Evaluation of the drug-drug interaction potential of treosulfan using a physiologically-based pharmacokinetic modelling approach

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Aims: The aim of this work is the development of a mechanistic physiologically-based pharmacokinetic (PBPK) model using in vitro to in vivo extrapolation to conduct a drug-drug interaction (DDI) assessment of treosulfan against two cytochrome p450 (CYP) isoenzymes and P-glycoprotein (P-gp) substrates.

Methods: A PBPK model for treosulfan was developed de novo based on literature and unpublished clinical data. The PBPK DDI analysis was conducted using the U.S. Food and Drug Administration (FDA) DDI index drugs (probe substrates) midazolam, omeprazole and digoxin for CYP3A4, CYP2C19 and P-gp, respectively. Qualified and documented PBPK models of the probe substrates have been adopted from an open-source online model database.

Results: The PBPK model for treosulfan, based on both in vitro and in vivo data, was able to predict the plasma concentration-time profiles and exposure levels of treosulfan applied for a standard conditioning treatment. Medium and low potentials for DDI on CYP3A4 (maximum area under the concentration-time curve ratio (AUCR_max = 2.23)) and CYP2C19 (AUCR_max = 1.6) were predicted, respectively, using probe substrates midazolam and omeprazole. Treosulfan was not predicted to cause a DDI on P-gp.

Conclusion: Medicinal products with a narrow therapeutic index (e.g., digoxin) that are substrates for CYP3A4, CYP2C19 or P-gp should not be given during treatment with treosulfan. However, considering the comprehensive treosulfan-based conditioning treatment schedule and the respective pharmacokinetic properties of the concomitantly used drugs (e.g., half-life), the potential for interaction on all evaluated mechanisms would be low (AUCR < 1.25), if concomitantly administered drugs are dosed either 2 hours before or 8 hours after the 2-hour intravenous infusion of treosulfan.

KEYWORDS
anticancer therapy, drug-drug interaction, inhibitor, modelling and simulation, pharmacokinetics

The authors confirm that there is no principal investigator for this study.

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1 | INTRODUCTION

Treosulfan (L-threitol-1,4-bis-methanesulfonate) is a prodrug of a bifunctional alkylating agent with cytotoxic, myeloablative and immunosuppressive properties applied in the treatment of ovarian cancer and conditioning therapy prior to haematopoietic stem cell transplantation.\(^1,2\) Indication-dependent, it can be administered intravenously or orally in single doses of 3-8 g/m\(^2\) every 2 weeks or intravenously at higher doses of 10-14 mg/m\(^2\) on 3 consecutive days. Treosulfan is structurally related to busulfan, from which it differs in two hydroxyl groups, leading to a different mechanism of alkylation.\(^3\) Treosulfan is nonenzymatically, pH-dependently converted into its monoepoxide and diepoxide transformation products (2S,3S)-1,2-epoxybutane-3,4-diol 4-methanesulfonate and (2S,3S)-1,2:3,4-diepoxybutane (S,S-EBDM and S,S-DEB, respectively). These mono- and diepoxide transformation products of treosulfan are considered to be the active cytotoxic species and are responsible for DNA alkylation, crosslinking of DNA and proteins, chromosomal aberration, and, finally, subsequent interference with various actions, including genotoxicity and induction of apoptosis.\(^4\) However, the levels of S,S-DEB in the whole body, including cell nuclei, are expected to be several orders of magnitude lower than that of S,S-EBDM.\(^5,6\)

Understanding drug-drug interactions (DDIs) is a critical part of the drug development process since a clinically relevant change in the exposure of a co-administered drug can lead to loss of efficacy or, conversely, an adverse drug reaction, depending on the therapeutic window of the victim drug.\(^7\) The DDI risk becomes important for anticancer drugs since these drugs are typically administered close to the maximum tolerated dose.\(^8\) About 20-30% of all adverse drug reactions have been reported to be caused by DDIs,\(^7\) contributing to 4% of overall death rates in cancer patients.\(^9\)

The evaluation of potential DDI events is instigated at an early stage in drug discovery with preclinical assessment and characterization using appropriate in vitro tools of human systems. Depending on the outcomes of a thorough risk assessment, formal clinical studies may be necessary to address labelling requirements and support prescribing information.\(^7\) The advances in modelling and simulation approaches (eg, physiologically-based pharmacokinetic (PBPK) models) have enabled a more quantitative perspective to better inform decision-making around DDI risk assessment and mitigation, a strategy that has evolved mainly from the fact that chronic drug therapy and polypharmacy are commonplace in many patient populations.\(^7\) PBPK models are mathematical models that mechanistically describe the pharmacokinetics (PKs) of xenobiotics based on their physicochemical properties and the physiology of the exposed species. They are typically composed of multiple compartments, each representing a separate organ or tissue, interconnected via transport rate equations representing the circulatory system of the body. Relying on a priori knowledge on partly independent physiological processes integrated within a mechanistic framework, PBPK models allow the prediction and description of absorption, distribution, metabolism, excretion (ADME) properties, and DDI of a drug.\(^10-13\)

What is already known about this subject

- Treosulfan is a prodrug of a bifunctional alkylating agent with cytotoxic, myeloablative and immunosuppressive properties applied in the treatment of ovarian cancer and conditioning therapy prior to hematopoietic stem cell transplantation.
- Previously conducted detailed in vitro studies did not completely exclude potential interactions between high plasma concentrations of treosulfan and CYP3A4, CYP2C19 or P-gp substrates.

