Quantitative assessment of the effects of the selective $S1P_1$ receptor modulator ponesimod using pharmacometric modeling and simulation

Dissertation

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Tell me and I forget.

Teach me and I may remember.

Involve me and I learn.

Benjamin Franklin

Publications included in this thesis

I   Population pharmacokinetics of ponesimod and its primary metabolites in healthy and organ-impaired subjects

Dominik Lott, Andreas Krause, Jasper Dingemanse, Thorsten Lehr

Eur. J. Pharm. Sci. 2016, 89:83-93. doi: 10.1016/j.ejps.2016.04.021

II   Impact of demographics, organ impairment, disease, formulation, and food on the pharmacokinetics of the selective $S1P_1$ receptor modulator ponesimod based on 13 clinical studies

Dominik Lott, Thorsten Lehr, Jasper Dingemanse, Andreas Krause

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III   Modeling the effect of the selective $S1P_1$ receptor modulator ponesimod on subsets of blood lymphocytes

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IV   Modeling tolerance development for the effect on heart rate of the selective $S1P_1$ receptor modulator ponesimod

Dominik Lott, Thorsten Lehr, Jasper Dingemanse, Andreas Krause

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Contribution report

Herewith, the author would like to declare his contributions to the publications I-IV included in this thesis. The author:

I   programmed the modeling data set, conducted the analysis, created the graphics, and wrote the manuscript.

II programmed the modeling data set, conducted the analysis, created the graphics, and wrote the manuscript.

III programmed the modeling data set, conducted the analysis, created the graphics, and wrote the manuscript.

IV programmed the modeling data set, conducted the analysis, created the graphics, and wrote the manuscript.

Table of Contents

Abbreviations

Abbreviation	Definition
-2LL	Minus two times the logarithm of the likelihood
AIC	Akaike information criterion
bpm	Beats per minute
C	Concentration
CL	Clearance
CNS	Central nervous system
CV	Coefficient of variation
EM	Expectation maximization
EMA	European Medicines Agency
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FO	First-order estimation method
FOCE	First-order conditional estimation method
FOCE-I	First-order conditional estimation method with interaction
GIRK	G-protein coupled inwardly rectifying potassium channel
GOF	Goodness-of-fit
GvHD	Graft-versus-host disease
IFN	Interferon
IIV	Inter-individual variability
IOV	Inter-occasion variability
M12	Ponesimod metabolite M12
M13	Ponesimod metabolite M13
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NK	Natural killer (cells)
NLME	Nonlinear mixed effects
o.d.	Once-daily dosing
OFV	Objective function value
pcVPC	Prediction-corrected visual predictive check
PD	Pharmacodynamics
PK	Pharmacokinetics

Abbreviation	Definition
PPMS	Primary progressive multiple sclerosis
PRMS	Progressive-relapsing multiple sclerosis
RA	Rheumatoid arthritis
RRMS	Relapsing-remitting multiple sclerosis
RSE	Relative standard error
RV	Residual variability
S1P	Sphingosine-1-phosphate
$S1P_{1-5}$	S1P receptor subtypes 1-5
SAEM	Stochastic approximation of expectation maximization
SLE	Systemic lupus erythematosus
SPMS	Secondary progressive multiple sclerosis
t	Time
TDM	Therapeutic drug monitoring
V	Volume of distribution
VPC	Visual predictive check

Graphical abstract
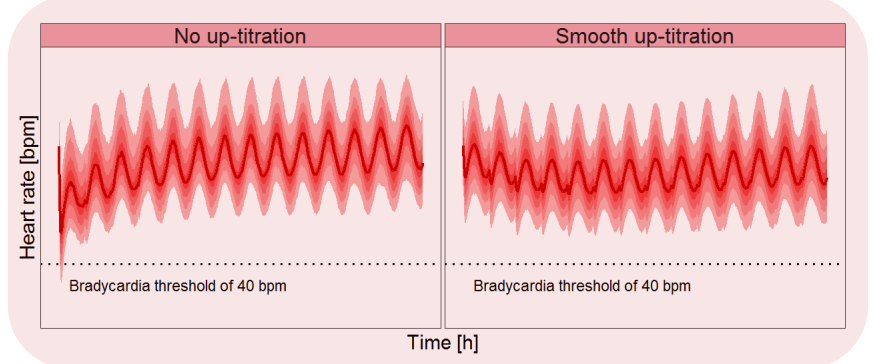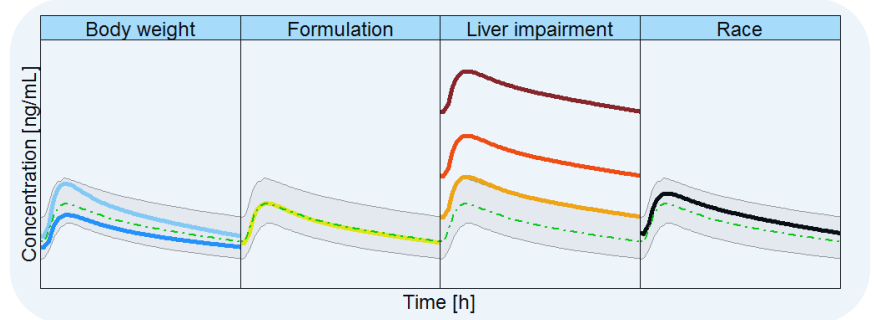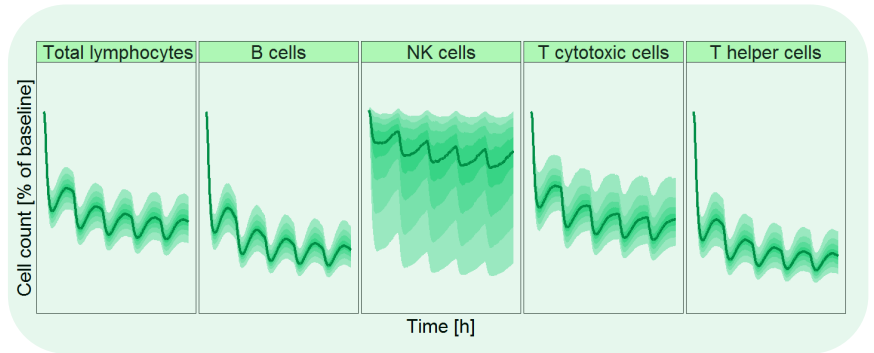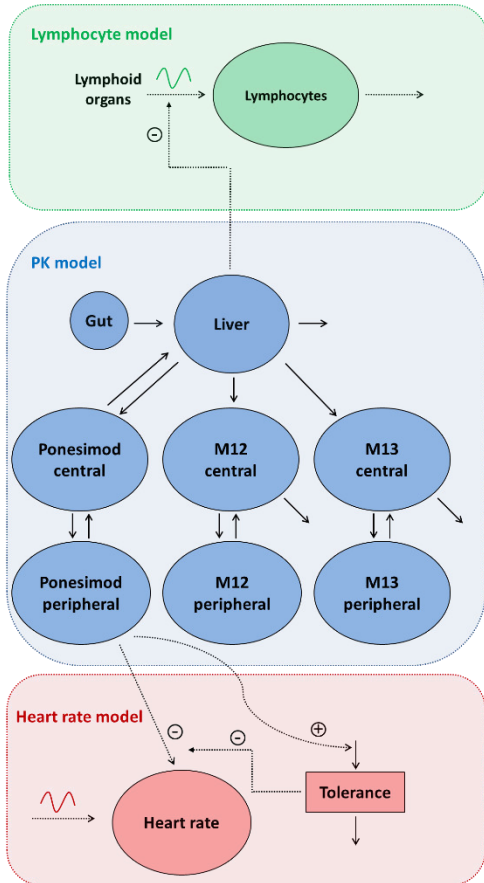
1. Introduction

1.1 Multiple sclerosis

Multiple sclerosis (MS), an inflammatory autoimmune disease of the central nervous system (CNS), is one of the most common and important neurological diseases (1, 2). It causes progressive neurological disability following demyelination and neuronal loss in the CNS and affects 2-2.5 million people worldwide (3). The main characteristics of MS are its broad spectrum of signs and symptoms, an unpredictable clinical course, and a variable prognosis. MS negatively affects life expectancy as well as quality of life (2). Thereby, frequent and progressive neurological disability often leads to social isolation and dependency on caregivers resulting in major personal, social, and financial consequences for patients, their families, and the health care system (4).

The prevalence of MS ranges from < 5 to > 100 cases per 100,000 individuals and is known to vary between races and geographical regions (2, 3, 5). The highest prevalence was reported for Northern Europe, Southern Australia, New Zealand, and North America (3). The relationship between geographical latitude and prevalence is thought to be attributed to environmental and genetic factors (3, 6). MS affects predominantly women between 20 and 50 years of age (3). Female to male ratios ranging from 2:1 (3) to 3:1 (2) were reported. The peak onset of the disease appears in the early thirties (3), while only 2-5% of patients diagnosed with MS are below the age of 16 (7).

The etiology of MS is still unknown. Genetic variations, environmental factors, and exposure to viruses such as the Epstein-Barr virus are suggested to be involved (8, 9). It is widely accepted that the disease involves the migration of autoreactive lymphocytes across the blood-brain barrier (2). These autoreactive cells falsely identify the myelin sheath of neurons in the CNS as an external threat to the body and cause demyelination, axonal loss, and gliosis (3). Under normal conditions, neurons are able to recover via remyelination mediated by oligodendrocytes (10). In MS, however, this process is ineffective due to gliosis and damaged oligodendrocytes. As a result, repeated damage results in the formation of scar-like plaques (also known as lesions) around the damaged axons. These plaques are primarily observed in the white matter of the brain and the spinal cord and can be visualized via magnetic resonance imaging (MRI).

MS can be characterized by two main clinical features, relapses (also called attacks or exacerbations) and disease progression (11). Relapses are considered the clinical manifestation of acute, focal inflammatory processes in the CNS that may translate to a large variety of neurologic symptoms, such as sensory disturbances, visual loss, etc. Disease progression, i.e., the progressive loss of neuronal

function, is thought to be the clinical expression of progressive neurodegeneration, i.e., demyelination, and axonal loss, as a result of incomplete recovery following relapses.

