SUPPLEMENTARY TEXT

Pharmacokinetic modelling

Method:

Busulfan concentrations were analysed by population pharmacokinetics within NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City, MD, USA) with an intel FORTRAN 10.0 compiler. The first order conditional estimates method with interaction (FOCE INTER) estimation was used with the minimum value of the objective function value (OFV), goodness of fit plots, condition number (<1000) and predictive checks used to arrive at suitable models during the model-building process. A significance level of $P<0.05$ was set for comparison of nested models. An additive residual variability (RV) model, equivalent to proportional RV structures on the normal scale, was used for the log-transformed data. Busulfan concentration profiles were modelled using one- and two- (ADVAN 1, 3) with first-order elimination from the central compartment. Secondary parameters including half-life and $AUC_{0-\infty}$ were calculated using standard pharmacokinetic formulae.

Once the structure of the models was established, between subject (individual) variability (BSV) was estimated on parameters where supported by the data. As there was one observation after subsequent doses on days 3 and 4, between occasion (dose) variability (BOV) was only able to be estimated for a single parameter to avoid over-parameterisation of the model. The bioavailability parameter was chosen given expected variability of bioavailability of oral busulfan. Models where the bioavailability of the subsequent doses were estimated were also assessed, while the population bioavailability of the first dose remained fixed at 1.

The effect of body size on pharmacokinetic parameters was investigated. Allometric scaling based on size was applied a priori, with coefficients of 1 and ¾ for clearance and volume parameters, respectively. Several measures of size were tested in the model building process to identify the most appropriate descriptor. These were total body weight (TBW), ideal body weight (IBW), adjusted body weight (ABW25 = IBW +0.25*(TBW-IBW) and ABW40 = IBW +0.4*(TBW-IBW)) and lean body mass[13]. Additionally, models with normal fat weight using an additional parameter FFAT were assessed; normal fat weight = fat free mass + FFAT x (total body weight – fat free mass) where FFAT represents the relative contribution of fat mass compared to fat free mass to the size covariate for each parameter.

Final model evaluation included goodness of fit plots as well as a non-parametric bootstrap and prediction corrected visual predictive check (pcVPC) performed using Perl speaks NONMEM (PsN). The bootstrap consistent of 1,000 samples with the median and 95% empirical confidence intervals (2.5th and 97.5th percentiles) summarised for each model parameter. The pcVPC was performed with 1,000 simulated datasets with the observed 10th, 50th, and 90th percentiles were plotted with their respective simulated 95% CIs to assess the predictive performance of the model and to assess for any major bias.

Results:

In the initial phase of modelling a two-compartment model did not result in an improved fit comparted to a one-compartment model. Therefore, the pharmacokinetic parameters were ka (absorption rate constant), V/F

(volume of distribution) and CL/F (clearance) with the latter two being relative to oral bioavailability (/F). Between subject variability in all three pharmacokinetic parameters was estimated as was the between occasion variability in relative bioavailability. There was no significant improvement in the model when additional parameters were included to estimate the difference in relative bioavailability of the subsequent doses compared to the first dose.

Incorporating allometric scaling with all tested size measures improved the fit of the model, as shown in Table S1. Of these, adjusted body weight (ABW40) performed best overall by reducing between subject variability (BSV) for both clearance (from 17.1 % to 15.0 %) and volume of distribution (from 12.8 % to close to 0 %). Given BSV for volume of distribution was close to zero it was not included in the final model. Due to its good fit, allometric scaling for adjusted body weight (ABW40) was included in the final model.

Final model parameters and bootstrap results are included in the supplementary material and demonstrate low degree of bias in parameter estimates. Goodness of fit plots and the prediction corrected visual predictive check (pcVPC) are presented in supplementary figures S1 and S2 respectively and demonstrate adequate fit and good predictive performance of the model.

SUPPLEMENTARY FIGURES

Figure S1. Goodness-of-fit plots for busulfan including observed concentration against population (A) and individual (B) predicted concentrations, and conditional weighted residuals against time from first dose (C) and population predicted concentrations (D).