What this study adds

- This study is the first physiologically-based pharmacokinetic (PBPK) model developed for treosulfan.
- This work leverages PBPKs to provide drug-drug interaction (DDI) guidance in the patient population under treatment with treosulfan.
- Using the probe substrates midazolam, omeprazole and digoxin for CYP3A4 and CYP2C19, medium and low potentials for DDI on CYP3A4 (AUCR = 2.23) and CYP2C19 (AUCR = 1.6) were predicted, while treosulfan was not assessed as an inhibitor of P-gp (AUCR < 1.25).

The objective of the presented analysis was to conduct a PBPK DDI analysis on CYP3A4, CYP2C19 and P-gp inhibition by treosulfan. The PBPK DDI analysis was conducted using the FDA DDI index drugs (probe substrates) midazolam, omeprazole and digoxin for CYP3A4, CYP2C19 and P-gp, respectively.

2 | METHODS

2.1 | Clinical data

The pharmacokinetic study population (clinical phase III trial MC-FludT.14/L Part I) consisted of 24 patients aged from 43 to 70 years with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who were randomised into the treosulfan arm and received preparative treatment prior to allogeneic hematopoietic stem cell transplantation. Treosulfan concentrations were determined in plasma and urine for the evaluation of pharmacokinetic parameters. The treosulfan plasma levels were measured by 20 blood samples (3 mL of whole blood each, total 60 mL) on day −6 (hours 0 [prior to the infusion]), 2 [immediately after the 2-hour treosulfan infusion], 2.5, 3, 4, 5, 6, 8 and 12 hours after the start of the treosulfan infusion), day −5 (hours 0 [immediately prior to the second treosulfan infusion], 2 [immediately after the second 2-hour treosulfan infusion]) and day −4 (hours 0 [immediately prior to the third treosulfan infusion],
2 [immediately after the third 2-hour treosulfan infusion], 2.5, 3, 4, 5, 6, 8, 12 and 24 hours after the start of the third treosulfan infusion (3 mL of whole blood each, total 60 mL). Urine samples were collected from 4-hour fractions of total urine volume collected for 72 hours during the three consecutive treosulfan treatment days (day −6 to day −4). These studies were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice. The final protocol and informed consent form were approved by the institutional review boards at the respective study sites. Informed consent was obtained from all volunteers before any study procedures were conducted.14

2.2 | Cytochrome P450 inhibition

Six treosulfan concentrations (ranging from 40 to 10,000 μM in water) and human liver microsomes (final concentration 0.25 mg/mL) were pre-incubated each for 30 min in the absence and presence of NADPH or underwent a 0 min pre-incubation15 (unpublished data). At the end of the pre-incubation period, probe substrates of the respective CYP isoforms and NADPH (1 mM) were added (final DMSO concentration 0.05%), and the samples were incubated for 5 min at 37 °C. The time-dependent inhibitors, furafylline (CYP1A2 inhibition), thiotepa (CYP2B6 inhibition), gemfibrozil 1-O-β-glucuronide (CYP2C8 inhibition), tienilic acid, (CYP2D6 inhibition), paroxetine (CYP2C19 inhibition), fluoxetine (CYP2C9 inhibition), probenecid (OAT1 and OAT3), verapamil (OCT2 and OCT1) and midazolam/testosterone.

A decrease in the formation of the metabolite compared to vehicle control was used to calculate an IC50 value (test compound concentration that produces 50% inhibition; Supporting Information Figures S13 and S14) for each experimental condition. The fold shift in IC50 was calculated using the following equation:

\[
\text{fold shift} = \frac{IC50(\text{minus})}{IC50(\text{plus})}
\]

where IC50 (minus) = IC50 determined from a 30-minute pre-incubation in the absence of NADPH and IC50 (plus) = IC50 determined from a 30-minute pre-incubation in the presence of NADPH.