MS can be classified into four different categories based on the frequency of relapses and the pattern of disease progression (11, 12):

- Relapsing-remitting MS (RRMS) – the most common form of MS (80-85% of the MS population (11)) that is characterized by repeated, clearly defined, acute relapses with full or partial recovery followed by periods without disease progression.
- Secondary progressive MS (SPMS) – initial RRMS followed by sustained progression of disability without periods of remission, characterized by fewer inflammatory and more pronounced neurodegenerative features. Approximately 65% of RRMS patients enter this stage of the disease (2).
- Primary progressive MS (PPMS) – continuous and steady increase in disability from onset without attacks.
- Progressive-relapsing MS (PRMS) – progression of disability from onset of the disease with occasional relapses of escalating severity.

MS is typically diagnosed by using McDonald's Diagnostic Criteria (13) that include the evaluation of different assessments such as description and frequency of attacks, number of lesions detected by MRI, and results from cerebrospinal fluid tests.

Currently, there is no cure for MS. Its treatment is symptomatic and includes therapies with disease-modifying drugs used to optimize long-term clinical outcomes. The aim of the therapies is to reduce the rate of relapses and to prevent or delay progression of the disease. The majority of drugs approved for the treatment of MS needs to be administered either by injection or infusion. In the past years, new drugs with oral administration were approved. An overview of drugs approved for the treatment of MS is given in Table 1.

MS treatment is usually initiated with first-line basic therapeutics, e.g., interferons (IFNs) and glatiramer acetate, and is escalated to more potent second-line therapeutic agents if first-line therapeutics do not sufficiently prevent disease progression. In general, second-line therapeutics are more potent but associated with more serious side effects compared to first-line therapeutics. The two monoclonal antibodies, alemtuzumab and natalizumab, and, in some cases, mitoxantrone, are at the top of the escalation hierarchy (14). Further drugs that are used off-label for the treatment of MS include corticosteroids, e.g., methylprednisolone, mainly used for the treatment of acute relapses (14), and immunosuppressants such as cyclophosphamide, methotrexate, and cyclosporine (15).

While immunosuppressants are highly toxic (nephrotoxicity [16], hepatotoxicity [17], and bone marrow suppression [18]) and increase the risk of opportunistic infections [19], IFNs and monoclonal antibodies need to be applied parenterally and are partly associated with autoimmunity [20]. In addition, existing therapies are only partly effective in reducing inflammatory tissue damage and preventing disease progression. Thus, in spite of the multiple disease-modifying therapies, a medical need towards more effective treatments with a better benefit-risk profile remains.

Until the late 1990's or early 2000's, the number of drugs available for the treatment of MS was limited. Since then, monoclonal antibodies and immuno-modulators (e.g., dimethyl fumarate, teriflunomide) were developed. In parallel, the orally active sphingosine-1-phosphate (S1P) receptor modulator fingolimod was developed and shown to significantly reduce the risk of disability progression as well as to improve MRI-related measures (e.g., brain lesions) [21]. Subsequently, several compounds, highly selective for the S1P receptor subtype 1 ($S1P_1$) playing a central role in lymphocyte trafficking [22, 23], were synthesized and administered to healthy subjects and patients. Ponesimod was the first selective $S1P_1$ receptor modulator tested in humans [24].

Table 1 Approved disease-modifying therapies for RRMS (United States).

Drug class	Approved drug(s)	Trade name	Year of approval	Route of administration
First-line therapies				
Interferons (IFNs)	IFN β-1a	Avonex®	1996	Intramuscular or subcutaneous
	IFN β-1a	Rebif®	2002	
	IFN β-1b	Betaseron®	1993	
		Extavia®	2009	
	Pegylated IFN β-1a	Plegridy®	2014	
Amino acid copolymer	Glatiramer acetate	Copaxone®	1996	Subcutaneous
Pyrimidine synthesis inhibitor	Teriflunomide	Aubagio®	2012	Oral
NFκB inhibitor	Dimethyl fumarate	Tecfidera®	2013	Oral
S1P receptor modulator	Fingolimod	Gilenya®	2010	Oral
Second-line therapies				
Monoclonal antibodies	Natalizumab	Tysabri®	2004	Intravenous
	Alemtuzumab	Lemtrada®	2014	
	Ocrelizumab	Ocrevus®	2017	
Immunosuppressant	Mitoxantrone	Novantrone®	2000	Intravenous

1.2 Sphinghosine-1-phosphate receptors

The adaptive immune system consists of highly specialized cells and processes that protect the body from pathogens. Its proper functioning relies on the constant circulation of lymphocytes between lymphoid organs and other tissues of the body. Lymphocytes mature in primary lymphoid organs such as bone marrow and thymus. Following maturation, they are released into the circulation and travel via blood and lymphatic system to survey the body for cognate antigens (25). In the secondary lymphoid organs, e.g., lymph nodes, Peyer's patches, and spleen, naïve lymphocytes can be activated via interaction with antigen-presenting cells. Activated immune cells need to egress the secondary lymphoid organs. T cells travel to the target tissue and antibody-secreting B cells migrate to the bone marrow (25, 26). The circulation of lymphocytes between blood, lymphatic system, and non-lymphoid tissues is regulated by S1P (27-29).

S1P is a lysosphingolipid signaling molecule, abundantly synthesized and secreted by many cell types, including endothelial cells, red blood cells, and platelets, that is involved in the regulation of numerous fundamental biological processes (28, 30). The pleiotropic effects of S1P are mediated by a family of five G protein-coupled receptors namely $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ (27). The different S1P receptors are expressed in a wide variety of tissues, with each subtype exhibiting a different cell specificity (27). Physiological and pathophysiological processes that involve S1P receptors include angiogenesis, cell migration, hearing, vasodilatation and vasoconstriction, airway hyper-responsiveness, and immune cell trafficking (Figure 1) (30, 31). The concentration of S1P within the lymph node parenchyma is low while it is very high in the adjacent lymphatic circulation (32, 33). As lymphocytes are able to sense the concentration gradient of S1P and migrate towards areas of higher S1P concentration, lymphocyte egress from primary and secondary lymphoid organs is dependent on the $S1P_1$ receptor (28, 34, 35).
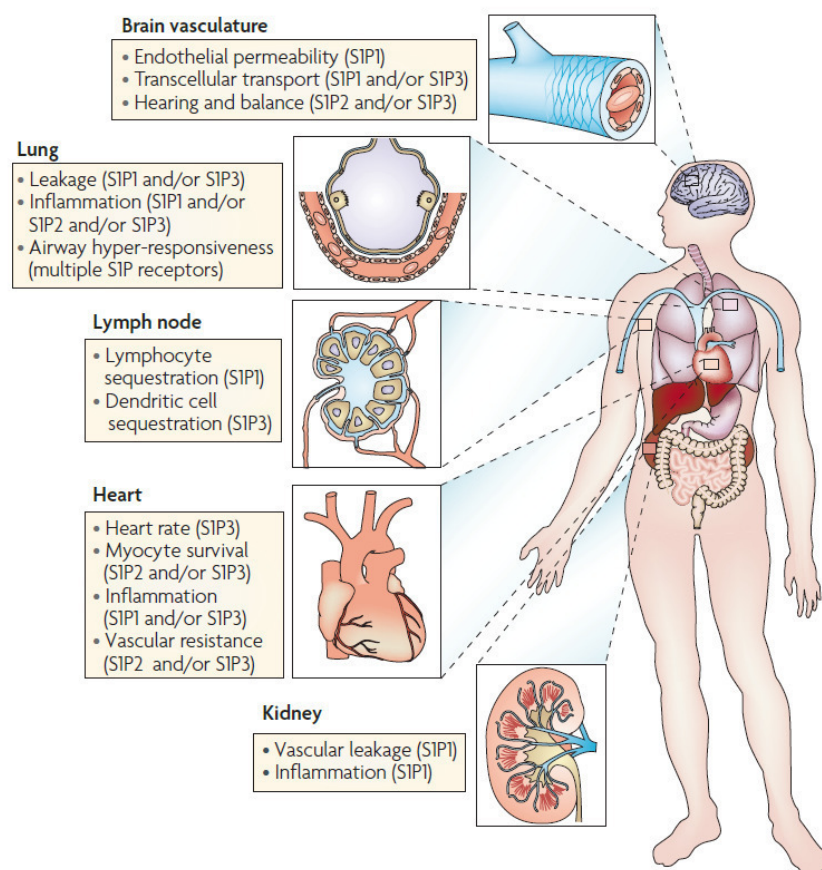
Figure 1 Expression of S1P receptors and their involvement in physiological and pathophysiological processes (reprinted with permission of Macmillan Publishers Ltd: [Nature Reviews Drug Discovery, https://www.nature.com/nrd] from article (31), copyright (2009)).

