope respectively). We also determined the accuracy of the corrected mGFR, and this was considered accurate when ≥80% of measurements were within 30% ($P_{30}$) and ≥50% were within 10% ($P_{10}$).

## Results

Bland-Altman analysis showed a -5.5% mean bias (95% CI -7.26, -3.80) and an absolute mean difference of -4.6 ml/min (95% CI -6.01, -3.31) in corrected mGFR when comparing the methods. Passing-Bablok regression revealed Y=0.95x-0.92 (slope 95% CI: 0.91, 1.00; Y-intercept 95% CI: −4.54, 2.13). The $P_{10}$ was 63% and the $P_{30}$ was 98%. For the ERPF the Bland-Altman analysis showed a +14.5% mean bias (95% CI 12.8, 16.2) and an absolute mean difference of +47.0 ml/min (95% CI 41.4, 52.7). Passing-Bablok regression revealed Y=1.26x−27.2 (slope 95% CI: 1.22, 1.31; Y-intercept 95% CI: -38.3, -16.6).

## Discussion/Conclusion

We compared the results of the "warm" and "cold" methods to determine kidney function. The $ERPF_{cold}$ showed a systematic and proportional overestimation in comparison to the $ERPF_{warm}$. This is probably due to a limitation of the warm method, which is reliant on the radiochemical purity of $^{131}$I-hippuran when measuring radiation of $^{131}$I (free $^{131}$I and $^{131}$I-hippuran), as opposed to the cold method which specifically measures hippuran [3]. No major differences were seen between $mGFR_{cold}$ and $mGFR_{warm}$. These results indicate that mGFR can be assessed from trace amounts of non-radioactive iothalamate and hippuran.

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# A POPULATION PHARMACOKINETIC STUDY TO EVALUATE DOXORUBICIN EXPOSURE ACROSS ALL AGE RANGES.

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## Background

Doxorubicin is an important compound in the treatment of solid tumours and haematological malignancies in children and adults. Despite decades of use, the effect of age on doxorubicin pharmacokinetics (PK) remains inconclusive. Also, limited data are available on patients <3 years and >70 years old. Therefore, in this study we combined PK data from infant, childhood, adults and elderly patients to develop a population PK model to further investigate the impact of age on the PK of doxorubicin across all ages.

## Methods

Data generated from prospective studies were combined to derive PK data from infants (<3 years), children (≥3 and <18 years), adults (≥18 and <70 years), and elderly (≥70 years).

For the initial model development, a three-compartment model with first order elimination was used. Firstly, several methods for body size scaling were tested, which included: fixed allometric scaling using body weight (BW), and scaling for body surface area or lean body weight using a power function with estimated exponents. Secondly, to investigate the effect of maturation in infants and children, a maturation function (Eq.1) was added on CL and on the volume of distribution of the central compartment (V1) and the two peripheral compartments (V2 and V3). Thirdly, ageing is associated with a decline in organ function and change in body composition. So, to investigate the effect of older age, an $E_{max}$ function was added (Eq.2) to CL, V1, V2, and V3.

$$F_{mat} = \frac{(\text{Age in weeks})_i^{\text{HILL}}}{(\text{Age in weeks})_i^{\text{HILL}} + \text{TM}_{50}^{\text{HILL}}} \quad (1)$$

$$P_i = P_{pop} * \left(1 - \left(\frac{\text{Age in years}}{\text{Age in years} + \text{TM}_{50}}\right)\right) \quad (2)$$

## Results

A total of 168 patients (30 infants, 33 children, 48 adults and 57 elderly) treated with doxorubicin were included in this analysis with a median age of 53.2 years (range 0.11 – 90 years) and 555 doxorubicin samples.

For body size scaling, BW with allometric scaling provided the best predictive model. Covariate analyses of the maturation function showed an effect on V1 and V2, whereas adding the $E_{max}$ function only had an effect on V3. Therefore, in the final model, age was added as a maturation function on V1 and V2 and $E_{max}$ function on V3. Based on the final model, typical CL was estimated 9.8 L/h for infants, 30.7 L/h for children, 48.2 L/h for adults and 53.4 L/h for elderly. This was not clinical relevant for the exposure of doxorubicin.

## Discussion/Conclusion

In summary, age had an effect on the volume of distribution (the central compartment and the two peripheral compartment), but had no statistically effect on the PK of doxorubicin. So, we recommend to keep the same dosing approach in patients, despite their age.

# ASSESSING ACCURACY OF CHATGPT IN RESPONSE TO QUESTIONS FROM DAY TO DAY PHARMACEUTICAL CARE IN HOSPITALS

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**Background**

The advent of Large Language Models (LLMs) such as ChatGPT introduces opportunities within the medical field. Nonetheless, use of LLM poses a risk when healthcare practitioners and patients present clinical questions to these programs without a comprehensive understanding of its suitability for clinical contexts. The objective of this study was to assess ChatGPT's ability to generate appropriate responses to clinical questions that hospital pharmacists could encounter during routine patient care.

**Methods**

Thirty questions from 10 different domains within clinical pharmacy were collected during routine patient care and after peer review presented to ChatGPT in a standardized format. For one question per domain, the effect of modifying the question on response was examined by introducing variations. Personification was changed, the questions were presented in Dutch, and the primary questions were regenerated. All responses from ChatGPT were independently evaluated by two senior hospital pharmacists, focusing on: the availability of an advice, accuracy and concordance.

**Results**

ChatGPT provided an advice in response to the 77% of the question. Accuracy was correct and complete for 26%, correct but incomplete for 22%, partially correct and partially incorrect for 30% and completely incorrect for 22% of the responses. All responses were considered concordant by the expert panel. The reproducibility was poor, with merely 10% remaining consistent upon regeneration of the primary question.

**Discussion/Conclusion**

While concordance of responses was excellent, the accuracy and reproducibility were poor. In its current form, ChatGPT should not be used to address questions encountered by hospital pharmacists during their shifts. The limited performance poses a safety risk rather than enhancing patient safety.

# A COMPLEX, INDIVIDUALIZED, ADHERENCE-IMPROVING PHARMACIST INTERVENTION TO REDUCE HYPERPHOSPHATEMIA IN HEMODIALYSIS PATIENTS

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**Background**
**P**hosphate-binding medication (PBM) demonstrates limited efficacy in reducing hyperphosphatemia in nearly 50% of hemodialysis patients, primarily due to suboptimal adherence. This suboptimal adherence can be attributed to barriers such as forgetfulness, complex treatment regimens, and a high pill burden. There is substantial interindividual variation in encountered barriers to adherence to PBM. However, a higher PBM pill burden is generally associated with lower adherence. Therefore we hypothesize that a reduction in PBM pill burden, combined with a complex, individualized, pharmacist intervention to address barriers to adherence, will enhance adherence to PBM and reduce hyperphosphatemia.

**Methods**
This was a prospective, pharmacist intervention study in 75 hemodialysis patients with hyperphosphatemia (>1.50 mmol/l) and a high pill burden of PBM (≥6 tablets sevelamer a day or equivalent) in the Franciscus Gasthuis and Vlietland Hospital in Rotterdam. The complex, individualized, adherence-improving intervention consisted of 3 pharmacist consultations with the patient at baseline, at 1-2 weeks, and at 3 months. At baseline, the Quick Barrier Scan (QBS, to investigate barriers to adherence), and MARS-5 (patient-reported adherence) were administered. At 1-2 weeks, results from the QBS and a PBM dose reduction were discussed. At 3 months, the intervention was evaluated and MARS-5 was repeated. The primary outcome parameter was the mean phosphate concentration in the 3 months after the start of the intervention versus the 3 months before. Secondary outcome parameters were PBM pill burden and patient-reported adherence at baseline and after 3 months. Data were statistically analyzed with SPSS version 28.0 (paired T-test for phosphate concentrations and PBM pill burden, Wilcoxon signed-rank test for patient-reported adherence).

**Results**
The mean (±SD) phosphate concentration was 1.99±0.34 mmol/L before versus 2.03±0.37 mmol/L after the start of the intervention ($p=0.268$). The mean PBM pill burden decreased from 8.5±3.0 to 5.6±2.6 units ($p<0.001$). Patient-reported adherence increased, although median adherence did not change (median 24, IQR 22-25 before vs IQR 23.25-25 after, $p=0.008$).

**Discussion/Conclusion**
Contrary to our hypothesis, the intervention did not reduce hyperphosphatemia. However, a major reduction in PBM pill burden as well as an increase in medication adherence were achieved. The intervention may improve clinical effectiveness and cost-effectiveness of PBM, but more prospective, controlled studies are needed to confirm these results.

# GENOTYPING OF ESKETAMINE DRUG TRANSPORTERS AND METABOLIZER IN PATIENTS WITH TREATMENT-RESISTANT DEPRESSION

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**Background:** Oral esketamine is currently investigated as an add-on treatment for treatment-resistant depression (TRD). Polymorphisms regulating the expression of CYP3A4, ABCB1 (PGP) and ABCG2 (BCRP) represent important genetic markers that might influence the pharmacodynamic (PD) and -kinetic (PK) aspects of oral esketamine as it is largely metabolized by enzymes like CYP3A4, while ABCB1 and ABCG2 are important drug efflux transporters. An analysis of the mutations of these biomarkers, shown here as minor allele frequency (MAF), has yet to be performed in a TRD patient cohort as compared to a healthy cohort.

**Methods:** 59 patients with TRD included in a study comparing oral esketamine to placebo were genotyped with regards to CYP3A4 (15389C>T), ABCB1 (3435C>T), ABCG2 (421C>A) and their variants. Statistical analysis of each variant was performed to determine the MAF of each polymorphism and was compared to a healthy population (n=527).

**Results:** CYP3A4 genotyping in the patient cohort was 52 (91.1%) for the common C/C homozygous wildtype variant, followed by 4 (7.0%) C/T heterozygous and only 1 (7.0%) volunteer with a (T/T) homozygous variant. Similar rarity of homozygous variance was found in ABCG2 (A/A n=0, C/A n=8 (13.6%), C/C=51(86.4%)) while ABCB1 showcased a more even distribution among its genotypes (T/T n=23 (39.7%), C/T n=25(43.1%), C/C n=10(17.2%)). Analysis for all three markers showcase a MAF for CYP3A4*22, ABCG2 and ABCB1 of 5.3%, 6.7% and 60.2% respectively. Comparing these to our larger reference population of 527 volunteers with corresponding MAF for CYP3A4, ABCG2 and ABCB1 of 6.3%, 12.4% and 50.2%.

**Discussion/Conclusion:** Comparisons of all three polymorphisms on their respective genotype MAF show no significant differences between patients with TRD and the healthy population. Published literature showcases similar MAF for CYP3A4 of 5.0% as our patient cohort, while ABCG2 and ABCB1 also show similar MAF of 14% and 52% respectively [1][2][3]. Based on these findings it could be argued that therapy resistance in TRD patients might be independent from these selected polymorphisms. Though for now no conclusions can be drawn of the effect of these different polymorphism configurations on esketamine PK or PD without additional data from the patient cohort.

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# PHARMACOGENETICS OF ANTIPSYCHOTIC INDUCED CORRECTED QT (QTC) INTERVAL PROLONGATION: A SYSTEMATIC REVIEW

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**Background** Psychotic disorders are associated with a large personal burden as well as social-economically, and are often treated with antipsychotics. The use of antipsychotics is frequently accompanied by adverse effects, contributing to adherence issues. Among these adverse effects, cardiac complications, such as QTc interval prolongation, are particularly concerning as they may lead to the so-called arrhythmia Torsade de Pointes and sudden cardiac death. The variability in the inter-individual response to antipsychotics may be partially attributed to genetic differences in drug metabolism, a field known as pharmacogenetics. The application of pharmacogenetics may be crucial for assessing the risk of antipsychotic-induced QTc prolongation.

**Methods** This study systematically evaluated literature to identify studies measuring antipsychotic-induced QTc prolongation prospectively while evaluating the influence of genetic variants. A comprehensive search in MEDLINE, PsycINFO, and Embase yielded N=12 eligible studies, including ten candidate gene studies, two genome-wide association studies (GWAS), and one study that combined both approaches.

**Results** Thirty-six genes were found to be significantly associated with antipsychotic-induced QTc prolongation, with eight identified through candidate gene studies and 30 through GWAS. Notably, only two genes (*CACNA1C* and *NUBPL*) were independently reported by different studies. Candidate gene analyses revealed significant associations with *CYP2D6, KCNQ1, EPB41L4A, LEP, NOS1AP, NUBPL, CACNA1C*, and *KCNH2*. However, some candidate gene studies failed to replicate earlier findings or did not yield significant results. The majority of studies (N=7) focused on patients with psychotic disorders, while others included healthy individuals or non-psychotic patients, with varying results. Discrepancies in sample sizes and the spectrum of investigated genes and alleles likely contributed to the heterogeneity in findings.

**Discussion/Conclusion** While our results suggest the involvement of several genes in antipsychotic-induced QTc prolongation, further research, particularly through well-powered GWAS, is needed to elucidate specific genetic variants' roles in cardiac risks associated with antipsychotic use. Identification of these variants may facilitate personalized pharmacotherapy by mitigating potentially harmful side effects, thus improving adherence and treatment efficacy.

# DISCREPANCY BETWEEN HOME MEDICATION AND MEDICATION AT INTENSIVE CARE DISCHARGE IN PATIENTS SURVIVING A PERIOD OF SEVERE ACUTE ILLNESS

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**Background**
During a period of acute illness requiring intensive care unit (ICU) admission, a substantial portion of home medication is discontinued. Part of this stopped medication needs to be reintroduced before ICU discharge. If indicated and not reintroduced, this could have significant consequences. The aim of this study was to gain insight into the discrepancy between home medication and medication at ICU discharge.

**Methods**
In this retrospective cohort study, the medical record of patients admitted to the ICU of a non-university teaching hospital between January 2020 and September 2022 were studied. We aimed for inclusion of 200 patients. Inclusion criteria: ICU admission ≥48 hours, and alive at discharge from the ICU (without palliative care), and with registered home medication at hospital admission. Exclusion criteria: previous ICU stay during same hospital admission, transfer from and to another hospital and patients on chronic ventilation with an elective ICU admission. Medication was classified according to ATC-classification.

**Results**
We included 200 patients (mean age 63.5 years, 63.0% male, surgical admission 24.0%). 49.0% of patients were ventilated and 52.0% had no additional nutrition. Mean APACHE4 score was 68.4 and mean maximum SOFA score was 5.4. Median length of ICU stay was 93.3 hours (IQR 69.4-141.7). Mean number of home medication on admission and at discharge ICU were 5.0 and 2.7, respectively. Table 1 shows percentages of most frequent stopped home medication. Of all stopped home medication at ICU discharge, 22.4% was incorrectly not reintroduced.

| Table 1 | Home | Discharge ICU | % stopped |
|---|---|---|---|
| Antihypertensives | 238 | 86 | 63,9% |
| Lipid modifying agents | 100 | 74 | 26,0% |
| Antithrombotic agents | 92 | 59 | 35,9% |
| Antidiabetics | 86 | 30 | 65,1% |
| Proton pump inhibitors | 83 | 77 | 7,2% |
| Vitamins/minerals | 64 | 29 | 54,7% |
| Psycholeptics | 53 | 26 | 50,9% |
| Antidepressants | 34 | 23 | 32,4% |
| Drugs for obstructive airway | 34 | 18 | 47,1% |
| Analgesics | 23 | 14 | 39,1% |
| Drugs for constipation | 22 | 17 | 22.7% |
| Total | 829 | 453 | 45,4% |

**Discussion/Conclusion**
Almost half of used home medication was stopped during a period of acute illness requiring ICU admission. For several home medication, failure to reintroduce could lead to significant consequences for patients and should be subject of future research.