2.3 | Efflux and solute carrier transporter inhibition

Treosulfan (concentrations up to 1000 μM) was tested as an inhibitor of the human transporters P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, OCT1, MATE1 and MATE2-K in various in vitro cell test systems, and of BSEP in membrane vesicles16 (unpublished data). Probe substrates loperamide (P-gp) and estrone 3-sulfate (BCRP), and the respective inhibitors cyclosporin A and novobiocin were incubated with the appropriate in vitro test system for a specific incubation time in the absence and presence of a range of concentrations of the test compound treosulfan. The rate of transport of the probe substrate, which was used to determine apparent permeability (Papp) was calculated according to the equation given below:

\[
Papp = \frac{\frac{dC}{dt}}{A 	imes C_0}
\]

where Papp is apparent permeability (cm/s × 10⁻⁶), dQ/dt is the rate of drug transport (pmol/s), A is the surface area of the membrane (cm²) and C₀ is the initial donor concentration (nM, pmol/cm²). The individual replica Papp determined in the apical to basolateral direction was subtracted from the corresponding Papp determined in the basolateral to apical direction to give a transporter-mediated net secretory flux value. Each replicate’s net secretory flux value was then converted to a percentage of the mean vehicle control transport activity, which was plotted against test compound concentration and subsequently fitted to calculate an IC50 value (concentration which produces 50% inhibition of vehicle control transport activity; Supporting Information Figure S15).

The positive inhibitor controls used for treosulfan SLC transporter assessment were rifamycin (OATP1B1), cyclosporin A (OATP1B3), probenecid (OAT1 and OAT3), verapamil (OCT2 and OCT1) and cimetidine (MATE1 and MATE2-K).

2.4 | PBPK model development

The PBPK analyses were performed using qualified installations of the PBPK software PK-Sim version 8.0 (http://www.open-systems-pharmacology.org/).17 The analytical approach is based on principles established in the European Medicines Agency (EMA) and/or FDA guidelines on the reporting of PBPK modeling and simulation (M&S).18,19 As the first step in model development, all available information on the drug regarding its ADME properties is gathered. This includes the drug-specific parameters used as input parameters and the characterisation of the organism or population.

2.5 | Treosulfan PBPK model development and qualification

Treosulfan is nonenzymatically, pH-dependently converted into its monooxepoxide and diepoxide transformation products S,S-EDBM and S,S-DEB, respectively. This transformation process is systemic within all blood and tissue compartments. Treosulfan is excreted in the kidneys via glomerular filtration, and the fraction of unchanged treosulfan excreted in patients’ urine is 15-40%.20 No other transport or secretion processes in renal clearance are indicated. In the PBPK model, treosulfan transformation was implemented using a non-specific systemic enzymatic process (required in PK-Sim to implement transformation rates of any kind). The process was implemented such that the “enzyme” is “expressed” uniformly across all systemic compartments. The expression level was arbitrarily set to 1 μmol/L (Figure 1). The reported glomerular filtration rate (GFR) of each
patient was set according to their reported creatinine clearance (Supporting Information Table S1). The model was otherwise parameterized according to the parameters listed in Table 1, and the simulation was created using the partition coefficient and permeability calculation method PK-Sim Standard.

The PBPK model of treosulfan PKs was developed using individual data from study MC-FludT.14/L Part I (Supporting Information Table S1). For model development and qualification, study subjects were divided into two groups based on their administration protocol. Subjects which received an infusion exactly every 48 hours, as specified in the protocol, were taken into the qualification set and subjects for which the time of administration deviated from this exact timing were taken into the training set. This was done to receive a homogeneous set of concentration-time profiles for the qualification to be compared against a population simulation.

For the model development, a parameter identification (PI) routine was set up to characterise interindividual variability using the training data set. The PI was executed in PK-Sim using the Levenberg-Marquardt algorithm. Within the PI, the rate of systemic transformation and the fraction excreted via glomerular filtration were estimated (Supporting Information Table S1). From the individual resulting estimates, the mean and standard deviation were calculated for use as “User Defined Variability” in the population simulation for the model qualification using the qualification data set.

The qualification of the developed PBPK model of treosulfan consisted of a population simulation of 200 subjects using ranges for age, gender, body weight and dosing information representative of the second half (qualification data set) of the study population. To evaluate the predictive performance of the PBPK model, the overall accuracy of the predicted PK parameters was assessed using the mean fold-error (MFE; the difference between predicted in silico and observed in vivo values from the qualification data set):

$$MFE = \frac{\text{PK parameter}_{\text{predicted mean}}}{\text{PK parameter}_{\text{observed mean}}}$$

The model was accepted when all predicted PK parameters were within 2-fold of the corresponding observed values (eg, MFE 0.5-2.0).23

2.6 | PBPK DDI analyses with index substrates

The simulations required to analyse the DDIs of treosulfan with the selected FDA DDI index substrate drugs midazolam,24,25 digoxin24,26 and omeprazole27,28 were conducted in PK-Sim. The IC50 inhibition constants listed in Table 1 were used in the model to predict the reversible inhibitory potential of treosulfan as a competitive inhibitor.

The recalculation of the IC50 values to $K_i$ values were conducted as outlined in Equation 4.29,30

$$IC_{50} = (1 + [S] \times f_u/K_m)K_i$$

$$K_i = IC_{50}/(1 + [S] \times f_u/K_m)$$
where IC₅₀ is the half-maximal inhibitory concentration, [S] is the (unbound) substrate concentration, Kₘ is the substrate concentration of half enzyme activity, Kᵢ is the dissociation constant of the inhibitor-enzyme complex and fᵤ is the fraction unbound (from buffer or microsomal protein).