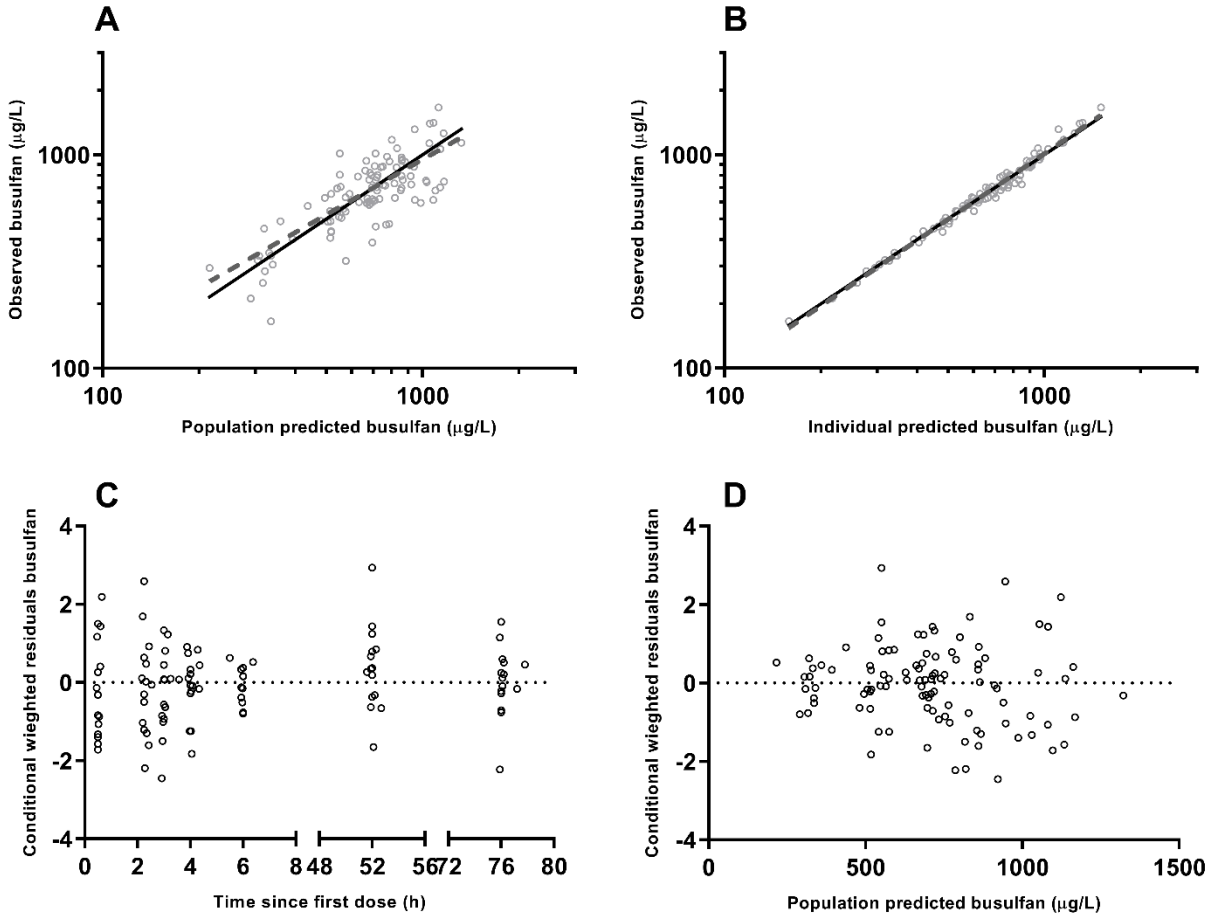
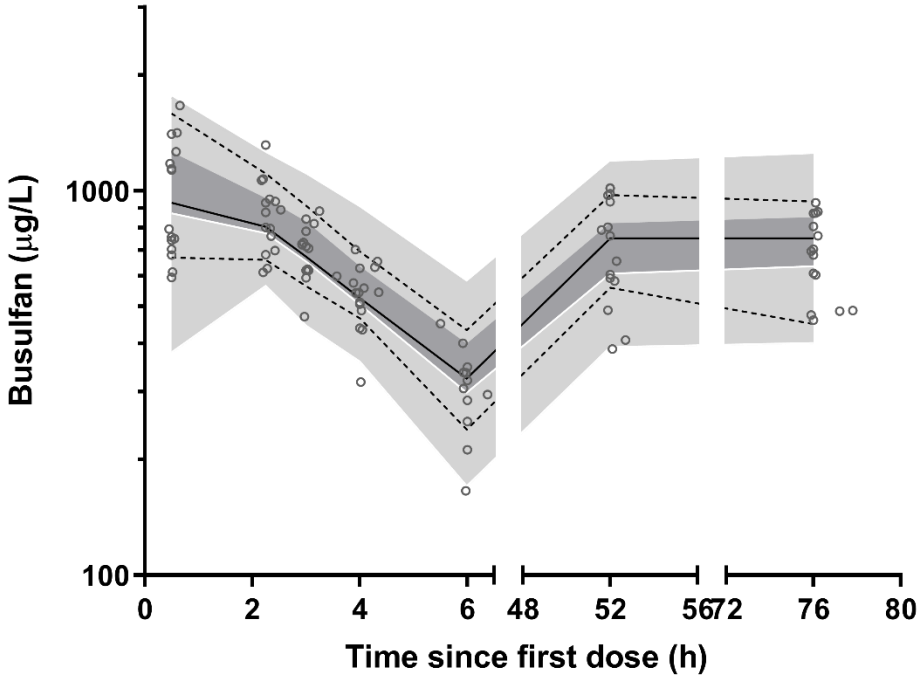