# IMPROVED FERTILITY IN WOMEN WITH RHEUMATOID ARTHRITIS AND A WISH TO CONCEIVE WHEN TREATED ACCORDING TO A TREAT TO TARGET APPROACH AIMED AT REMISSION.

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## Background

The duration of time required for conception, known as Time to Pregnancy (TTP), is prolonged in women diagnosed with rheumatoid arthritis (RA). RA-related factors that influence TTP were previously studied in the Pregnancy-induced Amelioration of RA (PARA) cohort, a cohort in the years 2002 - 2010. Those factors include high disease activity, daily nonsteroidal anti-inflammatory drug (NSAID) use and daily prednisone intake exceeding 7.5 mg. In the Preconception Counseling in Active RA (PreCARA) study, women who wish to conceive were followed in a clinical pathway with counseling and treatment according to a treat-to-target (T2T) approach, aimed at remission, but avoiding the use of NSAIDs and high those prednisone. To achieve this, sulfasalazine and hydroxychloroquine were prescribed and if needed, TNF-inhibitors (TNFi) or low dose prednisone was added.
Our aim is to investigate whether a T2T strategy, including TNFi use, in women RA in the PreCARA cohort is associated with a shorter TTP compared to women with RA the historical PARA cohort.

## Methods

In both cohorts, women were included before conception or during the first trimester of pregnancy. TTP was computed by measuring the interval between unprotected sexual intercourse and the onset of the last menstrual period. A comparative analysis of fertility outcomes between the PreCARA and PARA cohorts was conducted.

## Results

A total of 215 patients were included in the PreCARA study, while the former PARA study included 245 patients. In the PreCARA study, 3% of the patients did not take any medication during the preconception period, compared to 36% in the PARA study. During the preconception period, NSAIDs use was observed in 13% of PreCARA patients and 24% of PARA patients. In the PreCARA study, 23 of 96 (23%) patients that used prednisone, took daily doses over 7.5 mg, compared to 41 out of 85 (48%) PARA patients. In the PreCARA study, 53% of the patients used TNFi during the preconception period compared to only 9 patients (3%) in the PARA study. In the PreCARA study, the median TTP is 84 days in patients who got pregnant, in contrast to 196 days observed in the PARA study.

## Conclusion

TTP was shorter in PreCARA patients compared to PARA patients. This indicates that fertility is increased in women with RA when treated according to a T2T strategy, whilst avoiding NSAIDs and high doses prednisone.

# INTRAPERITONEAL PHARMACOKINETICS OF SYSTEMIC OXALIPLATIN, 5-FLUOROURACIL AND BEVACIZUMAB IN PATIENTS WITH COLORECTAL PERITONEAL METASTASES

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**Background:** Peritoneal metastases (PM) commonly occur in colorectal cancer patients. Systemic chemotherapy yields poor outcomes for these patients. It is hypothesized that traditional systemic chemotherapy is not very effective for this patient population. This study investigates to what extent the systemic anti-cancer therapy crosses the peritoneal barrier.

**Methods**: In a Phase I study, eighteen patients received systemic oxaliplatin, 5-FU, and bevacizumab. Plasma and peritoneal fluid samples were collected to measure drug concentrations. A non-compartmental analysis determined the Area Under the Curve (AUC) for oxaliplatin and 5-FU in both matrices. Intraperitoneal (IP) and intravenous (IV) exposure ratios were calculated, along with the bevacizumab concentration IP/IV ratio. The relationship between tumour load and IP/IV ratios, and the correlation between the IP/IV ratios of different treatments were assessed statistically.

**Results:** A total of 438 5-FU samples and 578 oxaliplatin samples were analysed in plasma and peritoneal fluid. Bevacizumab was quantified with 17 measurements in plasma and 15 measurements IP. Median IP/IV ratios were 0.143, 0.352 and 0.085 for 5-FU, oxaliplatin and bevacizumab, respectively. Oxaliplatin exhibited a longer IP half-life than 5-FU. A correlation was found between oxaliplatin and bevacizumab IP/IV ratios ($R=0.69$, $p=0.01$). No statistical correlations were found between the other investigated drugs.

**Conclusion/Discussion:** Our findings indicate that only a small percentage of systemically administered anti-cancer treatment reaches the IP cavity, questioning their efficacy against PM. This strengthens the hypothesis for repeated intraperitoneal chemotherapy to reach adequate anti-cancer drug levels.

# SAFETY AND PRELIMINARY PROTECTIVE EFFICACY OF IMMUNISATION WITH GENETICALLY ATTENUATED *PF*Δ*MEI2* (GA2) MALARIA PARASITES IN HEALTHY DUTCH VOLUNTEERS.


## Authors

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## Background

The recent malaria resurgence highlights the need for an effective vaccine now more than ever. Live attenuated sporozoites induce >90% protection in clinical trials. Strategic genetic attenuation (GA) combines biosafety with potentially high efficacy. We therefore created a late-arresting *Plasmodium falciparum* (GA2: *Pf*NF54Δ*mei2*) that aborts development at the time of transitioning to the blood stage, after 6-7 days of liver stage.

## Methods

We conducted a phase 1/2a partially double-blind placebo-controlled clinical trial, assessing the safety, tolerability and preliminary protective efficacy of GA2 delivered by mosquito bite (MB).
After a dose-escalation stage in which participants were exposed once to 15 or 50 GA2-MB, exposure of GA2-MB was compared to its predecessor GA1-MB (arresting at early liver stage) and infectivity controls. Participants were exposed to 50 GA2-MB, GA1-MB or uninfected MB three times, each at 28-day intervals. Three weeks later, all participants underwent controlled human malaria infection (CHMI) by means of 5 MB infected with the unattenuated homologous *Pf* isolate (3D7).

## Results

The GA2 parasite was found to be safe and well-tolerated with no breakthrough infections at the tested doses.
Immunisation with GA2 resulted in 89% (8/9) protection, whereas only 13% (1/8) of GA1-immunized participants and none (0/3) of the infectivity controls were protected.
GA2- and GA1-immunised participants exhibited a significant increase in anti-*Pf* circumsporozoite protein (CSP) antibodies compared to baseline, yet titres did not differ between experimental groups and between protected and non-protected individuals. In contrast, we did not detect significant antibody responses against apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1), two antigens expressed predominantly during the parasite blood-stage.

## Discussion/Conclusion

Our results provide the pivotal proof-of-concept that longer liver stage development of attenuated whole sporozoites results in significantly enhanced protection against CHMI and warrant further investigation of T cell mediated immunity and evaluation of GA2 as a promising *Pf* vaccine candidate.

# BEING BORN SMALL FOR GESTATIONAL AGE HAS IMPORTANT INFLUENCE ON VANCOMYIN CLEARANCE AND REQUIRES ADAPTED DOSING

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**Background** The pharmacokinetics (PK) of drugs in neonates vary widely due to maturation with gestational age (GA), birthweight (bBW), and postnatal age (PNA). Newborns that are born small for gestational age (SGA) have been largely ignored in PK studies. We aim to determine the influence of being born SGA on the clearance (CL) of vancomycin in neonates and quantify its influence on exposure when dosing vancomycin according to two guidelines.

**Methods** For vancomycin, previously published data from 437 (pre)term neonates with a median (range) GA of 30 (23-41) weeks, PNA of 12 (1-31) days, bBW of 1310 (385-4680) g, and current bodyweight (cBW) of 1375 (415-4860) g were available[1,2]. Using Fenton growth charts, 100 (22.9%) neonates were identified as SGA (i.e., bBW < 10th percentile of the bBW for their GA). In the overall population, the median Zscore for bBW for GA was -0.3 (-3.3 – 3.8). We used a previously published two compartment model[3], in which a covariate analysis was performed studying the influence of SGA and Z-score, in addition to a model with PNA and either bBW or GA on CL. The dosing guideline of the Dutch Pediatric Formulary and a previously developed model-based dosing guideline[3] were evaluated for their performance.

**Results** SGA was a significant covariate for vancomycin CL with its influence depending on the other covariates in the model. In a model with bBW and PNA, CL was 30% higher in SGA neonates compared to AGA neonates ($p<0.001$). In a model with GA and PNA, vancomycin CL was 28% lower ($p<0.001$) in SGA vs AGA neonates. Overall, the model with bBW, PNA, and SGA described the data better than the model with GA, PNA, and SGA ($p<0.01$) and was therefore selected for the simulations. When neonates are dosed according to the Dutch Pediatric Formulary, the AUC is generally too low (< 400 mg*h/L), particularly in SGA neonates. When vancomycin is dosed according to the model-based dosing guideline[3], the calculated AUC is within the target range (400-600 mg*h/L) for most neonates, except for SGA neonates in the lowest weight quartile (median cBW of 800 g).

**Discussion/Conclusion** Being born SGA has a significant impact on vancomycin clearance. For both dosing guidelines, SGA neonates show lower vancomycin concentrations vs AGA neonates of the same cBW, with the ultimate influence being dependent on bBW and PNA of the individual. The previously published model-based dosing[3], results in more appropriate AUC in both AGA and SGA neonates.

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# SAFE TREATMENT WITH PEMETREXED IN LUNG CANCER PATIENTS WITH RENAL IMPAIRMENT USING FOLINIC ACID RESCUE: RESULTS OF THE IMPROVE-I CLINICAL STUDY

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**Introduction:** Pemetrexed is a cornerstone in the treatment of non-small cell lung cancer (NSCLC), as a part of first- and second-line chemo(immuno)therapy. Renal impairment is a common comorbidity in NSCLC patients. Approximately 30% of patients have a creatinine clearance in which pemetrexed is currently contraindicated, corresponding to an estimated 1000 patients yearly in the Netherlands who are withheld pemetrexed treatment. Because toxicity is driven by a time-above-threshold concentration [1], an innovative strategy is needed to enable therapeutic pemetrexed exposure while minimizing the risk of (hemato)toxicity in patients with renal impairment.

**Methods:** We performed a 3+3 intra-patient pemetrexed dose escalation study in patients with eGFR <45 mL/min. Pemetrexed was dosed on eGFR to stepwise reach a target exposure of 164 mg*h/L (therapeutic exposure in patients with normal renal function). This dose was considered 100% [2]. Additionally, folinic acid prophylaxis for toxicity was administered as 45 mg Q6H from day 2-15 of the 21-day cycle. The primary endpoints of this study were safety and pharmacokinetics, in line with regulatory guidelines.

**Results:** Six patients were included with an eGFR varying between 26-41 mL/min. The first cohort was successfully escalated to 100% pemetrexed dose to reach target exposure. Median exposure at the final dose was 176 mg*h/L (range 136-193 mg*h/L) and, thus, all patients the predefined target AUC (164 mg*/L ±25%). All patients completed at least four cycles of treatment and treatment was generally well-tolerated. Grade III/IV neutropenia, anemia and thrombocytopenia occurred in 3/24, 2/24 and 3/24 cycles, respectively. Grade I/II anemia was very common (15/24 cycles). However, 5 out of 6 patients already had grade I/II anemia at baseline. Treatment delay occurred over 4 cycles, divided over 3 patients. This was due to pancytopenia in (2 cycles,1 patient), elevated serum creatinine (1 cycle, 1 patient) and thrombocytopenia (1 cycle, 1 patient). After 4 cycles, treatments responses were as follows; 1 complete and 2 partial responses, 1 stable disease and 2 progressive disease.

**Conclusion:** The safety profile matched that of patients with normal renal function. With this study we showed that folinic acid rescue therapy in combination with renal function-based dosing to reach target exposure, enables safe pemetrexed treatment in patients with renal impairment. As our results comply with regulatory guidance, our strategy can be directly implemented in the clinic.

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# ADRB2 GENOTYPE GUIDED TREATMENT FOR CHILDHOOD ASTHMA: AN INDIVIDUAL PATIENT DATA META-ANALYSIS OF THE PACT AND PUFFIN TRIAL

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**Background**

Long-acting beta2-agonists (LABA) are commonly used to treat asthma, however, some children do not respond to LABA. This might be due to variation in the A allele of rs1042713 (Arg16 amino acid) in the ADRB2 gene encoding the beta2 receptor. We investigated whether Arg16Gly genotype-guided treatment improved asthma-related outcomes.

**Methods**

We recruited Dutch and Swiss 6-18-year-old children (PUFFIN) and 12-18 year olds from England and Scotland (PACT) with uncontrolled asthma despite inhaled corticosteroids (ICS) and performed an individual patient data meta-analysis of the two randomised controlled trials. Patients requiring step-up were randomised to genotype-guided treatment or standard care with a follow-up of at least 6 months. Genotype-guided treatment consisted of adding LABA (Gly16Gly) or montelukast (Arg16Arg/Arg16Gly; PACT), or adding LABA (Gly16Gly) or doubling ICS (Arg16Arg/Arg16Gly; PUFFIN). The primary outcome was change in asthma control. Secondary outcomes were change in exacerbation rate and time to exacerbation. Repeated measures mixed models and cox regression were used for statistical analyses.

**Results**

59 out of 102 (PUFFIN) and 59 out of 91 (PACT) children had at least one A allele. These 193 children were included. There was no difference in asthma control between both arms. Genotype-guided treatment resulted in lower asthma exacerbation rates (-0.075 (95%CI -0.15 - -0.00, p=0.050)) and a trend towards longer time to exacerbation (HR 0.66 (95%CI 0.41-1.06, p=0.085)) compared to standard care.

**Discussion/Conclusion**

Genotype-guided step-up treatment for children with uncontrolled asthma on ICS may lower asthma exacerbation rates.

# MEDICATION ADHERENCE DO DIRECT ORAL ANTICOAGULANTS: EXTENT AND IMPACT OF SIDE EFFECTS

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**Background**

Arterial and venous thromboembolism are a leading cause of mortality. Direct oral anticoagulants (DOACs) are highly effective in both stroke prevention and prevention of venous thrombotic events. Medication adherence is a prerequisite for optimal protection against thromboembolic complications. Recent studies have shown that good adherence cannot be taken for granted for DOACs. In this cross-sectional study adherence among DOAC users was investigated and associations between beliefs about medication, perceived side effects and adherence were explored.

**Methods**

We included 100 randomly selected adult DOAC users visiting one of the two participating Dutch community pharmacies in the summer of 2020.

The self-reported adherence (primary outcome) was assessed with the Medication Adherence Rating Scale-5 (MARS-5) using three different cut-off scores. Beliefs about DOACs were assessed with the Beliefs about Medicine Questionnaire Specific (BMQ-S), while side effects and side effect burden were assessed with a self-developed questionnaire based on the Lareb Intensive Monitoring (LIM) system.

**Results**

Of the participants, 9% reported non-adherence on the primary MARS-5 cut-off score <24. For the MARS-5 scores <23 and <25 non-adherence percentages of, respectively, 3 and 33% were calculated. Associations were found between adherence and both side effects and side effect burden, regardless of the MARS-5 cut-off score. Bruising and minor bleeds were the most reported side effects (both 20%). For all patients the necessity beliefs outweighed the concern beliefs. No associations were found between adherence and either gender, indication, DOAC or dosage.

**Discussion/Conclusion**

This study confirms that adherence in patients on DOACs cannot be taken for granted. High necessity beliefs do not guarantee good adherence, as side effects impair adherence even in patients having high necessity beliefs. Therefore we recommend that both physicians and pharmacists evaluate both adherence and side effects with their patients using DOACs on a regular base.

# The CHAPATI feasibility study: a pharmacokinetically guided dose-escalation study of vincristine in Kenyan children with cancer.