Requalification and DDI analysis of the adopted midazolam PBPK model as a substrate of CYP3A4: The published and qualified model for midazolam has been adopted for this analysis, and recreated and resimulated to verify a correct model adoption. The simulations from the adopted model have been reproduced identically.

Requalification/verification of the adopted digoxin PBPK model as a substrate of P-gp: The published and qualified model for digoxin has been adopted for this analysis, and recreated and resimulated to verify a correct adaptation. The simulations from the adopted model have been reproduced identically.

Requalification/verification of the adopted omeprazole PBPK model as a substrate of CYP2C19: The published and qualified model for omeprazole has been adopted for this analysis, and recreated and resimulated to verify a correct adoption. The simulations from the adopted model have been reproduced identically.

2.7 In silico DDI design

Simulations with mean representative individuals have been conducted to predict plasma concentrations and DDI AUC and Cₘ₉₅ ratios for a single oral dose (2 mg) of midazolam or multiple oral doses (0.5 mg once-daily (QD)) of digoxin or multiple oral doses (20 mg QD).

### TABLE 1 Compound properties used as input parameters for the treosulfan PBPK model

| Parameter       | Value     | Reference               |
|-----------------|-----------|-------------------------|
| MW (g/mol)      | 278.29    | DrugBank                |
| Compound type   | Neutral   |                         |
| Log Pₒ,ₘ (--)   | -1.58     | Literature²⁰           |
| fᵤ (--)         | 1         | Literature²⁰           |
| B:P (--)        | 0.88      | Literature²¹           |
| Vₛₛ (L/kg or L)| 17-34 L   | Literature²⁰           |

**Transport**
- Tubular reabsorption: ...
  - Accounted for in estimated GFR fraction

**Elimination**
- CLₑ (mL/min): 33 ± 6
  - Estimated (literature²⁰: 39 to 88)
- GFR fraction (--) : 0.95 ± 0.51
  - Estimated (literature²⁰: 0.41 ± 0.22)

**DDI parameters**
- IC₅₀ CYP3A4 (μM): 1870
  - Internal CYP inhibition assay (substrate: midazolam, 2.5 μmol incubation)
- Kᵢ CYP3A4 (μM): 1213
  - Calculated from IC₅₀ and fᵤ,ₘic (Equation 4; substrate: midazolam [Kₘ = 2.73])
- fᵤ,ₘic midazolam: 0.55
  - Predicted by PK-Sim (0.5 mg/mL HLM)
- IC₅₀ CYP2C19 (μM): 972
  - Internal CYP inhibition assay (substrate: mephenytoin, 25 μmol incubation)
- Kᵢ CYP2C19 (μM): 778
  - Calculated from IC₅₀ and fᵤ,ₘic (Equation 4; substrate: mephenytoin [Kₘ = 100 μmol²²])
- fᵤ,ₘic mephenytoin: 0.98
  - Predicted by PK-Sim (0.5 mg/mL HLM)
- IC₅₀ P-gp (μM): 3000
  - Internal CYP inhibition assay (substrate: loperamide, 25 μmol incubation)
- Kᵢ P-gp (μM): 1774.51
  - Calculated from IC₅₀ and fᵤ,ₘic (Equation 4; substrate: loperamide [Kₘ = 36.2; source: Cyprotex])
- fᵤ,ₘic loperamide: 1
  - Assumed (worst-case scenario for DDI)
- fᵤ,ₘic treosulfan: 1
  - Predicted by PK-Sim

Abbreviations: B:P, blood-plasma ratio; CLₑ, renal clearance; F, bioavailability; fᵤ, fraction unbound in plasma; fᵤ,ₘic, fraction unbound in microsomal assay; GFR, glomerular filtration rate; HLM, human liver microsomes; IC₅₀, half maximal inhibitory concentration; Kᵢ, inhibitor constant; Kₘ, Michaelis constant; log P, lipophilicity; MW, molecular weight; Vₛₛ, volume of distribution at steady state.

### TABLE 2 Predicted variation in treosulfan pharmacokinetics for a variation in body size

| Time  | Mean  | SD     | CV%  |
|-------|-------|--------|------|
| 2 hr  | 23.20 | 5.000  | 21.50|
| 12 hr | 0.54  | 0.051  | 9.44 |
| 24 hr | 0.15  | 0.030  | 21.20|

Note: Listed are the mean, standard deviation (SD) and coefficient of variation (CV%) for treosulfan pharmacokinetics in short (both thin and heavy) and tall (both thin and heavy) individuals, reflecting variation in body surface area. The corresponding simulated variability in pharmacokinetics is shown in Figure 3.

where IC₅₀ is the half-maximal inhibitory concentration, [S] is the (unbound) substrate concentration, Kₘ is the substrate concentration of half enzyme activity, Kᵢ is the dissociation constant of the inhibitor-enzyme complex and fᵤ is the fraction unbound (from buffer or microsomal protein).
of omeprazole in the absence of treosulfan and following the standard treosulfan conditioning treatment, where treosulfan is given as 2-hour intravenous infusions of either 10 or 14 g/m² on three consecutive days (total dose 30 or 42 g/m²).