$S1P_1$ receptor modulators cause $S1P_1$ receptor internalization mediated via the endosomal pathway (36), reducing the number of $S1P_1$ receptors on the cell surface. As a consequence of the reduced number of functional $S1P_1$ receptors on the cell surface, lymphocytes lose their ability to detect the S1P concentration gradient and are unable to migrate out of lymphoid tissue into the lymphatic and vascular circulation (34, 35). This leads to low lymphocyte counts in peripheral blood and prevents recruitment of lymphocytes, e.g., autoreactive T cells, to the sites of inflammation (Figure 2) (24, 37, 38). Blockade of lymphocyte egress from lymphoid organs is reversible upon withdrawal of $S1P_1$ receptor modulators in that sequestered lymphocytes can return into the circulation. As immune cells are only prevented from reaching the target site, i.e., the site of inflammation, and not killed, this approach of immuno-modulation offers potential advantages over existing therapies currently used for the treatment of autoimmune disorders (39). In addition, T-cell mediated processes such as macrophage recruitment, tissue invasion, cytokine release, and killing of cells are suppressed while B-cell mediated generation of antibodies, functioning of neutrophils and monocytes as well as activation of T cells via antigens are not affected (40-43).
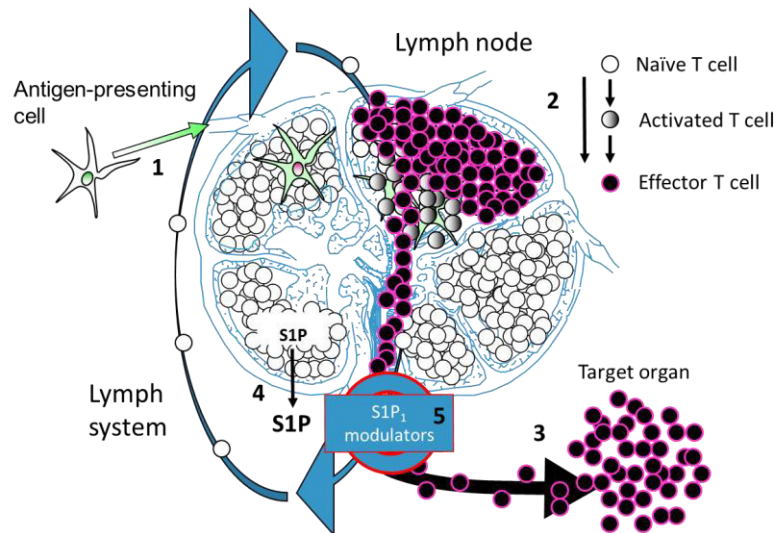
Figure 2 Mechanism of action of S1P receptor modulators: If antigen-representing cells reach the lymph node (1), they can activate T cells (2) which, in turn, enter the systemic circulation to travel to the target organ (3) where they can cause tissue damage. The egress of lymphocytes from the lymph nodes is regulated via the gradient of S1P (4). $S1P_1$ receptor modulators block the egress of activated T cells, i.e., effector T cells, and thus prevent them from reaching the target organ (5) (adapted from (23) with permission of https://www.tandfonline.com/)

Due to their ability to partially "shut down" the immune system, S1P receptor modulators are developed for the treatment of diseases with an autoimmune component in which lymphocytes play a critical role such as MS (21, 44, 45), psoriasis (46), and systemic lupus erythematosus (SLE) (47). Other target diseases include rheumatoid arthritis (RA) (48), ulcerative colitis (49), and graft-versus-host disease (GvHD) (50). Fingolimod, approved by the FDA in 2010 for the treatment of RRMS, was the first S1P receptor modulator on the market. Due to their mode of action, several S1P receptor modulators have been developed for the treatment of autoimmune diseases, including fingolimod (FTY-720), ponesimod (ACT-128800), ozanimod (RPC-1063), amilesimod (MT-1303), ceralifimod (ONO-4641), siponimod (BAF312), and cenerimod (ACT-334441). These mainly differ with respect to selectivity and affinity for the different S1P receptor subtypes (23). Fingolimod is a non-selective S1P receptor modulator as it targets all subtypes (except for $S1P_2$) with comparable affinity, while ponesimod is selective for the $S1P_1$ receptor, the subtype critically involved in immune cell trafficking.

1.3 Ponesimod

Ponesimod is an iminothiazolidinone derivate (Figure 3) that targets the $S1P_1$ receptor with high selectivity (22). The high selectivity and the favorable pharmacokinetic (PK) profile, i.e., rapid absorption (24), and the compatibility with a once-daily (o.d.) dosing regimen (51), supported its selection for clinical development. Administration of ponesimod results in substantial and rapid reversible reduction of peripheral blood lymphocyte count via sequestration of lymphocytes in lymphoid organs (24). It has been shown to be efficacious in the treatment of RRMS (45) and moderate-to-severe chronic plaque psoriasis (46), and has the potential to be used for the treatment of other diseases in which lymphocytes are critically involved such as SLE, RA, and GvHD.
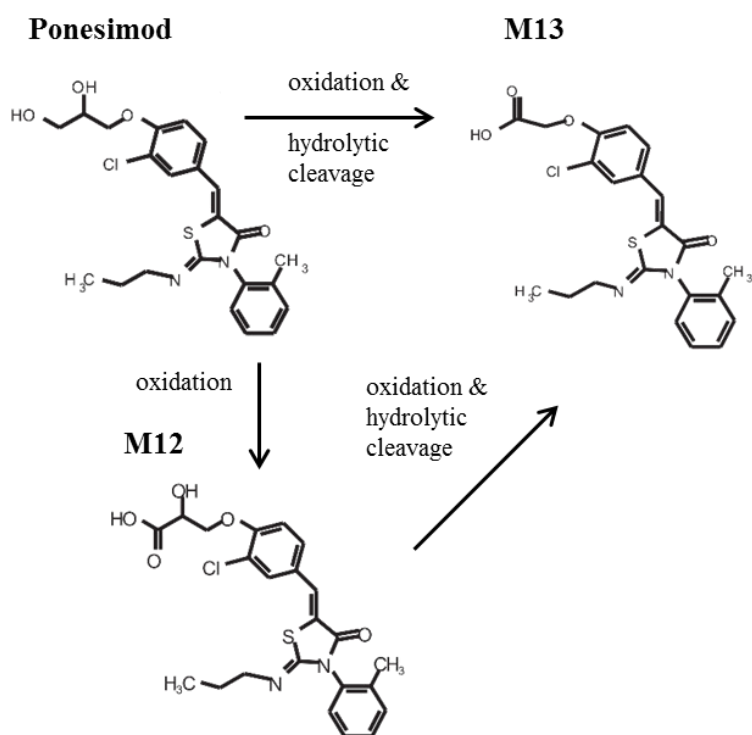
Figure 3 Chemical structures of ponesimod, M12, and M13 including the proposed metabolic scheme of ponesimod to M12 and M13 (reprinted with permission of Elsevier: [European Journal of Pharmaceutical Sciences, https://www.journals.elsevier.com/european-journal-of-pharmaceutical-sciences/] from article (52), copyright (2016)).

Ponesimod has been investigated in a large number of clinical studies assessing single- and multiple-dose safety, tolerability, PK, and pharmacodynamics (PD) in healthy subjects (24, 51, 53-61), patients with RRMS (45), and patients with moderate-to-severe chronic plaque psoriasis (46). Healthy subjects were treated with single doses of up to 75 mg (24) and multiple doses of up to 100 mg o.d. for 22 days (54, 55), while subjects with RRMS and psoriasis were treated with up to 40 mg o.d. for up to 4 years

and up to 28 weeks, respectively. Further studies with ponesimod included otherwise healthy subjects with mild-to-severe hepatic impairment and moderate-to-severe renal impairment (62). Currently, ponesimod is undergoing phase 3 clinical development in subjects with RRMS in 2 ongoing studies that are planned to include 1100 and 600 patients, respectively (63, 64).

The PK profile of ponesimod following oral administration is characterized by rapid absorption with a median time to reach peak plasma concentrations of 2-5 h (24, 51, 54, 56, 62, 65), high absolute bioavailability of 83.8% (53), low variability between subjects (24), and a terminal half-life of about 32 h (24, 51). The accumulation at steady state, reached within 5 days following repeated dosing, is approximately 2.3-fold (51). During its clinical development, ponesimod was investigated as different formulations (56). Ponesimod is extensively metabolized but the enzymes involved in its metabolism are not well characterized. Its two primary metabolites *in vivo*, M12 and M13, correspond to 8.1% and 25.7% of the total drug-related exposure and are pharmacologically inactive (60). M12 is formed of ponesimod via oxidation. M13 can be formed via oxidation and hydrolytic cleavage either of ponesimod directly or of M12 (Figure 3). Experiments in human liver microsomes and hepatocytes do not suggest the involvement of cytochrome P450 enzymes in the formation of M12 and M13. Fecal excretion was found to be the major route of elimination of ponesimod while urinary excretion was minor (60).

The PD effects of ponesimod include total lymphocyte count reduction, a transient decrease in heart rate following treatment initiation, and a reduction in pulmonary function, i.e., a decreased forced expiratory volume in 1 second (FEV_1) (24, 41, 45, 51, 54). While lymphocyte count reduction is the desired pharmacological effect, reductions in heart rate and FEV_1 are undesired side effects. The effect on FEV_1 is only marginal and considered not clinically relevant at therapeutic doses. Heart rate reduction, however, already starts following the administration of relatively low doses of ponesimod (24). This effect is transient, dose-dependent, and primarily occurs upon treatment initiation (51, 57, 61). Heart rate effects were also reported for fingolimod (21, 37, 38) and other S1P receptor modulators (66, 67) and suggested to be a class effect mediated via $S1P_1$ and $S1P_3$ receptors linked to G protein-coupled inwardly-rectifying potassium channels (GIRKs) (68, 69). With repeated dosing, heart rate reduction disappears due to development of tolerance associated with desensitization of the S1P receptor system via receptor internalization (23, 70, 71).

A particular challenge in the clinical development of ponesimod was to balance its desired effect, i.e., lymphocyte reduction, with its undesired effect, i.e., heart rate reduction. Such optimization of the benefit-risk ratio requires a proper understanding of the PK and PD properties as well as their relationship and should not only include the average subject but also "extremes", i.e., individuals that

respond very differently compared to the average population. Thus, quantification of variability in PK and PD and understanding of its sources are of great importance. Pharmacometric approaches allow to describe and link PK and PD as well as to understand and quantify the variability between individuals. Therefore, they offered a valuable tool to foster the clinical development of ponesimod to further increase the understanding of its PK and PD, and their relationship.

1.4   Pharmacometrics and its role in drug development

Pharmacometrics, also often referred to as modeling and simulation, is the science of quantitative pharmacology (72). It is more formally defined as "the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug's pharmacokinetic and pharmacodynamic behavior" (73). One key element of pharmacometric approaches is the quantitative description of a drug's dose-concentration-response relationship. This relationship is a fundamental component in clinical pharmacology as it determines how frequent and at which dose a treatment needs to be administered (74). Its importance was already realized by Paracelsus who wrote "Poison is in everything … the dosage makes it either a poison or a remedy" (75). Using pharmacometric approaches, the relationship between dose and concentration over time is described by PK models, while PD models describe the relationship between concentration and response. Hereby, response can be the desired clinical effect, undesired adverse ("side") effects, or the change in a biomarker indicating a change in underlying biological processes.