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**Background** Vincristine-induced peripheral neuropathy (VIPN) is very rare in Kenyan children with cancer. Black children more often express cytochrome P450 (CYP) 3A5, which metabolizes vincristine more efficiently than CYP3A4, leading to lower blood concentrations. This has been associated with both a reduced VIPN risk but also with higher relapse rates. We performed a pharmacokinetically guided dose-escalation study of vincristine in Kenyan children with cancer. Here we report the results of the feasibility study.

**Methods** Eligible patients were between 5-14 years old and treated at a tertiary referral center. Exclusion criteria were severe malnourishment, hyperbilirubinemia, pre-existent mental retardation and VIPN. Plasma samples were collected at 1, 1.5 and 4 hours after i.v. push vincristine administration and shipped to the Netherlands for analysis with liquid chromatography-mass spectrometry (LC-MS/MS). Vincristine exposure was assessed with a previously developed pharmacometric nomogram. A 20% dose increase was recommended for participants with low exposure and no VIPN, hyperbilirubinemia and severe malnourishment.

**Results** Ten out of fifteen participants were diagnosed with acute lymphoblastic leukemia (ALL). The median age was 9.3 years. Low exposure to vincristine was observed in one participant and a dose increase was implemented. Vincristine exposure was significantly higher in the Kenyan participants than in the European patients included in the pharmacometric nomogram: median plasma concentrations (ng/ml) and interquartile range (IQR) on time 1: Europeans = 3.89 (5.96), Kenyans = 9.81 (8.19), p-value<0.001, time 2: Europeans = 2.37 (0.95), Kenyans = 8.67 (6.15), p-value<0.001, and time 3: Europeans = 1.99 (3.07), Kenyans = 12.4 (9.41), p-value<0.001). None of the participants developed VIPN.

**Discussion/Conclusion** Kenyan participants had high exposure to vincristine but did not develop VIPN. A PK explanation for the lack of VIPN in this population seems unlikely. Considering the very low incidence of VIPN in this population, conventional dose-escalation based on toxicity is an option that must be considered.

# REAL WORLD PHARMACOKINETICS OF TEZACAFTOR IN CHILDREN WITH CYSTIC FIBROSIS USING TEZACAFTOR-IVACAFTOR: THE SYM-CF STUDY

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**Introduction -** The clinical efficacy of the cystic fibrosis transmembrane conductance regulator (CFTR) combination drug, tezacaftor-ivacaftor, is variable. Some children with cystic fibrosis (cwCF) respond, while others do not or have side effects. The inter-individual variability (IIV) seems large and therefore we hypothesize that specific groups of cwCF are over- or undertreated. Very little is known about the pharmacokinetics (PK) of these drugs, especially in the pediatric population. Understanding of the PK of tezacaftor may provide more insight into the exposure-response relationships and its IIV. The aim of this study was to evaluate the PK profile of tezacaftor and assess the area under the curve (AUC) in cwCF in a real world setting.

**Methods -** In this prospective, multi-center, observational with interventional sampling PK study, 21 cwCF aged 6-17 years using tezacaftor-ivacaftor as chronic CF treatment were included between May 2021 and August 2022. PK samples were taken at T=0, 4 and 8 hours after administration of tezacaftor-ivacaftor using dried blood spot (DBS) sampling at home. During regular outpatient hospital visits patients were sampled at random time points with DBS or venous sampling. The gathered data were used for external validation of population PK models available in the registration documents. In addition, a new population PK model was developed using nonlinear mixed-effects modelling.

**Results -** The two models described in the registration documents (sponsor and reviewer model) did not properly describe the PK of tezacaftor in our cohort, with mean prediction errors of 61.5 (95%CI -96.1 – 219.2) and -44.5% (95%CI -116.8 - 27.9), respectively. Thus, a novel population PK model was developed. The PK of tezacaftor, and its main metabolite tezacaftor-M1, were best described by 1-compartment models with first-order absorption and elimination. Bodyweight-based allometric scaling was applied with fixed exponents of 0.75 and 1.00 on CL/F and V/F, respectively. Mean (SD) calculated tezacaftor AUCs were compared with mean AUCs found in the product information: 67.4 (11.4) vs 97.1 (35.8), 91.5 (24.1) vs 107 (30.1) and 57.8 (11.8) vs 58.9 (17.5) mg·h/L for the different age and weight groups 12-17yo, 6-11yo ≥30kg and 6-11yo <30kg, respectively.

**Conclusions -** The predictive performance of the population PK models found in the registration documents poorly described our pediatric data, with significant over- and under prediction. Hence, a novel population PK model with real world data in cwCF was developed. Especially in adolescents (12-17yo) we found lower mean AUCs in our population compared to the reference AUCs. More research on the exposure-response relationship is needed to identify patients are possibly over- or undertreated.

# FENTANYL ROTATION FROM SUBCUTANEOUS TO TRANSDERMAL ADMINISTRATION: A VALIDATION OF CURRENT PRACTICE

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**Background:** Fentanyl is a potent opioid that is widely used to treat cancer-related pain. Subcutaneous (SC) administration of fentanyl allows for rapid dose titration. After establishing the optimal SC dose of fentanyl for adequate pain relief, patients typically rotate towards transdermal (TD) fentanyl patches. The prior dosing scheme, continuing SC administration 12h post-rotation, led to elevated fentanyl levels and toxicities. SC administration is now discontinued directly after application of the patch. Consistent fentanyl exposure is crucial for safe and effective treatment. The aim is to establish bioequivalence pre- and post-rotation with the updated dosing regimen.

**Methods**: In this observational, single-center cohort study, hospitalized patients with cancer pain who were rotated from SC to TD fentanyl using a 1:1 dose conversion and were treated with SC fentanyl for at least 36 hours, were included. The primary endpoint was to establish bioequivalence (confidence interval (CI) of exposure was within the 0.8 - 1.25 interval) in fentanyl exposure 12h pre- and 12h post-rotation. Using a population pharmacokinetic (POP-PK) model, the area under the curve (AUC) was quantified pre- and post-rotation and compared by a paired t-test after log transformation using 4 – 5 fentanyl plasma samples per patient. The exponent of the observed difference and the 90% CI bounds provided the geometric mean ratio and its corresponding CI. Secondary endpoints assessed changes in patient-reported pain scores and adverse events (AE) (nausea, vomiting, obstipation, dry mouth, transpiration, myoclonia, drowsiness, hallucinations, and confusion) pre- and post-rotation using Wilcoxon signed rank test.

**Results**: Between December 2021 and September 2023, 31 evaluable patients were enrolled in the study (median age 64 years, median BMI 26.1 kg/m$^2$, median dose of SC fentanyl 75 µg/h (range 12-200)). The geometric mean ratio between post- and pre-rotation AUC was 0.98 (90% CI 0.97, 0.99). The related CI falls well within the acceptable bioequivalence boundaries. The range of the ratios per patient between post- and pre-rotation fentanyl exposure was 0.89 and 1.07. The overall mean pain scores differed significantly ($p = 0.037$) pre- ($3.81 \pm 1.93$) and post-rotation ($3.61 \pm 2.05$). No significant differences in patient-reported AEs pre- and post-rotation were found except for nausea and transpiration ($p = 0.022$). Pre-rotation and post-rotation mean scores for nausea were $1.52 \pm 0.74$ and $1.29 \pm 0.63$ whereas these were $1.55 \pm 0.92$, and $1.25 \pm 0.54$ for transpiration. Nonetheless, the clinical relevance of the differences pre- and post-rotation are limited as the changes are less than 20%.

**Conclusion:** With the use of the updated rotation scheme, using a 1:1 dose conversion and discontinuation of SC fentanyl directly after rotation, fentanyl exposure pre- and post-rotation was bioequivalent and pain scores and AEs pre-and post-rotation were stable.

# PRECISION-CUT PLACENTA SLICES AS AN EX VIVO MODEL FOR PLACENTA TOXICITY: A VIABILITY STUDY

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**Background**
To study drug toxicity in the placenta, several in vivo, ex vivo and in vitro models have been developed. These all have their own limitations. A new approach to examine drug toxicity in placental tissue is the precision-cut slicing (PCS) technique. The aim of this study is to provide insights in the viability of the different cell types in the precision-cut placental slices during incubation.

**Methods**
Small strips (5x5x15 mm) of both the fetal and maternal side of eight placentas were excised and sliced using a Krumdieck tissue slicer. Slices were incubated with Williams' E Medium + glutaMAX supplemented with gentamicin and glucose. ATP/protein and morphology assessments were performed to assess tissue viability.

**Results**
The amount of ATP increased during incubation up to 24 hours in both maternal ($p = .0097$) and fetal ($p = .0512$) tissue slices when compared to unincubated slices, indicating that these slices were viable. The layer of syncytiotrophoblasts remained intact, but the incubated tissue presented a higher fraction of villous stroma cells with karyorrhexis compared to placenta slices without incubation.

**Discussion/Conclusion**
This study demonstrates that precision-cut placenta slices remain viable when incubated in medium, and implies that the precision-cut placenta slices can be used as an ex vivo model to study drug toxicity in the placenta.

# POPULATION PHARMACOKINETICS OF INOTUZUMAB OZOGAMICIN IN PEDIATRIC RELAPSED/REFRACTORY B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA – RESULTS OF STUDY ITCC-059

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**Background**
Inotuzumab ozogamicin (InO) is an antibody-drug conjugate approved for treating relapsed/refractory (R/R) B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in adults. Pediatric pharmacokinetic (PK) data of InO are lacking. This study is the first to examine the population PK (POPPK) of InO in pediatric R/R BCP-ALL patients and to assess the PK at the pediatric recommended phase 2 dose (RP2D).

**Methods**
InO serum concentrations were analyzed using non-linear mixed effects modeling (NONMEM, version 7.5.0). Clinical data was collected from adult non-Hodgkin's lymphoma, adult BCP-ALL, and pediatric BCP-ALL patients. A published adult InO POPPK model, was adapted to describe the pediatric data by examining the changes in the structural model and covariate effect (body size, age, disease, etc.).

**Results**
8924 InO serum concentrations were analyzed. InO disposition was described by a two-compartment model with a linear ($CL_1$) and a time-dependent clearance ($CL_t$); where $CL_t$ is described as $CL_t = CL_2 * e^{(-kdes*Time)}$, $CL_2$ is the initial value of $CL_t$, and kdes is the first-order decay coefficient. $CL_1$ is thought to reflect the Fc receptors-mediated monoclonal antibodies clearance; while $CL_t$ relates to the target-mediated clearance, which decreases over time as tumor burden reduces. For R/R BCP-ALL patients, increasing age was associated with a decreasing kdes of $CL_t$, reflecting that the target-mediated drug clearance declines more rapidly in children. In pediatric BCP-ALL trial participants, the median [interquartile range] cumulative area under concentration-time curve (AUC) was significantly higher among responders (n = 42) versus non-responders (n = 10) at the end of first cycle (26.1 [18.9 - 35.0] vs. 10.1 [9.19 - 16.1], $*10^3$ ng*h/mL, $p < 0.001$). From simulations performed at the RP2D, InO exposure (26.6 [17.9 - 37.0] , $*10^3$ ng*h/mL) reached similar level as observed in responding pediatric trial participants.

**Discussion/Conclusion**
In conclusion, the PK profile of InO in pediatric R/R BCP-ALL patients was well described by our model. Our results support the current dosing strategy based on BSA. Children receiving the RP2D achieved a desirable cumulative AUC at the end of the first cycle; additionally, the RP2D has been reported to be well tolerated in previous pediatric trial reports. Therefore, no dose adjustment is required in pediatric R/R BCP-ALL patients for clinically reasons.

# ACCEPTANCE OF INTERVENTIONS TO IMPROVE PHARMACOTHERAPY: COMPARISON BETWEEN SPECIALIZED CLINICAL PHARMACISTS AND NON-SPECIALIZED CLINICAL PHARMACISTS

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**Background**
Hospital pharmacists who are integrated in the medical team on a hospital ward can reduce drug-related problems, such as prescribing errors, by proposing interventions to improve pharmacotherapy. However, when this specialized hospital pharmacist is not available, pharmaceutical care will be conducted by another, non-specialized, hospital pharmacist with less clinical experience for that specific patient group**.** Our aim is to determine the acceptance rate of pharmacists' interventions, in a setting with specialized clinical pharmacists on the wards, and to compare the acceptance rate between specialized clinical pharmacists and non-specialized clinical pharmacists. Besides, we will investigate whether other characteristics of the clinical pharmacists as well as characteristics of the prescriber, patient, drug or the intervention itself are associated with the acceptance rate.

**Methods**
A retrospective cross sectional study was conducted to assess acceptance of pharmacist interventions by prescribers, based on the analysis of all electronic prescription orders in June 2021. Prescribing errors were assessed by a medical doctor and a clinical pharmacist. Acceptance of prescribing errors was defined as resolution of the error within 24 hours after detection. Primary outcome was the acceptance rate of the interventions.

**Results**
In total**,** 145574 medication prescriptions were newly made or altered and 448 prescribing errors were detected, leading to 421 interventions. Of these interventions, 392 (93.1%) interventions were accepted. The acceptance rate of interventions proposed by specialized clinical pharmacists did not differ from the acceptance rate of interventions from non-specialized hospital pharmacists (94.4% versus 91.9%, p=0,145 (chi-square test)). Neither other characteristics of the pharmacist, prescriber, patient, involved drug nor the intervention itself were associated with acceptance.

**Discussion/Conclusion**
The vast majority of the interventions, proposed by specialized clinical pharmacists as well as non-specialized clinical pharmacists, were accepted by prescribers and resulted in the resolution of prescribing errors. The acceptance rate did not differ between specialized clinical pharmacists and non-specialized clinical pharmacists. Given the high acceptance rate, clinical pharmacists should focus on additional strategies to further improve pharmacotherapy, in research as well as daily clinical practice.

# EXPLORING ALTERNATIVE DOSING REGIMENS FOR AVELUMAB WITH HELP OF POPULATION PHARMACOKINETIC MODEL SIMULATIONS

**Authors**
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## Background

Avelumab is a PD-L1 inhibiting monoclonal antibody (mAb), indicated for renal cell, urothelial cell and Merkel cell carcinoma. Initially, the registered dosing regimen was 10 mg/kg Q2W. Later, this has been adjusted to 800 mg Q2W. In December 2022, the Food and Drug Administration (FDA) released criteria on supporting alternative dosing regimens for PD-1 and PD-L1 inhibitors based on in silico modelling[1]. The criteria state maximal percentages for mean differences in AUC, $C_{trough}$ (both max. 20% lower) and $C_{max}$ (max. 25% higher) between an alternative dosing regimen and the initially approved dosing regimen. We applied these criteria to explore alternative dosing regimens for avelumab. Dosing regimens consisting a lower administered dose and dosing regimens with a prolonged dosing interval were of main interest, because these could lead to cost-savings and a lower patient burden (by reducing hospital visits).

## Methods

With a previously developed population pharmacokinetic model[2], the following dosing regimens were simulated using NONMEM v7.5: 10 mg/kg Q2W, 400 mg Q2W, 600 mg Q2W, 800 mg Q2W, 1200 mg Q4W and 1600 mg Q4W. This was done using a set of 500 virtual patients with representative European demographic data, which was created with the PopGen virtual human population generator.

## Results

The adjusted registered 800 mg Q2W regimen complied with the FDA criteria. Besides this regimen, only the 600 mg Q2W regimen complied with the FDA criteria. For the registered indications, the simulated mean AUC, $C_{trough}$ and $C_{max}$ of this regimen were 10-12% lower compared to the initially approved regimen (10 mg/kg Q2W). The other simulated regimens did not comply with the criteria. In the 400 mg Q2W regimen, the mean AUC and $C_{trough}$ levels were 40-42% lower in comparison. In both the Q4W regimens, the mean AUC levels complied with the criteria, but the $C_{trough}$ levels were more than 60% lower, and the $C_{max}$ at least 63% higher in comparison.