Figure S2. Prediction corrected visual predictive checks for busulfan with observed 50th (solid line), and 10th and 90th (dotted lines) percentiles within their simulated 95% CI (grey shaded areas) are shown with overlying the data points (○).



SUPPLEMENTARY TABLES

Table S1. Change in objective function value (OFV) and between subject variability (BSV) of clearance (CL/F) and volume of distribution (V/F) with use of different size measures for allometric scaling.

Model	Change in OFV	BSV in CL/F (%)	BSV in V/F (%)
Base (no allometric scaling)	N/A	17.1	12.8
Body weight	-14.642	15.7	8.1
Adjusted body weight (ABW40)	-19.339	14.6	~0
Adjusted body weight (ABW25)	-15.685	15.5	5.4
Ideal body weight	-6.113	16.0	13.1
Lean body weight	-18.588	14.8	~0
Normal fat weight	-16.886	15.7	4.7

Table S2. Final model population pharmacokinetic estimates and final model bootstrap results for busulfan.

	Final model	
Parameter	Mean	Bootstrap median [95% CI]
Objective Function Value	-274.866	-281.254 [-326.774 - -243.811]
Structural model parameters		
k_a (/h)	2.84	2.82 [1.89 - 4.6]
CL/F (liters/h per 70kg)	13.6	13.5 [12.5 - 14.9]
V/F (liters per 70kg)	48.3	48.2 [44.6 - 52.2]
Bioavailability	1	FIXED
Variable model parameters (shrinkage%)		
BSV in CL/F	15 (6)	14 [5 - 20]
BSV in k_a	72 (13)	70 [43 - 105]
BOV in F	15 (1,14, 46)	15 [10 – 20]
RV (%)	7	7 [6 - 9]

k_a (absorption rate), CL/F (clearance), V (volume of distribution), BSV (between subject variability), BOV (between occasion/dose variability) and RV (residual variability). IIV and RV are presented as $100\% \times \sqrt{variance\ estimate}$.

Table S3. Simulation intervals comparing total body weight and adjusted body weight (ABW40) based dosing for busulfan area under the curve (AUC) from 1,000 simulated patients with BMI ranging from 15 to 50.

BMI	TBW dosing			ABW40 dosing		
	Below target	Within target	Above target	Below targe	Within target	Above target
15	68.0%	32.0%	0%	11.4%	80.8%	7.8%
20	18.0%	78.6%	3.4%	6.8%	82.4%	10.8%
25	2.9%	72.7%	24.4%	7.0%	79.8%	13.2%
30	0.2%	48.3%	51.5%	4.6%	79.9%	15.5%
35	0.1%	22.3%	77.6%	1.9%	76.5%	21.6%
40	0%	9.9%	90.1%	2.5%	73.6%	23.9%