UNIVERSITAT DE BARCELONA

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BACHELOR'S DEGREE FINAL PROJECT

Faculty of Pharmacy and Food Science
University of Barcelona

DEVELOPMENT OF A POPULATION PHARMACOKINETIC MODEL FOR BUSULFAN IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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MAIN FIELD : BIOPHARMACY & PHARMACOKINETICS

SECONDARY FIELDS : PHARMACOLOGY & THERAPEUTHICS
PHYSIOLOGY & PATHOPHYSIOLOGY

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ABBREVIATIONS

AEMPS: Agencia Española del Medicamento y Productos Sanitarios

AIC: Akaike Information Criteria

ATC: Anatomical, Therapeutic, Chemical Classification System

BBB: Blood-Brain Barrier

BPV: Between Patient Variability

CCV: Constant Coefficient of Variation

CL: Clearance

CML: Chronic Myeloid Leukemia

CRAI: Learning and Research Resource Center (Centre de Recursos per l'Aprenentatge i la Recerca)

CV: Coefficient of Variation

CWRES: Populational Conditional Weighted Residuals

DV: Dependent Value

EMA: European Medicines Agency

FDA: Food and Drugs Administration

FOCE: First Order Conditional Estimation

GVHD: Graft-Versus-Host Disease

GVT: Graft-Versus-Tumor

HSCT: Hematopoietic Stem Cell Transplantation

HDC: Hight-Dose Conditioning

HPLC: High-Performance Liquid Chromatography

ICO: Catalan Oncology Institute (Institut Català d'Oncologia)

IIV: Interindividual Variability

IOV: Interoccasion Variability

IPRED: Individual Predicted Observations

IRES: Individual Residuals

IV: Intravenous

IWRES: Individual Weighted Residuals

LOQ: Limit of Quantification

MOFV: Minimum Objective Function Value

NCBI: National Center for Biotechnology Information

NMC: Non-myeloablative Conditioning

NM-TRAN: NONMEM Translator

NONMEM: Non-linear Mixed Effects Models

NTI: Narrow Therapeutic Window drugs

OBS: Observed Concentrations

OFV: Objective Function Value

PCIA: Adjusted Ideal Body Weight (Pes Corporal Ideal Ajustat)

PK: Pharmacokinetics

POPPK: Population Pharmacokinetics

PPRED: Population Predicted Concentrations

RE: Residual Error

RIC: Reduced Intensity Conditioning

SD: Standard Deviation

SOC: Standard of Care

SOP: Standard Operating Procedure

TAD: Time After Dose

TBI: Total Body Irradiation

TBW: Total Body Weight

TK: Tyrosine Kinase

V: Volume

VPC: Visual Predictive Check

1. SINOPSI

L'ús del Busulfan com a agent quimioteràpic ha anat desapareixent a mesura que noves i millors alternatives terapèutiques han sortit al mercat. Actualment s'utilitza en algunes malalties mieloproliferatives i en l'acondicionament previ al Transplantament de Progenitors Hematopoètics. Durant el desenvolupament d'aquest projecte hem utilitzat dades de 98 pacients prenent Busulfan via oral (1mg/Kg/6h) conjuntament amb altre medicació concomitant en l'esmentat acondicionament per desenvolupar un model farmacocinètic poblacional per predir concentracions plasmàtiques en pacients.

Es va obtenir un model monocompartimental que ajustava la seva variabilitat interindividual (IIV) per l'Aclariment (CL/F) amb un Valor Típic de 11.0 L/h i pel Volum de distribució (V/F) amb un Valor Típic de 42.7 L. Ambdós paràmetres ajustaven millor les dades utilitzant la covariable "Pes Corporal Ideal Ajustat" per explicar part de l'error residual. El model definitiu va passar dos processos de validació interna i serà utilitzat properament en la unitat de farmacocinètica clínica de l'Institut Català d'Oncologia amb la intenció de poder detectar aquells pacients que arribarien a concentracions tòxiques i evitar efectes adversos derivats del seu ús.

SYNOPSIS

The applications of Busulfan as a chemotherapeutic agent has been disappearing over the years as better therapeutic alternatives have been released. Nowadays it is mainly used for some myeloproliferative diseases and the conditioning process previous to Hematopoietic Stem Cell Transplantation. During this project, data was collected from 98 patients taking Busulfan for the aforementioned conditioning, which were further used to develop a population pharmacokinetic model aiming to predict plasma concentrations of the drug in patients.

A one-compartment model best described the data, which adjusted its interindividual variability for clearance (CL/F) with a Typical Value of 11.0 L/h, and for the distribution volume (V/F) with a Typical Value of 42.7 L. Both parameters showed a better fit to the data by the application of the Adjusted Ideal Body Weight covariate to explain the residual error. The final model went through two internal validation processes and will be used henceforth in the clinical pharmacokinetic unit of the Catalan Oncology Institute targeting those patients with potential to achieve toxic concentrations and avoid adverse events derived from its use.

2. FIELD JUSTIFICATION

This project rests upon three pillars. The main field of study is Biopharmacy & Pharmacokinetics. The whole project is mainly focused on the development of a population model of Busulfan, so a deep research and insight had to be acquired to understand its concepts and being able to analyze the output information provided by the model-building software with a critical point of view. Pharmacokinetics was also basic to learn the behavior of the drug regarding its absorption, distribution, metabolization, and excretion in the human body.

The secondary fields were for one: Physiology & Physiopathology which was crucial to understand the cellular bases of myeloproliferative diseases, specially blood cancers (leukemias, lymphomas, myelomas, myelodysplastic syndromes, etc.) and the implications, and consequences derived from all stages of the Hematopoietic Stem Cell Transplantation (HSCT). Pharmacology & Therapeutics knowledge was required to interpret the role of Busulfan and other concomitant medication in the conditioning procedure previous to HSCT, and the immunosuppressive therapy after the transplant. Furthermore, both disciplines allowed a better comprehension of the potential side effects of the studied drugs, and its optimal therapeutic range.

3. INTRODUCTION

3.1. Busulfan

Busulfan is a bifunctional alkylating agent chemically named 1,4-butanediol dimethanesulphonate. It is not a structural analog of nitrogen mustards. The ATC classification given by AEMPS ("Agencia Española de Medicamentos y Productos Sanitarios") is L01AB01, inside the alkylsulphonates alkylating drugs according to the Spanish regulation. Nowadays, there are two available presentations of the drug: an oral 2 mg tablet or a powder for infusion. Busulfan was developed as a cancer treatment by Glaxo-Smith-Kline in 1959. It is a small lipophilic molecule (MW: 246.29 g/mol; LogP = -0.5; Solubility: 69 mg/mL) which presents as a white, needle-like crystal.

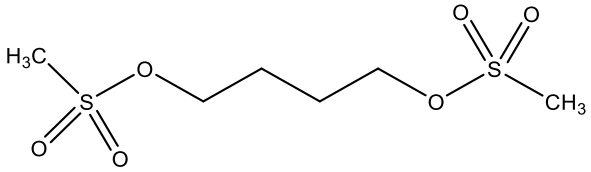

Figure 1.1. Busulfan chemical structure.

3.1.1. ADME

Busulfan has a variable permeability due to its low solubility in water. When given orally, the assimilated fraction is around 0.8 but it presents a high inter-individual variability due to age, weight, and time of dosage.(1)(2) Hassan et.al.(3) and Schuler et.al. (4)performed a comparison between intravenous and oral doses of busulfan and estimated an 80%(SD: 19, range 22-120%) bioavailability in infants and adults and a 70% (range 44-94) in adults only (23-54 years). Due to its lipophilic nature, busulfan reaches the cerebrospinal fluid at a concentration equal to those in plasma by crossing the Blood-Brain Barrier (BBB). Even though it has a neglectable reversible protein binding (about 7%), it has a relevant irreversible bonding to albumin (about 30%).(5)

The drug is not typically excreted unaltered in urine (only 1%) as a result of extensive metabolization in liver by glutathione S-transferase and spontaneous conjugation to glutathione. About 30% of the metabolite is then excreted in urine, and the rest is largely oxidized in the liver although not excreted in feces.(12) The main pharmacokinetic parameters have been widely studied over the years since its approval. The available literature has been reviewed and summarized in Table1.2.1 and 1.2.2.

Table 1.2.1. Integrated busulfan oral PK parameters from literature.(3,13–18)

ORAL	Units	Range	SD
AUC	mg·h/mL	5.90 - 8.26	0.94
CL/F	L/h	10.5 - 12.8	1.54
$t_{1/2}$	h	2.33 – 3.39	0.26
V/F	L/Kg	0.56 – 0.66	0.04
Ka	h^{-1}	0.02- 0.055	1.02

Table 1.2.2. Integrated busulfan intravenous PK parameters from literature.(4,6–11)

IV	Units	Range	SD
AUC/D	mg·h/D	5.99 - 9.45	1.6
CL	L/h	8.05 - 11.44	2.14
$t_{1/2}$	h	2.44 - 3.38	0.26
V	L/Kg	0.55 - 0.57	0.02

Albeit it is considered residual, some studies performed in rats suggest a role of the microsomal fraction in the metabolism of busulfan at high doses, causing an enhanced activity of N-nitrosodimethylamine demethylase (NDMA-demethylase), an enzyme largely related to human CYP2A6 and CYP2E1.(19–22)

3.1.2. Pre-existing Pharmacokinetic Models of Busulfan

Since its approval, some Pharmacokinetic Models have been developed. These models vary in the number of patients and the location where the study was performed. Six models have been constructed based on data from intravenous busulfan, an easier approximation in view of the erratic bioavailability of oral busulfan. Oral busulfan models are therefore sparse but three could be reviewed.

The results of the PK parameters obtained in the preexisting models are summarized in Table 1.2.1. and 1.2.2.

Table 1.2.3.Comparation of population PK parameters of Busulfan models in literature.(23–28)

Intravenous	N	Nº. CMT	TVCL (L/h)	TVV1(L)	TVV2 (L)	TVQ (L/h)
McCune J et.al	1610	2	12.4	13.9	29.9	135.2
Salinger D et.al	37	1	12.5*	50.6*	-	-
Wang Y et.al.	207	1	12.9	48.3	-	-
Choi B et.al	36	1	11.0	42.4	-	-
Kawazoe A et.al.	54	2	11.8	13.0	31.9	88.1
Wu X et.al.	53	1	11.8	48.2	-	-
Median	-	-	12.0	46.3		111.6
SD	-	-	0.6	2.8		23.5

**Calculated assuming a 70 kg subject. Original data was normalized by weight.*

Table 1.2.4. Comparation of population PK parameters from oral Busulfan models in literature. (29–31)

Oral	N	Nº. CMT	TVCL/F (L/h)	TVV/F (L)	TVKa (h^{-1})	Tlag (h)
De Castro F. et.al.	29	2	12.3	48.8	3.98	0.20
Hadjibabaie M. et.al.	30	2	13.4	42.6	-	-
Takamatsu Y. et.al.	71	2	10.7*	48.6*	2.93	-
Median	-	-	12.1	46.6	3.45	-
SD	-	-	1.1	2.8	0.52	-

**Calculated assuming a 70 kg subject. Original data was normalized by weight.*

We observe that estimated parameters are very similar in both oral and intravenous (IV) models when oral parameters were normalized by the absorbed fraction.