## Discussion/Conclusion

The results of the simulations validate the use of the 600 mg Q2W dosing regimen. The use of this regimen lowers the costs by 25% and still results in an adequate exposure to avelumab. The Q4W regimens did not comply with the FDA criteria. The reported half-life of avelumab is 6-7 days. This half-life is relatively low in comparison to other mAbs, and makes extending the dosing interval inadvisable. In further research, the exposure-effect relationship of avelumab should be studied more intensively. New insights about this relationship would support further evaluations to optimize avelumab dosing and to the possible application of therapeutic drug monitoring (TDM).

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# Abstract book

# SCIENTIFIC MEETING

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

Part 2:

ABSTRACTS

# WHOLE BODY PHYSIOLOGICAL BASED PHARMACOKINETIC MODEL TO EXPLAIN A PATIENT WITH DRUG-DRUG INTERACTION BETWEEN VORICONAZOLE AND FLUCLOXACILLIN

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**Background:** Subtherapeutic plasma concentrations of voriconazole were observed in a patient with Staphylococcus Aureus sepsis and probable pulmonary aspergillosis when voriconazole was administered concomitantly with flucloxacillin. After switching our patient to posaconazole, therapeutic concentrations were reached.

**Methods:** The aim of this study was to first test our hypothesis that flucloxacillin competes with voriconazole for binding to albumin *ex vivo*, leading to low total concentrations in plasma. Then a whole body physiologically-based pharmacokinetic model (WBPBPK) was applied to predict the mechanism of action of the drug-drug interaction (DDI). The model included non-linear hepatic metabolism.

**Results:** The unbound voriconazole concentration remained unchanged in plasma after adding flucloxacillin, thereby rejecting our hypothesis of albumin-binding site competition. The WBPBPK model was able to adequately predict the plasma concentration of both voriconazole and posaconazole over time in healthy volunteers. Upregulation of CYP3A4, CYP2C9, and CYP2C19 through the PXR gene by flucloxacillin resulted in decreased voriconazole plasma concentrations, reflecting the DDI observations in our patient. Posaconazole metabolism was not affected by changes through the PXR gene, which agrees with the observed plasma concentrations within the target range in our patient.

**Conclusion:** In conclusion, the *ex vivo* experiments reported that the unbound voriconazole plasma fraction remained unchanged after adding flucloxacillin. The WBPBPK model describes the potential mechanism driving the drug-drug and drug-disease interaction of voriconazole and flucloxacillin, highlighting the large substantial influence of flucloxacillin on the PXR gene and the influence of infection on voriconazole plasma concentrations. This study substantiates the reason for why it is safe to switch to posaconazole during flucloxacillin treatment.

# PHOTOSWITCHABLE SMALL-MOLECULE LIGANDS TO OPTICALLY MODULATE CHEMOKINE RECEPTORS

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**Background:** Photopharmacology allows the optical modulation of protein activity with light-responsive molecules such as azobenzene derivatives. This technology can be used to investigate the biological function of G protein-coupled receptors (GPCRs). In this study we developed functionally light-responsive ligands to optically modulate chemokines receptors such as CXC chemokine receptor 3 (CXCR3) and atypical chemokine receptor 3 (ACKR3). Those GPCRs play crucial role in T-cell function and are associated with inflammatory diseases and cancer.

**Methods:** Design and synthesis of photoswitchable compounds were inspired by the CXCR3 antagonist VUF11211 and a patent of an ACKR3 agonist.
Phtoswitchable CXCR3 antagonist VUF16338 was characterized in radioligand binding assay. Activation of CXCR3 by CXCL11 and inhibition of 10 nM CXCL11-induced CXCR3 activity by VUF16338, was measured by [$^{35}$S]GTPγs accumulation assay and NanoBit-based $G\alpha_{i1}$ recruitment.
Photoswitchable ACKR3 agonis was characterized in NanoBRET binding assay using fluorescently labeled CXCL12-AF647. β-arrestin2 recruitment to the ACKR3 after stimulation with VUF25471 wad detected by NanoBit complementation.

**Results:** The CXCR3 photoswitchable ligand VUF16338 has been identified as a key compound from a library of eleven analogs. VUF16338 inhibits CXCL11-induced G protein activation by CXCR3 with a 10-fold inhibitory potency shift between dark and irradiated states. For ACKR3 VUF25471 has been selected as tool compound and has been found to recruit β-arrestin2 to ACKR3 with 10-fold potency shift between dark and irradiated states

**Conclusion:** VUF25471 compound is the first photoswitchable ligand for ACKR3.VUF16338 and VUF25471 are a valuable tool to investigate the role of CXCR3 and ACKR3 in biological functions, respectively.

# CO-TRIMOXAZOLE-INDUCED PERTURBATION OF THE PLACENTAL BARRIER: UNRAVELING THE IMPACT ON IL-6 AND THE MAPK SIGNALING PATHWAY

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**Background:** During pregnancy, the placenta is the only link between the fetus and mother and as a barrier, protects the fetus against the harmful compound circulating in maternal blood. Interleukin-6 (IL-6) and mitogen-activated protein kinase (MAPK) are known for the effect on placenta barrier via affecting the cell junctional complex. Considering the antibiotics (co-trimoxazole/CTX) as a common gestational prescription, we explore the impact of CTX on tight junction genes in human placental cells, followed by evaluating the potential effect on IL-6 and MAPKinase.

**Methods:** Human placenta choriocarcinoma BeWo cells were cultured in a 24-well plate and treated with CTX at concentrations of 5 µg/ml and 50 µg/ml. Bulk RNA-sequencing was conducted, and over-representation analysis using the enricher tool was performed. Functional gene sets from KEGG, Reactome, and gene ontology were obtained through the msigdbr R package. Significant pathways and gene ontologies (p adj $< 0.05$) were compared across different groups. The effect of CTX on IL-6 was measured in both mRNA and protein level using qPCR and ELISA respectively. Furthermore, the study also examined the impact of CTX on MAPK activation by conducting western blotting for phosphorylated ERK, JNK, and p-38 using specific antibodies.

**Results:** CTX has a notable impact on the genes involved in cell-cell junctions (GO_CC enrichment analysis). CTX modulated the expression of 14 tight junctions genes significantly. Most of these genes were decrease, however, in contrast to what was expected, claudins (CLDNs) as paracellular localized proteins like CLDN4 significantly increased which was confirmed with both mRNA and protein. Furthermore, CTX decreased the expression of IL6 in both mRNA and protein level significantly. Interestingly, CTX activate the MAPK/ERK signaling pathway without any effect on MAPK/JNK and MAPK/p38.

**Conclusion:** CTX modulates tight junction genes that are crucial for maintaining placental integrity. The reduction effect of CTX on placental IL6 production and its activation effect on MAPK/ERK pathway, is indicative for a decreased placental integrity. This decrease in placental integrity may elevate fetal exposure to harmful compounds in maternal blood.

# RECOMMENDATIONS FOR A (PHARMACO) GENETIC SAMPLING METHOD IN PATIENTS FOLLOWING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Background:** Allogenic hematopoietic stem cell transplantation (HSCT) is one of the most widely applied curative treatment for both malignant and non-malignant haematological diseases. While complete chimerism is aimed for in patients receiving HSCT, this is accompanied with a challenge when assessment of recipient germline DNA is required. We provides recommendations for a pharmacogenetic sampling method based on a case report of a patient following HSCT in which pharmacogenetic analysis was performed on different biological samples.

**Methods:** Pharmacogenetic analysis was requested at Erasmus MC for a patient intolerant for several medications. This patient revealed a medical history of chronic lymphocytic leukemia treated with allogenic HSCT. We performed pharmacogenetic analysis on DNA derived from peripheral blood (pre- and post- allogenic HSCT) and post-transplantation buccal swab and hair follicles.
DNA isolation from buccal swab is regarded as the golden standard for recipient germline DNA after allogenic HSCT at Erasmus MC. DNA from peripheral blood, the buccal swab and hair (10 hair follicles) was extracted using automated extraction methods. We compared results from the different samples on 6 relevant pharmacogenetic genes using available modalities. Additionally, short tandem repeat analysis was performed on DNA extracted from post-transplantation peripheral blood samples, buccal swabs and hair to detect contamination of these samples with donor DNA. Patient provided written informed consent for additional DNA analysis and publication of the results.

**Results:** For peripheral blood and the buccal swab, post-transplantation DNA showed identical genotypes for CYP2C9*1/*1, CYP2C19*1/*2, CYP2D6*2/*9, CYP3A4*1/*1, VKORC1-1639TT, SLCO1B1*1/*5. However, pre-transplant DNA from peripheral blood showed different alleles for CYP2D6 *1/*2 and SLCO1B1*1/*1. For hair samples, 69.2 ng DNA could be extracted (n=10). As the amount of DNA from hair was low we only analysed the alleles that were different between the donor and recipient. DNA profile from hair follicles showed identical CYP2D6 *1/*2 and SLCO1B1*1/*1 genotypes with the pre-transplantation peripheral blood sample. DNA isolated from the buccal swab was contaminated with 36.7% of recipient DNA, while DNA samples isolated from hair follicles showed a 100% match with the pre-transplant peripheral blood DNA.

**Conclusions:** For pharmacogenetic profiling after allogenic HSCT, DNA samples from buccal swabs are contaminated with donor DNA, which could lead to incorrect pharmacotherapy conclusions. Analysis of pre-transplant peripheral blood is preferred. As an alternative for post-transplant analysis, DNA from hair follicles can be used. The lower yield of DNA from hair, however, limits application for broad pharmacogenetic testing.

# FIRST STEPS IN DEVELOPING A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR CHILDREN WITH OBESITY IN SIMCYP

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**Background:** The escalating rates of obesity pose a significant health challenge, particularly in children. While it is acknowledged that both obesity and developmental factors impact drug disposition in children, the precise interplay remains unclear. Altered pharmacokinetics and the lack of individualized dosing guidelines puts children with obesity at risk of treatment failure or drug toxicity.
To address this issue, physiologically-based pharmacokinetic (PBPK) models, mathematical models that integrate information on physiology and drug properties, hold promise for guiding optimal drug dosing in this population. This study aims to develop a population model for a PBPK model of children with obesity.

**Methods:** In this study, the first paediatric obesity population model in the PBPK software package Simcyp was developed by modifying the existing population parameters that describe anatomy and physiology for healthy children. The population model was modified by incorporating available information on obesity-related physiological changes in children. This included adjusting the model code for body weights based on BMI ranges from WHO Child Growth Standards. Furthermore, organ volumes were adapted with published organ-specific scaling factors. In order to capture variability, next to mean values also coefficients of variation were included.

**Results:** The adapted Simcyp model successfully represents a virtual population of children with obesity, with weights and BMIs aligning with the target population. In addition, simulated kidney and liver weights were accurately predicted, with 1531 of 1600 simulated organ weights falling within the 95% prediction interval of the autopsy reports.

**Conclusion:** While these developments mark significant progress in creating a PBPK model for children with obesity in Simcyp, further refinement is essential. For example, incorporating information on obesity-related changes in renal and liver function is paramount to enhancing PBPK models for future applications in model-informed dosing.

**Iron(ing) out neurodegeneration: targeting ferroptosis pathways to unravel novel research techniques and therapeutic avenues in tau-related parkinsonisms**

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**Background:** Parkinsonism is a spectrum of neurodegenerative disorders, characterised by motor-related deficits, caused by degeneration of dopaminergic neurons in the substantia nigra (SN). Although Parkinson's disease (PD) is the most common type of parkinsonism, definite diagnoses are only possible post-mortem which, due to the clinical similarity of different parkinsonisms, often leads to misdiagnoses. In this project, we focus on Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD) parkinsonisms, and their pathological hallmark, tau. Both alpha-synuclein (aS), PD hallmark, and tauopathies have been linked to ferroptosis pathways. Ferroptosis is a non-apoptotic, iron-induced, form of cell death, thought to be linked to age-related neurodegenerative diseases and thus, exploring its underlying mechanisms could further our understanding of PSP/CBD pathophysiology. Our objectives were: (1) Improve research from a technical point by fine-tuning a new tool, digital spatial profiling (DSP), in postmortem tissues and, for the first time, explore spatial transcriptomics in dopaminergic neurons with and without pathological inclusions. (2) DSP will consequently deepen our understanding of ferroptosis-related pathways at a molecular level. (3) Study ferroptosis-related markers in relevant cell lines with a novel tau-protofibril model and (4) intervene via pharmacological manipulation of ferroptosis.

**Methods:** The ferroptosis pathway will be quantitatively evaluated in human post-mortem SN surviving neurons with and without tau, using DSP. The relationship between tau and ferroptosis will also be tested in primary neurons and differentiated dopaminergic neurons with tau-protofibrils, using pharmacological ferroptosis inhibitors/stimulants, to evaluate viability and tau accumulation.

**Results:** In support of the DSP novel approach, preliminary data using pixel density scoring indicated differential expression of ferroptosis-related markers in tau-labelled neurons within the SN of post-mortem PSP/CBD brains, compared to age-matched controls and PD patients. However, it is still unclear if pathology is a cause or a consequence of ferroptosis in the progression of parkinsonism. Analysis of the ferroptosis pathway in vitro/vivo will help understand the relationship between ferroptosis and tau in PSP/CBD progression. Furthermore, comparing the output of tau with existing aS data will help elucidate differences (and commonalities) between parkinsonisms at a molecular level.

**Conclusion:** Ferroptosis seems to be a key component of parkinsonism progression and a possible target for novel therapeutic avenues. Hence why we study ferroptosis in surviving/live neurons which will offer insight into which components could confer this cell death resilience, despite ferroptosis machinery being seemingly active. As previously mentioned, tau-related parkinsonisms are often overlooked due to their clinical similarity to PD, consequently leading to misdiagnoses and the regression in funding/research of PSP/CBD, ultimately leaving patients without adequate care. Thus, with our state-of-the-art approach, it is our hope that the findings of this project will reveal new research methods to study neurodegeneration and ferroptosis, as well as unravel novel aspects of parkinsonism pathophysiology.

# UNDERSTANDING THE ROLE OF MITOCHONDRIAL-CONTAINING EXTRACELLULAR VESICLES (MITOEVS) IN NEURODEGENERATIVE DISEASES

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**Background:** Extracellular vesicles (EVs), a type of cell-released lipid bilayer-delimited particles, have emerged as important mediators of intercellular communication in the brain. Another important messenger, mitochondrion is reported to be a possible cargo inside EVs that is called mitoEVs. The alteration of mitochondrial biogenesis is frequently linked to the progression of neurodegeneration. Moreover, mitochondria can be used as in several therapeutic approaches. However, it is unclear which role mitoEVs play in neurodegenerative diseases.

**Methods:** In this study, we employ size exclusion chromatography (SEC) and ultracentrifugation (UC) techniques to isolate mitoEVs from conditioned medium (CM) and brain tissues. Characterization of the isolated mitoEVs is carried out using BCA protein assay, Dot-blot, and nanoparticle tracking analysis (NTA). LC-MS is utilized to investigate the protein composition within mitoEVs. Furthermore, a luminescent ATP detection assay is utilized to demonstrate the functional integrity of mitochondria within EVs isolated from fresh brain tissue.