2.8 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.31,32

3 RESULTS

3.1 Clinical data

Geometric mean profiles of treosulfan plasma concentrations were similar on day −6 and day −4. As was expected, the maximum concentrations were observed at the end of infusion after 2 hours. Model-independent pharmacokinetic parameters were calculated from plasma concentrations of treosulfan using noncompartmental procedures separately for day −6 and day −4. The observed $C_{\text{max}}$ in the total population was slightly lower on day −4 (434.6 μg/mL [SD 17.5%]) than on day −6 (470.5 μg/mL [SD 18.5%]) in the total population. AUC was slightly higher on day −4 (1449 μg*h/mL [SD 17%]) than on day −6 (1424 μg*h/mL [SD 18%]). The apparent half-life was 1.86 hours (median, range 1.12 to 2.56 hours) on day −6 and 1.93 hours (median, range 1.51 to 3.83 hours) on day −4. Altogether, 294 urine samples were analysed for treosulfan. Urine samples were planned to be collected for 72 hours starting after the first treosulfan dose on day −6. However, several of the later samples are missing so that 10 patients were excluded from the pharmacokinetic evaluation of treosulfan urine concentrations on day −4, no patient was excluded on day −5 and two patients were excluded on day −6. About 40% of an administered dose was excreted in urine at 130.38 mL/min (coefficient of variation [CV%] = 31.98%).

3.2 Nonclinical data

Treosulfan did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6 or CYP3A4 using testosterone as the substrate under any of the pre-incubation conditions tested and therefore no IC₅₀ or fold shift values were calculated.15 On the other hand, treosulfan returned a fold shift of 0.933 and 0.841 against CYP2C19 and CYP3A4 using midazolam as the substrate, respectively. Reversible IC₅₀ values of 972 and 1870 μM for CYP2C19 and CYP3A4 were obtained using midazolam as the substrate, respectively.

The transporter inhibitory potential of treosulfan was assessed in various in vitro cell test systems and transporter-expressing membrane vesicles to determine IC₅₀ values.16 Under the assay conditions tested, treosulfan was determined to be an inhibitor of the probe substrate transport mediated via P-gp and MATE2-K, but not via OATP1B1, OATP1B3, OAT1, OAT3, OCT2, OCT1, MATE1 and BSEP. The IC₅₀ value for P-gp inhibition was determined as 3000 μM. The IC₅₀ value for MATE2-K inhibition was determined as 8210 μM (standard error of the fitted IC₅₀ value = 1200 μM). As the observed IC₅₀ > > predicted $C_{\text{max}}$, MATE2-K inhibition was deemed as clinically not relevant. The corresponding $K_i$ values as derived with Equation 4 are 1213 μM for CYP3A4, 972 μM for CYP2C19 and 1774.51 μM for P-gp (Table 1).

3.3 PBPK model development and qualification

Parameter identification for the development of the treosulfan PBPK model for each patient in the training data set resulted in individual estimates for the rate of transformation of treosulfan and its renal clearance (Supporting Information Table S1). Both the pharmacokinetics of treosulfan and the amount of treosulfan excreted in the urine were captured well within the model and the simulated concentration-time profiles of all individual simulations from the training set are shown in Supporting Information Figures S1-S10. The use of measured creatinine clearance as an input parameter for the glomerular filtration rate did not predict renal excretion well, as estimates for GFR fraction in the model and measured creatinine clearance used as the baseline for the glomerular filtration rate are strongly negatively correlated ($r = −0.88$).

The population simulation for model qualification adding the mean and standard deviation from the individual resulting estimates for the rate of transformation and GFR fraction as “User Defined Variability” underpredicted renal clearance and slightly overpredicted PKs (Figure 2). Increasing mean GFR fraction by 70% in this group accurately predicts both renal excretion and PKs (data not shown), indicating a difference in baseline renal excretion but not in the transformation rate of treosulfan between the two groups (training vs qualification data set).

In a further investigation on variability, the PBPK model was used to assess changes in PK due to variations in physiology and GFR, as in a previous population PK (popPK) analysis of treosulfan PK,33 body surface area (BSA) was identified as the major covariate but (measured) GFR was not. To assess the variability of treosulfan between individuals with strong differences in body size, the PBPK model was used to simulate treosulfan PK for a variation in body size in individuals with short (both thin and heavy) and tall (both thin and heavy) physique. Resulting changes in PK in the PBPK model were low for variations in body size but high for low values of GFR (GFR < 50% of baseline; Figure 3).