3.1.3. Clinical Uses

The two methanesulphonate groups of busulfan are highly reactive. They are mostly hydrolyzed by water in the internal fluid releasing methanesulphonyl cations, but also can cross-react with proteins (that act as a nucleophile) due to the electrophilic properties of the α-carbons of the drug. Then, the generated carbonium anions can alkylate DNA and have a fatal effect in cells.(12)

Nowadays its use is restricted to a few myeloproliferative diseases owing to its myelosuppressive effects. For instance, it is used in Polycythemia Vera as a second-line drug for hydroxyurea refractory or intolerant, showing a broader activity versus this myeloproliferative disease than α-peg-interferon and ruxolutinib, and a long-term hematological control or even remission. It has even been compared to hydroxyurea,

and large safety and clinical data has been collected from busulfan as a first-line therapy.(32–37)

Busulfan was the first treatment of Chronic Myeloid Leukemia (CML), improving the effectivity of concomitant radiotherapy and the quality of life, but not increasing the overall survival of patients.(38) Since 1983 this drug is not commonly used for CML since it has been long substituted by α-interferon, TK inhibitors (imatinib, dasatinib, and nilotinib), and Hematopoietic Stem Cell Transplantation (HSCT).(39,40)

Hematopoietic Stem Cell Transplantation (HSCT) is used as therapy for many hematological diseases either malignant or not. This therapeutic approach has been improved over the past decades, enhancing its success rate and reducing its mortality.(41)

According to the American Society for Bone Marrow Transplantation, HSCT (autologous and/or allogenic) is Standard of Care in adults for the following diseases:

Table 1.2.5. Indications for HSCT as Standard of Care in malignant and non-malignant diseases. (42,43)

Malignant diseases	
Acute Myeloid Leukemia	Diffuse large B cell Lymphoma**
Acute Lymphoblastic Leukemia	Follicular Lymphoma
Chronic Myeloid Leukemia	Mantle cell Lymphoma
Myelodysplastic syndromes	Lymphoplasmatic Lymphoma**
T-cell Lymphoma	Cutaneous T cell Lymphoma
Chronic Lymphocytic Leukemia	
Burkitt's Lymphoma	
Hodgkin Lymphoma**	**Non-malignant diseases**
Anaplastic Large Cell Lymphoma**	Aplastic anemia
Myelofibrosis and myeloproliferative	Sickle cell disease
Plasma Cell disorders	Thalassemia
Acute Promyelocytic Leukemia**	Polycythemia Vera
Some solid tumors*	Other rare indications

**Only autologous HSCT for Germ Cell Tumor, and Ewing's Sarcoma*
***Except after the first complete response (CR1).*

Before transplantation, there is usually a conditioning regimen performed in the host, that may vary according to their characteristics or the established medical procedures in the hospital. The main three types of conditioning therapies were named in 2006 by the Center for International Blood and Marrow Transplant Research:

— Myeloablative or high-dose conditioning (HDC) is usually performed in adult patients with hematological malignancies due to its immunosuppressive activity to better tolerate the donor's hematopoietic progenitors, and antineoplastic properties reducing the malignancy burden. It is carried out with alkylating agents, or total body irradiation (TBI) plus alkylating agents. High dose busulfan is usually used due to its effect in either dividing or non-dividing cells although it must be given concomitantly with other agents such as cyclophosphamide, Thiotepa, thymoglobulin, or fludarabine, since it has a limited effect in mature lymphocytes. The standard doses of busulfan are 1 mg/kg (oral) or 0.8 mg/kg (IV) every 6 hours for 4 days.(44–46)

— Non-myeloablative (NMC) and reduced-intensity chemotherapy (RIC) conditioning regimens produce mild cytopenia and makes them suitable for older, pediatric patients that are not candidates for high-dose regimens. Initially a donor-host graft chimerism is produced, and eventually evolves into full donor graft in the following days (in RIC) or months (in NMC). The difference between the two of them is that RIC uses doses of alkylating agents that cause a cytopenia that requires from stem cell support to resolve whereas the NM does not. The therapeutic approach is different from myeloablative conditioning, since it relies on the graft-versus-tumor effect, meaning that the donor's immune system will attack the tumor cells recognizing them as non-self-cells and attack them. The doses in RIC are usually 30% lower than HDC (busulfan, melphalan, and Thiotepa are the most common alkylating agents in RIC). In NMC, low-dose combinations of fludarabine + cyclophosphamide + thymoglobulin, cladribine + cytarabine, or fludarabine + cytarabine + idarubicin have shown the best outcomes. TBI conditioning at lower radiation doses is also used in these cases.(47,48)

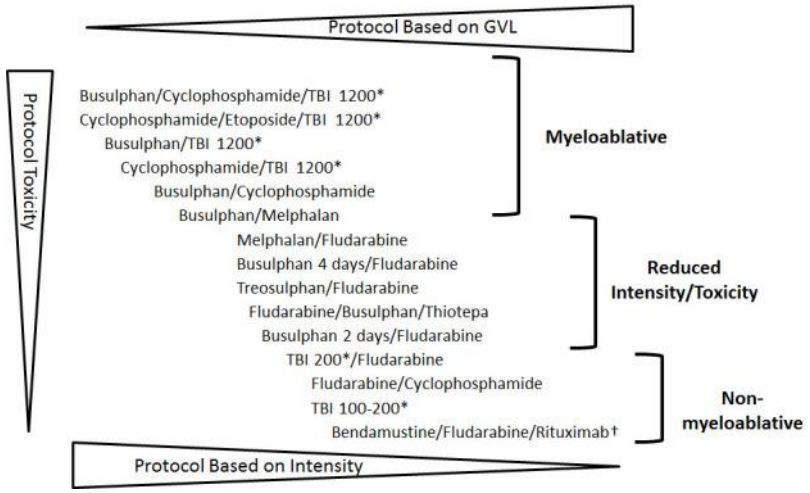

Figure 1.2.2. Therapeutic approximation to the different HSCT condition regimen.(42)

After the transplant, the host must receive chronic immunosuppressive therapy to avoid graft-versus-host disease. This happens when the immune system of the donor recognizes the cells of the host as targets and attacks them. The balance between GVT effect and GVHD must be considered in RIC and NMC regimens. Modified grafts HSCT are being developed in rats to avoid GVHD while preserving GVT effect performing TBI and anti-thymocyte globulin.(49,50)

The most common complication of HSCT is disease relapse, a lower life expectancy, and a higher incidence of long-term complication of different natures compared to disease-free population. Secondary cancers such as post-transplant lymphoproliferative disorders resulting of an Epstein-Barr virus infection, secondary myelodysplastic syndrome, acute myeloid leukemia (5-10% hosts), and other solid tumors. Organ-specific impairments might arise due to chronic GVHD, TBI sequels, or long-term treatment with immunosuppressors like corticoids and calcineurin inhibitors. Furthermore, due to the immunosuppression, HSCT patients are susceptible of infections by opportunistic bacteria.(51)

Indirect effects in the quality-of-life of the hosts are the psychological effects. They often have difficulties reentering the laboral market with lower rates of job finding in the following five years after the intervention, and in their reintroduction in society, partly due to sexual dysfunction, fertility impairments, anxiety, depressive symptoms and negative body image, although it seems to be improving over the years.(52,53)

3.2. Population pharmacokinetics

During the 20th century, different approaches were developed to describe the Pharmacokinetic-Pharmacodynamic behavior of a drug in humans and predict its efficacy. Even though pharmacometrics started in 1920's with Widmark, it gained recognition in the 1960's decade. Pharmacometry aims to characterize, understand, and predict the pharmacokinetics and pharmacodynamics of a drug, quantify its uncertainty, and standardize the decision-making process in clinical practice.

Nowadays, a pharmacometric assessment is crucial during the clinical studies, and has a great impact on its development. Initially, pharmacometry was intended to obtain information regarding the disposition of a drug in each individual (individual pharmacokinetics), but it was later replaced by the need to estimate population parameters and how they diverged into different subgroups. Hence, S. Beal and L. Sheiner, conceived the aforementioned *Population Pharmacokinetics* (POPPK). Both FDA ("Guidance for Industry: Population Pharmacokinetics") and EMA ("Guideline on reporting the results of Population Pharmacokinetic analyses") have developed standardized guidelines advising the possible situations where POPPK may be of service. It is considered highly advantageous in drugs with a narrow therapeutic window and/or targeting a very heterogeneous population.

It can also be used in Phase I dose ranging studies where a small group is tested, or Phase II studies, where information is key in how the drug will be used in Phase III studies and a moderately higher number of subjects are used. Furthermore, a combination of Phase I and Phase II data may provide high-quality evidence of the drug on account of wide dose-range and number of patients of the resulting combined model.(54,55)There are two main approximations for the pharmacometrician to explore:

— The Naïve Pooled Data approach treats all the observations as if it came from a single patient. It is an easy approach but drops a great deal of information by leaving the population variabilities unexplored and uncharacterized. Furthermore, it provides a poor estimation of parameters. Its use is limited in clinical practice since it does not account for physiological changes.

— The Two-Stage approach uses traditional pharmacokinetics to estimate the basic parameters of the drug for each individual using non-linear regression technics. The second stage summarizes the mean, variance and covariance of the parameters and how they are affected by covariates. This method usually overestimates the residual error. The working unit is the patient and so there must be sufficient data of each individual to calculate the parameters. With the results and patient's data, concentrations may be predicted using a Bayesian estimation.

— The Nonlinear Mixed Effect Modelling (NONMEM) approach uses the population as a whole, which makes it suitable for situations where the patient input is sparse, unbalanced and/or fragmentary. In just one step, this approach calculates the population pharmacokinetic parameters, its variance, and covariance while accounting for individual characteristics that may be used as covariates to a better explanation of the model. This approach requires the use of specific software, being NONMEM the gold-standard.(56)

In the populational models using NONMEM software's nomenclature, for each structural parameter, there is a fixed value expressed with the letter THETA (θ) that represents the Typical Value (TV) of the population. Depending on the specified basic model we might have different structural parameters. On the other hand, we have two random-effect values for each parameter: ETA (η) that accounts for Between Patient Variability (BPV) and Interoccasion Variability (IOV) and which variation is represented by OMEGA (ω^2), and EPSILON (ε) that represents the unexplained difference between predicted and observed values and which variance is defined by SIGMA (σ^2).(57)

For BPV and IOV the variation can be explained by different random effects models each one describing the supposed behavior of it. The main models are: the additive error model that assumes the same magnitude of variability regardless of the value of the parameter (constant standard deviation); the Constant Coefficient Variation (CCV) maintains the same CV value making the standard deviation increase when the mean population value increase; the most used model is the Exponential model which assumes the variability follows a log-normal distribution.

There are also error models for random variability that aim to explain its behavior. The main ones are: The Additive Variation; the Constant Coefficient Variation (CCV); the Additive plus CCV which combines the two previous models, so the Additive model dominates at low values whereas the CCV dominates at larger values; and Log-error model assumes a log-linear distribution of the residual values. (56,58)

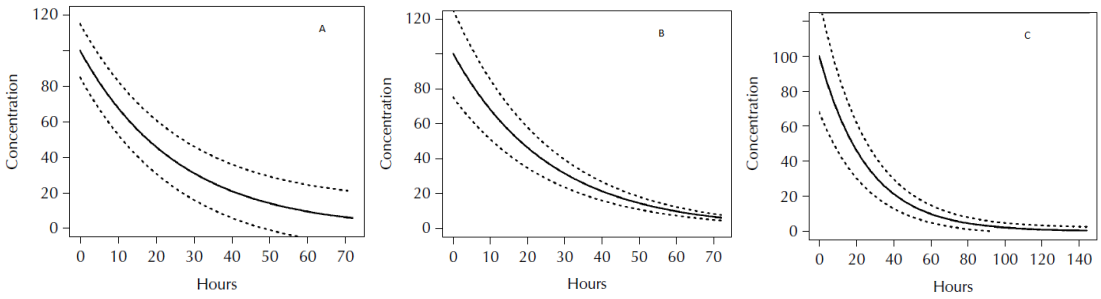

Figure 1.3.1. A) Additive error model. B) CCV error model. C) Additive + CCV error model.(56)

4. OBJECTIVES

The main objectives of this final degree project are:

1. Comprehend population pharmacokinetic models of drugs, especially in Narrow Therapeutic Index drugs (NTIs). This objective will be accomplished starting from basic pharmacokinetic knowledge acquired from academic formation.

2. Acquire a comprehensive insight of NONMEM® software as a tool in Nonlinear Mixed Effects Models and its potential impact in the current gender gap in clinical trials.

3. Development of a Population Pharmacokinetic Model of Busulfan employing data collected from the Catalan Oncology Institute (ICO) patients subjected to conditioning therapy for Hematopoietic Stem Cell Transplantation.

4. Confirm the predictive performance of the resulting model with appropriate validation methods.

5. MATERIALS AND METHODS

5.1. Bibliographic methods

For this project, we performed an exhaustive research employing articles and books extracted from the PubMed section of NCBI, and the CRAI ("Centre de Recursos per l'Aprenentatge i la Recerca") searching tool "Recercador+" available for students of the University of Barcelona (UB). Due to the long period of time that busulfan has been in the market, there were few new publications regarding its pharmacokinetics. Information regarding gender inequality in clinical trials using the aforementioned tools was also gathered to assess its current situation in science. Books were obtained from the CRAI library or provided by the Unit of Biopharmacy and Pharmacokinetics of the Pharmacy and Food Science Faculty.

The "Population Pharmacokinetics Studies" postgraduate course was taken in order to further understand the theoretic bases of the discipline and the coding language of the NONMEM. Furthermore, the assistance to several "R®" seminars was required to interpret NONMEM output and to execute the preliminary data analysis.

5.2. Screening and analytical methods

Using the ICO's Standard Operating Procedure (SOP) "Acondicionamiento para TPH" (PNT-TPH-004), the appropriate dosage of Busulfan and other concomitant drugs for the conditioning treatment in HSCT was applied to the 103 patients that were included in this project. All the subjects were about to receive an HSCT either autologous or heterologous, but the conditioning treatment varied depending on the base illness to be treated.

All patients received concomitant phenytoin 24 hours before starting Busulfan to avoid seizures, a common adverse event of this drug. Patients who used a conditioning treatment without Busulfan were not included in the project. Also, this model only used oral administration data and all patients dosed with intravenous busulfan were discarded from de database leaving 98 patients left to build the model.

Two sample tubes with 3 mL of blood in EDTA of each patient were collected at time zero (before dosing) to be used as a blank, and at 1.5, 3, 4, and 6 hours posterior to the first dose. The date and hour, alongside the information of the patient, the dose, and the concomitant medication were introduced in the template available in SOP “Protocol de Monitorització del Busulfà (1ªDosis)”. Samples from the subsequent doses were obtained at times 2- and 6-hours post-administration according to the SOP “Protocol de Monitorització del Busulfà (Altres)”. Both templates are available in ANNEX 2.