**Results:** To investigate the involvement of mitoEVs in Alzheimer's disease (AD), we isolated particles from the conditioned medium (CM) of neural progenitor cells with a PSEN1 mutation and from their isogenic control. A total of 24 fractions were obtained through size exclusion chromatography (SEC), among which fractions 8-12 tested positive for both EVs marker (CD9) and mitochondria marker (TIM23) as per Dot-Blot results. Protein concentrations in fractions 8-12, determined by BCA assay, were approximately 20ug/ml. Comparing the biogenesis of mitoEVs between AD and its isogenic control, we observed no statistical differences in vesicle sizes and concentrations. However, LC-MS analysis revealed significant disparities in mitochondrial proteins within AD and isogenic mitoEVs, with the latter exhibiting a higher abundance of mitochondrial proteins. Using ultracentrifugation (UC), we attempted to isolate vesicles from fresh mouse brain tissue, demonstrating their capability to produce ATP. By combining UC and SEC techniques, we observed a greater ATP production in mitoEVs compared to those isolated solely through UC, suggesting an enrichment effect when both methods are utilized in tandem.

**Conclusions:** We successfully isolate mitoEVs from CM and brain tissue using SEC and UC. The mitochondrial protein contents of CM-mitoEVs hold promise as potential biomarkers for Alzheimer's disease (AD). Additionally, the capacity of fresh brain mitoEVs to produce ATP presents potential therapeutic avenues for brain diseases.

# CHEMOKINE RECEPTORS ON THE MOVE; ACKR3 IS SECRETED FROM BREAST CANCER CELLS ON EXTRACELLULAR VESICLES

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**Background:** Elevated expression of the chemokine receptors CXCR4 and ACKR3, both binding CXCL12, is detected in a wide range of tumors, including breast cancer. Yet, the molecular mechanisms by which the CXCL12/CXCR4/ACKR3 axis contributes to the pathogenesis are not fully understood. In particular, the role of the atypical chemokine receptor 3 (ACKR3) has proven difficult to dissect. Unlike its canonical counterpart CXCR4, ACKR3 displays a primarily intracellular localisation, but its lack of G protein activation has led to a decoy or ligand scavenging model, that requires refining.

**Methods:** Using immunofluorescent confocal imaging we probed ACKR3 localization in breast cancer cells, and it colocalization with various organelle markers that might shed light on functionality. Isolation of secreted components was performed by differential centrifugation, followed by western blot detection of protein cargo. To investigate possible functionality, a chemotaxis assay was set-up using microfluidic devices, coupled with live-cell microscopy.

**Results:** ACKR3 colocalizes intracellularly with the late endosomal marker LAMP1, indicative of a role beyond mere receptor recycling. To probe possible endosomal exocytosis via extracellular vesicles, the secretome of the cells was differentially centrifuged, and material of expected EV size range analysed by Western blotting and ELISA, both confirming the presence of ACKR3. In a chemotactic assay, blockade of ACKR3 with nanobodies hamper the ability of aggressive breast cancer cells to migrate.

**Conclusion:** The secretion of ACKR3 on EVs appears to be a hallmark of breast cancer cells. Treatment of these cells with ACKR3-blocking nanobodies impairs the ability of the cells to migrate, suggestive of a role for EV-ACKR3 in chemokine gradient control. Such a sequestration mechanism may regulate chemokine availability, and hence cell-interactions, within in the local microenvironment. Pharmaceutical development should consider the implications of such a new ‘pool’ of extracellular chemokine receptors as potential targets, or indeed unintentional ‘sinks’ of therapeutic agents once delivered.

# COUNTERACTING ANGIOTENSINOGEN SMALL INTERFERING RNA-MEDIATED ANTIHYPERTENSIVE EFFECTS WITH REVERSIR

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**Objective:** Administering small interfering RNA (siRNA) targeting liver angiotensinogen (AGT) effectively depletes circulating AGT, leading to a sustained blood pressure reduction for up to six months post-single injection. However, emergency situations may require a rapid increase in angiotensin levels. REVERSIR (reverse siRNA silencing) emerges as a promising approach to counteract AGT-siRNA effects.

**Design and methods:** Spontaneously hypertensive rats underwent a 3-week AGT-siRNA treatment, followed by REVERSIR doses at 1, 10, or 20 mg/kg. After a week of REVERSIR, a re-dose of AGT-siRNA assessed its post-REVERSIR effectiveness. Additionally, the impact of REVERSIR with a 3-week valsartan treatment (4 mg/kg per day) was examined. Arterial pressure was measured via radiotelemetry, circulating AGT, and renin by enzyme-kinetic assays, and liver AGT mRNA levels by quantitative PCR.

**Results:** Baseline mean arterial pressure (MAP) was 144 ± 1 mm Hg. AGT-siRNA reduced MAP by ~16 mm Hg and circulating AGT by >95.9%, with a 25-fold increase in circulating renin. All REVERSIR doses restored MAP to baseline within a week. Notably, 10 and 20 mg/kg fully restored circulating AGT and renin to baseline, while 1 mg/kg allowed ~50% AGT restoration with renin above baseline. Liver AGT mRNA changes mirrored circulating AGT alterations. A second AGT-siRNA dose, on top of 1 mg/kg REVERSIR, replicated the initial dose's MAP effect. On top of 10 mg/kg REVERSIR, the second dose resulted in ~50% of the first dose's MAP effect. Valsartan, like AGT-siRNA, reduced MAP similarly, inducing a 30-fold renin increase and 39.6% circulating AGT reduction. Adding REVERSIR to valsartan did not alter this outcome.

**Conclusion:** REVERSIR, demonstrating dose-dependent efficacy, ameliorates AGT reduction induced by AGT siRNA, normalizing MAP. Notably, MAP normalization persists with partially recovered AGT levels (~50%), likely due to upregulated renin maintaining normal angiotensin generation. REVERSIR has no impact on valsartan-induced AGT reduction. Post-REVERSIR, a second AGT siRNA dose effectively lowers MAP to the same extent as the initial dose. Therefore, REVERSIR is a rapid and highly selective tool for emergency AGT elevation, allowing AGT siRNA re-introduction after patient stabilization, leading to blood pressure reduction once again.

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

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**Background:** US28 is a viral G protein-coupled receptor (vGPCR) encoded by the human cytomegalovirus. In glioblastoma, a fast-growing and aggressive form of brain cancer, it has been demonstrated that US28 expression enhances oncogenic signaling pathways (onco-modulation). 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. The notion that GPCRs can control mechanisms behind EV secretion and cargo selection is not entirely knew. We propose that US28 modulates EV secretion and/or composition via pathways that require elucidation to further understand the GPCR-mediated onco-modulation.

**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. For co-localization studies of US28 with 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 factors of US28 that are responsible for EV secretion and/or cargo selection.

**Results:** Our results showed the presence of US28 in small EVs derived from glioma cells. This was confirmed via a luminescence assay where US28 is tagged to a nano-luciferase protein, allowing to delineate the molecular determinants of US28 responsible for its incorporation into EVs. Our data demonstrated a partial dependency on Gq proteins, the predominant G protein through which US28 signals. A comprehensive EV marker panel indicated an US28-dependent change in either EV quantity or content upon its expression in glioma cells. Our data demonstrated an increase in numerous reported cargo molecules: CD9, CD81, CD63, HSP70 and LAMP1 in the EVs derived from $US28^+$ cells compared to EVs derived from $US28^-$ cells. Further, US28+ cells appear to decrease CD63 and LAMP1 levels compared to glioma cells lacking US28 expression, two markers US28 co-localizes with. Lastly, no increase was detected for TSG101 and Alix in the EVs derived from $US28^+$ cells compared to EVs derived from $US28^-$ cells. These are two proteins involved in the ESCRT-dependent EV biogenesis pathway, suggesting that US28 might employ another EV biogenesis machinery.

**Conclusion:** Our findings identify US28 as a regulator of EV secretion and/or cargo selection, changes that may aid viral immune evasion and explain its reported oncomodulatory role.

# (*S*)-OPTO-PROP-2 ANALOGS TO DYNAMICALLY CONTROL $\beta_2$-ADRENERGIC RECEPTOR SIGNALING WITH LIGHT

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**Background:** Beta-blockers are used for numerous acute and chronic conditions such as various heart problems, hypertension, and glaucoma. These beta-blockers antagonize agonist-induced $\beta_2$-adrenergic receptor activity. Previously, we have reported the azologization of the FDA-approved beta-blocker propranolol to yield the photoswitchable antagonist (*R/S*)-opto-prop-2 that displays 600-fold increased binding affinity upon illumination with 360 nm light. This research aims to design and characterize photoswitchable (*S*)-Opto-prop-2 analogs that switch to high-affinity *cis* isomer upon illumination of the *trans* isomer with red-shifted wavelengths and appropriate half-lives. Such photoswitchable antagonists would ultimately allow local control of their bioactivity in the body.

**Methods:** For this research, various photoswitchable (*S*)-Opto-prop-2 analogs were designed, synthesised, photochemically characterized, and pharmacologically tested in a FRET-based EPAC biosensor assay to measure $\beta_2$-adrenergic receptor-induced cAMP production. All photoswitchable ligands were tested in both agonist and antagonist modes in one.

**Results:** The illumination wavelengths required to obtain the *cis* isomer are mostly 520 nm. Conversely, *cis* isomers are illuminated at 434 or 520 nm to revert to *trans* isomers. As a result, most of the ligands can be illuminated with longer wavelengths than UV light. The $PSS_{cis}$ and $PSS_{trans}$ values ($PSS_{cis}$ % = the percentage of *cis* isomers after illumination of *trans* isomer) are found to be above 50%. The half-lives of the ligands are long enough to perform pharmacological assays.
All photoswitchable ligands antagonized isoprenaline-induced $\beta_2$-adrenergic receptor activity. Further pharmacological research is currently ongoing.

**Conclusion:** This study provides possible candidates for photoswitchable beta-blockers with appropriate half-lives and lower-energy switching wavelengths compared to (S)-Opto-prop-2.

# PHARMACOLOGICAL CHARACTERIZATION OF SEVEN HUMAN HISTAMINE H3 RECEPTOR ISOFORMS

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**Background:** The histamine $H_3$ receptor ($H_3R$) regulates as a presynaptic G protein-coupled receptor the release of histamine and other neurotransmitters in the brain, and is consequently a potential therapeutic target for neuronal disorders. The human $H_3R$ encodes for seven splice variants that vary in the length of intracellular loop 3 and/or the C-terminal tail but are all able to induce heterotrimeric $G_i$ protein signaling. The last two decades $H_3R$ drug discovery and lead optimization has been exclusively focused on the 445 amino acids-long reference isoform $H_3R$-445. However, except for some basic characterization, the other 7 transmembrane $H_3R$ isoforms have so far been largely understudied. The aim of this study is to evaluate the pharmacological properties of eighteen $H_3R$ reference ligands including agonists and inverse agonists on all 7TM $H_3R$ isoforms. Through this comprehensive evaluation, our primary objective is to enhance our understanding of $H_3R$ pharmacology and lay the groundwork for more effective drug discovery programs.

**Methods:** The affinity of 18 reference $H_3R$ agonists and inverse agonists for the seven $H_3R$ isoforms was determined by radioligand competition binding assays on the HEK293T cell membrane transiently expressing each $H_3R$ isoform. Furthermore, the ligand-induced signaling with respect to the intracellular cAMP level was measured by a FRET-based EPAC cAMP biosensor with co-expression of $H_3R$ isoforms stably expressed in HEK293 cells.

**Results:** The $H_3R$-453, $H_3R$-415, and $H_3R$-413 isoforms display similar binding affinities for all ligands as the $H_3R$-445. However, increased agonist binding affinities were observed for the three shorter isoforms $H_3R$-329, $H_3R$-365, and $H_3R$-373, whereas inverse agonists such as the approved anti-narcolepsy drug pitolisant (Wakix®) displayed significantly decreased binding affinities for the latter two isoforms. This opposite change in binding affinity of agonist versus inverse agonists on $H_3R$-365 and $H_3R$-373 is associated with their higher constitutive activity in a cAMP biosensor assay as compared to the other five isoforms.

**Conclusion:** In this study, we pharmacologically characterized for the first time all seven $H_3R$ isoforms by determining their binding affinities for reference histamine $H_3$ receptor agonists and inverse agonists. The observed differences in pharmacology between longer and shorter $H_3R$ isoforms should be considered in future drug discovery programs.

# INTERPLAY BETWEEN THE IMMUNE SYSTEM AND HUMAN TROPHOBLASTS: A SYSTEMATIC REVIEW AND RISK OF BIAS ANALYSIS OF IN VITRO STUDIES

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**Background:** An increasing amount of evidence suggests that immune responses may affect trophoblast functioning, which in turn may play a role in gestational disorders and fetal development. This systematic review offers the first summary of in vitro studies on the interplay between the immune system and human trophoblasts, in conjunction with a risk of bias analysis.

**Methods:** A search in Pubmed and Embase yielded 110 relevant studies. To accompany the narrative review section of this paper, study characteristics were extracted and presented in the characteristics sheet of a systematic map. In addition, all 5171 relevant measurements were extracted and presented in an easy to navigate datasheet in the systematic map.

**Results:** Primary trophoblasts were the most commonly used cell type, but trophoblast subtypes were not always defined. Similarly, the exact natures of trophoblast cell lines were sometimes unclear. Cytokines and Toll-like receptor agonists were often used as interventions, but most studies focused on a select few substances such as tumor necrosis factor-α and lipopolysaccharide. In regard to the outcome parameters, some important trophoblast functions, such as hormone production and barrier formation were underrepresented.
Whether or not risk of bias was high varied strongly between types of bias. Risk of selection bias, for example, was usually low. However, none of the included papers mentioned blinding or plate randomization. Only a select few papers mentioned passage numbers, use of vehicle control or conflict of interest.

**Conclusion:** Better characterization of trophoblast subtypes and a broader range of studied interventions and outcome parameters would contribute to a more complete understanding of trophoblast responses to immune stimuli. Additionally, researchers should be thorough and pay close attention when setting up and writing down their methodologies, in order to improve the reproducibility and translatability of the evidence.

# APOLIPOPROTEIN E DEFICIENCY IS ASSOCIATED WITH DIMINISHED HEPATIC CYP2B10 ACTIVITY AND INCREASED SUSCEPTIBILITY FOR BUPROPION-INDUCED LOCOMOTOR DYSFUNCTION IN MICE

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**Background:** Metabolism by hepatic cytochrome P450s (CYPs) is a major route involved in the clearance of most drugs in clinical use. Studies in human subjects suffering from non-alcoholic fatty liver disease have suggested that changes in lipid metabolism may contribute to inter-individual differences in CYP activity. In the current study, we tested the hypothesis that a genetic disruption of lipid uptake by hepatocytes not only results in hypercholesterolemia but also induces relevant changes in drug metabolism.

**Methods:** Gene expression profiling was performed on livers (n=4/5) obtained from female genetically hypercholesterolemic LDL receptor knockout mice, apolipoprotein E knockout (APOE KO) mice, and normolipidemic wild-type (WT) C57BL/6 controls (age: 10-12 weeks). Changes in CYP transcript levels were verified on the protein activity level ex vivo using liver microsomes (n=9). The relevance to bupropion plasma clearance was tested in vivo (n=7) using HPLC-UV detection in additional groups of female APOE KO and WT mice upon intraperitoneal administration of 50 mg/kg bupropion.