For all PBPK models the predicted and observed pharmacokinetic exposure parameters of midazolam, digoxin, omeprazole and treosulfan after ascending intravenous and oral doses in healthy adults and cancer populations are summarized in Figure 4. The simulated exposure parameters for all populations were consistent with the observed values. The MFE values for the AUC from time of the
first dose to the last sampling time point ranged from 1.0 to 1.33 for midazolam, from 1.2 to 1.5 for digoxin, from 0.86 to 1.32 for omeprazole and from 1.33 to 1.53 for treosulfan. The MFE determined for $C_{\text{max}}$ ranged from 1.5 to 1.8 for midazolam, from 1.1 to 1.6 for digoxin, from 1.0 to 1.5 for omeprazole and from 1.36 to 1.4 for treosulfan. The MFE values for AUC and $C_{\text{max}}$ were well within 0.5 and 2 when comparing the observed and predicted exposure parameters and PK profiles for single and multiple doses (Figure 4).

### 3.4 | Simulations of concentration-time profiles of DDI substrates

Simulations of the midazolam DDI scenario resulted in the AUC and $C_{\text{max}}$ ratios as documented in Table 3 and depicted in Figure 5. Both $C_{\text{max}}$ and AUC of midazolam were slightly increased during concurrent use of treosulfan. According to the predicted AUC and $C_{\text{max}}$ ratios by the PBPK model, treosulfan can be classified as a weak inhibitor of CYP3A4 ($1.25 \leq \text{AUCR} \leq 2$). However, as concurrent use of drugs with the intravenous conditioning treatment is unlikely, it was evaluated how the predicted DDI ratios change during concomitant use of a CYP3A4 substrate (ie, midazolam) in treosulfan conditioning treatment if the drugs are not given simultaneously. The effect of a time difference in dosing of midazolam of $-2$, $-1$, $+1$, $+2$, $+3$, $+4$ and $+8$ hours was evaluated with respect to the changes in the predicted DDI ratios (Table 4). The outcomes show a significant dependency of the DDI potential on dosing times. If midazolam is not given within 2 hours prior to or 8 hours after the 2-h intravenous treosulfan treatment, the DDI potential can be significantly decreased ($\text{AUCR} < 1.25$). The maximum AUCR for midazolam ($\text{AUCR} = 2.23$) is reached if the time difference in dosing of midazolam is $+1$ hour, dropping below a “moderate” interaction potential ($\text{AUCR} < 2$) when dosed concomitantly or 2 hours after treosulfan injection.

Simulations of the digoxin DDI scenario resulted in the AUC and $C_{\text{max}}$ ratios as documented in Table 3 and depicted in Figure 5. Both $C_{\text{max}}$ and AUC of digoxin did not increase during concurrent use of treosulfan. According to the predicted AUC and $C_{\text{max}}$ ratios by the PBPK model, treosulfan likely cannot be classified as an inhibitor of P-gp (AUCR $< 1.25$). Given the predicted low interaction potential on P-gp substrates by treosulfan, no additional evaluations were conducted.
Simulations of the omeprazole DDI scenario resulted in the AUC and C\text{max} ratios documented in Table 3 and depicted in Figure 5. Both C\text{max} and AUC of omeprazole were slightly increased during concurrent use of treosulfan. According to the predicted AUC and C\text{max} ratios by the PBPK model, treosulfan can be classified as a weak inhibitor of CYP2C19 (1.25 ≤ AUC\text{R} < 2). As for midazolam, the effect of a time difference in dosing of omeprazole of /C\text{0}, 2, /C\text{0}, 1, +1, +2, +3, +4 and +8 hours was evaluated with respect to the changes in the predicted DDI ratios. For omeprazole, the outcomes also show a significant dependency of the DDI potential on dosing times. If omeprazole is given within 2 hours prior to or 3 hours after the 2-hour intravenous treosulfan treatment, a significant (“weak”) interaction could be expected (1.25 ≤ AUC\text{R} < 2 with maximum AUC\text{R} = 1.60), otherwise the potential for interaction is low.

4 | DISCUSSION

The final PBPK model for treosulfan, based on both in vitro and in vivo data, was able to generate plasma concentration-time profiles and exposure levels of treosulfan matching the treosulfan conditioning treatment of patients, where treosulfan is given as 2-hour intravenous infusions of 14 g/m² on 3 consecutive days (total dose 42 g/m²). The model was developed by optimising the systemic transformation rate and the GFR fraction to identify individual variability in the systemic transformation and renal clearance of treosulfan for model prediction and qualification. While model predictions for qualification were underpredicting drug PKs, this could be explained with the concomitant treatment with diuretics in the population used for qualification or a higher volume of fluids administered to this group of hematopoietic stem cell transplantation (HSCT) patients possibly leading to a higher rate of renal clearance. The general low variability of PKs across both anthropometric properties and GFR in patients without severe renal impairment may be used as a justification for a sparse sampling approach in future clinical analyses.

Patients enrolled across studies with treosulfan had either no or mild renal impairment or moderate impairment in rare cases. For patients with no or only mild renal impairment, the measured creatinine and derived observed renal clearance are not well correlated with renal excretion, and correlation is only significant for severe renal impairment. Furthermore, concomitant use of diuretics during the study may further invalidate the a priori measured creatinine clearance values as valid input parameters for the PBPK model. Although the effects of diuretic use on GFR are transient and complex, they might partly explain why the measured renal clearance is not a significant covariate in the popPK studies summarized in earlier simulation studies. A further explanation could be high-volume fluid infusions in HSCT patients.