The samples were preserved in the fridge at 4ºC until they were sent to the Santa Creu i Sant Pau Hospital Biochemistry Laboratory to be analyzed with a HPLC validated method with a Limit of Quantification (LOQ) of 100 ng/mL.

5.3. Pharmacokinetic modelling methods

5.3.1. Excel database building

The information used to build the database was obtained from the Pharmacy Service of the Catalan Oncology Institute. Dr. Carmen Muñoz Sanchez and Dr. Nuria Gonzalo Diego compiled data from 103 patient being administered with either oral or intravenous Busulfan conditioning before undergoing a Hematopoietic Stem Cell Transplant.

The aim of the Excel database was providing understandable information for the pharmacokinetic analysis program NONMEM, which uses a specific coding. This step was key to obtain consistent data from NONMEM.

We used in our table preserved names that NONMEM provides for certain measurements:

- **ID**: subject identifier, all records of the subject must be continuous or NONMEM treats them as different subjects.
- **TIME**: represents the moment in which an event occurs and must always be present, positive and increasing. This parameter may be substituted in plotting and analysis by Time After Dose(TAD).
- **AMT**: this parameter represents the amount of drug given to the patient in a specific record. AMT must be zero when a DV value is present.
- **DV**: this is the dependent value, which is concentration (ng/mL) in our case. This value must be zero when a dose (AMT) is given to the subject. DV values under the LOQ were hidden from the dataset.
- **MDV**: the missing data value parameter is used to indicate the absence of a DV record value. MDV = 0 when there is a DV value, and MDV = 1 when its missing.

— **EVID**: this parameter refers to the type of record. EVID = 0 is used for observation events, EVID = 1 for dose events, EVID = 2 for other type of event records, and EVID = 3 and 4 is for reset of the conditions of the system.
— **CMT**: compartment parameter indicates in which compartment is happening the record. In our case, CMT = 1 described oral dose records and CMT = 2 was IV doses or observational records.
— **OCC**: occasion parameter is used to separate different curves observations in the same patient.

ADDL and II were not included in our table because based on the protocol for plasma concentration sampling, we mostly had measurements of the first curve, punctual measurements to assess dose adjustments during the conditioning, and there were no dosing interval variations.

Regarding the covariates (non-preserved parameters), we used shortened names because programs such as Xpose4 may misread the table with long headers. The covariates are grouped in two:

— Continuous covariates:

- **AGE**
- **WEIGHT**
- **PCIA** (Adjusted Ideal Body Weight)
- **HGT** (Height)
- **AREA** (Body Surface)

— Categorical covariates:

- **GEND** (Gender): where Man = 0, and Woman = 1.
- **FHEP** (Altered Hepatic Function): where NO = 0, and YES = 1. Parameters that were considered as an alteration were: out-of-range AST, ALT, GGT, FA, and/or Bilirubin levels.
- Assessing concomitant conditioning drugs was deemed of interest in order to search for pharmacokinetic interactions. Drugs were added as individual covariates and coded as NO = 0, and YES = 1:

  FLUD (Fludarabine)

  CICLO (Cyclophosphamide)

  TIMO (Thymoglobulin

  TIO (Thiotepa)

- **INDICATION**: not all patients suffered from the same illness, but there was a mixture of oncological problems we intended to explore individually during our population PK model. There was a myriad of them, hence we created 6 generic groups under this covariate:

1. Lymphoblastic Acute Leukemia
2. Myeloid Chronic Leukemia
3. Myeloid Acute Leukemia
4. Hodgkin Lymphoma
5. Non-Hodgkin Lymphoma
6. Multiple Myeloma / Waldenström Myeloma

5.3.2. Data exploration

Building a population PK model required an understanding and familiarization with the data. This was achieved using the statistical software R (v.3.5.2), specifically R package: Xpose4 (v.4.6.1). This software allowed us to analyze the patients as a group and portray its characteristics. It is also useful to see trends and identify outliers. We also generated exploratory histograms, dot plots, and scatterplots for covariates, that defined correlations between these, and helped us understand the results of the covariate model building in latter steps.

5.3.3. Model Building

The process of modelling was sequential. The initial step, once the data had been assessed, was writing the control file in a ".mod" text file with NONMEM coding language. The control file, also named NM-TRAN (NonMem TRANslator), is the framework that contains the commands we want NONMEM to perform, for instance calling the dataset, or executing the appropriate subroutines. The file is very sensitive to misspelling and additional characters that can disrupt the proper run of the desired model. The NM-TRAN was defined in a series of "Blocks", always preceded by a "$" sign. The basic blocks are:

1. $PROBLEM (also PROB or PRO) which contained the name of the file or its characteristics. When developing a model, hundreds of control files were crated with slight variations between them. Therefore, it was useful to specify the change, in less than 160 characters in a unique line, in this part of the control file.
2. $INPUT allowed us to state the headers to NONMEM and recognizing them as either variable or covariate. This block had to be in a single row and each header was separated from the other with one blank space. NONMEM allows a maximum of 50 active variables per run. If our dataset had had more, we could have introduced them all, and then introduce "=DROP" in the variables that were not needed. There are reserved names, these are: ID, DATE, DAT1, DAT2, DAT3, TIME, DV, AMT, RATE, SS, II, ADDL, EVID, MDV, CMT, PCTM, CALL, CONT, L1, and L2.
3. $DATA indicated to the program the name of the file we were using in the run. Typically, it also indicates where the file is located, but in our case, we indicated it to the program itself in the command window. Additionally, we used the "IGNORE=#" command to indicate that when a "#" sign was found in the dataset, it had to be ignored. We used this to hide the headers, outlier patients or observations.

4. $SUBROUTINES is a block dedicated to specifying the structure and desired parametrization. It states the number of compartments of the model with the ADVAN, and the parameters that were used in the subroutine. There are multiple possible combinations to explore with NONMEM.

5. $PK contained the appropriate parameters required by the ADVAN/TRANS subroutine. This block contained different rows allowing a clearer expression of the code. For each parameter, we gave a THETA number to its typical value and, in the second row, we defined the behavior of its interindividual variability (additive variation, constant coefficient variation, or exponential variation).

   The predicted concentrations were produced in mg/mL. Since our data was set in other units (ng/mL), we used a correction factor “S2” of 1000 (S2=V/1000).

   When the covariate model is engaged with, we introduced them in this block next to the THETA row with IF-THEN structures for categorical covariates or multiplying THETA for a normalized parameter (parameter divided for its mean value).

6. $ERROR defines the residual error (RE) specifications for the model in several rows. Using the statement “IPRED=F” we indicated that F is the Individual Predicted Concentrations values. The block also contains the parameters IRES (Individual Residuals) that were the observed concentration minus the IPRED, and the parameter IWRES (Individual Weighted Residuals) which was IPRED wheighted with a parameter. We equaled “W” to either 1 (for additive error), F (for Constant Coefficient of Variation error), or IPRED=LOG(F) plus W=1 (for log transformed additive error).

7. $THETA was the block dedicated to set the initial estimate parameters of the THETAs. There were as many $THETA rows as parameters in $PK. The initial values were extracted from literature. They had to be written in brackets with or without upper and lower limits for said value. We lower-limited our parameters to zero. We also fixed some THETAs value such as the bioavailability (TVF) to 1 since we did not include intravenous patients in the model and therefore, we could not have calculated it.

8. $OMEGA was used to introduce the initial estimates for ETA variance (ω^2). Generally, when there is a lack of information regarding its variability, we assume a 50% expressed as Coefficient of Variability.

$$CV = \sqrt{\omega^2} \times 100 \quad \rightarrow \quad 0.5 = \sqrt{\omega^2} \quad \rightarrow \quad \omega^2 = 0.25$$

9. $SIGMA was used to introduce the initial estimates for EPSILON variance (σ^2). Generally, when there is a lack of information, we proceed as in $OMEGA block.

$$CV = \sqrt{\sigma^2} \times 100 \quad \rightarrow \quad 0.5 = \sqrt{\sigma^2} \quad \rightarrow \quad \sigma^2 = 0.25$$

10. $ESTIMATION was a single row block to state the estimation method. We used a First-Order Conditional Estimation (FOCE), which estimated population parameters and individual-specific parameters in a single step conditioning them during the process of minimization.

11. $COVARIANCE (also COV) indicated a further post-processing of data after the minimization process to calculate standard errors, the covariance matrices, the correlation matrices, and the inverse of variance-covariance matrices for each estimated parameter.

12. $TABLE rows controlled the output, creating tables with detailed results of desired parameters in files separated from the ".lst" general result file. The name of this file was established in this row, and it's recommended that it matches the name of the run to facilitate the check-out in further steps. We requested 4 $TABLE for each of our runs: one for categorical covariates (catabxxx), one for continuous covariates (cotabxxx), one for parameters (patabxxx), and one for residual parameters along with TAD and IPRED (sdtabxxx).

The building of a model dwelled in constructing a basic model and adding one change at a time. Two models with one change between them are denominated nested models. Each model had its own Objective Function Value (OFV), that was provided by NONMEM. Minus twice the logarithm of the difference between two nested models follows a χ^2 distribution. Therefore, we were able to establish numerical p values based on this distribution. An ΔOFV < - 3.84 equals to a p-value of 0.05, an ΔOFV < - 7.88 equals to a p-value of 0.005, and an ΔOFV < -10.83 equals to a p-value of 0.001. Another sign of improvement is the reduction of the ETA variance (ω^2) of at least 10%.

The comparison of two non-nested models, for example one-compartment versus two-compartment models, must be performed with the Akaike Information Criteria which was calculated for the two models. The best model was the one with the lowest value. This method favors simple models with less variables (k).

$$AIC = 2k - 2Ln(OFV)$$

A number of control files to define the structural part of our model (base model) were written. This part consisted in determining which parameters suffer a significative interindividual variability reduction by decreasing the OFV at least -7.88. This part of the model optimization usually has great variations in the OFV, due to the great flexibility and poorness-of-fit of previous models. We established that at this level, the OFV parameter was enough to accept or dismiss a change in the model.

Once the structural part had been selected, we began to apply the covariates to the model one at a time and check each Minimum Objective Function Value. In this 2nd step, called "Forward", we accepted a change with a p-value<0.05 and a ΔETA_1(%) or ETA_2(%) of at least 10%. The acceptance criteria were less restrictive than the previous stage being that each significant covariate was re-challenged in the 3rd stage of the process called "Backwards". The Backwards process eliminated the first covariate added to the final model, and that covariate was considered significant if the OFV worsened at least 10.83 points. This process is necessary because the adding covariates in a certain sequence does not provide the same results as another, even though the final OFV is the same, as a result of the flexibility of the structural initial model.

5.3.4. Model Validation

The final model obtained after the Backwards process was asserted to ensure its predictive ability. There are two general kind of validations: external and internal. External validation was not performed during this process since data from other studies or subjects have not been acquired though it may be done in the future.

We ran internal validation procedures that did not require previous segregation of subjects from the dataset, accounting that all subjects were introduced to build a better model. The two arrangements we used to challenge our model were:

— **Visual Predictive Check (VPC)**: an internal validation process that uses the characteristics of each patient to perform a given number of simulations for each observation using a determinate model. We executed one thousand simulations using the best fitting model.

  The program generated the simulated values using the ω^2 and σ^2 matrices applied to each subject observation but keeping the structural and covariate model parameters unaffected. The final control file had to be slightly changed to perform this process. Firstly, the initial estimates for the $THETA, $OMEGA, and $SIGMA block were substituted and fixed for the estimated values given by the output datafile of the final model. Secondly, the $ESTIMATION and $COV were replaced by the $SIMULATION block which contained the number of simulations (SUBP=1000) and a 7-figure number (SEED=1234567) which was the starting number for random sampling number generation.

  The results were exported to an output Excel compatible datafile with the median and confidence intervals (5^{th} and 95^{th}) of all simulations and the median and confidence intervals (5^{th} and 95^{th}) of all subject observations at each time after dose. The model was considered valid if the real values were within the confidence intervals and the median of the simulations. With Xpose4 (v.4.6.1) package in R (v.3.5.2) we performed a visual plot with these values.

— **Bootstrap**: an internal validation process that digress from the previous one in the generation of datasets. Bootstrap created datasets called “subsets”, with the same number of subjects by randomly resampling patients from the original dataset and applying the model to them. The newly generated subsets might not have all the subjects from the original dataset and might have repeated subjects.

  The performance of the bootstrap procedure was obtained by generating one thousand subsets and obtaining the overall median and a 95^{th} interval confidence (percentile 2.5^{th} and 97.5^{th}) of every parameter: OFV, THETAs(1-4), ETAs (1 and 2), OMEGAs, and SIGMA. The median of the original dataset parameters must be enclosed by these intervals to be considered satisfactory.