**Results:** The hepatic gene expression profile of the major drug metabolizing variants CYP3A11 (human CYP3A4), CYP1A2, CYP2C29 (human CYP2C19), CYP2D22 (human CYP2D6), (human CYP2B6), CYP2A4/5 (human CYP2A6), and CYP2E1 was not affected by LDL receptor deficiency. In contrast, APOE deficiency was associated with a 25- to 41-fold decrease ($P<0.001$) in CYP2B10 gene expression levels. In agreement, the efficacy of APOE KO microsomes to convert the CYP2B6/10 substrate bupropion into hydroxybupropion was 8-fold reduced as compared to wild-type microsomes ($P<0.001$). As a result, the formation of the metabolite hydroxybupropion was reduced (AUC: -46%; $P<0.001$) and the plasma clearance of bupropion profoundly impaired (AUC: +228%; $P<0.001$) in mice lacking APOE. Importantly, the elevated bupropion plasma levels in APOE KO mice were associated with an adverse drug reaction, as visual locomotor dysfunction was observed 40 to 60 minutes after bupropion administration.

**Conclusion:** Apolipoprotein E deficiency is associated with diminished hepatic CYP2B10 activity and increased susceptibility for bupropion-induced locomotor dysfunction in mice.

# WITH INCREASING ANTIBIOTIC RESISTANCE ADULTS NEED HIGHER DOSES: DO KIDS AS WELL?

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**Background:** Despite the European Committee on Antimicrobial Susceptibility Testing's (EUCAST) redefined 'susceptible, increased exposure' category, it still remains unclear how the corresponding adult dosing recommendations should be translated to paediatrics. Therefore, we aimed to assess the probability of target attainment (PTA) of the EUCAST 'susceptible, increased exposure' and the Dutch Paediatric Formulary (DPF) dose for severe infections for three worst-case drug-bug combinations with physiologically-based pharmacokinetic (PBPK) modelling. If the PTAs are less than 90%, we performed additional PBPK simulations to determine model-informed dosages.

**Methods:** For ceftazidime (CAZ), cefuroxime (CXM), and ciprofloxacin (CIP), PBPK models were extracted from literature and all models were verified with adult and paediatric clinical pharmacokinetic data. Thereafter, we assessed the PTAs using the following targets: unbound plasma concentration above the minimal inhibitory concentration (MIC) at 100% of the dosing interval (100%$f$T>MIC) for CAZ and CXM, and the area under the curve (AUC) over MIC ratio ≥125 for CIP. PTAs were determined for the worst-case scenarios of each drug-bug combination (i.e., CXM for *E. coli* (epidemiological cut-off (ECOFF) 8 mg/L), CAZ for *P. aeruginosa* (ECOFF 8 mg/L), and CIP for *P. aeruginosa* (ECOFF 0.5 mg/L)) for recommended EUCAST 'susceptible, increased exposure' and DPF dosages. Additional dose simulations were performed in case a PTA was less than 90% to find the optimal model-informed dose.

**Results:** Model verification against available clinical data proved successful for all three antibiotics. Simulations in adults showed that in worst-case scenarios no patient will reach its therapeutic target with the current EUCAST 'susceptible, increased exposure' dosages for CIP and CXM. For CAZ, the PTA was 62%. For paediatric patients, the DPF dosages of CIP and CAZ resulted in a worst-case PTA of up to 38% and 75%, respectively. Only the DPF dose of CXM in 1 to 4-month-old infants resulted in a satisfactory PTA of 92-99%. Worst-case model predictions indicate that the dosing frequency (CAZ and CXM) and total daily dose (for all three antibiotics) should be increased to reach a PTA >90%. CAZ and CXM could also be given via continuous infusion.

**Conclusion:** PBPK simulations showed that for almost every age group, including adults, intended therapeutic targets were not achieved in worst-case scenarios with the current recommended EUCAST and DPF dosages for CAZ, CXM, and CIP. Model-informed dosages were developed for such cases, although in some cases an alternative antibiotic may be preferred over the predicted required dose increase.

# EVALUATING CXCL12 SCAFFOLDING INTERACTIONS

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**Background:** 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). 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. Chemokines play critical roles in regulating immune responses, wound healing, angiogenesis, haematopoiesis, and embryogenesis. However, they are also implicated in the development and progression of various pathological conditions, such as cancer metastasis. Although targeting chemokine receptors has been a widely explored approach in drug discovery, targeting chemokines directly has been underexplored. In this research we aim to explore the interactions of CXCL12 with known interacting molecules and to assess the therapeutic potential of CXCL12-directed ligands.

**Methods:** We used Nano-Bioluminescence Energy Resonance Transfer (Nano BRET) and reporter gene assays to assess the modulatory effects of CXCL12-directed ligands on CXCL12 function. Moreover, we produced CXCL12 (variants) using *E.coli* to further characterize the CXCL12-directed ligands. To assess their therapeutic potential we set up new relevant cell models. As endothelial cells are known to contain various CXCL12 binding molecules (CXCR4, ACKR3 and heparan sulfate) we characterized endothelial cells, derived from human-induced pluripotent stem cells, as a model system.

**Results:** We validated the obtained methodology using molecules that are known to bind the CXCL12 and its receptors. The methods seem to be suitable for characterizing CXCL12 ligands, however, the tested CXCL12 ligands seem to have a low inhibitory potency on CXCL12 function.

**Conclusions:** We established a methodological framework to characterize CXCL12 ligands. Using the new methodologies, we are now exploring new CXCL12 ligands which we aim to characterize for their therapeutic potential.

# REACHING BEYOND THE UV SPECTRUM – A PHARMACOLOGICAL PROFILE OF A HISTAMINE H1 RECEPTOR RED-SHIFTED PHOTOCAGED ANTAGONIST

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**Background**: Photopharmacological modulation of G protein-coupled receptors (GPCRs) offers new exciting ways to modify ligand on-target activity in a spatio-temporal manner. One strategy involves the introduction of a photosensitive moiety to a pharmacologically active compound, thereby blocking its bioactivity (photocaging). The active compound can then be irreversibly uncaged with light, restoring biological activity. However, most investigated photoligands rely on UV light for photo-modulatory effects, which can be damaging for living cells. In addition, longer wavelengths provide better tissue penetration, but are lower in energy, hereby opening doors for more efficient real-time photo-modulation of ligand activity, while being less invasive for the system. In this work, the FDA-approved histamine H1 receptor (H1R) antagonist desloratadine was caged with BODIPY to impair its binding affinity, which could be restored by illumination with red (560 nm) light.

**Methods**: Radioligand binding, non-dynamic H1R NFAT-Luc reporter gene assay, *in situ* uncaging in an imaging-based functional assay.

**Results:**

| Assay | Pharmacological parameter | Parental desloratadine | Caged desloratidine | Uncaged desloratidine | Δ | |
|---|---|---|---|---|---|---|
| Competition binding | $pK_i$ ± SEM | 9.0 ± 0.1 | <6 | 8.5 ± 0.1 | >2.5 | |
| Reporter gene response | $pIC_{50}$ ± SEM | 8.7 ± 0.0 | <5 | 8.1 ± 0.1 | > | |

**Conclusion:** BODIPY-caged desloratadine is shown to have more than 300 times lower affinity than desloratidine, has no activity in an NFAT-driven reporter gene assay and can be uncaged during live-cell confocal functional imaging assays. Our research shows not only that histamine activity can be modulated by light in the presence of a photocaged antagonist, but also offers the potential of *in situ* activity modulation of H1R by light in living cells.

# VASOPROTECTIVE EFFECT OF THE MODIFIED 6-CHROMANOL SUL-138 DURING VASCULAR AGING-INTERPLAY OF MITOCHONDRIA AND ENDOTHELIUM-DERIVED HYPERPOLARIZATION

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**Background:** Vascular aging is marked by decreased vasodilation due to lower nitric oxide bioavailability caused by dysfunctional mitochondria. Nitric oxide loss can partially be compensated by endothelium-derived hyperpolarization (EDH) during aging. Another postulated adaptation to vascular aging is an alteration in mitochondrial calcium-signalling leading to increased EDH in aged vessels. Modulation of mitochondrial function is therefore a potential treatment target to alleviate age-related vascular dysfunctions. In this study, we investigated the effect of chronic treatment with the modified 6-chromanol SUL-138, a reverse electron flux inhibitor, in vascular aging mouse models. Accelerated aging in mice was induced by KO of DNA repair endonuclease ERCC1, leading to DNA damage accumulation in the target cells. We investigated the effect of SUL-138 in the accelerated aged endothelial (EC-KO) and vascular smooth muscle cell (SMC-KO) mouse models. We hypothesize that SUL-138 might be able to prevent vascular dysfunction and accompanied end organ dysfunctions.

**Methods:** EC-KO, SMC-KO and healthy littermates received SUL-138 (30 mg/kg/day) or vehicle chow for 8 weeks until euthanasia at the age of 22 weeks, where pronounced vascular dysfunctions in the KO animals are expected. Arteries were isolated and *ex vivo* vascular function assessed in wire myograph setups. Mitochondrial function was measured in seahorse assays. Protein and mRNA were isolated from tissues to perform Western Blot and qPCRs. Arterial stiffness was assessed *in vivo* with pulse wave velocity measurements of abdominal aorta.

**Results:** The chronic treatment with SUL-138 prevented vascular dysfunction in the EC-KO mouse model and increased vasodilation in SMC-KO mice by enhancing EDH-contribution. Mitochondrial function was increased after treatment with SUL-138. Furthermore, arterial stiffness, increased in SMC-KO mice, was normalized to littermate control level by SUL-138. Finally, the accompanied end organ dysfunctions, namely salt-wasting tubulopathy in EC-KO mice were attenuated by SUL-138.

**Conclusion:** The chronic treatment with SUL-138 prevented the development of age-associated vascular dysfunctions including decreased vasodilation, mitochondrial dysfunctions, vascular stiffness, as well as related end organ dysfunction. Vasodilation was modulated by enhanced EDH. This seem to be an additional mechanism of SUL-138 and according to our knowledge the only drug with this capacity which opens new fields of application.

# DEACTIVATION OF A CANONICAL CHEMOKINE RECEPTOR: CCR9 SIGNAL DURATION IS GOVERNED BY INTERNALIZATION EFFICIENCY

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**Background:** The chemokine receptor CCR9 mediates T-cell maturation and immune cell migration from the thymus to the small intestine following gradients of the chemokine CCL25. Receptor dysregulation is associated with a variety of inflammatory bowel diseases as well as cardiovascular disease, arthritis, and others. Additionally, CCR9 and CCL25 overexpression has been observed in numerous types of malignant tumors and is corelated with metastasis to the colon. CCR9 signaling is well described through G proteins, but the specific mechanisms leading to receptor deactivation are less understood. GPCR signaling is canonically terminated by phosphorylation by GPCR kinases (GRKs) and subsequent arrestin binding which blocks further signaling and promotes desensitization through internalization. The specifics of this process for CCR9 remain to be elucidated and may present new avenues for addressing CCR9 mediated diseases. Here we resolve the underlying mechanism, the effectors involved, and the primary mechanisms of signal cessation.

**Methods:** Using BRET-based biosensors for G protein activation, arrestin recruitment, and internalization, we resolved the signaling pathways of CCR9 and the effectors that coordinate these responses. The contributions of specific GRKs and arrestins were identified by testing receptor activation in CRISPR-Cas9 knockout cells.

**Results:** Our results present that, while CCR9 activates G proteins in response to CCL25, the effect is muted compared to other GPCRs. Despite recruiting arrestins, CCR9 deactivation is primarily mediated by internalization, which occurs in an arrestin-independent manner. Instead, efficient internalization depends on GRKs and robust G protein activation is observed in GRK-knockout cells.

**Conclusion:** In summary, though a canonical GPCR, CCR9 shows impaired G protein activation due to rapid and efficient internalization, which is driven by GRK phosphorylation of the receptor C-terminus. The ramifications and therapeutic potential of CCR9 deactivation mechanisms will be discussed along with potential caveats.

# THE LINK BETWEEN AMYLOID β AND FERROPTOSIS PATHWAY IN ALZHEIMER'S DISEASE

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**Background:** Alzheimer's disease (AD), currently affecting millions of people worldwide, is the most prevalent form of dementia. Treatment strategies aiming to interfere with the formation of amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs), the two major AD hallmarks, have shown modest or no effect. Recent evidence suggests that ferroptosis, a type of programmed cell death caused by iron accumulation and lipid peroxidation, contributes to AD pathogenesis. Here we evaluate if Aβ is associated with ferroptosis pathways and whether ferroptosis inhibition could attenuate Aβ-related effects. The existing link between ferroptosis and AD has been largely based on cell culture and animal studies, while evidence from human brain tissue is limited.

**Methods:** Positive pixel density scoring was performed on immunohistochemically stained post mortem BA17 sections. The human brain cortical organoids (cBOs) were generated from the iPSCs (AD4 harbouring PSEN1-ΔE9 mutation, isogenic control corrected for PSEN1-ΔE9 mutation and healthy control stem cell lines) and differentiated up to day 100. Statistical significance of difference between groups was determined using one-way ANOVA ($*p < 0.05$).

**Results:** We revealed that the progression of AD pathology was accompanied by decreased expression of nuclear receptor co-activator 4 (NCOA4) and glutathione peroxidase 4 (GPX4) in the grey matter (GM). The expression of ferroportin, NCOA4, GPX4, 4-hydroxy-2-nonenal (4HNE) and cytochrome C was significantly lower, while the expression of ferritin was higher in the Aβ plaque area compared to the immediate vicinity of the non-Aβ tissue in AD brain tissue. Taken together, these findings indicate a relationship between Aβ-related processes and the expression of ferroptosis-related proteins. Additionally, ferroptosis inhibition with ferrostatin-1 prevented Aβ pathology, decreased lipid peroxidation and restored iron storage in human AD iPSCs-derived cBOs. Differential expression analysis of AD organoids compared to isogenic controls indicate activation of the ferroptotic pathway.

**Conclusion:** This further supports the previously suggested link between Aβ-related pathology and ferroptosis. Determining the causality between development of Aβ plaques and deregulation of molecular pathways involved in ferroptosis is crucial for developing potential therapeutic interventions.

# INFLUENZA A VIRUS INFECTION CAUSES LIPID PEROXIDATION IN LUNG EPITHELIAL CELLS

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**Introduction:** Influenza viruses are the most common cause of acute respiratory infections, which can be highly contagious and can lead to severe illness in high-risk groups. Influenza virus infection begins by infecting epithelial cells lining the upper respiratory tract, causing cell death and disruption of the epithelial barrier. In severe cases, the virus can also infect the lower respiratory tract and damage the alveoli. An important pathogenic factor during infection is oxidative stress in the host cells. Recent studies show that swine influenza A virus causes depletion of glutathione, an important ROS scavenger, resulting in a ferroptosis-like cell death. The aim of this study is to investigate the involvement of ferroptosis during influenza A virus infection in bronchial and alveolar epithelial cells.

**Methods:** Bronchial (BEAS-2B) and alveolar (A549) epithelial cells were infected with influenza A virus A/PR8/(H1N1) at a multiplicity of infection (MOI) of 1 or treated with RSL-3 (200 nM). Ferrostatin (Fer-1, 5μM) pre-treatment was added 1h before infection. Infected and control cells were collected after 24 hours post-infection (h.p.i.). Malondialdehyde (MDA), a marker of lipid peroxidation, was quantified following the thiobarbituric acid reactive substances assay (TBARS).

**Results:** IAV infection causes a significant increase in lipid peroxidation in both bronchial and alveolar epithelial cells. Pre-treatment with Fer-1, a ferroptosis inhibitor, during 1 hour prior to the infection reduces the lipid peroxidation in the IAV-infected cells, however the decrease in lipid peroxidation was not significant. This might be due to the short pre-treatment time with the ferroptosis inhibitor.

**Conclusion:** Influenza A virus infection a causes lipid peroxidation in bronchial and alveolar epithelial cells. Lipid peroxidation is a marker of ferroptotic death, suggesting the involvement of ferroptosis during IAV infections.