Pharmacometric models most commonly involve mathematical formulas for the characterization of processes such as absorption, distribution, metabolism, and excretion. These formulas contain parameters that are to be estimated during the modeling step based on the available data (76). This estimation is empirical and data driven (77). Data from *in vitro* experiments and preclinical and clinical studies can be included. Once parameters are estimated, they can be used to predict future outcomes such as the drug effect following administration of a dose that has not been clinically tested (76). This simulation step often helps to select the doses or dosing regimens for future studies. Once more data has been generated, e.g., due to the conduct of further studies, these can be integrated and parameters re-estimated.

The administration of the same drug to different individuals usually results in different exposures and responses. It is an important component of clinical studies to identify and quantify this so-called inter-individual variability (IIV) which can be associated with a subject's physiological characteristics such as body weight, height, age, sex, etc. (78). Population modeling, first introduced by Sheiner *et al.* 1972 (79), enables to identify and describe the relationship between subject-specific characteristics (in

modeling most commonly called covariates) and observed drug exposure or response. Application of population PK modeling is a milestone in the evolution of modeling and simulation as controlling variability in drug exposure is important to improve a drug's safety and efficacy (78).

Over the past 40 years, the field of modeling and simulation rapidly evolved due to advances in computer hardware and software, improved analytical methods, and an increased interest from pharmaceutical industry, academia, and regulatory bodies (80). Today, modeling and simulation is used from preclinical to late-stage clinical development (77, 78, 81) to create a better and more rapid understanding of a drug's safety and efficacy to develop new therapies more efficiently with regard to time and costs (77) (Figure 4). However, the application of modeling and simulation is not limited to drug development but can also be used for dose individualization (personalized medicine), therapeutic drug monitoring (TDM), and determining the dose to be used in special populations, e.g., in pediatrics (75). Since 2000, the integration of pharmacometric analyses in submissions to the US Food and Drug Administration (FDA) dramatically increased and was shown to influence drug approval and labeling (82). Due to its potential to positively influence drug development, the FDA strongly recommends the use of pharmacometric analyses (83) and provides guidance on how to conduct these (84). Similar documents were published by the European Medicines Agency (EMA) (85) underlining the importance of pharmacometric analyses.
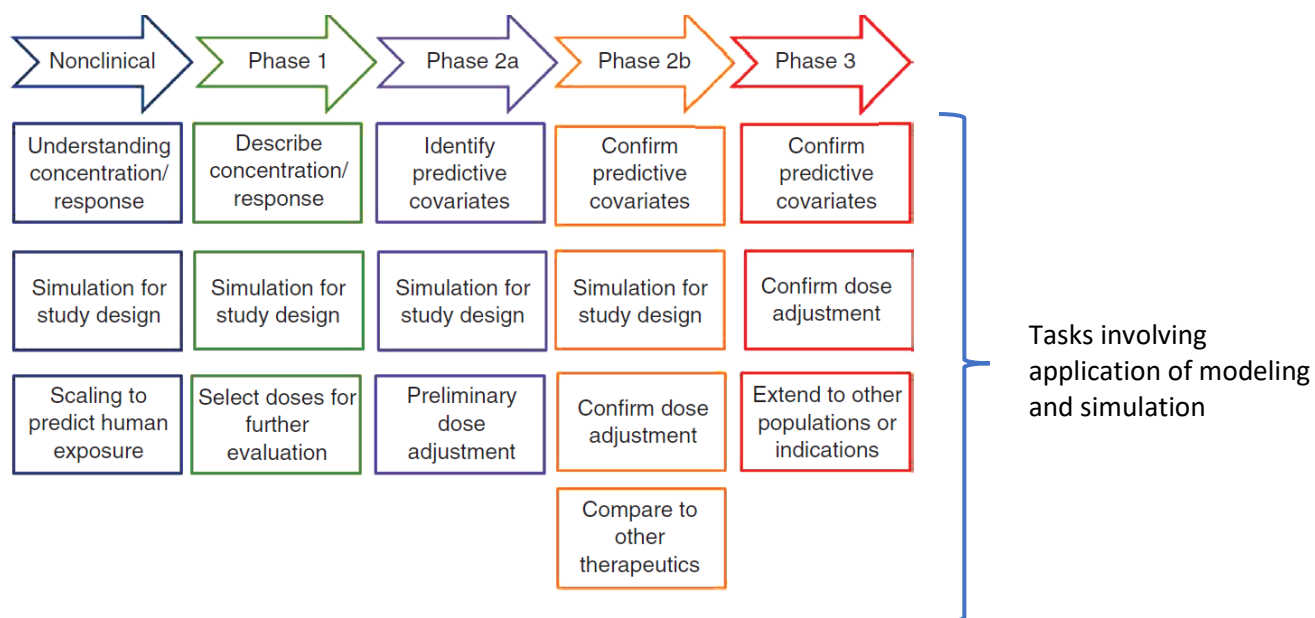

Figure 4 Application of modeling and simulation during drug development (reprinted with permission of Wiley: [CPT: Pharmacometrics and Systems Pharmacology, http://ascpt.onlinelibrary.wiley.com/] from article (78), copyright (2012)).

2. Objectives of the thesis

The overall aim of this thesis was to support the clinical development of ponesimod to ultimately provide patients a safe and efficacious treatment. The questions that were to be addressed as projects of this thesis are presented in the following. Each of the projects was published in a peer-reviewed scientific journal.

Project I

The results of a study in subjects with different levels of hepatic impairment showed that ponesimod, M12, and M13 concentrations are increased depending on the severity of impairment. As this study only included ponesimod administration as single dose, the accumulation of the three analytes following repeated dosing, which might be important for safety evaluations, was unknown. It was the aim of this project to develop a population PK model that characterizes the PK of ponesimod, M12, and M13 including IIV. In addition, the influence of covariates, in particular the different levels of organ impairment, were to be assessed. The established model was to be used to simulate dosing scenarios not clinically tested, i.e., multiple-dose administration in organ-impaired subjects, to evaluate the need for dose adaptation.

Project II

Due to the dose-dependent PD effects of ponesimod, proper characterization and understanding of its PK properties and their relation to the dose administered were of major importance. In addition, potential differences in PK between individuals, caused by subject-specific characteristics (covariates) such as demographic variables or disease, need to be evaluated and quantified. The objectives of this project were to develop a comprehensive population PK model including data from 13 clinical studies, to characterize the concentration-time profile of ponesimod including IIV. In addition, the effect of key demographic variables and disease on the PK of ponesimod and their contributions to IIV were to be assessed. The results of this analysis were used to evaluate the clinical relevance of the covariates.

Project III

Reduction of circulating lymphocytes is thought to be key in the treatment of autoimmune disorders with ponesimod. Therefore, proper understanding of this PD effect and its link to the concentration-time profile, and in turn, the dose of ponesimod, e.g., which dose is required to induce the desired lymphocyte reduction, is important. As the involvement of specific lymphocyte subsets varies between different autoimmune diseases, exploring the relationship between PK and PD on the level

of lymphocyte subsets contributes to better understand the drug's potential for the treatment of different autoimmune diseases. This project aimed at developing a PK/PD model that describes the effect of ponesimod on total lymphocyte counts, B cells, T helper cells, T cytotoxic cells, and natural killer (NK) cells. The maximum possible reduction and the variability associated with the effect of ponesimod on different lymphocyte subsets were to be determined.

Project IV

Ponesimod administration dose-dependently reduces heart rate upon treatment initiation. Following repeated dosing, this effect disappears due to tolerance development enabling the usage of up-titration regimens to mitigate pronounced first-dose effects. Different up-titration regimens were investigated during the clinical development of ponesimod. However, clinical studies are expensive and the number of different possible up-titration scenarios innumerable. A PK/PD model, however, enables *in silico* investigation of all thinkable regimens. In addition, such models allow quantification of variability between individuals associated with the effect of ponesimod on heart rate. Proper description of this variability is of particular importance as treatment optimization should not only be tailored to the average patient but also include "extremes", e.g., patients with very low or high baseline heart rate values. Thus, the objective of this analysis was to develop a population PK/PD model that characterizes the effects of ponesimod on heart rate including development of tolerance and identification of covariates that influence these. The model was to be used to simulate and compare various up-titration regimens with respect to occurrence of very low heart rate values, i.e., bradycardia (heart rate < 40 beats per minute [bpm]). The regimen that includes up-titration of ponesimod to the target dose of 20 mg o.d. with the least pronounced heart rate effects was to be identified.

3. Methods

3.1 Population modeling - background

As outlined in Section 1.4, being able to describe and understand a drug's dose-concentration-response relationship is important to ensure the administration of the right drug, at the right dose, to the right patient, at the right time via the right route, known as the 5 R's of medication management (74). Pharmacometric approaches are widely used to address this question by using PK/PD models, which, in a broad sense, are a simplified representation of a system, e.g., the body (78). These approaches comprise individual subject models and population models. Theoretically, each individual's PK and PD data can be analyzed separately. However, such analyses rarely allow to make statements about the variability between individuals which is important to provide a safe and efficacious treatment to all patients. Here, population modeling aiming at describing PK and/or PD for a group of individuals rather than for a single individual comes into place (78, 86).

Historically, methods such as the "naïve pooled approach" and the "two-stage approach" were used to analyze the data of a population. While naïve pooling ignores differences between individuals by assuming that all data arise from the same subject, the two-stage approach, during which individual parameters are first estimated and then summarized, can lead to biased results, e.g., due to missing samples or poor compliance (78, 86). Although these methods, in a strict sense, represent population approaches, the terms "population approach" or "population model" most commonly refer to nonlinear mixed-effects (NLME) modeling, developed by Sheiner *et al.* (79). The NLME modeling approach allows to simultaneously estimate population mean parameters, IIV, inter-occasion variability (IOV), and covariate effects that explain and quantify variability between subjects to a certain extent (78). The advantage of NLME modeling is that it allows pooling of data from different studies with different properties (doses, number of observations, treatment durations, etc.). Thus, studies in which as few as one sample was taken per subject ("sparse data") (87), studies with many samples per individual ("rich data"), and studies that include both, sparse and rich sampling, can be analyzed jointly (88). Pooling of different studies enlarges the variety of subject characteristics that can be included as covariate effects into the model and therefore increases the likelihood to identify sources of variability in a drug's PK and/or PD. As NLME modeling techniques were the pharmacometric approach used for all analyses included in this thesis, this technique will be explained in more detail in the following section.