6. RESULTS

6.1. PRELIMINARY DATA EXPLORATION

The retrospective chart included all subjects treated with Busulfan as a medullar depletion before an HSCT between 2012 to 2018. Data was collected from 103 patients, 98 of which were used to build the pharmacokinetic model.

The preliminary assessment of data begins with the demographic and biochemical characteristics of our patients, which are summarized in Table 5.1.1.

Table 5.1.1. Summary of Median (Minimum, Maximum) values of the target population.

Variable	Units	Median	Range
SEX (M/F)	N	56/43	-
AGE	Years	52.02	20 - 69
WEIGHT	Kg	73.72	36 - 119
PCIA (Adjusted Ideal Body Weight	Kg	64.21	41.78 – 91.08
HEIGHT	m	1.67	1.46 – 1.92
AREA	m^2	1.82	1.25 – 2.38
GOOD HEPATIC FUNCTION (YES/NO)	N	62/37	-
ADDITIONAL IMMUNOSUPRESSION			-
FLUDARABINE	N	90	-
CYCLOPHOSPHAMIDE	N	52	-
TIMOGLOBULINE	N	19	-
TIOTEPA	N	2	-

N= number of patients

A total of 523 plasma concentrations were obtained from 98 patients. Each patient was sampled according to the SOPs. The administered dose to a given subject was calculated based on its weight at reason of 1 mg/kg every 6 hours. Patients 4, 30 and 69 received a dose of Acetaminophen, which has a described interaction with Busulfan. Other medications with possible interactions such as metronidazole, CYP3A4 inhibitors antifungals, and antipsychotics were checked.

We observed the behavior of Busulfan with a Concentration (ng/mL) vs TAD (Time After Dose) plot displayed in Figure 5.1.1.

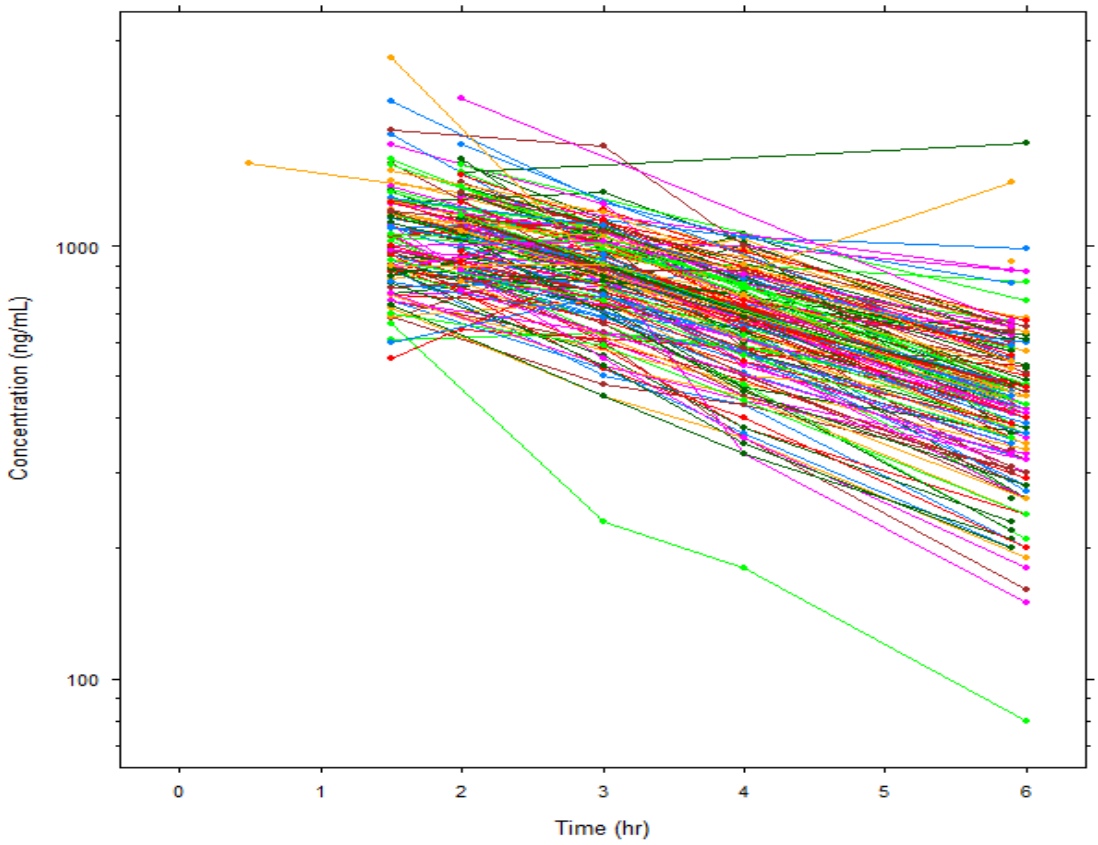

Figure 5.1.1. Individual DV vs TAD plot during exploratory data analysis.

During the preliminary exploration, the medium and range of the continuous covariates was examined, alongside their homogeneity by performing dot plots for each one. Our data provided a homogeneous dispersion, enhancing the accuracy of predictions of the model in high and low values of each covariate. The resulting dot plots are shown in the Figure 5.1.2.

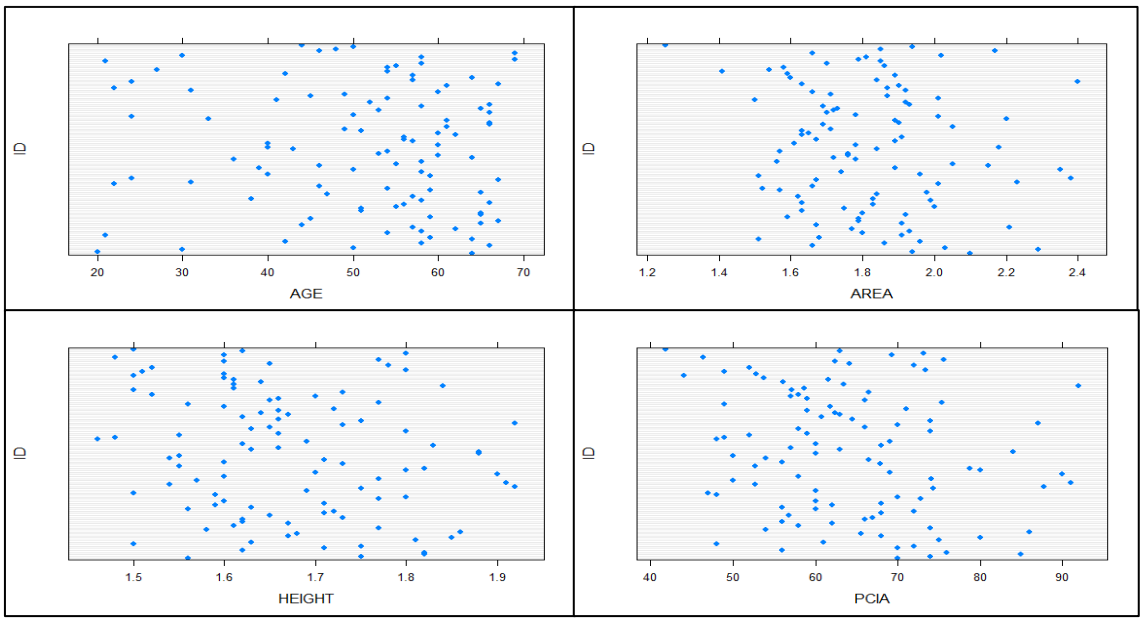

Figure 5.1.2. Dispersion of continuous covariates

In order to conclude the initial covariate checkout, we performed a scatterplot confronting each variable with the rest to determine correlations. When two covariates have correlation, they are not likely to enhance the model when applied together. The scatterplot is shown in Figure 5.1.3 and includes only AGE, WEIGHT, PCIA, HEIGHT and AREA. We expected correlation between anthropometric measurements, and the outcome further confirmed it.

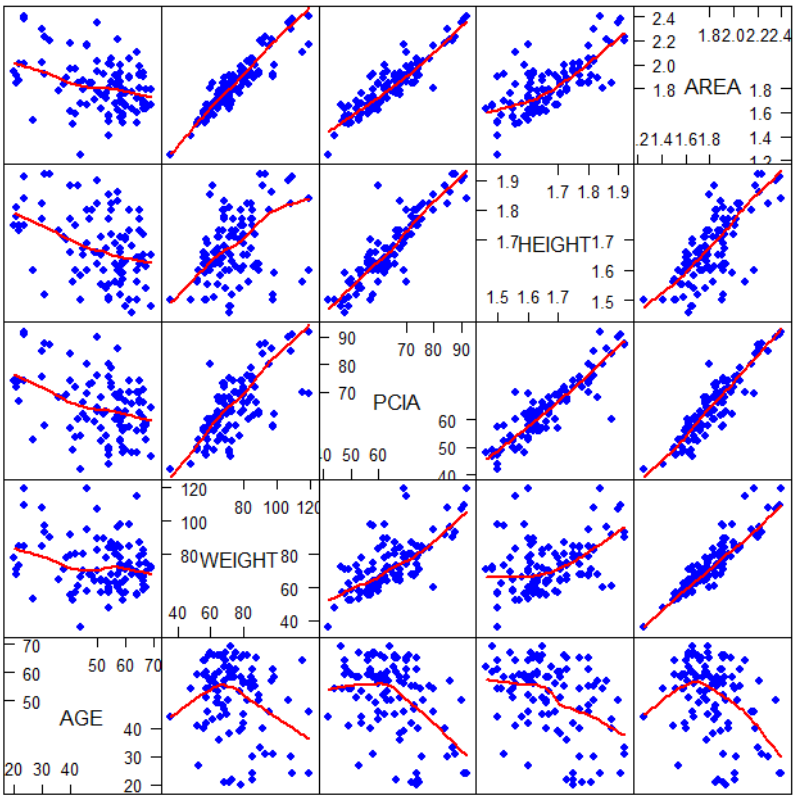

Figure 5.1.3. Scatterplot matrix of continuous covariates.

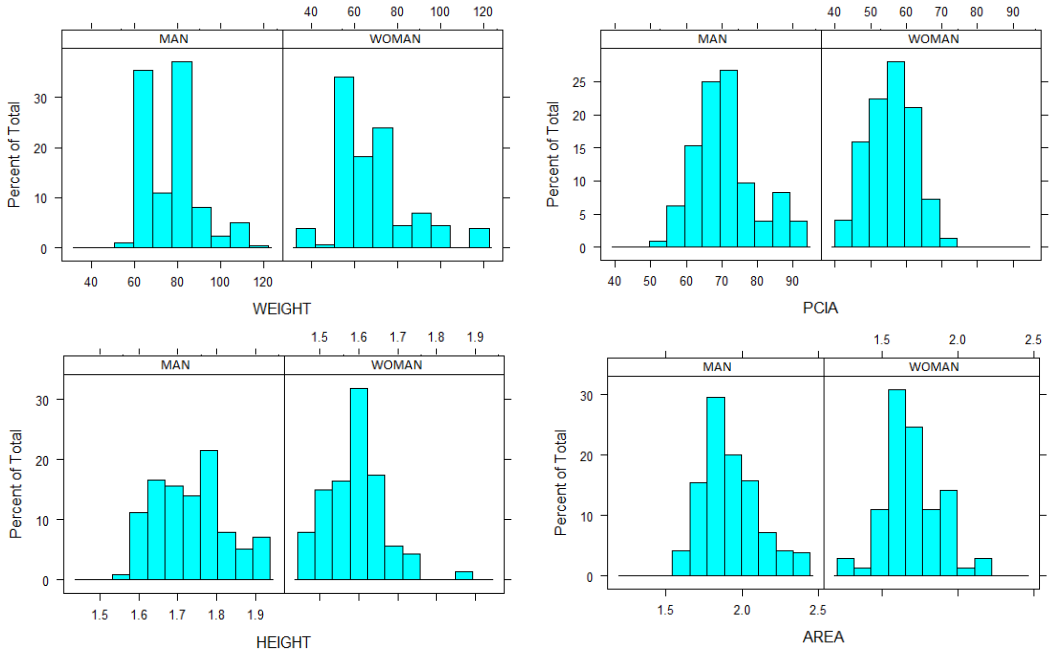

Figure 5.1.4. Antropometric differences between gender. There is different median values for the represented covariates

6.2. MODEL DEVELOPEMENT

6.2.1. Base model development

The first step in PK modelling is finding the base model that best describes our data. We began with the simplest one-compartment extravascular model and evolved to models with higher complexity. To clearly represent the results of this stage of the process we standardized the NONMEM's output in Tables.

The decision to preserve a change in this step is, as previously stated, a reduction in the Objective Function Value of 7.879 which equals a p value <0.005. The results of this process are displayed in tables 5.2.1 and 5.2.2

Table 5.2.1. One-compartment base models.