# PHARMACOLOGICAL DISRUPTION OF ENDOGENOUS CXCR4 OLIGOMERS HAS ANTI-TUMORIGENIC EFFECTS IN HEMATOLOGICAL MALIGNANCIES.

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**Background:** The human chemokine receptor CXCR4 is an important regulator of the tumor-microenvironment in various hematological malignancies, inducing pro-survival signaling and tumor homing to lymphoid organs. In heterologous systems, CXCR4 is shown to organize into clusters. However, it remains elusive to what extent such oligomers affect malignancies, and if so whether this can be pharmacologically targeted.

**Methods:** In this study, we describe nanobody-/BRET-based detection of endogenous CXCR4 oligomers in hematological cancer cells. This method was further validated using CRISPR-Cas9 CXCR4 KO cell lines and chemically-induced dimerized CXCR4-FK506-binding protein (FKBP) domain fusion proteins. Direct stochastic optical reconstruction microscopy (dSTORM) single-molecule imaging was conducted to investigate CXCR4 oligomer stoichiometry on hematological cancer cells. Cluster disrupting capabilities of CXCR4 antagonists were characterized using BRET-based sensors and spatial intensity distribution analysis (SpIDA). Consequences of CXCR4 cluster disruption were investigated by using phosphoproteomics and subsequent cell killing assays in mantle cell lymphoma (MCL) cell lines and primary chronic lymphocytic leukemia (CLL) patient-derived PBMCs. In addition, the intrinsic effects of CXCR4 antagonists on proliferation and viability of patient-derived CLL PBMCs are investigated in a 3D spheroid model.

**Results:** CXCR4 oligomerization is expression-depended and can be disrupted in hematological cancer cell lines and primary CLL/MCL cultures by small molecule CXCR4 antagonists IT1t and AMD070 and various antibody fragments. Analysis of the phosphoproteome revealed CXCR4 oligomer-driven activation of signaling pathways involved in survival. Consequently, CXCR4-monomerizing ligands inhibited sensitized Non-Hodgkin lymphoma cells to cell death-inducing Bcl-2 inhibitor clinical drug Venetoclax and prevented growth of CLL patient-derived spheroids.

**Conclusion:** Our findings illustrate an important role of CXCR4 oligomers in cell survival and suggest selective targeting thereof has therapeutic potential in non-Hodgkin lymphomas.

# UNLOCKING LONGEVITY: METFORMIN ENHANCES LIFESPAN, AND RENAL AND ENDOTHELIAL HEALTH-SPAN IN MOUSE MODEL OF VASCULAR AGING

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**Background:** Vascular aging is a critical contributor to cardio/cerebrovascular diseases, with chronic low-grade inflammation and senescence implicated as key factors. However, the effectiveness of anti-inflammatory therapies in preventing cardiovascular aging remains underexplored. Here, we hypothesized that long-term metformin therapy, a safe biguanide with anti-inflammatory properties used in type 2 diabetes treatment, could prevent age-associated pathological changes.

**Methods:** Using vascular age-accelerated mouse models based on endothelium-selective Ercc1 DNA repair gene excision (EC-KO), we administered metformin (150mg/kg/day) or vehicle from 10 to 20 weeks of age. In-vivo and in-vitro assessments of kidney, vascular, cardiac, and endothelial function were performed.

**Results:** EC-KO mice exhibited a significantly lower survival rate compared to littermates (LM) (70.5% vs 100%) by the study conclusion (week 20). Notable, metformin treatment increased the survival rate of EC-KO mice to 88.8%. EC-KO mice also displayed increased water intake and urine volume compared to LM ($P=0.001$), which was completely restored by metformin treatment ($P=0.002$), indicating renal protection. Furthermore, endothelium-dependent vasodilation was decreased in EC-KO mice ($p<0.01$), but chronic metformin treatment restored this function, thanks to enhanced nitric oxide-cGMP signalling.

**Conclusion:** Our study highlights endothelial cell aging as a significant contributor to increased mortality and impaired nitric oxide-mediated endothelium-dependent vasodilation. Metformin therapy emerges as an effective intervention for slowing vascular aging and preventing endothelial dysfunction. Further investigations are warranted to elucidate the underlying mechanisms driving its health and longevity benefits.

# EXPLORING CHEMOKINE BINDING TO GPR182 AND RECEPTOR ACTIVATION USING BIOLUMINESCENCE-BASED APPROACHES

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**Background:**Atypical chemokine receptors (ACKRs) modulate other chemokine and non-chemokine receptors and serve exciting therapeutic targets[1]. GPR182 is the newest identified atypical chemokine receptor (ACKR5) with important functions in the tumour microenvironment mediated via CXCR3 cross-talk and modulation of immune cell migration[1]. Whilst, it has been shown that GPR182 can bind and internalise a range of chemokines[2,3], a detailed molecular characterisation of chemokine-receptor binding and receptor signalling has not yet been reported.

**Methods:**NanoBRET and NanoBiT bioluminescence-based technologies were used to probe ligand-GPCR interactions and GPCR activation.

**Result:**We characterised CXCL12 chemokine-receptor interactions both in terms of affinity and binding kinetics and further, elucidated the negative modulation of receptor conformation by Na+ ions. CXCL12-AZ488 and CXCL12-AZ594 bounds to Nluc-tagged GPR182 with Kd of 11.51 ± 3.4 nM and 16.90 nM ± 6.61 respectively (means ± S.D. of 3 individual experiments). Furthermore, unlabelled human CXCL12 and human CXCL10 displaced tracer binding with pKis of 7.78 ± 0.4 and 6.5 ± 0.2 respectively. In addition, GPR182 scavenged CXCL12-AF647 ligand over time confirming its function of a decoy receptor. The receptor proved to be constitutively active based on its high basal arrestin interactions. Finally, we probed if the receptor could be modulated by non-chemokine ligands, previously shown to bind other atypical and typical chemokine receptors.

**Conclusion:**To conclude, GPR182 acts as a constitutively active chemokine scavenging receptor. Next, we aim to probe the pharmacology of the receptor further including receptor trafficking and recycling and receptor modulation by small-molecule ligands.

# DISTINCT G PROTEIN AND β -ARRESTIN RECRUITMENT TO SEVEN HISTAMINE H3 RECEPTOR ISOFORMS.

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**Background:** The histamine H3 receptor (H3R) regulates, as a presynaptic G protein-coupled receptor (GPCR), the release of histamine and other neurotransmitters in the brain and is consequently a potential therapeutic target for neuronal disorders. The human H3R gene encodes for seven splice variants that conserve the seven transmembrane helical domains but vary in the length of intracellular loop 3 and/or C-terminal tail. All seven splice variants are able to induce heterotrimeric Gi protein-mediated signaling. The last two decades, drug discovery and lead optimization has been focused on the reference H3R-445 isoform, whereas all other isoforms were neglected. Here we setup G protein and β-arrestin recruitment assays to quantify their interaction with activated H3R isoforms in real time.

**Methods:** The recruitment of the mini-Gi protein, β-arrestin1, or β-arrestin2 to agonist-activated H3R isoforms is performed using NanoBiT technology. To this end, LgBiT or SmBiT is fused to C-terminal tail of hH3R isoforms, whereas the complementary NanoBiT fragment is fused to β-arrestin1/2 or the mini-Gi protein. Recruitment of mini-Gi protein or β-arrestin1/2 to the H3R isoforms brings the LgBiT and SmBiT fragments in close proximity allowing reconstitution of a functional Nanoluc luciferase enzyme.

**Results:** First the assay was optimized for SmBiT/LgBiT configuration between the receptor and transducers. Mini-Gi protein recruitment could be observed for all isoforms upon agonist stimulation with 10 μM histamine. However, the H3R-373, -365 and -329 isoforms constitutively recruited mini-Gi protein, which could be decreased by the inverse agonist pitolisant. Upon 10 μM histamine stimulation the β-arrestin1/2 recruitment to the H3R-445, -415, -413 isoform results in stable signal over time, whereas a unique transient response for H3R-365 and -329 isoforms has been observed. Lastly, H3R-453 and -373 isoforms displayed no β-arrestin1/2 recruitment.

**Conclusion:** hH3R isoforms has determined to have no β-arrestin1/2 preference. Additionally, NanoBiT assay allows observation of unique isoform dependent kinetic profiling. The H3R-373, -365, and -329 isoforms constitutively recruit mini-Gi proteins.

# INDUCING RECEPTOR DEGRADATION AS A NOVEL APPROACH TO TARGET CC CHEMOKINE RECEPTOR 2 (CCR2)

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**Background:** CC chemokine receptor 2 (CCR2) is a G protein-coupled receptor highly expressed in immune cells. Dysregulation of CCR2 has been linked to inflammatory or immune diseases, such as atherosclerosis, multiple sclerosis and cancer. Yet, all CCR2 inhibitors developed so far have failed in clinical trials, which warrants the development of novel strategies to target this receptor. A novel approach to inhibit protein function is to induce proteasomal degradation of the target protein with the use of PROteolysis TArgeting Chimeras (PROTACs). Before we embark on the development of CCR2 PROTACs, we aimed to investigate whether proteasomal degradation can be chemically induced for CCR2, and thus, if this approach can be used therapeutically.

**Methods:** To investigate the amenability to receptor degradation, we designed and characterized a CCR2-HaloTag-HiBiT fusion protein. This system allowed us to induce degradation with HaloPROTAC3, a tool PROTAC that selectively degrades HaloTag fusion proteins. To measure protein levels, we set up a luminescence-based assay, which relies on the complementation of the HiBiT tag with an LgBiT subunit to generate a luminescent enzyme. In addition, we used a label free, impedance-based functional assay (xCELLigence) to determine the functional effect of inducing CCR2 degradation.

**Results:** Luminescence assays showed that treatment with HaloPROTAC3 leads to ~30-60% reduction in CCR2 levels, which was not observed after treatment with negative control or with traditional CCR2 antagonists ($n \geq 3$). Finally, xCELLigence assays showed that inducing CCR2 degradation results in a reduced functional response after agonist stimulation, similar to the remaining response after treatment with CCR2 inhibitors ($n=4$).

**Conclusion:** In conclusion, our results indicate that CCR2 is amenable to targeted degradation. In addition, these assays allowed us to shed light on the degradation pathways and degradation kinetics of CCR2 by HaloPROTAC3. Finally, the assays from this project pave the way for the development of CCR2 PROTACs as a novel strategy to target this receptor in a variety of diseases.

# TYPICAL MEETS ATYPICAL - VIRUS-ENCODED GPCRS US28 AND UL78 WORK IN TANDEM TO HIJACK THE HIPPO PATHWAY VIA DISTINCT MECHANISMS

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**Background:** Human cytomegalovirus (HCMV) is a common DNA herpesvirus that is widely prevalent in the human population. In healthy individuals, HCMV infection usually induces few to no symptoms. However, recent findings indicate that HCMV is oncomodulatory, i.e. changes the progression of cancer in infected patients. HCMV exhibits these oncomodulatory effects by encoding proteins that hijack the signaling network of its host cells. Two of these proteins are the HCMV-encoded viral GPCRs (vGPCRs), US28 and UL78. Both US28 and UL78 modulate the Hippo pathway, which controls cell proliferation and organ growth. As dysregulation of the Hippo pathway has been linked to cancer, its modulation by these two vGPCRs may be a crucial determinant of HCMV-mediated oncomodulation.
In this project, we aim to investigate the Hippo pathway modulation by US28 and UL78, gain insight into the molecular signaling mechanism behind it, and develop nanobodies to potentially modulate it.

**Methods:** We used a TEAD reporter gene assay to monitor Hippo pathway modulation in HEK293T cells, combined with multiple pharmacological inhibitors and G protein knockout cell lines to determine crucial signaling components. We employed various mutated receptor constructs of US28 and UL78 to determine structural elements of these receptors essential for signaling. Finally, we identified nanobodies to target UL78 and modulate its signaling capacity.

**Results:** Our findings show that while both US28 and UL78 lead to an increase in the activity of TEAD, the transcription factor controlled by Hippo, they do so via two distinct mechanisms. US28 couples to G proteins of the Galphaq/Galpha12 family, which are known to lead to Hippo modulation. Conversely, UL78 is an atypical GPCR that does not signal to Galphaq/G12, and seems to increase TEAD activity via a different, undescribed mechanism. We show that UL78 signaling remains intact when Galpha proteins are inhibited or absent. Using mutational studies, we identified the receptor regions essential for UL78-mediated signaling, and developed a toolbox of nanobodies to modulate its activity.

**Conclusion:** Taken together, our findings demonstrate how two vGPCRs, one classical and one atypical, work in tandem to hijack the Hippo pathway. Our findings not only uncover a GPCR signaling pathway that is independent of Galpha activation, but also provide essential insight into how HCMV mediates its oncomulatory functions.

# TNF INHIBITOR USE INCREASES BIRTHWEIGHT IN PREGNANT WOMEN WITH RHEUMATOID ARTHRITIS INDEPENDENTLY OF THE SOLUBLE FMS-LIKE TYROSINE KINASE-1/PLACENTAL GROWTH FACTOR RATIO

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**Background:** To study whether the use of TNF inhibitors (TNFi) by pregnant women with rheumatoid arthritis (RA) affects soluble Fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), or their impact on birthweight.

**Methods:** sFlt-1 and PlGF were measured in all trimesters of pregnancy in the Preconception Counseling in Active RA study, and were compared according to the use of TNFi. The association of sFlt-1 and PlGF with birthweight in relation to TNFi was determined.

**Results:** 158 women were included, of whom 52.5% used TNFi during pregnancy. Both sFlt-1 and PlGF increased during pregnancy, whereas their ratio declined. Taking into consideration the trimester-related variation in levels of sFlt-1 and PlGF, after correction for relevant confounders, the sFlt-1/PlGF ratio was not significantly different between patients that did, or did not use TNFi (sFlt-1/PlGF ratio in the second trimester compared to the first trimester: estimated change 8.17, CI 2.54 – 26.29, p = 0.79; sFlt-1/PlGF ratio in the third trimester compared to the first trimester: estimated change 6.25, CI 1.73 – 22.50, p = 0.25). In women that did not use TNFi, birthweight was significantly lower (3180 vs. 3302 grams; p=0.03), and sFlt-1 displayed a negative correlation with birthweight (r=-0.462, p<0.001) and birthweight percentile (r=-0.332 , p=0.008). In TNFi users, these correlations were absent.

**Conclusions:** TNF inhibitor use increases birthweight in pregnant women with rheumatoid arthritis independently of the sFlt-1/PlGF ratio.

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

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**Background:** Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a leukodystrophy with
onset in infancy, characterized by chronic brain oedema (van der Knaap et al., 2012). In most patients, pathogenic variants in either the *MLC1* or *GLIALCAM* gene are disease causing (Leegwater et al., 2001; Lopez-Hernandez et al., 2011). Recently we have identified two dominant variants in *GPRC5B* in three unrelated MLC patients without MLC1 or GlialCAM defects. These variants result in amino acid duplications in transmembrane helix 4 (Ile176dup and Ala177dup) and increased expression levels in patient-derived cells (Passchier et al., 2023). GPRC5B is an orphan class C GPCR with no identified endogenous ligand. Three GPRC5B splice variants have been reported: the canonical sequence (GPRC5B(1)), a longer isoform transcribed from an upstream start codon (GPRC5B(2)) (Brauner-Osborne & Krogsgaard-Larsen, 2000), and a brain specific isoform with a distinct C-tail (GPRC5B(BR)) (Cool et al., 2010). GPRC5B has been shown to constitutively increase NFkB signalling through its interaction with the tyrosine kinase Fyn (Freundt et al., 2022; Kim, 2012), which could be used as a possible screening method for GPRC5B activation. To design a screening method for the discovery of ligands or modulators of GPRC5B, expression of the three isoforms and mutants is assessed, as well as their interaction with MLC1 and their signalling.