3.2 Nonlinear mixed-effects modeling

NLME models, from now on synonymously used with "population models", consist of several components: the structural model, the statistical model, and the covariate model (88). The structural model ("fixed effects") describes the time course of a measured response for the mean population with the use of algebraic or differential equations. The statistical model accounting for and quantifying unknown variability in the response parameter can be differentiated into "random effects", i.e., IIV and IOV, and residual variability (RV). Covariate models aim at explaining IIV by subject-specific characteristics such as demographic variables or disease characteristics. The simultaneous estimation of fixed and random effects and the nonlinear relationship between the different components of the model gave the approach the name NLME modeling (88). A typical population PK model can be written as:

$$Y = f(x; \Theta, \Omega, K, \Sigma, z) \tag{3.1}$$

The observations Y are described as a function of the vectors x, Θ, and z and the matrices Ω, K, and Σ. The design parameters such as dose (amount and times of administration) and time are comprised in x, the parameters of the structural model (population-typical parameters) in Θ, and the parameters describing the covariates in z. The components of the statistical model, i.e., IIV , IOV, and RV are represented by Ω, K, and Σ, respectively. The single components of population models are illustrated in Figure 5 and explained in more detail in the following.
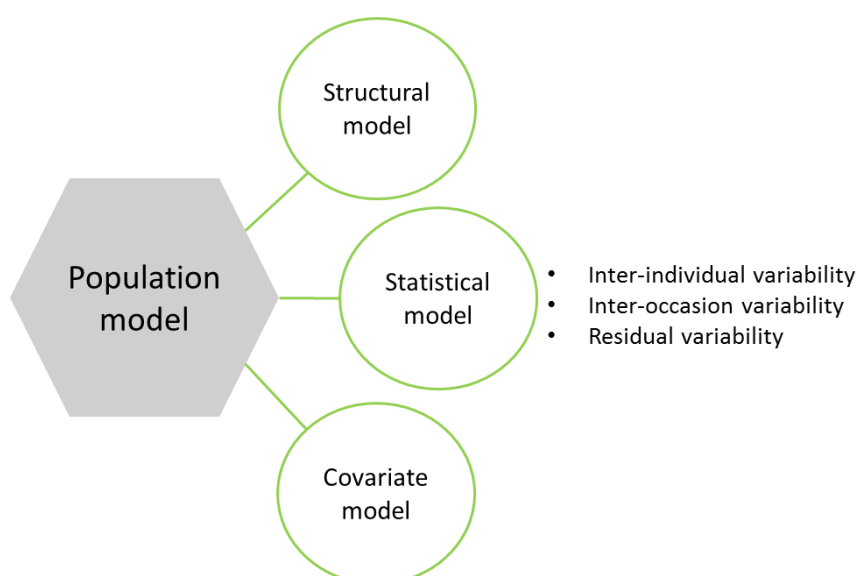

Figure 5 Components of nonlinear mixed-effects models.

3.2.1 Structural model

The structural model aims at describing the central tendency of the observed variable with the use of mathematical equations. These can either be algebraic or differential equations (78, 88). The algebraic expression describing the concentration following a single intravenous injection with a 1-compartment model is shown in Equation 3.2:

$$C(t) = \frac{\text{Dose}}{V} \cdot e^{-\frac{CL}{V} \cdot t} \qquad (3.2)$$

This model describes the relationship between the dependent variable, concentration (C), and the independent variable, time (t), with the parameters dose, clearance (CL), and (apparent) volume of distribution (V). If systems become more complex, they cannot be stated as algebraic equations but need to be described by using differential equations. A more general structural model can be defined as:

$$Y(t) = f(x; \Theta) \qquad (3.3)$$

where the observations Y are described as a function of the design parameters x (e.g., dose and time) and the parameters of the structural model Θ. The selection of the structural model is usually the first step in the development of a population model.

Different structural models such as 1-, 2-, and 3-compartment models with different absorption models can be investigated to describe a drug's PK. Using a 1-compartment model, the entire body is treated as one giant "bucket". This can be observed if drug concentrations in plasma and all tissues to which the drug is distributed rapidly and simultaneously reach equilibrium. Using 2 compartments, the central compartment usually represents the blood stream and organs that are well perfused while poorly perfused organs, e.g., fat tissue, are treated as the peripheral compartment (89).

PD models can range from rather simple E_{max} models to very complex (semi)-mechanistic models incorporating mechanistic aspects of a drug's PD response, e.g., circadian variation, receptor internalization, or tolerance development. Two basic PD models frequently used to establish PK/PD relationships are direct and indirect response models. Indirect models often need to be used as drug concentrations are measured in plasma, while the effect is often dependent on the drug concentration at the effect site, e.g., in tissue. This leads to a delay in response that needs to be accounted for. PD models can not only be developed for continuous but also for non-continuous response variables such as the occurrence of adverse events (the number of events or time to occurrence of the first event). The probability of such events can be described using time-to-event and logistic regression models (89).

3.2.2 Statistical model

The statistical model aims at describing and quantifying the variability in the population. The concept of variability is important for the development of safe and efficacious dosing, in particular for drugs with a narrow therapeutic window. For such drugs, high variability quickly leads to either toxic or subtherapeutic exposure. In addition, random effects allow to estimate an individual's parameters, e.g., the volume of distribution V for a particular individual. Different sources of variability are distinguished: IIV, IOV, and RV. While IIV refers to the difference between an individual's model parameter and the population-typical value, IOV reflects changes within the same individual. RV accounts for the difference between model-predicted (already accounted for IIV and IOV) and observed values. This difference is called residual error (72).

Inter-individual variability

PK model parameters are commonly modeled on a logarithmic scale as they are assumed to be lognormally distributed, i.e., log(x) follows a normal distribution such that exp(x) is strictly positive. This prevents the occurrence of negative values that are often physiologically implausible, e.g., a negative drug clearance. The individual model parameter θ_i for the ith individual is thus given as:

$$\theta_i = \theta_{pop} \cdot \exp(\eta_i) \tag{3.4}$$

with θ_{pop} being the population-typical parameter and η_i the individual deviation from the population-typical parameter for the ith individual. Across the population being evaluated, η_i is assumed to be normally distributed with mean 0 and variance ω^2 (72). If the random effects of different parameters are assumed to be uncorrelated, the variance terms of all IIV parameters represent the diagonal elements of the variance-covariance matrix Ω. Off-diagonal elements can be implemented if correlations between parameters are included into the model. In population analyses with lognormally distributed parameters, the variability is often reported as coefficient of variation (CV%) which can be calculated as:

$$CV\% = \sqrt{\exp(\omega)^2 - 1} \cdot 100 \tag{3.5}$$

In the analyses that are part of this thesis, IIV was *a priori* added on all model parameters and removed depending on the precision with which the parameter was estimated (Section 3.2.5) and the value (magnitude) of the parameter itself.

Inter-occasion variability

Model parameters for the same individual can change over time due to many factors, e.g., due to fluctuations in body weight or aging (in particular in children), or presence of an infection. Regular and

predictable changes can be accounted for by time-varying covariates. If the reasons for a change, e.g., in PK, within the same individual are unknown, this change in variability is suggested to reflect intra-subject variability (also called within-subject variability). If individuals were observed at different occasions, e.g., during different periods of a crossover study, this random variability can be accounted for and is referred to as IOV (72). If data were collected at O occasions, o = 1, 2, ... , O, the model can be written as:

$$\theta_i = \theta_{pop} \cdot \exp(\eta_i + \kappa_1 OCC_1 + \kappa_2 OCC_2 + \cdots + \kappa_O OCC_O) \qquad (3.6)$$

with κ_1 being the deviation from the population mean due to differences at occasion 1, κ_2 the difference due to occasion 2 etc. If the data were collected at the oth occasion, OCC_O is coded as 1 and 0 otherwise. κ_O is assumed to be normally distributed with mean 0 and variance $\pi_\kappa{}^2$ (72). If IOV is observed but not implemented in the model, this variability is reflected in the residual error term. In some cases, not accounting for IOV might lead to biased results (90).

Residual variability

All variability remaining after controlling for different sources of variability is lumped into RV (88). This remaining variability is the difference between individual model-predicted values and observed data and can originate from measurement variability, model misspecification, or inaccuracies in dosing history. Different models accounting for RV can be assessed. Most commonly either additive, proportional or combined error models are used. The residual error (ϵ) is assumed to be normally distributed with mean 0 and variance σ^2. The set of all residual error components builds the residual error matrix Σ. Additive error models assume that σ^2 is constant over the range of observed data while for proportional error models σ^2 increases with larger values (72). Combined error models comprise an additive and a proportional component. A commonly used strategy in selecting the residual error model is to start with a combined error model and to then further simplify it. For example, if one of the terms, either the additive or the proportional term, is close to 0, it can be removed from the model (i.e., set to 0). The model obtained following selection of the structural and statistical model is often referred to as "base model".

3.2.3   Covariate model

Identification of covariates that explain variability in the dependent variable represents one of the key elements of population analyses. Covariate analyses are most commonly performed following selection of the base model. Covariates are subject-specific characteristics that are either assumed to be continuous (e.g., body weight, height) or categorical (e.g., sex, race) and can be further classified into intrinsic and extrinsic factors (72). Intrinsic covariates such as age, height, sex, and race generally

do not change within a short period of time, whereas extrinsic covariates such as concomitant medications and compliance to study drug might change during the course of a clinical study.