RUN Nº	CHANGE		OFV	ΔOFV	CHANGE DECISION
Run 101	PK model IIV RE	One compartment None Additive	6664.37	-	-
Run 102	PK model IIV RE	One compartment CL Additive	6203.41	-460.96	**ACCEPTED**
Run 103	PK model IIV RE	One compartment Cl + V Additive	6079.16	-124.25	**ACCEPTED**
Run 104	PK model IIV RE	One compartment CL + V Proportional	5931.11	-148.05	**ACCEPTED**
Run 105	PK model IIV RE	One compartment CL + V + Ka Proportional	5924.65	-6.46	DISCARDED
Run 106	PK model IIV RE	One compartment CL + V + TLAG Proportional	ND	-	-
Run 107	PK model IIV RE	One compartment CL + V + TLAG lim=0 Proportional	5931.15	+0.04	DISCARDED
Run 108	PK model IIV RE	One compartment CL + V Additive– Proportional	6207.71	+276.60	DISCARDED

.

At first sight, run105 provided the lowest OFV (5924.65) but the application of IIV in Ka did not produce a significant reduction compared to run104 (5931.11). Therefore, run104 was our candidate among the one-compartment pool of models.

Two-compartment model were attempted to obtain a better description of the distribution-elimination process. The results are shown in Table 5.2.2.

Table 5.2.2. Two-compartment base models.

RUN N.º	CHANGE		OFV	ΔOFV	CHANGE DECISION
Run 109	PK model IIV RE	Two compartments CL Additive	7016.65	-	-
Run 110	PK model IIV RE	Two compartments CL + V Additive	6616.39	-400.26	**ACCEPTED**
Run 111	PK model IIV RE	Two compartments CL + V Proportional	6598.83	-17.56	**ACCEPTED**
Run 112	PK model IIV RE	Two compartments CL + V + Q Proportional	6207.34	-391.49	**ACCEPTED**
Run 113	PK model IIV RE	Two compartments CL + V + Ka Proportional	NULL	-	DISCARDED
Run 114	PK model IIV RE	Two compartments CL + V + Vss Proportional	NULL	-	DISCARDED

Once the process had finished, we compared the best one-compartment model with the best two-compartment model using the AIC. Model 104 had an AIC = -13.37, a lower value than run 112 value AIC = -11.46.

6.2.2. Covariate model development

The influence of the covariates in the model was scrutinized in this step. Based on the acceptance limits stated in the methodology we accepted the covariates that had a significant positive effect explaining either clearance or distribution volume interindividual variability.

Table 5.2.3. Covariates effects on clearance.

RUN N.º	COVARIATE CHANGE		OFV	ΔOFV (%)	ETA 1	ΔETA 1 (%)	CHANGE DECISION
Run 104	**BASE MODEL**		**5931.11**	**-**	**0.104**	**-**	**-**
Run 115	CL	WEIGHT	5917.01	-14.10	0.0889	-14.51	**ACCEPTED**
Run 116	CL	PCIA exp θ	5914.62	-16.49	0.0869	-16.44	**ACCEPTED**
Run 116(1)	CL	PCIA	5914.46	-17.65	0.0856	-17.69	**ACCEPTED**
Run 117	CL	HGT	5923.71	-7.4’	0.0954	-8.30	DISCARDED
Run 118	CL	AREA	5914.56	-16.55	0.0864	-16.90	**ACCEPTED**
Run 119	CL	AGE	5984.74	+53.62	0.186	+78.84	DISCARDED
Run 125	CL	GEND	5920.00	-11.11	0.0919	-11.63	**ACCEPTED**
Run 126	CL	GOOD HEPATIC FUNCTION	5929.31	-1.80	0.102	1.92	DISCARDED
Run 127	CL	FLUDARABINE	5930.12	-0.99	0.102	1.92	DISCARDED
Run 128	CL	CYCLO	5929.49	-1.62	0.102	1.92	DISCARDED
Run 129	CL	TYMOGLOBULIN	5926.19	-4.92	0.0981	5.67	DISCARDED
Run 130	CL	THIOTEPA	5931.09	0	0.104	0	DISCARDED
Run 131	CL	INDICATION	5928.88	-1.23	0.101	2.88	DISCARDED

Table 5.2.4. Covariates effects on distribution volume

RUN N.º		COVARIATE CHANGE	OFV	ΔOFV (%)	ETA 2	ΔETA 2 (%)	CHANGE DECISION
Run 104		**BASE MODEL**	**5931.11**	**-**	**0.0597**	**-**	**-**
Run 120	V	WEIGHT	5909.46	-21.65	0.0441	-26.13	**ACCEPTED**
Run 121	V	PCIA exp θ	5900.96	-30.15	0.0381	-35.18	**ACCEPTED**
Run 121(1)	V	PCIA	5900.96	-30.53	0.0386	-35.34	**ACCEPTED**
Run 122	V	HGT	5916.64	-14.47	0.0481	-19.43	DISCARDED
Run 123	V	AREA	5903.14	-27.97	0.0395	-33.83	**ACCEPTED**
Run 124	V	AGE	6010.73	+79.62	0.1690	+183.08	DISCARDED
Run 132	V	GEND	5919.22	-11.89	0.0505	-15.41	**ACCEPTED**
Run 133	V	GOOD HEPATIC FUNCTION	5930.99	-0.12	0.0596	-0.16	DISCARDED
Run 134	V	FLUDARABINE	5930.39	-0.724	0.0590	-1.17	DISCARDED
Run 135	V	CYCLO	5931.08	-0.03	0.0598	+0.16	DISCARDED
Run 136	V	TYMOGLOBULIN	5930.95	-0.16	0.0597	0	DISCARDED
Run 137	V	THIOTEPA	5930.01	-1.10	0.0594	0.50	DISCARDED
Run 138	V	INDICATION	5920.49	-10.62	0.0514	-13.90	**ACCEPTED**
Run 142	V	INDICATION HODGKIN LYMPHOMA	5921.87	-9.24	0.0528	-11.56	**ACCEPTED**

The BPV of the clearance and distribution volume of busulfan was better explained by the PCIA, the AREA and the GENDER. Patients with Hodgkin Lymphoma showed a significant difference in volume compared with the rest of indications.

The building of multivariate models was executes and the results were exposed in Table 5.2.5. and Table 5.2.6.

Table 5.2.5. Mixed covariate effects on clearance models.

RUN N.º	COVARIATE CHANGE		OFV	ΔOFV	ETA 1	ΔETA 1 (%)	CHANGE DECISION
Run 145	CL	GEND + PCIA	5914.50	-0.04	0.0868	+1.40	DISCARDED
Run 146	CL	GEND + AREA	5911.71	-2.85	0.0868	+0.46	DISCARDED
Run 148	CL	PCIA + AREA	5930.82	+16.36	0.104	+21.49	DISCARDED

Table 5.2.5. Mixed covariate effects on distribution volume models.

RUN N.º	COVARIATE CHANGE		OFV	ΔOFV	ETA 2	ΔETA 2 (%)	CHANGE DECISION
Run 149	V	GEND + PCIA	5900.30	-0.66	0.0379	-1.81	DISCARDED
Run 151	V	GEND + AREA	5900.41	-2.73	0.0381	-1.29	DISCARDED
Run 152	V	LH + PCIA	5893.70	-7.26	0.0348	-9.84	**ACCEPTED***
Run 154	V	LH + AREA	5896.03	-7.11	0.0357	-9.62	**ACCEPTED***
Run 155	V	PCIA + AREA	5917.69	+14.55	0.0514	+30.12	DISCARDED

PCIA and AREA were the covariates that showed a better fit for the model. When tested univariately they provided a similar reduction of the objective function value, but together worsened the model. To finalize the covariate model building, all significant covariates were tested multivariately to build the final model. The results are exposed in Table 5.2.6.

Table 5.2.6. Mixed effects on clearance plus distribution volume models.

RUN N.º	COVARIATE CHANGE		OFV	ΔOFV	CHANGE DECISION
Run 156	CL V	PCIA LH + PCIA	5876.02	-17.68	**ACCEPTED**
Run 157	CL V	AREA LH + PCIA	5876.41	-17.29	**ACCEPTED**

There was little difference between AREA and PCIA when applied in the model. It was decided to keep PCIA as our covariate. A correlation of ETAs was suggested by the exploration of the final model. However further analysis determined a poor correlation between ETA 1 and ETA2 despite the graphic scatterplot trend line indications.

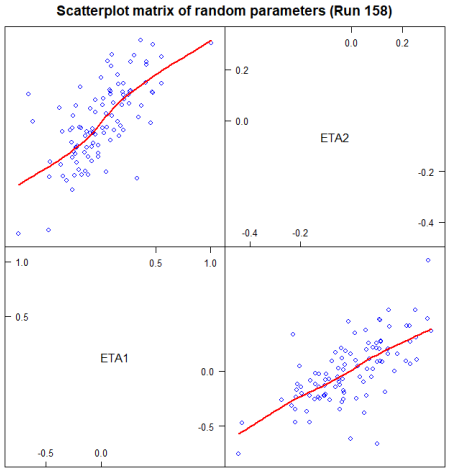

Figure 5.2.1. Scatterplot Matrix of ETAs. r2=0.544.

The resulting model, Run158, was the best fit. Its characteristics are shown in table 5.2.7.

Table 5.2.7. Forward best-fitting model results.

Run 158	CL V ETA1 ETA2	PCIA LH + PCIA BLOCKED	5855.66	vs. Run 142 -38.04 vs. Run 156 -20.36	**ACCEPTED**

With this model, we ended the Forward process of model selection. Due to the model's flexibility, adding covariates in a predefined sequence may be different than another and for this reason we had to do a "Backwards" process which consisted in deconstructing the model eliminating the first covariate introduced in the final model and to observe if an increase (worsening) higher than 10 points in the OFV was induced. This would mean that such covariate has at least p<0.001 statistical significance.

Table 5.2.8. Backward elimination process results.

RUN N.º	COVARIATE CHANGE		OFV	ΔOFV	CHANGE DECISION
Run 159	CL V ETA1 ETA2	PCIA PCIA BLOCKED	5864.39	+8.63	NOT SIGNIFICANT
Run 160	CL V ETA1 ETA2	- LH + PCIA BLOCKED	5879.72	+24.06	**SIGNIFICANT**
Run 161	CL V ETA1 ETA2	PCIA LH BLOCKED	5895.46	+39.8	**SIGNIFICANT**

The results obtained in the backward process showed that separating patients with Hodgkin Lymphoma from the others in the model was not significant enough and therefore it was discarded from the model.

The final model is a one-compartment model with two non-correlated ETAs, one in Clearance and one in Distribution Volume, both affected by PCIA covariate. With this, we finalized the model enhancement and proceeded to the next step.

The Typical Values of the THETAs, and ETAs is shown in the table 5.2.9.

Table 5.2.9. Typical Values of PK parameters in the definitive populational model.

Run 162	F	Ka (h^{-1})	CL/F (L/h)	Vd/F (L)
Value	FIXED = 1	1.47	11.0	42.7
ETA	-	-	0.0895	0.0394
ETA correlation	-	-	$\omega_{2,1}^2$=0.0322 / r^2=0.544	
CV (%) of IIV			29.9	19.85

6.2.3. Internal Model Validation

Results of the two internal validation methods (prediction visual predictive check and bootstrap) are shown in Figure 5.5.2 and Table 5.2.11 respectively.

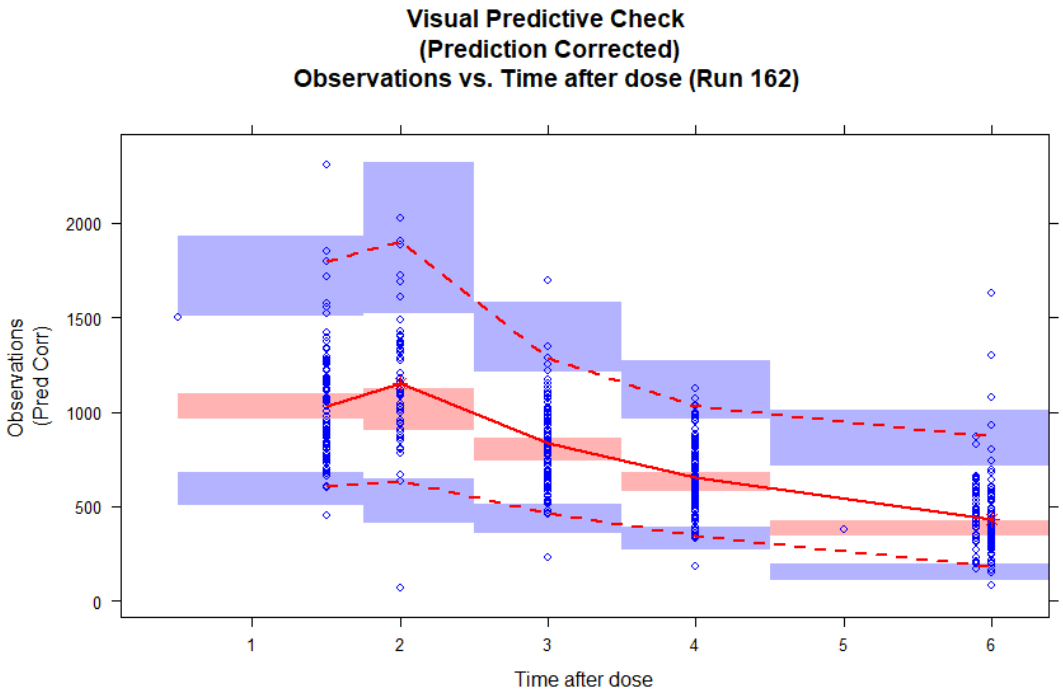

Figure 5.2.2. Time After Dose vs Observations of Simulated and Real populations. Pink areas enact as the median values, and the blue areas represent the 5th and 95th percentiles values of the simulations. The red lines embody the results of the observed real concentrations of the original population, the median is the continuous line and the dashed lines are the 5th and 95th percentiles.