The model was used prospectively to predict the likely outcome of DDI with treosulfan during conditioning treatment as an inhibitor of CYP3A4, P-gp and CYP2C19 using midazolam, digoxin and omeprazole as corresponding substrates. The DDI scenarios were simulated using the developed PBPK model of treosulfan in combination with previously developed and published PBPK models of the substrates available as templates from the Open Systems Pharmacology (OSP) GitHub model template repositories. The recalibration of the IC\text{50} values to K\text{i} values resulted in K\text{i} values of 1213 μM for CYP3A4, 972 μM for CYP2C19 and 1774.51 μM for P-gp (Table 1). In
FIGURE 4  Comparison between simulated and observed pharmacokinetic parameters from several studies in the literature for different populations. (A) and (B) show the predicted vs observed. (C) and (D) show adaptations of a Bland-Altman plot where the y axis is plotted as the ratio (instead of the difference). Solid lines represent line of unity; dashed lines represent 2-fold difference. Literature data for the different compounds are digoxin: Greiner et al.\textsuperscript{34} Kramer et al.\textsuperscript{35} Hayward et al.\textsuperscript{36} Oosterhuis et al.\textsuperscript{37} (extract, full qualification in evaluation report\textsuperscript{26}); midazolam: Smith et al.\textsuperscript{38} Chung et al.\textsuperscript{39} (extract, full qualification in evaluation report\textsuperscript{25}); omeprazol: Andersson et al.\textsuperscript{40,41} Regardh et al.\textsuperscript{42} (extract, full qualification in evaluation report\textsuperscript{22}). HV, healthy volunteers.

TABLE 3  Predicted AUC and $C_{\text{max}}$ values, and the calculated DDI AUC and $C_{\text{max}}$ ratios for simultaneous dosing of a single oral dose of 2 mg of midazolam, daily oral dose of 20 mg of omeprazole and daily oral dose of 0.5 mg of digoxin in the absence of treosulfan and following the standard treosulfan conditioning treatment where treosulfan is given as 2-hour intravenous infusions of either 10 or 14 g/m$^2$ on 3 consecutive days (total dose 30 or 42 g/m$^2$).

| Drug    | Treosulfan dose | AUC (μmol·min/l) | $C_{\text{max}}$ (μmol/l) | AUC ratio | $C_{\text{max}}$ ratio |
|---------|-----------------|------------------|---------------------------|-----------|------------------------|
| Midazolam | None            | 4.18             | 0.028                     | ...       | ...                    |
|         | 10 (g/m$^2$)   | 7.09             | 0.038                     | 1.70      | 1.35                   |
|         | 14 (g/m$^2$)   | 8.14             | 0.041                     | 1.95      | 1.46                   |
| Digoxin  | None            | 6.67             | 7.73e-3                   | ...       | ...                    |
|         | 10 (g/m$^2$)   | 7.04             | 8.38e-3                   | 1.00      | 1.08                   |
|         | 14 (g/m$^2$)   | 7.32             | 8.86e-3                   | 1.10      | 1.15                   |
| Omeprazole | None          | 100.0            | 0.66                      | ...       | ...                    |
|         | 10 (g/m$^2$)   | 147.8            | 0.92                      | 1.40      | 1.39                   |
|         | 14 (g/m$^2$)   | 160.8            | 0.99                      | 1.60      | 1.50                   |
conclusion, the DDI analysis with sensitive index substrates midazolam, digoxin and omeprazole for CYP3A4, P-gp and CYP2C19 predicted a weak (1.25 ≤ AUCR < 2) to moderate (2 ≤ AUCR < 5) interaction for CYP3A4, a weak interaction for CYP2C19 and a negligible (AUCR < 1.25) interaction for P-gp.

Clinical PK data used for PBPK modelling originated from adult patients treated with a 2-hour infusion of 14 g/m² treosulfan. Meanwhile, the approved treosulfan conditioning schedule has been modified for adult patients with malignant diseases. The recommended treosulfan dose for these patients has been reduced to 3 × 10 g/m², postponing the start of treatment to day −4 prior to allogeneic HSCT. The modified dose and treatment regimen resulted from the observation of a significantly prolonged duration of neutropenia and inherent serious infectious complications in the initial treosulfan arm compared with the reference arm in the pivotal randomized phase III study MC-FludT.14/L Part I.14 The difference in duration of neutropenia between the two study arms, however, can be predominantly attributed to the fact that the treosulfan conditioning treatment started 2 days earlier (on day −6) than the intensity-reduced busulfan reference treatment (on day −4). After modification of the treosulfan dose and application schedule (MC-FludT.14/L Part II),14,46 the duration of neutropenia was still significantly longer in the treosulfan arm compared with the reference arm (median difference of 1.5 days). This difference most probably reflects the earlier demonstrable effect of the higher myelotoxic potential even of the reduced treosulfan dose compared with the intensity-reduced busulfan reference treatment and—to a lesser extent—dose-dependent myelotoxic properties of the two treosulfan doses. This is also verified by the steeper decline of neutrophil counts after treosulfan treatment, whereas the time to neutrophil regeneration after allogeneic transplant was identical in both study arms. Finally, the modified (“reduced toxicity”) treosulfan treatment regimen resulted in a significant reduction of nonrelapse mortality without increased incidence of relapse or progression after allogeneic transplantation, while acute nonhaematological organ toxicities were comparable between the two treatment arms. However, in case of malignant paediatric or nonmalignant transplant indications, including treosulfan exposure to 3 × 14 g/m², are frequently applied.47–49