In the analyses that are part of this thesis, covariates were included into the model as power relationships to restrict the range to positive values. Continuous variables were centered to a value close to the median of the respective covariate in the analyzed population. The individual volume of distribution V_i for the ith subject can be defined as:

$$V_i = V_{pop} \cdot \left(\frac{\text{body weight}_i}{\text{median(body weight)}}\right)^{\theta_{V,\text{body weight}}} \tag{3.7}$$

with V_{pop} being the population-typical body weight and $\theta_{V,\text{body weight}}$ the covariate parameter that characterizes the relationship between (apparent) volume of distribution and body weight. The effect of categorical covariates on a model parameter was implemented as difference to a reference group, typically the most frequent group. The volume of distribution for group female, V_{female}, with male as the reference group is given as:

$$V_{female} = V_{male} \cdot \exp^{\theta_{V,\text{female}}} \tag{3.8}$$

with V_{male} denoting the volume of distribution for the reference group male and $\theta_{V,\text{female}}$ describing the difference in typical volume of distribution between male and female.

The first step in a covariate analysis is most commonly the selection of so-called "candidate covariates" which are the covariates that are to be investigated for their relationship to specified model parameters. These are often selected graphically by plotting individual model parameter estimates using the base model against covariates (scatterplots for continuous variables and box-and-whisker plots for categorical variables). Furthermore, physiological plausibility, e.g., the association between volume of distribution and body weight, prior knowledge about the metabolism of the drug, and reports from literature can be used to select candidate covariates.

In a next step, the selected candidate covariates are statistically tested for their significance towards the specified model parameters. This is done in a univariate manner, i.e., one covariate on one model parameter at a time (univariate forward selection). All covariates that are found to be statistically significant (on a specified level, e.g., $p < 0.05$) are added to the model to form the "full covariate model". Subsequently, covariates are removed step by step (backward elimination) until all remaining terms are significant based on a more stringent statistical criterion (e.g., $p < 0.01$).

The information gathered during covariate analyses can be used to answer questions such as "does the exposure change with age?" or "can patients with low and high body weight be administered the

same dose?" Thus, the results can be used for dose adaptions based on identified covariates or to show that dose adaptations are not warranted.

3.2.4 Parameter estimation

Estimation of model parameters is a central element of the modeling endeavor. The aim of this process is to find, for a given model, the set of parameters (Θ, Ω, K, Σ) that best describes the observed data on the population and the individual level. To obtain the "best parameters", the maximum likelihood approach is commonly used. The likelihood reflects the probability that for a given set of parameters the observed data might arise from the specified model. Minus twice the logarithm of the likelihood (-2LL) is a metric that indicates how well model-predicted and observed data correspond (for a given set of estimated parameters). In software packages such as NONMEM, the objective function value (OFV), which is proportional to -2LL, is often used (72). Thereby, the lowest OFV corresponds to the maximum likelihood (lowest -2LL) and indicates the "best fit" (78, 88). To maximize the likelihood, equivalent to minimizing -2LL, model parameters are iteratively changed until the set of "best parameters" is identified. In the following, OFV and -2LL are used synonymously.

Different mathematical algorithms can be used to find this set of parameters. For the projects included in this thesis, the stochastic approximation of expectation maximization (SAEM) algorithm implemented in the software package Monolix (91) was used. This algorithm is a stochastic implementation of the expectation-maximization (EM) algorithm (92) that consists of two steps, the E-step (expectation) and the M-step (maximization). During the E-step, the expected value of the likelihood, given the observed data and a set of parameters, is determined. The M-step "updates" the parameter estimates to maximize the likelihood. E- and M-step are alternatingly repeated until convergence is reached. In the SAEM algorithm, the E-step is replaced by a stochastic approximation of the non-observed individual model parameters. Monolix combines the SAEM with a Markov Chain Monte Carlo procedure (93, 94) that allows for rapid convergence towards the solution by generating multiple random samples per individual and iteration during the E-step. A particular advantage is that it generally identifies the global optimum (the minimum of -2LL).

Other methods frequently used for maximum likelihood estimation are the first-order (FO) approximation and the first-order conditional estimation (FOCE). Both of these methods implemented in the software package NONMEM use a Taylor series approximation (72) to obtain the maximum likelihood estimates. While FO linearizes the likelihood based on the population parameters and assesses the individual parameters *a posteriori* after minimization, FOCE linearizes the likelihood for each individual at the individual's maximum likelihood parameter estimates. Thus, FOCE is generally preferable but more time consuming compared to FO (95). To account for correlation between IIV and

RV, FOCE can be used with an interaction option (FOCE-I). Results based on FO, FOCE, and FOCE-I can depend on the selection of the starting values.

For the work presented here, the SAEM algorithm implemented in Monolix version 4.3.1 (and higher) was used due to its generally rapid convergence and its insensitivity to the choice of initial parameters (93, 94).

3.2.5 Model evaluation

During model development, models are evaluated and compared regarding their ability to describe the observed data, aiming at selection of the model with the most suitable properties. Most commonly, such evaluation is based on statistical criteria such as the OFV and graphical analyses using diagnostic plots. In addition, the purpose of the model needs to be taken into consideration. This is of particular importance as, due to the fact that models are a simplified representation of a system, essentially, each model is "wrong" (96). However, it needs to be "useful" for the purpose it was built for.

Objective function value

The OFV (Section 3.2.4) is generally used as statistical criterion for model comparison. As the OFV is approximately chi-square (χ^2)-distributed (72), a decrease of > 3.84 for one additional parameter (1 degree of freedom) is referred to as statistically significant on a significance level of $p < 0.05$. However, this so-called likelihood ratio test can only be used if the models are nested, i.e., if the simpler model is obtained by setting a particular parameter of the more complex model to a fixed value. To compare non-nested models, the Akaike information criterion (AIC) (88, 97) can be used. This measure accounts directly for the number of parameters by balancing the better model fit against the higher complexity of the model (larger number of parameters).

Precision of parameter estimates

The precision of the parameter estimates can be evaluated based on the relative standard error (RSE%) of the estimates. The RSE% can be calculated from the Fisher information matrix which itself is derived from the maximum likelihood estimates (the set of parameters for which -2LL is minimized). Low RSE% values indicate high precision and high RSE% values low precision. The latter is often associated with model over-parameterization (72, 88).

Alternatively, the non-parametric bootstrap can be used to estimate standard errors. In this method, replicates (e.g., 500) of the original data set are created by randomly sampling the same number of individuals from this data set with replacement. Model parameters are estimated for each of the new

data sets and the obtained bootstrap estimates are summarized with their standard deviation which, in turn, is used to estimate the standard error of the parameter estimates (72).

For the analyses presented in this thesis, models that included RSE values of > 40% (for population parameters) were not considered acceptable.

Shrinkage

Shrinkage is often observed with sparse data when the information available does not suffice to estimate individual model parameters. In this case, the variance of the individual model parameters shrinks towards 0 and, in turn, the individual parameters tend towards the population-typical parameters. For example, individual absorption can shrink towards the population-typical parameter if only limited data were collected during the absorption phase. This phenomenon, also referred to as η-shrinkage, needs to be considered when evaluating model diagnostics that are based on individual predictions. Shrinkage is assessed for each parameter individually. The rule of thumb threshold for shrinkage is that 20-30% should not be exceeded (98, 99).

Goodness-of-fit plots

Goodness-of-fit (GOF) plots are graphical analyses routinely performed during the development of a population model to compare model-predicted to observed values. The aim of these plots is to check for potential misspecifications in the model, e.g., if low or high values are systematically over- or under-predicted or variability is large for particular subsets of the data. The following GOF plots were routinely generated and evaluated:

- Observations versus population predictions
- Observations versus individual predictions
- Population- and individual-weighted residuals versus time or time after (first) dose
- Population- and individual-weighted residuals versus population and individual predictions

Population predictions are model predictions based on the population-typical parameters, whereas individual predictions additionally account for IIV. Plots comparing observations and model predictions are considered adequate if the data show random and uniform scattering around the line of identity, the diagonal (y=x). Residual plots should show the same scattering of data points around y=0.

Visual-predictive checks

Visual predictive checks (VPCs) are used to graphically evaluate the predictive performance of a model with respect to central tendency and variability (100, 101). The final model is used to simulate a large

number of data sets based on the "design" of the original data set. The simulated data are then visually compared to the observed data by assessing if the 10th, 50th, and 90th percentiles of the observed data are within the 80% prediction interval of the quantile (typically shown as shaded areas). The standard VPC can be extended to the prediction-corrected VPC (pcVPC) that corrects for different study designs and a wide range of covariates in the original data set by normalizing the observed and simulated data using the population predictions (102, 103).

3.2.6 Simulations

Simulations are important to evaluate the performance of pharmacometric models (i.e., simulations should be "similar" to the data). Furthermore, model simulations can be used to simulate doses and dosing regimens that were not clinically tested. Simulations can include doses that lie within the bounds of the original data (interpolation) and doses that are outside these bounds (extrapolation). Extrapolation requires a good understanding of the model properties and its limitations (78). Simulations enable to quickly answer "what if" questions and can be used for dose individualization and extrapolation to vulnerable populations in which clinical studies are ethically questionable, e.g., neonates.

Deterministic simulations are used to simulate population-typical profiles without accounting for variability. They are often used to simulate covariate effects and to explore the model properties.

In contrast, stochastic simulations account for IIV and, if included, for IOV and RV. Sets of individual model parameters are randomly sampled considering the population-typical parameters and the distribution of the random effects. Due to the inclusion of variability, the range of expected outcomes can be assessed. This is of particular importance when evaluating safety and efficacy of a drug. Thus, the percentage of subjects that experience a specific adverse event can be predicted for a given dosing regimen and, if required, the regimen can be modified.

3.2.7 Data set programming

Programming of the modeling data set is very important for the subsequent modeling analysis. Often, information about dosing, PK, PD, and demographics are stored in different source data sets that need to be combined into one modeling data set which needs to fulfil certain requirements. This task needs to be done carefully as inaccurate data might negatively influence the model building process and in fact result in wrong input data and thus wrong results. The modeling data sets used for the analyses included in this thesis were programmed using the software package R version 3.0.2 (and higher) (104).