Table 5.2.11. Bootstrap results compared to original population PK parameters.

	$\overline{X}_{original}$	$\overline{X}_{sample}$	2.50%	97.50%	% difference
OFV	5860.51	5857.06	5552.04	6194.43	0.06
THETA(1)→ F	1	1	1	1	0 (Fixed)
THETA(2)→ Ka	1.467	1.451	1.184	1.839	1.08
THETA(3)→ CL/F	10.982	10.976	10.395	11.584	0.05
THETA(4)→ V/F	42.697	42.714	39.716	45.686	-0.04
OMEGA(1,1)	0.0882	0.0883	0.0597	0.1235	-0.10
OMEGA(2,1)	0.0319	0.0313	0.0117	0.0566	1.66
OMEGA(2,2)	0.0386	0.0387	0.0230	0.0574	-0.10
SIGMA(1,1)	0.0291	0.0293	0.0218	0.0361	-0.73

7. DISCUSSION

The main objective of creating and developing a functional populational pharmacokinetic model has been accomplished throughout the 1st semester of 2019 as shown in the Results. For this purpose, knowledge of Nonlinear Mixed Effects Models and in NONMEM® software coding language was acquired.

A total of 98 patients were included in the current analysis, with 523 plasma concentrations values analyzed and 363 doses administered. Demographic characteristics of the studied population showed a proper balance between genders, close to 50% with a slightly predominance of male in the population. A wide range of variation was observed for ages and anthropometric covariates (Figure 5.1.2). There was a significant number of patients (62 out of 98) with some alteration of the hepatic function. The liver plays a major role in the metabolism of busulfan, so it was a variable that had to be taken in consideration. All patients took at least one concomitant medication alongside busulfan. Only two patients took Thiotepa concomitantly. Although no interaction had been described between Thiotepa and Busulfan, this co-medication was tested as covariate on Bulsulfan clearance and neither statistical nor clinical influence was found. Therefore, this was deemed irrelevant.

The pharmacokinetics of Busulfan was best described by a one-compartment model with first order absorption and elimination processes. This was in agreement with previous reported models developed after either oral or intravenous administration of Busulfan.(24,27,29–31,59) Only McCune J. et al., and Kawazoe A. et al. described the pharmacokinetics of busulfan with a two-compartment model. This discrepancy may be explained by the fact that these authors modelled intravenous data and a more intensive sampling schedule around the distribution phase was achieved.

The final model provided the pharmacokinetic parameter values normalized by bioavailability (F), due to the lack of intravenous data in the modelling process. Clearance was estimated as 11.0 L/h, the distribution volume was 42.7 L, the half-life was 2.69 h, the apparent elimination rate constant (Ke) was 0.257 h-1, and the absorption rate constant was 1.47 h^{-1}. These results agreed with those of the available literature, also developed form oral data, that reported a clearance (CL/F) around 12 L/h, and distribution volume (V/F) of 46 L.(24,27,29–31,59) Actually, according to McCune et al and Kawazoe et al, after its intravenous administrations busulfan showed a two-exponential pharmacokinetic behavior with two disposition phases.(23,26) Therefore, once in the body Busulfan distributes from the central compartment to the peripheral according to its lipophilic nature. Unfortunately, this behavior could not be characterized with our model, but the achieved model allowed a robust estimation of the plasma clearance.

Regarding to the absorption rate constant of our model (1.47 h^{-1}), it was different from other previous studies (2.9 – 4.0 h^{-1}).(29,31)There is a significant disagreement between the absorption rate constant, presenting a large variability between the reviewed models (CV = 15%), as well as in the clinical assays (CV = 45.9%). These differences could be due to the different sampling schedules applied during the absorption phase in these studies.

The interindividual variability could be included in Clearance and Volume with resulting values of 29.9 % and 19.8 % respectively. The only covariate identified as statistically significant was Adjusted Ideal Body Weight (PCIA) which reduced the OFV in 17.65 units ($p<0.001$) and led to a reduction of the BPV in clearance of 17.69%. When tested in distribution Volume, PCIA further decreased the OFV in 30.53 units ($p<0.001$) and its BPV a 35.34%. This was in agreement with other authors who also found a significant role of anthropometric covariates such as Total Body Weight, Body Surface Area, Adjusted Ideal Body Weight, Fat Free Mass and/or Actual Body Weight on clearance, on distribution volume, or both. Other studies also found Sex, Age, and ALT values as significant covariates. (23,24,61–64,25–31,60)

The effect of gender was not perceived so clearly in our model. Although during the "Forward" process, GENDER (Sex) was statistically significant, it could not be retained in the model combined with anthropometric covariates. This fact does not deny the differences between man and women, because , actually, these differences are accounted by differences in PCIA values in the model.

The variable "Type of Indication" was tested as a covariate (especially those with Hodgkin Lymphoma in front of the other indications) and it was also found significant during the forward inclusion. But, no significant increase in the OFV was observed during the backwards elimination so it had to be removed from the model. It is worth mentioning the study of Hadjibabaie M. et.al. which described the effect of the targeted disease on the Clearance of Busulfan. Unlike the aforementioned author, we only observed a trend for the influence of this covariate on the distribution Volume.

The predictive capability of the model was confirmed by the Prediction Corrected Visual Predictive Check. According to Figure 5.2.2. The comparison of distributions of observed and simulated data suggested that the 50%, 5% and 95% percentiles of the observed data were within the 95% prediction intervals of the corresponding percentiles of the simulated data. There was a slight underestimation of the mean concentration value around 2 hours after administration.

The stability of the model and precision of the parameter estimates were confirmed by the bootstrap methods with relative deviations between the population values and the mean values estimated by this method lower than 1.5% in all the cases. Moreover, the populational values were within the 95% confidence interval given by bootstrap.

An external validation should be carried out to further confirm the predictive power of the model before its application to the clinical practice as a support tool for dose calculations in the target population.

8. CONCLUSIONS

Through the attendance to several lessons about population pharmacokinetics we gained sufficient insight to start developing our project. By means of a review of preexisting literature, we were able to comprehend the actual importance and applicability of POPPK models in the clinical practice, especially as a support tool during therapeutic drug monitoring of drugs with high interindividual variability and narrow therapeutic index. Moreover, this approach allows to overcome the limitations associated with the routine clinical practice when a low number of samples per patient is available.

Among the different population analysis approaches, we focused on the non-linear mixed effects models. Despite its complexity, this methodology is highly powerful due to its great descriptive and predictive capability. Furthermore, the use of this approximation is a gender-friendly tool that allows the pharmacometrician to account for differences between man and woman, whether directly or indirectly through anthropometric measurements.

The PK parameters of the final model were similar to those of previously reported models, between patient variability associated with parameters and residual errors associated with concentrations also presented a low variance. All the population pharmacokinetic parameters estimated were physiologically meaningful and the estimated interindividual and residual variabilities were acceptable. Furthermore, goodness-of-fit plots showed the descriptive capability of the model and the two applied internal validation techniques showed its predictive capability.

In the second semester of 2019, the model will be translated to the Catalan Oncology Institute (ICO) and used to predict concentrations based on the specific anthropometric measurements of new patients subjected to HSCT (external population). From the collected data from several new patients an external validation will be performed to confirm its predictive capability and to evaluate its potential use as a support tool to either calculate the initial doses or if required for dose tailoring during the therapeutic drug monitoring .

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ANNEX I: GOODNESS-OF-FIT COMPARATIVE EVALUATION OF DEVELOPED MODELS

INITIAL MODEL (Run 101)

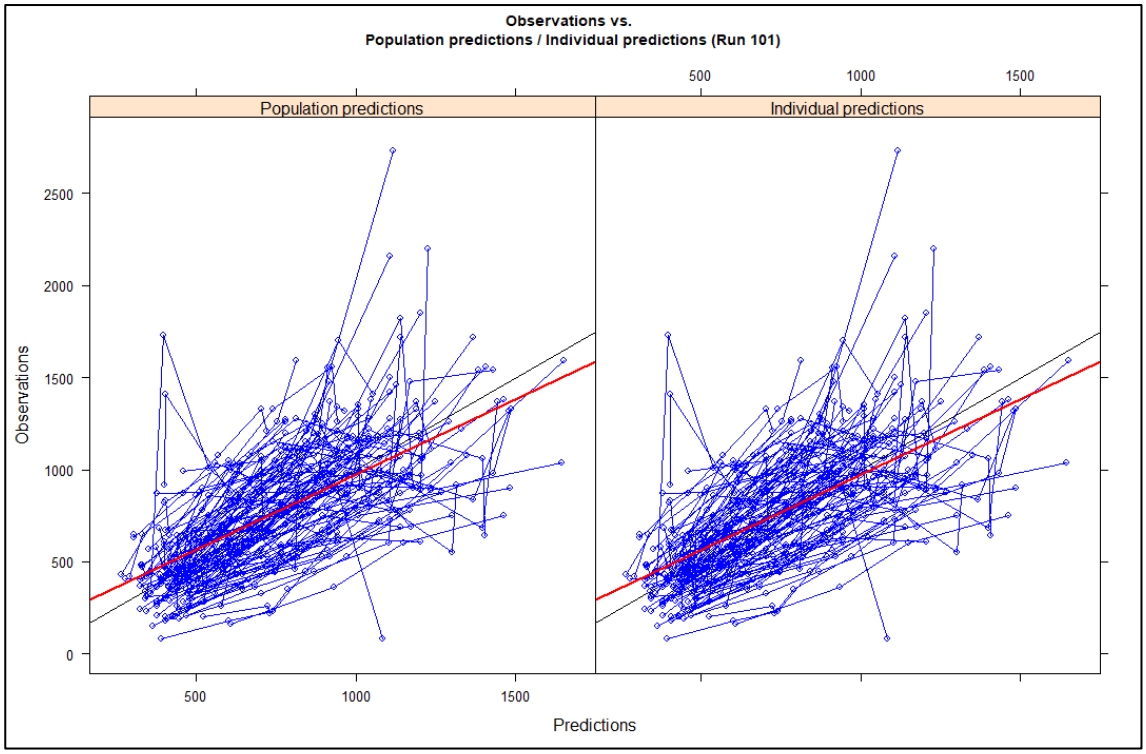

Figure A.1. Population Predicted Concentrations and Individual Predicted Concentrations vs Observed Concentrations

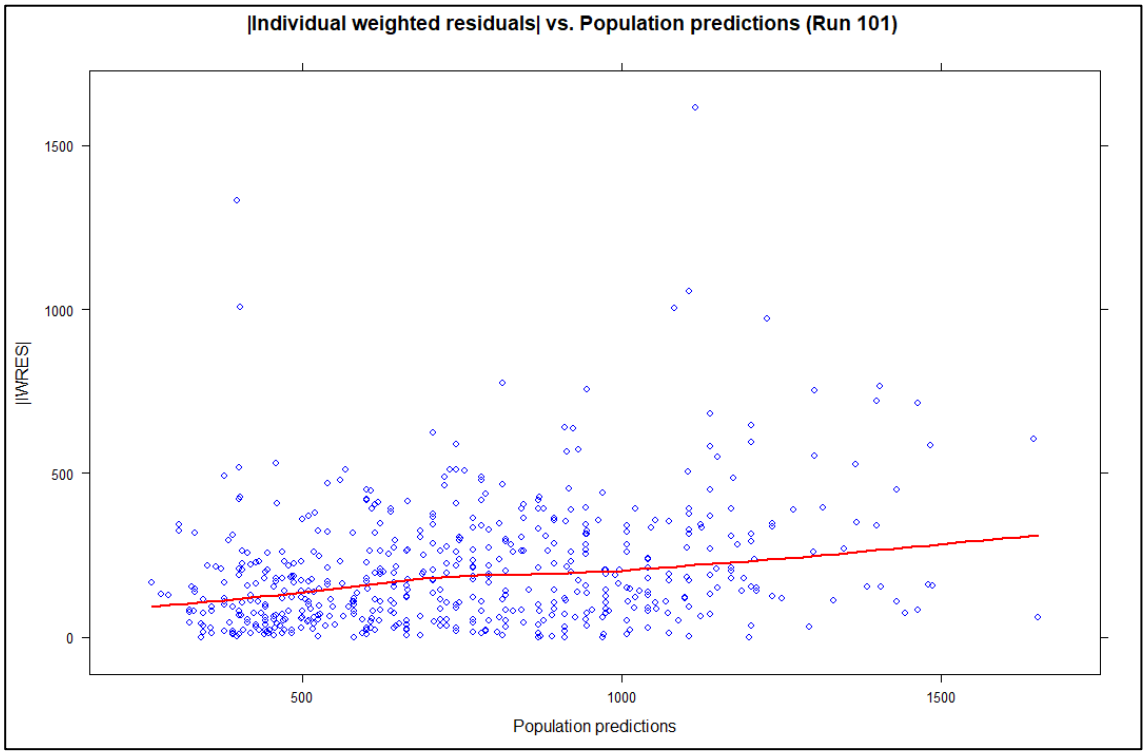

Figure A.2. Absolute value of Individual Weighted Residuals vs Population Predicted Concentrations.