**Methods:** Double-tagged GPRC5B isoforms and mutants with an extracellular N-terminal HA-tag and an intracellular C-terminal fluorescent protein (mVenus) or bioluminescent luciferase (Nluc) fusion were generated to investigate their expression, protein-protein interaction, and signalling in transiently transfected HEK293T cells.

**Results:** GPRC5B(1) and GPRC5B(BR) isoforms are readily expressed at the cell surface of HEK293T cells, whereas, expression of the long isoform GPRC5B(2) was hampered. Both GPCR5B(1) and GPCR5B(BR) isoforms can interact with co-expressed MLC1 protein in HEK293T cells as assessed by bioluminescence resonance energy transfer, and the propensity of this interaction was not affected by Ile176dup and Ala177dup mutations. NFkB signalling and Fyn interaction by GPRC5B(1) and GPRC5B(BR) is being investigated.

**Conclusion:** GPRC5B(1) and (BR) isoforms and MLC-related mutants are expressed and interact with MLC1 protein in HEK293T cells. We currently setup assays to measure GPRC5B activity to identify small molecule modulators of GPRC5B.

# THE EFFECT OF HYPERGLYCEMIA ON VASCULAR AGING: A MOUSE MODEL TO TRIGGER CARDIO-RENAL DYSFUNCTION AND BIOENERGETIC SHIFT

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**Background:** Hyperglycemia is commonly observed in the aging population and is a driver of cardiovascular and renal dysfunction, as well as further metabolic derailment and organ dysfunction that feature aging. We hypothesized that hyperglycemia accelerates non-atherosclerotic vascular aging (NAVA) through impaired cardiovascular performance and renal dysfunction via bioenergetic mechanisms.

**Methods:** Using vascular age-accelerated mouse models with endothelium-selective Ercc1 DNA repair gene excision (EC-KO) and the corresponding littermates (LM), we administered either streptozotocin (STZ) (50mg/kg/over 5 consecutive days, intraperitoneal) or vehicle at 8 weeks of age. Urine and blood samples were collected in 24-hour metabolic cage sessions at 10 and 18 weeks old. One week before sacrifice, echocardiography was performed to assess cardiac function. After sacrifice, thoracic aorta was used for myography, and kidneys were used for mitochondrial high-resolution respirometry.
Mice were considered diabetic with blood glucose >14 mmol/l (LM non diabetic: 6.9±0.5; EC-KO non-diabetic: 6.3±0.6; LM diabetic (LM+HG): 22.9±1.35 and EC-KO diabetic (EC-KO+HG):18.2±3.6 mmol/l).

**Results:** Both LM and EC-KO diabetic mice showed polyuria at both 10 and 18 weeks old compared to their non-diabetic controls ($p < 0.01$). However, water and food intake were significantly increased only in LM+HG mice. At 18 weeks of age, there were no discernible differences in body weight among the groups, except for the lower body weight observed in the LM+HG group compared to the LM group. Renal function declined in EC-KO+HG mice indicated with increased plasma creatinine compared EC-KO non diabetics at 18 weeks of age. Albumin creatinine ratio is augmented in all diabetic mice compared to their non-diabetic control. Mitochondrial oxygen consumption rate (OCR) in the kidney is higher only in LM+HG mice after addition of oligomycin, while maximal respiration capacity FCCP-induced is higher in both aging and hyperglycemia. Furthermore, endothelium-dependent vaso-relaxation is impaired in the aorta of EC-KO+HG mice compared to EC-KO ($p < 0.02$). Cardiac output is decreased in both LM+HG and EC-KO+HG mice compared to the non-diabetic controls ($p = 0.01$).

**Conclusions:** In conclusion, the introduction of hyperglycemia atop accelerated aging exacerbated cardiovascular dysfunction and CKD progression, accompanied by alterations in mitochondrial metabolism.

# SCRAB: A NOVEL SINGLE FLUOROPHORE BIOSENSOR TO STUDY SPATIOTEMPORAL DYNAMICS OF CXCR4 RECEPTOR ACTIVITY

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**Background:** The CXC chemokine receptor 4 (CXCR4), as a Class A G protein-coupled receptor (GPCR), becomes a major therapeutic target for cancer treatment. Nowadays, the development of more efficacious and selective CXCR4 pharmacological modulators is of great clinical interest, which largely hinges on a better understanding of ligand-induced receptor structural dynamics. Recently, a Förster resonance energy transfer (FRET)-based CXCR4 biosensor has been developed to spatiotemporally resolve the receptor activation. However, due to the demanding labeling process and a relatively small dynamic range (~ 3%), we set out to develop a novel single-color biosensor based on the label-free circularly permutated green fluorescent protein (cpGFP) module to study receptor conformational changes more efficiently. The biosensor is further named as the single-color chemokine receptor activation biosensor (SCRAB).

**Methods:** To perform sensor characterizations, we used single-cell epi-fluorescence microscopy, which also coupled with air-pressurized perfusion system in order to locally stimulate the HEK293T cells expressing the SCRABs with a panel of ligands, ranging from the agonist CXCL12 to CXCR4-specific antagonists, including a novel CXCR4 inverse agonist JM#173.

**Results:** The SCRAB displays a moderate fluorescence increase ($\Delta F/F_0$%, ~20.8%, n = 134 cells) and an activation kinetics with time constants ($\tau$) of ~ 2.0s (n = 134 cells) in response to the cognate agonist CXCL12. With a high CXCL12-induced signal specificity, two SCRAB mutants, N119S (n = 33 cells) and $\Delta$1-23 N-terminal truncation (n = 28 cells), showed impaired responses of ~8.4% and ~6.2%, respectively, towards CXCL12 as anticipated because 1) N119S imparts constitutive activity to the CXCR4 2) N-terminal is important for CXCL12 binding. The SCRAB, along with its N119S mutant, are further employed to characterize other CXCR4-bound small molecule ligands with distinct efficacies at the G protein level, and the SCRABs' fluorescence changes, after ligand treatments, are strongly correlated with the receptor pharmacology assessed by the Gi2 FRET sensor.

**Conclusion:** The novel SCRABs, particularly the SCRAB-N119S, would potentially become good single-cell ligand characterization tools to screen ligands with diverse pharmacological effects due of their abilities to confidently reflect the ligand intrinsic efficacy at the G protein level. With better spatiotemporal resolutions, SCRABs directly unravelled the dynamics of ligand-induced CXCR4 conformation modulations.

# ABSENCE OF TISSUE TRANSGLUTAMINASE REDUCES AMYLOID-BETA PATHOLOGY IN APP23 MICE

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**Background:** Alzheimer's disease (AD) is characterized by amyloid-beta (Aβ) aggregates in the brain (1). Targeting Aβ aggregates is a major approach for AD therapies, although attempts had little to no success so far. A novel treatment option is to focus on blocking the actual formation of Aβ multimers (2). The enzyme tissue transglutaminase (TG2) is abundantly expressed in the human brain and plays a key role in post-translational modifications in Aβ resulting in covalently cross-linked, stable and neurotoxic Aβ oligomers (3). **Aim.** In vivo absence of TG2 in the APP23 mouse model may provide evidence that TG2 plays a key role in development and/or progression of Aβ-related pathology.

**Methods:** Here, we compared effects on Aβ pathology in the presence or absence of TG2 using 12-month-old wildtype, APP23, and a crossbreed of the TG2-/- mouse model and APP23 mice (APP23/TG2-/-) (see figure 1). Based on established milestones in the progression of AD pathology within the model (e.g. first appearance of plaques, cognitive deficits and progression of wide-spread Aβ pathology), 12-month-old animals were selected. The 12-month-old mouse group consisted of APP23 (n=7), WT (n=4), APP23/TG2-/- (n = 7) and TG2-/- (n=2). All animal procedures meet the requirements as stated in the EU Directive 2010/EU/63. The experimental procedure using the above-mentioned mice were carried out in accordance with the animal welfare body of the VU University and approved by the local Animal Care and Use Committee.

**Results:** Using immunohistochemistry, we found that the number of Aβ deposits were significantly reduced in the absence of TG2 compared to age-matched APP23 mice. To pinpoint possible TG2-associated mechanisms involved in this observation, we analysed soluble brain $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$ and/or $A\beta_{40/42}$ ratio, and mRNA levels of human APP and TG2 family members present in brain of the various mouse models. In addition, using immunohistochemistry both beta-pleated sheet formation in Aβ deposits and the presence of reactive astrocytes associated with Aβ deposits were analysed.

**Conclusion:** We found that absence of TG2 reduces the formation of Aβ pathology in the APP23 mouse model, suggesting that TG2 may be a suitable therapeutic target for reducing Aβ deposition in AD.

# THE SECRETOME OF PULMONARY MICROVASCULAR ENDOTHELIAL CELLS SUPPORTS ALVEOLAR EPITHELIAL GROWTH

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**Background and Aims:** Human Pulmonary microvascular endothelial cells (HPMECs) orchestrate alveolar epithelial regeneration/repair with unclear mechanisms. This study aimed to identify the role of the HPMECs-derived secretome consisting of extracellular vesicles (EVs) and soluble factors (SFs), on the regenerative potential of alveolar epithelial progenitors.

**Methods:** Lung organoids were co-cultured with epithelial progenitors and fibroblasts and/or HPMECs. HPMECs-derived EVs ($10^9$ particles/ml) or SFs (30 μg/ml) were added to the organoid culture. In another setup, HPMECs were pretreated with 5% cigarette smoke extract (CSE) or 200 μg/ml diesel exhaust particles (DEP) for 48h. Proteomics analysis was performed on HPMEC-derived EVs and SFs. RNA sequencing (RNA-seq) was performed on pulmonary endothelial cells (ECs) from mice exposed to CS or air.

**Results:** The presence of HPMECs or HPMEC-derived EVs or SFs significantly increased alveolar organoid number respectively. Proteomics analysis on HPMEC-derived EVs and SFs revealed the presence of proteins related to VEGFA-VEGFR2, NRF2, WNT, and BMP signaling. CSE or DEP impaired the supportive function of HPMEC-derived secretome to form lung organoids. RNA-seq analysis showed decreased expression of *Bmp1*, *Bmp4,* and *Bmp6* on ECs in response to CS. Treatment with recombinant BMP6 supported alveolar organoid formation, whereas BMP1 or BMP4 failed to do so.

**Conclusion:** HPMECs support alveolar organoid growth, which is likely mediated by the secretion of EVs and SFs. Multiple secreted factors, including BMPs, are involved in this supportive function. These data suggest an important role of HPMECs and its secretome as orchestrators in distal lung repair.

# PHARMACOLOGICAL PROFILING FOR CCR5 ANTAGONISTS

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**Background:** CC chemokine receptor 5 (CCR5), belongs to the class A family of G protein-coupled receptors (GPCRs). Like most chemokine receptors, CCR5 has multiple endogenous ligands and is involved in many inflammatory and autoimmune diseases, making it a compelling therapeutic target. Notably, CCR5 also serves as a primary co-receptor for human immunodeficiency virus (HIV) infection. So far, only one CCR5 orthosteric antagonist, Maraviroc, is currently available in the market for the latter indication.
Intracellular allosteric antagonists are an interesting novel avenue to target receptors, as they bind to distinct binding sites from endogenous/orthosteric ligands. Thereby, they do not compete with but modulate the affinity/efficacy of endogenous ligands, and lead to insurmountable antagonism. Hence, a deeper understanding of promising CCR5 orthosteric and allosteric antagonists at the pharmacological level before the clinical application is needed.

**Methods:** For this study, three known orthosteric antagonists (Maraviroc, TAK779, and BMS-813160) and six intracellular allosteric antagonists from diverse chemical scaffolds were selected (CCR2-RA-[*R*], JNJ-27141491, LUF7722, LUF7654, LUF7684, and LUF7686). These were then profiled in different functional assays, i.e. [$^{35}$S]GTPγS binding, β-arrestin recruitment, and whole-cell impedance assay, yielding potency values of the antagonist set. Moreover, two CCR5 endogenous ligands, CCL3 and CCL5, were used in all assays to assess the so-called probe-dependence of these antagonists.

**Results:** All nine antagonists inhibit CCR5 in different functional assays when stimulated by chemokines CCL3 and CCL5, with $pIC_{50}$ values ranging from 5.8 to 10. Of note, the orthosteric antagonists were in general 100-3000 fold more potent than the allosteric antagonists.
Among the allosteric compounds, LUF7686 was the most potent in inhibiting [$^{35}$S]GTPγS binding ($pIC_{50}$ 7.0 with CCL3, and $pIC_{50}$ 7.2 with CCL5), while LUF7721 was the most potent in inhibiting the β-arrestin recruitment ($pIC_{50}$ 7.6 with CCL3). Interestingly, while CCR2-RA-[*R*] and JNJ-27141491 exhibited lower potencies, these compounds displayed a preference for inhibition of CCR5 upon induction by CCL5 than by CCL3 in the [$^{35}$S]GTPγS binding assay.

**Conclusion:** In conclusion, this study provides a comprehensive pharmacological characterization of CCR5 orthosteric and allosteric antagonists. Hence, it provides valuable insights to the understanding of CCR5 antagonism, and ultimately helps the development and clinical utilization of CCR5 antagonists.

# INCORPORATION OF ACTIVE TRANSPORT AND METABOLISM INTO CNS PBPK MODEL FOR CLOZAPINE BRAIN PHARMACOKINETIC PREDICTIONS

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**Background:** Clozapine, an atypical antipsychotic medication, is utilized in the treatment of treatment-resistant schizophrenia, a severe neuropsychiatric disorder linked with substantial morbidity and mortality. Despite its efficacy, clozapine is associated with numerous adverse effects, including agranulocytosis, myocarditis, and metabolic syndrome. Therefore, comprehensive pharmacokinetic (PK) and/or pharmacodynamic (PD) monitoring of clozapine in-vivo is imperative. Monitoring plasma clozapine levels has been highlighted as crucial for preventing adverse effects resulting from elevated concentrations. However, given that clozapine primarily acts within the central nervous system (CNS), understanding its unbound concentration at the brain target site is paramount. While brain microdialysis offers precise data, it can be applied in rats, while ethical constraints prohibit its use in the human brain. Consequently, positron emission tomography (PET) scans are commonly employed to monitor clozapine PK in the human brain. PET imaging, however, does not accurately reflect unbound clozapine concentrations, nor distinguishes between intracellular and extracellular regions. Thus, there is a pressing need for predictive models of brain clozapine PK to enhance understanding and refine treatment strategies.

**Methods:** Building upon previous models, we extended our translational CNS physiologically-based pharmacokinetic (PBPK) rat model (LeiCNSPK3.0) to incorporate brain metabolism (model 1). Then, model 1 was extended by active transport (model 2) for clozapine and extrapolated to humans based on known human enzyme and transporter activities.

**Results:** By integrating active transport and considering metabolic pathways, model 2 successfully predicted clozapine concentration profiles in brain extracellular fluid (brainECF) in rats, and offers a more comprehensive understanding of clozapine PK in the rat brain compared to previous models. Besides, we predicted clozapine PK profile in human brainECF with a rough validation range.

**Conclusion:** Model 2 enhances our comprehension of clozapine PK in the rat brain, providing the possibility to extrapolate to the human brain, and may facilitate the prediction of deviations under various conditions such as drug-drug interactions, environmental factors, or genetic polymorphisms. This enhanced understanding could inform personalized treatment strategies and mitigate adverse effects associated with clozapine therapy.