4. Results

4.1 Publication I: Population pharmacokinetics of ponesimod and its primary metabolites in healthy and organ-impaired subjects (doi: 10.1016/j.ejps.2016.04.021)

4.2 Publication II: Impact of demographics, organ impairment, disease, formulation, and food on the pharmacokinetics of the selective $S1P_1$ receptor modulator ponesimod based on 13 clinical studies

(doi: 10.1007/s40262-016-0446-8)

4.3 Publication III: Modeling the effect of the selective S1P$_1$ receptor modulator ponesimod on subsets of blood lymphocytes (doi: 10.1007/s11095-016-2087-x)

4.4 Publication IV: Modeling tolerance development for the effect on heart rate of the selective S1P$_1$ receptor modulator ponesimod (doi: 10.1002/cpt.877)

5. Conclusions

Ponesimod was shown to be an efficacious therapeutic agent for the treatment of RRMS and is currently undergoing phase 3 clinical development. Results are expected in 2019. Due to its cardiac effects, optimization of the benefit-risk ratio, i.e., maximizing total lymphocyte count reduction while keeping the incidence of undesired heart rate effects low, was one of the key elements in its clinical development. The analysis of individual studies frequently does not allow to describe a drug's PK and PD properties in a quantitative manner. Pharmacometric modeling and simulation, however, allow pooling of multiple studies which enables the conduct of more robust analyses including identification and quantification of sources of variability. Furthermore, PK/PD models enable to study *in silico* dosing scenarios that were not clinically tested allowing to rapidly answer "what if" questions which in turn saves time and resources. Due to these advantages modeling and simulation were extensively used throughout the clinical development of ponesimod.

A population PK model describing the PK of ponesimod and its primary metabolites M12 and M13 including covariates was successfully developed in project I. Hepatic impairment was found to significantly influence the elimination of ponesimod, M12, and M13 as well as metabolite formation. Renal function was not identified as statistically significant covariate and thus dose adaptation is not indicated in case of real impairment. The model was used to simulate steady-state concentration-time profiles of ponesimod, M12, and M13 following repeated dosing to predict the steady-state exposure to the three analytes in subjects with different levels of hepatic impairment. Subjects with severe hepatic impairment were predicted to have an approximately 3-, 9-, and 3-fold higher exposure to ponesimod, M12, and M13, respectively, compared to healthy subjects. The model enabled predicting drug accumulation following repeated dosing and thus provides a useful tool for safety evaluations and potential dose adaptations in subjects with hepatic impairment.

The influence of subject-specific characteristics on the PK of ponesimod was further investigated in project II in which more than 13700 concentration measurement from 680 subjects were pooled from 13 clinical studies to enable the conduct of an extensive covariate analysis. The PK model developed was shown to accurately predict the concentration-time data of ponesimod including IIV and the effect of identified covariates that largely explained IIV. The model was used to visualize the effect of the identified covariates and to compare it to the magnitude of the remaining IIV. Moderate and severe hepatic impairment were the only variables that influenced the PK of ponesimod beyond the IIV. Thus, it can be concluded that other covariates identified as statistically significant such as body weight, race, age, sex, drug formulation, etc., do not affect the PK of ponesimod to a clinically relevant extent

and, in turn, do not require dose adaptation. Dose adaptation scenarios for subjects with severe hepatic impairment were successfully simulated using the model. A key strength of the model is its solid source data from 13 clinical studies including 680 individuals and more than 13700 concentration measurements that reflect the PK information collected for ponesimod over a decade.

The population PK model developed in project II was used to establish the relationship between ponesimod concentration and lymphocyte count reduction in project III. Indirect response I_{max} models were shown to accurately describe the effect of ponesimod on total lymphocyte count and lymphocyte subsets such as B cells, T helper cells, T cytotoxic cells, and NK cells. Model-based simulations showed that these lymphocyte subsets respond differently to ponesimod treatment with B cells and T helper cells being more responsive compared to T cytotoxic cells and total lymphocyte count. In addition, the response of NK cells to ponesimod treatment was shown to be highly variable between individuals, while the effect of ponesimod on other lymphocytes such as B cells was less variable. These first population PK/PD models developed for S1P receptor modulators on the level of lymphocyte subsets offer a valuable tool for the interpretation and analysis of upcoming results from ongoing clinical studies. In addition, dosing scenarios not clinically tested can be simulated and used to support the planning of future studies.

A population PK/PD model that linked ponesimod concentration to its effects on heart rate was developed in project IV. The results of the population PK model built in project II were used as basis to establish the PK/PD relationship. A model with circadian rhythm, tolerance compartment, and drug effect implemented as I_{max} relationship with I_{max} decreasing with increasing tolerance was shown to accurately describe the effect of ponesimod on heart rate. Model-based simulations showed that the first-dose effect of ponesimod on heart rate increased with dose and reached a plateau at a dose of about 80 mg. Repeated dosing resulted in less prominent heart rate decreases as result of tolerance development. Tolerance maintenance upon treatment interruption allows for treatment continuation after several days of drug holiday without pronounced heart rate effects. The model was used to simulate and compare different up-titration regimens with respect to the occurrence of bradycardia (heart rate < 40 bpm). Slow gradual up-titration to the target dose of 20 mg o.d. was found to mitigate pronounced first-dose effects on heart rate and is considered favorable compared to regimens with high initial doses. No covariate was found to significantly influence the effect of ponesimod on heart rate indicating no evidence for the need of dose adaptation based on subject-specific characteristics.

This work describes the first population PK/PD model characterizing heart rate effects of S1P receptor modulators on the basis of human data. The model can be used to study *in silico* various up-titration regimens that were not clinically tested.

In summary, the analyses conducted during the course of this thesis demonstrate the successful integration of pharmacometric modeling and simulation in the clinical development of the selective $S1P_1$ receptor modulator ponesimod. The dose-concentration-response relationship of ponesimod was described and sources of variability quantified using pharmacometric approaches, i.e., population modeling. The models were developed based on pooled data from up to 13 clinical studies including single-dose, multiple-dose, and up-titration studies with various doses and dosing regimens. Data from healthy subjects, subjects with mild, moderate, and severe hepatic impairment as well as moderate and severe renal impairment, and data from psoriasis and MS patients were included. Overall, > 13700 ponesimod concentration, > 1300 lymphocyte (including subsets), and > 42500 heart rate measurements, collected during more than 10 years of clinical research, were included. The resulting models were used to optimize the benefit-risk ratio in the treatment with ponesimod. Furthermore, the results of these analyses can be used to warrant the need for dose adaptation based on demographic variables and to discuss the need for cardiac monitoring. All these aspects were important during the clinical development of ponesimod and will be of value when ponesimod is submitted for approval.

6. Summary

Ponesimod is a drug that is currently undergoing phase 3 clinical development for the treatment of relapsing-remitting multiple sclerosis. Reduction of circulating lymphocytes, the desired effect, needs to be balanced with an undesired decrease in heart rate upon treatment initiation. Pharmacometric modeling and simulation were used to describe the pharmacokinetics (PK) and pharmacodynamic (PD) of ponesimod and their relationship including identification and quantification of sources of variability.

A population PK model describing the concentration-time profile of ponesimod including inter-individual variability and the influence of covariates was developed based on pooled data from 13 clinical studies. The only covariate found to influence the PK of ponesimod to a clinically relevant extent was hepatic impairment. The model was demonstrated valuable to develop dose adaptation scenarios.

Simulations based on a PK/PD model describing the effect of ponesimod on lymphocytes including subsets showed that the effect of ponesimod varies for different lymphocyte subsets regarding both, the absolute effect and the associated variability. A PK/PD model describing the effect of ponesimod on heart rate was proven useful to compare and optimize dosing regimens regarding the occurrence of bradycardia. The models can be used to study *in silico* dosing regimens that were not clinically tested and provide a robust basis to discuss the need for dose adaptation and cardiac monitoring.

7. Zusammenfassung

Ponesimod ist ein Arzneistoff, der sich in Phase 3 der klinischen Entwicklung zur Behandlung von schubförmig-remittierender Multiplen Sklerose befindet. Die erwünschte Reduktion zirkulierender Lymphozyten muss gegen eine unerwünschte Senkung der Herzfrequenz, die zu Behandlungsbeginn auftritt, abgewogen werden. In dieser Arbeit wurden mathematische Modelle entwickelt, um diese Effekte zu beschreiben und deren Einflussfaktoren zu identifizieren und zu quantifizieren.

Ein Modell, das den Konzentrations-Zeit Verlauf von Ponesimod inklusive Variabilität und Einflüssen von Kovariaten beschreibt, hat gezeigt, dass Leberinsuffizienz die einzige Kovariate ist, welche die Pharmakokinetik von Ponesimod in klinisch relevantem Ausmaß beeinflusst. Modellbasierte Simulationen erwiesen sich hier als nützlich um eine Dosisanpassung durchzuführen.

Simulationen basierend auf einem Modell, das den Effekt von Ponesimod auf Lymphozyten beschreibt, haben gezeigt, dass sich der maximale Effekt und die Variabilität des Effektes zwischen verschiedenen Lymphozyten Untergruppen unterscheidet. Ein Modell, das den Effekt von Ponesimod auf die Herzfrequenz charakterisiert, wurde verwendet um verschiedene Dosierungsschemata zu vergleichen und bezüglich des Auftretens von Bradykardie zu optimieren. Diese Modelle können dazu verwendet werden verschiedene Dosierungsschemata mittels Simulationen zu testen und um die Notwendigkeit von Dosisanpassungen und Überwachung der Herzfrequenz zu Behandlungsbeginn zu diskutieren.