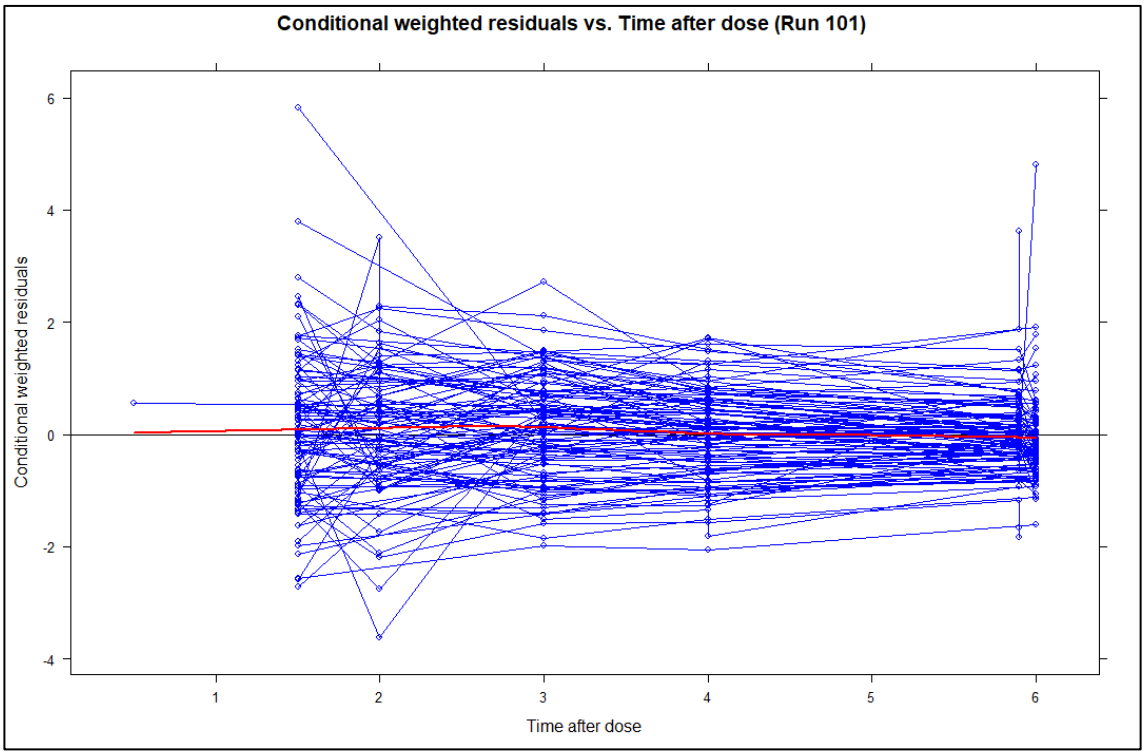

Figure A.3. Conditional Weighted Residuals versus Time After Dose.

The Figure A.1 shows an underestimation of the predictive values respecting the observations at the lower values, whereas an overestimation can be seen in the higher values. The errors of the model have to be corrected in the following steps of the process. This model has yet to explain the Between Patient Variability and the effects of the covariates that remain uncharacterized as a residual error.

The Figure A.2 shows a great IWRES (Individual Weighted RESidual), a representation of the unexplained errors between the predictions and the observations. This model shows a poor performance explaining the residual errors, giving a high absolute value of IWRES along the predicted values.

The Figure A.2 shows a dispersion of CWRES (Conditional Weighted RESiduals). The range of dispersion is from 6 to -4, with a central tendency around zero. The range is wider than desired and have to be narrowed to accept the model.

BASIC MODEL (Run 104)

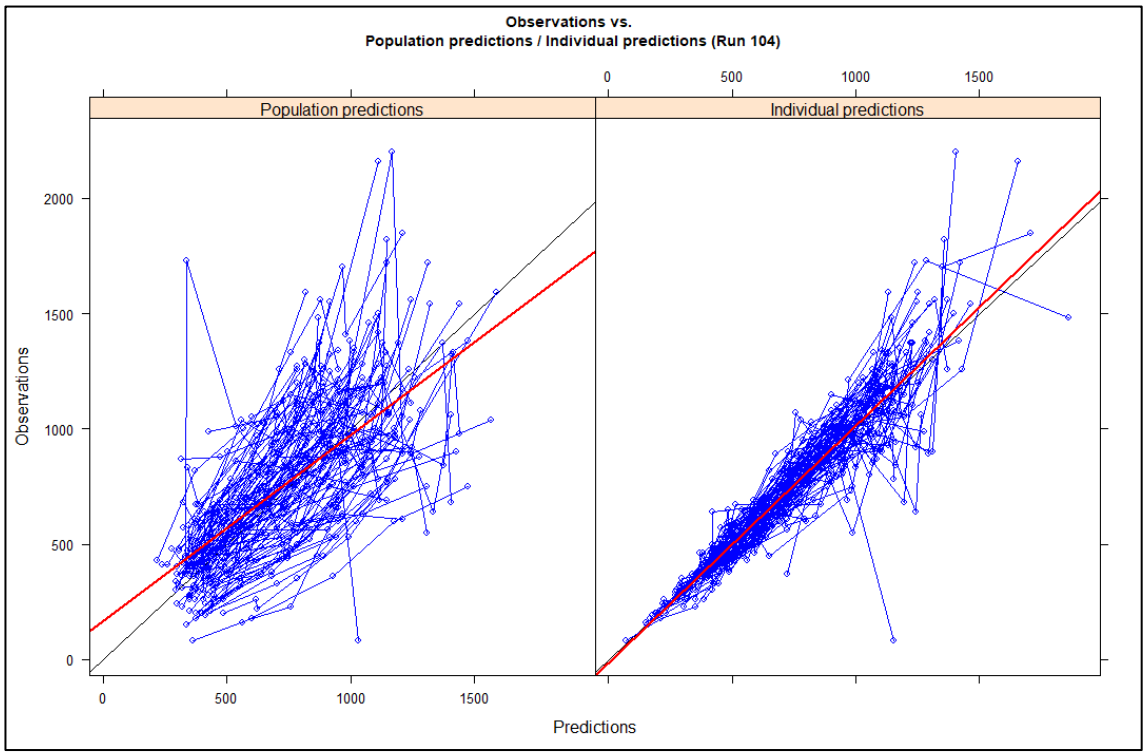

Figure B.1. Population Predicted Concentrations and Individual Predicted Concentrations vs Observed Concentrations

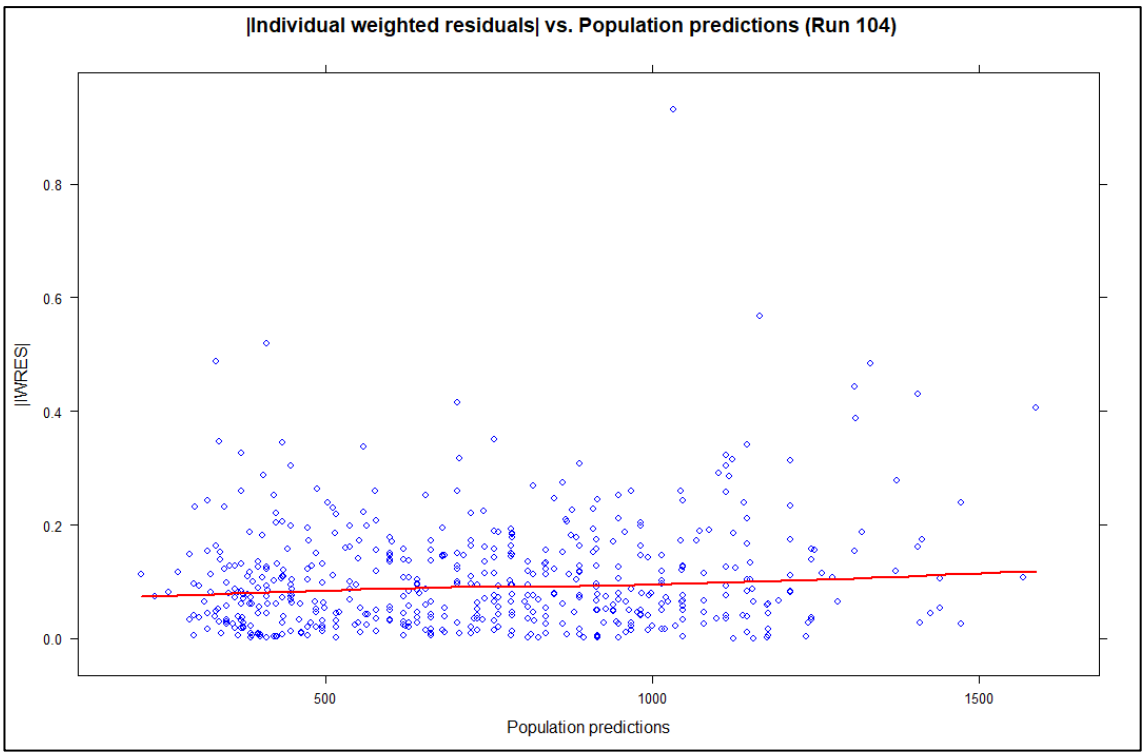

Figure B.2. Absolute value of Individual Weighted Residuals vs Population Predicted Concentrations.

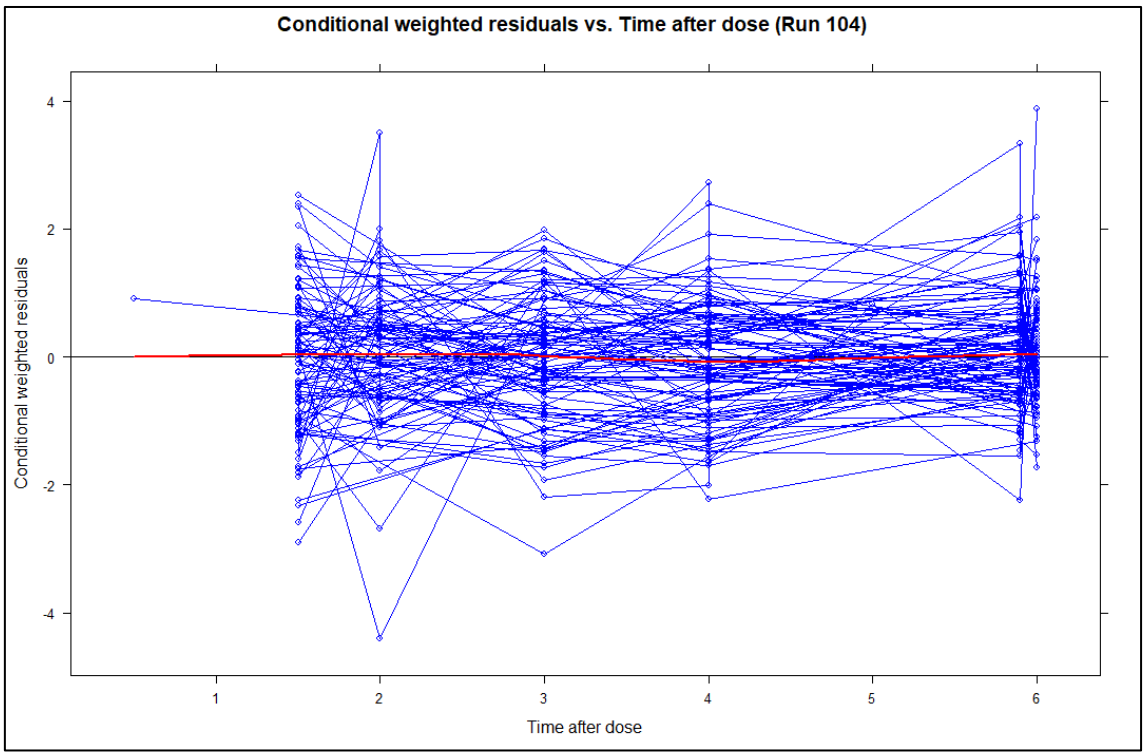

Figure B.3. Conditional Weighted Residuals versus Time After Dose.

Figure B.1 representing the fit of the model to the real observations, seems to have improved the Population predictions vs Observations, although the underestimation at low values and overestimation at high values persists. On the other hand, the Individual predictions vs Observations show a great enhancement on the model performance with an almost perfect fit. IPRED vs OBS is mainly affected by Between Patient Variability (BPV), whereas PPRED vs OBS is affected by BPV and the residual error. That explains why there is such a good fit of the first but not the second. The development of the basic model aims to explain BPV, that is now out of what was the residual error in Figure A.1.