Irrespective of the reduced treosulfan dose of 10 g/m² recommended for older and comorbid adult AML and MDS patients, and a correspondingly diminished potential for DDI, the PBPK-based analysis still predicts a weak inhibitor potential (1.25 ≤ AUCR < 2) for treosulfan with both CYP3A4 and CYP2C19.

The risk for DDI varies depending on the timing of concomitant dosing. The maximum AUCR for midazolam (AUCR = 2.23) is reached if the time difference in dosing of midazolam is +1 hour,

**TABLE 4** Predicted drug-drug interaction (DDI) AUC and $C_{\text{max}}$ ratios for a single simultaneous and time-shifted oral doses of 2 mg of midazolam and 20 mg of omeprazole during the standard treosulfan conditioning treatment

| Drug    | DDI ratio | Time of administration | Simultaneous | +1 h | +2 h | +3 h | +4 h | +8 h |
|---------|-----------|------------------------|--------------|------|------|------|------|------|
| Midazolam | AUC ratio (−) | 1.22 | 1.34 | 1.95 | 2.23 | 1.96 | 1.65 | 1.48 | 1.22 |
|          | $C_{\text{max}}$ ratio (−) | 1.03 | 1.03 | 1.46 | 1.80 | 1.73 | 1.50 | 1.37 | 1.17 |
| Omeprazole | AUC ratio (−) | 1.24 | 1.46 | 1.60 | 1.49 | 1.30 | 1.19 | 1.14 | 1.10 |
|          | $C_{\text{max}}$ ratio (−) | 1.00 | 1.23 | 1.50 | 1.48 | 1.29 | 1.18 | 1.12 | 1.09 |

Note. Treosulfan is given as 2-h intravenous infusions of 14 g/m² on 3 consecutive days (total dose 42 g/m²).
dropping below a “moderate” interaction potential (AUCR < 2) when
dosed concomitantly or 2 hours after treosulfan injection. If omepra-
zole is given within 2 hours prior to or 3 hours after the 2-hour
intravenous treosulfan treatment, a significant (“weak”) interaction
could be expected (1.25 ≤ AUCR < 2), with maximum AUCR = 1.60,
otherwise the potential for interaction is low. The interaction poten-
tial can be reduced to “no interaction” (AUCR < 1.25) if the con-
comitantly used drugs tested in our model are dosed 2 hours before
or 8 hours after the 2-hour intravenous infusion of treosulfan. To
minimise risk, however, medicinal products with a narrow therapeu-
tic index (eg, carbamazepine and doxefetilde) that are substrates for
CYP3A4 and CYP2C19 should be carefully considered and
monitored during treatment with treosulfan. However, it has to be
considered that the potential for DDIs after treosulfan infusion is
rather limited compared to, for example, busulfan, 50 supporting the
generally reported excellent tolerability of treosulfan-based condi-
tioning treatment.

5 | CONCLUSION

Due to a potentially weak DDI, medicinal products with a narrow
therapeutic index that are substrates for CYP3A4 and CYP2C19
should be carefully considered and monitored when administered
simultaneously with treosulfan-based conditioning therapy. Consid-
ering the complexity of conditioning treatment and the respective
PK properties of the concomitantly used drugs (eg, half-life), theo-
retically the interaction potential on all evaluated mechanisms can be
reduced to “no interaction” (AUCR < 1.25) in the case where con-
comitantly used drugs are dosed 2 hours before or 8 hours after the
2-hour intravenous infusion during the treosulfan conditioning
treatment.

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COMPETING INTERESTS

S.S., F.S.M. and P.B. are employees of eqqLABS GmbH, the contract
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B. and R.A.H. report no conflict of interest. S.B., J.B. and C.H. are
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GmbH at the time of the investigation.

CONTRIBUTORS

S.S., J.B., C.H., S.B. and A.R. designed the research. D.W.B. and
R.A.H. provided the clinical PK data of clinical trial MC-FludT.14/L
Part I. S.S. developed the PBPK model and algorithms. S.S., P.B. and
F.S.M. developed the compound files, ran simulations and analysed
the data. All authors wrote, reviewed and approved the final
manuscript.

DATA AVAILABILITY STATEMENT

PBPK models for midazolam, digoxin and omeprazole are available on
https://github.com/Open-Systems-Pharmacology/OSP-PBPK-Model-
Library.

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