Figure B.2 shows a great reduction of IWRES values along the PPRED values since the BPV has been explained. We changed from an absolute value of IWRES of hundreds to decimal values. The next step was to further characterize the residual error using the covariate.

Figure B.3. shows a narrower dispersion of CWRES over TAD, ranging from 4 to -4 with a central tendency line at zero.

FINAL MODEL (Run 162)

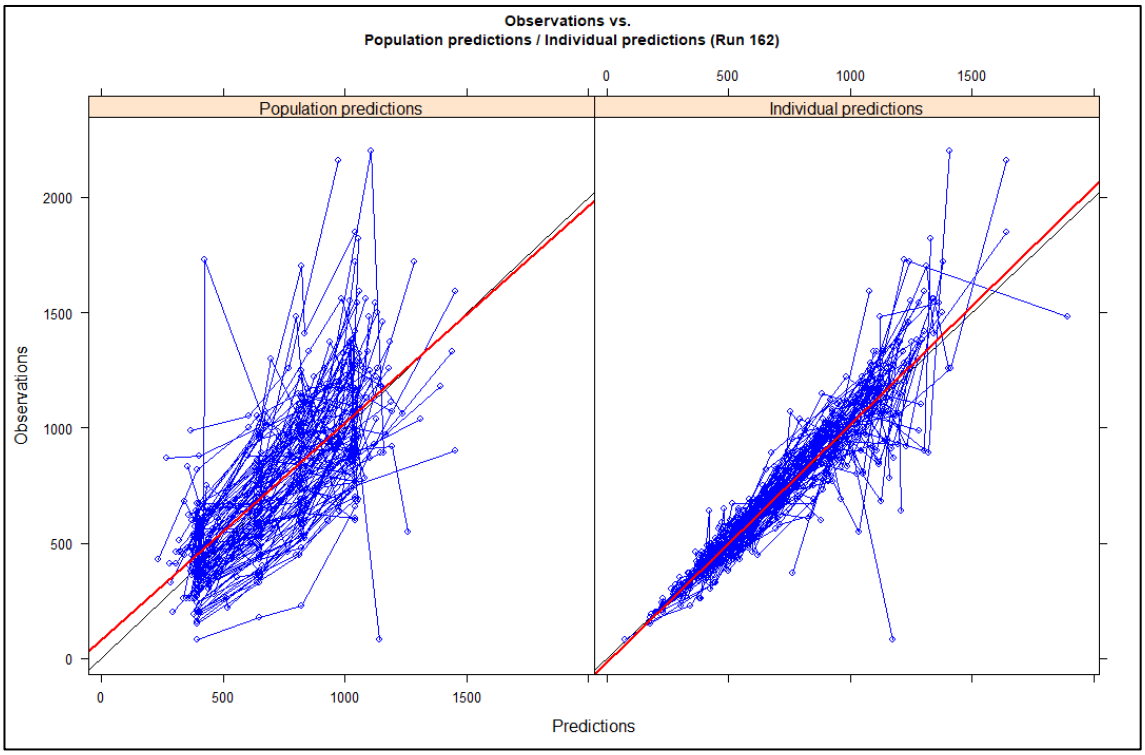

Figure C.1. Population Predicted Concentrations and Individual Predicted Concentrations vs Observed Concentrations

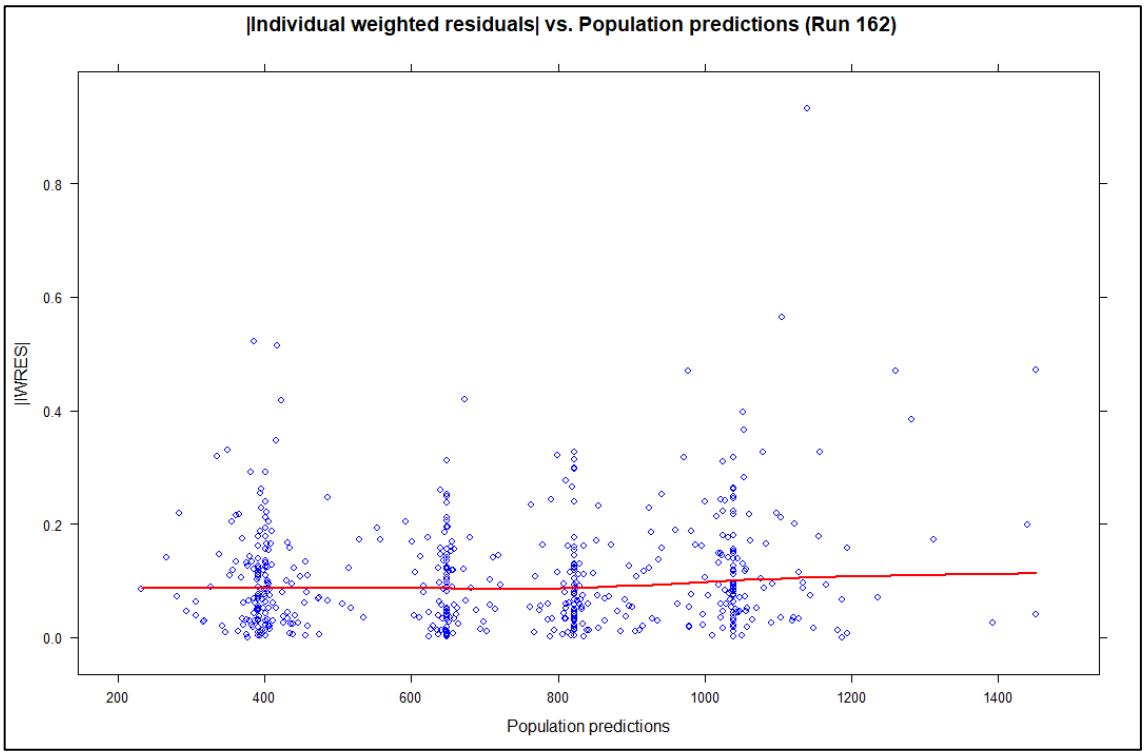

Figure C.2. Absolute value of Individual Weighted Residuals vs Population Predicted Concentrations.

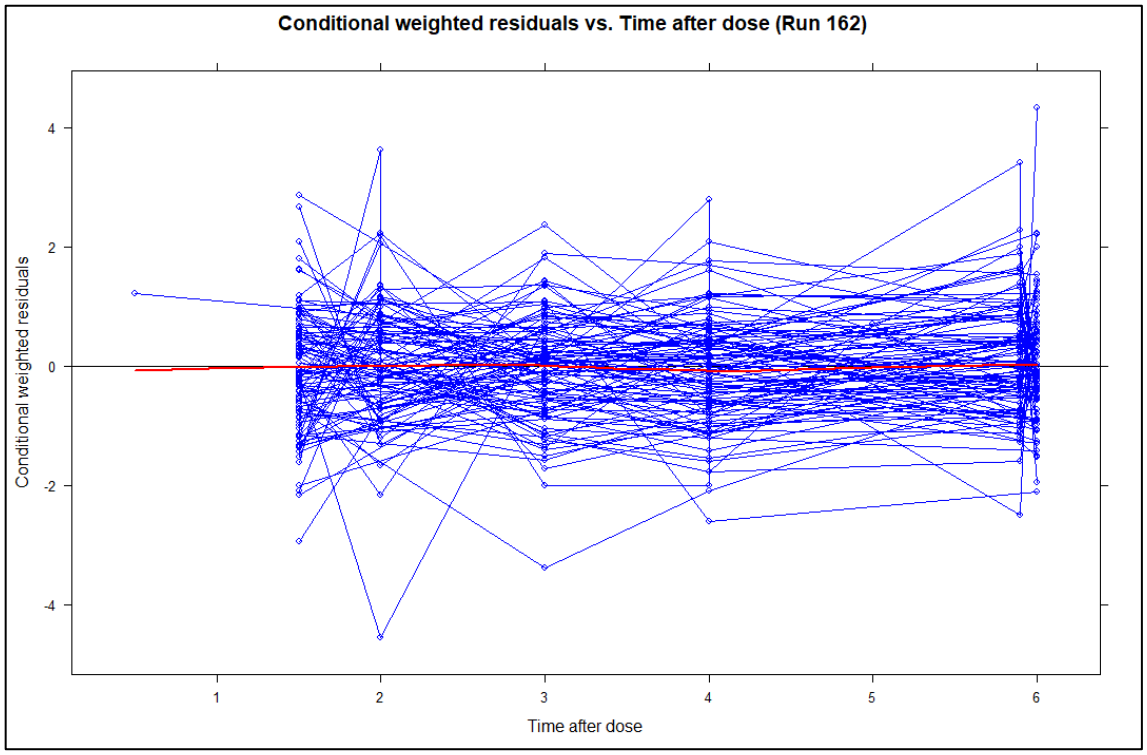

Figure A.3. Conditional Weighted Residuals versus Time After Dose.

Figure C.1 represents the PPRED vs OBS and IPRED vs OBS plots of the best model, once the covariates have been testes and properly combined to characterize the residual values. The overestimation at higher values has been corrected and a light underestimation persist al lower values, which it's not expected to have relevance in the performance of the model, since values that low usually are not of clinical relevance.

Figure C.2 shows little variation of absolute IWRES value, but the dispersion of the dots has been reduced and grouped. This plot represents the residuals that were not explained by the final model.

Figure C.3 representing the CWRES vs TAD shows a narrow range mostly between 3 and -3, but mostly between 2 and -2, with a tendency at zero. Punctual CWRES values lay outside the 3,-3 range but it is still acceptable.

ANNEX II: SOPs FOR PLASMA SAMPLING

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MONITORITZACIÓ BUSULFÀ ENDOVENOSA PROTOCOL Al·loTIR
Full Recollida de Dades d'Extracció
Servei de Farmàcia - Servei d'Hematologia Clínica

Nom Pacient:		**Pes (kg):**		**S.C (m^2):**	
Història Clínica:		**Alçada (cm):**		**Edat (anys):**	
Identificació Hospital:					

Busulfà EV Infusió 2hores (0,8 mg/kg/6 h) 10 Dosis	**Dosi Total Administrada**	**Data Inici (dd/mm/aa)**	**Hora Inici Infusió (hh:mm)**	**Hora Finalització Infusió (hh:mm)**

Extraccions de Sang en Tubs EDTA – Utilitzar una Via Perifèrica
Volum Mínim 2mL/Tub - Consevar en NEVERA!!!
Registrar Hora d'Extracció en els Tubs !! Extreure 2 Tubs per Mostra
Enviar Mostres al Laboratori Bioquímica – Hospital Santa Creu i Sant Pau

Nº Mostra	Hora Teòrica d'Extracció	Data Real d'Extracció (dd/mm/aa)	Hora Real d'Extracció (hh:mm)
1	Pre-Dosi (Blanc)		
2	1,5 Hores Post-Inici Infusió		
3	3 Hores Post-Inici Infusió		
4	4 Hores Post-Inici Infusió		
5	6 Hores Post-Inici Infusió		

Medicació Concomitant Destacable:		
Fàrmac	**Posologia**	**Via d'Administració**

Alteracions Funcionals Destacables/Altres Observacions:		
Nom:	**Signatura:**	**Data:**

Consultar qualsevol dubte amb el Servei de Farmàcia de l'Hospital Duran i Reynals. Institut Català d'Oncologia.
Tel: 93-260-78-08

ICO
Institut Català d'Oncologia

MONITORITZACIÓ BUSULFÀ ORAL-NIVELLS COMPROVACIÓ
Després de la Cinquena dosi de Busulfà Administrada
Full Recollida de Dades d'Extracció
Servei de Farmàcia-Servei d'Hematologia Clínica

Nom Pacient:		**Pes (kg):**		**S.C (m^2):**	
Història Clínica:		**Alçada (cm):**		**Edat (anys):**	
Identificació Hospital:					

	Dosi Administrada (mg)	Data Inici (dd/mm/aa)	Hora d'Administració (hh:mm)
Busulfà Oral:			

Extraccions de Sang en Tubs EDTA - Volum Mínim 2mL/Tub - Conservar en NEVERA!!!

Registrar Hora d'Extracció en els Tubs !! Extreure 2 Tubs per Mostra

Enviar Mostres al Servei de Bioquímica - Hospital de Santa Creu i Sant Pau

Nº Mostra	Hora Teòrica d'Extracció	Data Real d'Extracció (dd/mm/aa)	Hora Real d'Extracció (hh:mm)
1	2 Hores Post-Administració		
2	6 Hores Post-Administració		

Medicació Concomitant Destacable:

Fàrmac	Posologia	Via d'Administració

Alteracions Funcionals Destacables/Altres Observacions:

Nom:	Signatura:	Data:

Consultar qualsevol dubte amb el Servei de Farmàcia de l'Hospital Duran i Reynals. Institut Català d'Oncologia.
Tel: 93-260-78-08