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9. Supplementary material

9.1 Supplementary material for publication I: Population pharmacokinetics of ponesimod and its primary metabolites in healthy and organ-impaired subjects

Figure S1 Residual diagnostics ponesimod: Individual-weighted residuals (IWRES, left column) and normalized prediction distribution errors (NPDE, right column) versus model-predicted concentrations (top and middle row), density estimate (bottom row). Colors indicate studies.
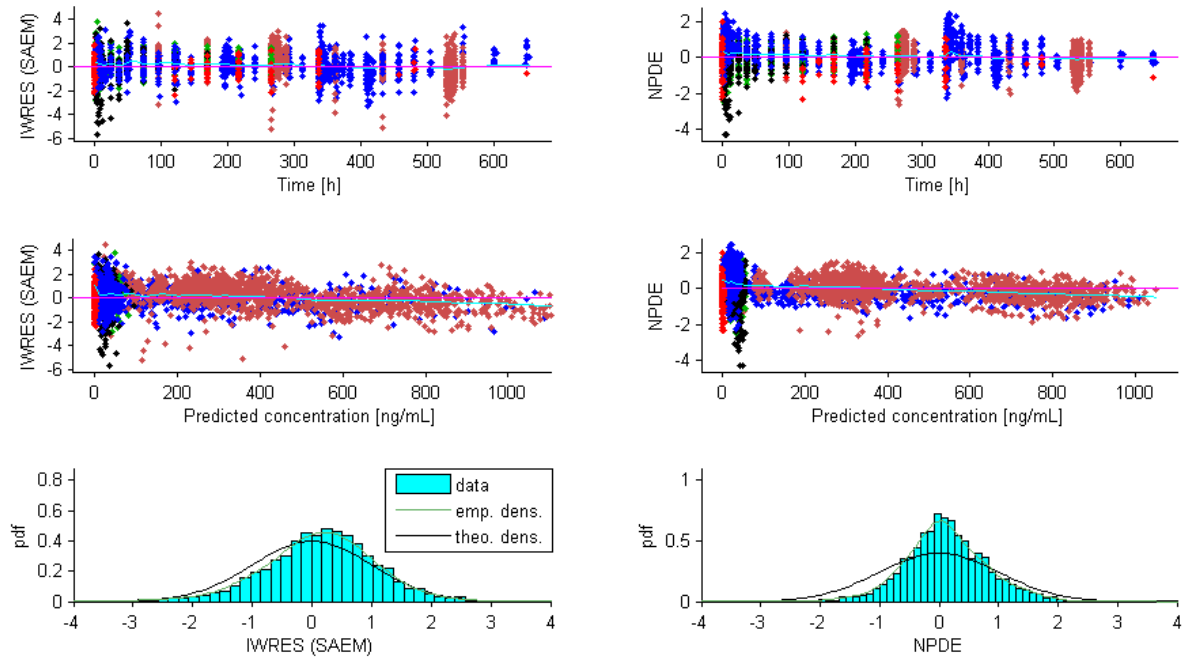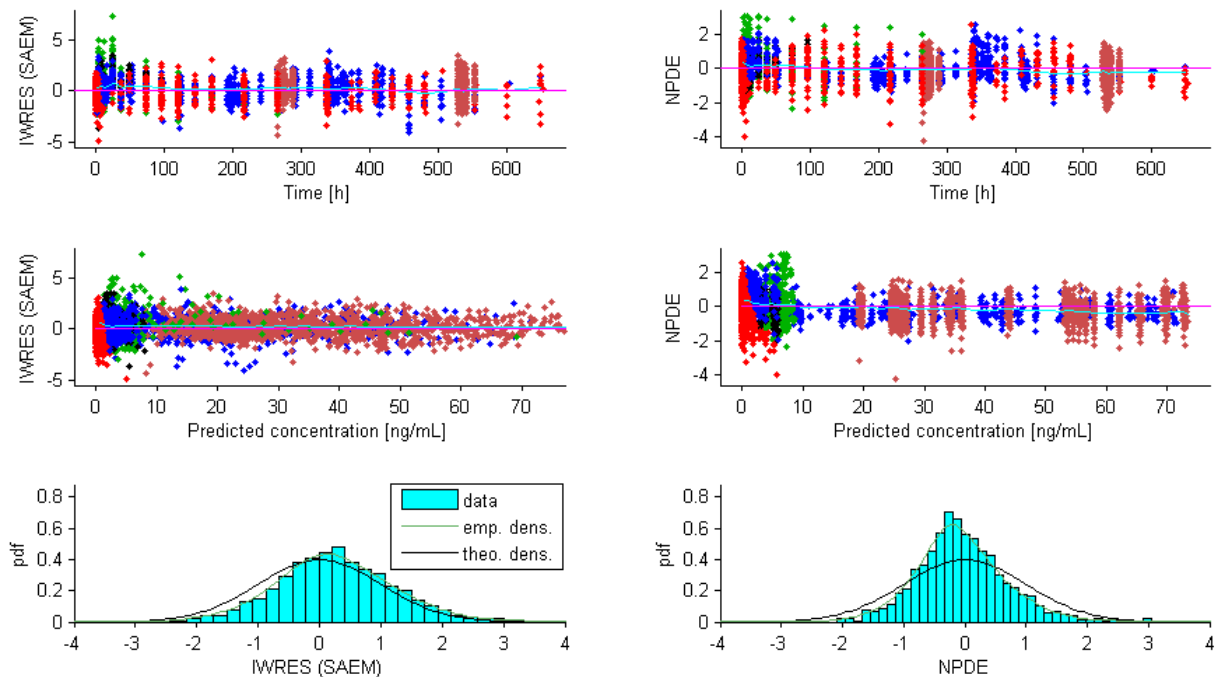

Figure S2 Residual diagnostics M12: Individual-weighted residuals (IWRES, left column) and normalized prediction distribution errors (NPDE, right column) versus model-predicted concentrations (top and middle row), density estimate (bottom row). Colors indicate studies.

Figure S3 Residual diagnostics M13: Individual-weighted residuals (IWRES, left column) and normalized prediction distribution errors (NPDE, right column) versus model-predicted concentrations (top and middle row), density estimate (bottom row). Colors indicate studies.
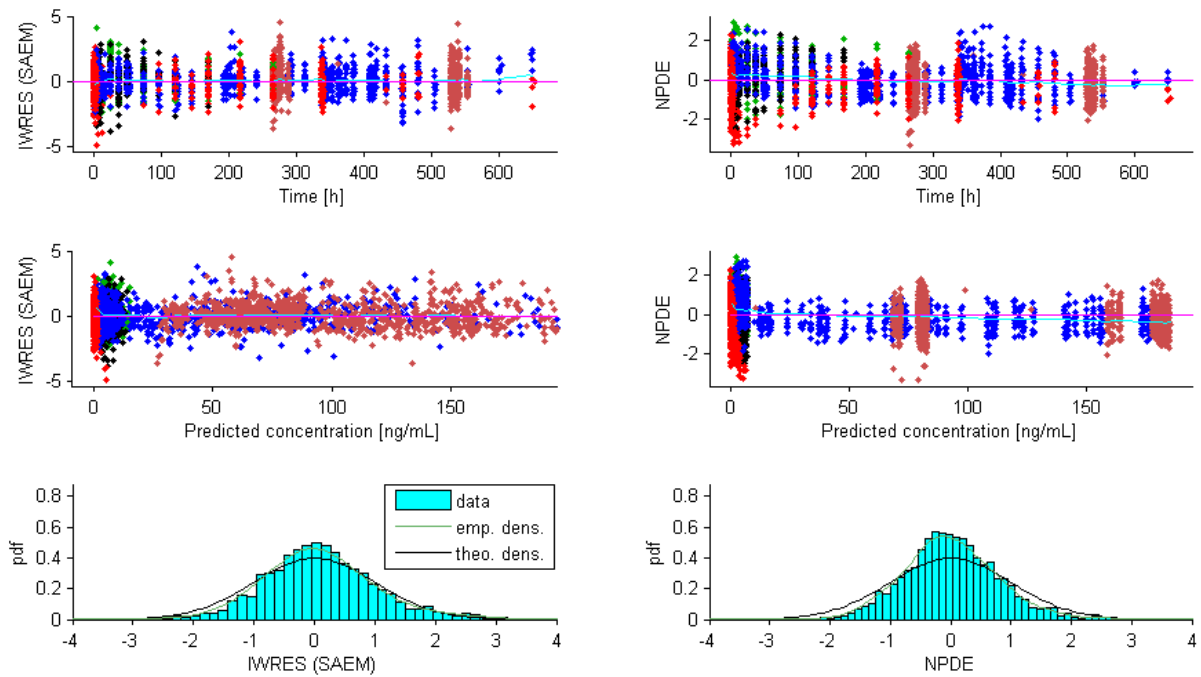
9.2 Supplementary material for publication III: Modeling the effect of the selective S1P$_1$ receptor modulator ponesimod on subsets of blood lymphocytes

Table S1 Population parameter estimates of the PK model of ponesimod according to Lott et al**.** (22)

Parameter	Description	Estimates (rse%)
T_{lag} (h)	Absorption lag time	0.40 (6)
Tk_0 (h)	Duration of the zero-order absorption process	0.58 (5)
Fr	Fraction absorbed via zero order	0.15 (8)
k_a (1/h)	Absorption rate constant	0.93 (7)
V_c/F (L)	Apparent central volume of distribution	165 (2)
Body weight on V_c	Covariate effect of body weight on V_c	0.85 (4)
V_p/F (L)	Apparent peripheral volume of distribution	107 (4)
V_p/F (L)	V_p for a subject of race Black	67 (11)
Body weight on V_p	Covariate effect of body weight on V_p	0.69 (21)
Q/F (L/h)	Apparent inter-compartmental flow	21 (11)
CL/F (L/h)	Apparent clearance	6.64 (1)
Body weight on CL	Covariate effect of body weight on CL	0.42 (10)
Inter-individual variability (% CV)		
IIV T_{lag}		43 (8)
IIV Tk_0		56 (8)
IIV Fr		62 (10)
IIV k_a		61 (6)
IIV V_c/F		22 (6)
IIV V_p/F		29 (9)
IIV Q/F		10 (244)
IIV CL/F		26 (3)
Residual error terms		
a	Additive error	0.006 (28)
b	Proportional error	0.21 (1)

rse% relative standard error, %CV coefficient of variation (percentage), IIV inter-individual variability

Figure S1 Individual profiles for total lymphocyte count, B cells, T helper cells, T cytotoxic cells, and NK cells in placebo subjects versus clock time.
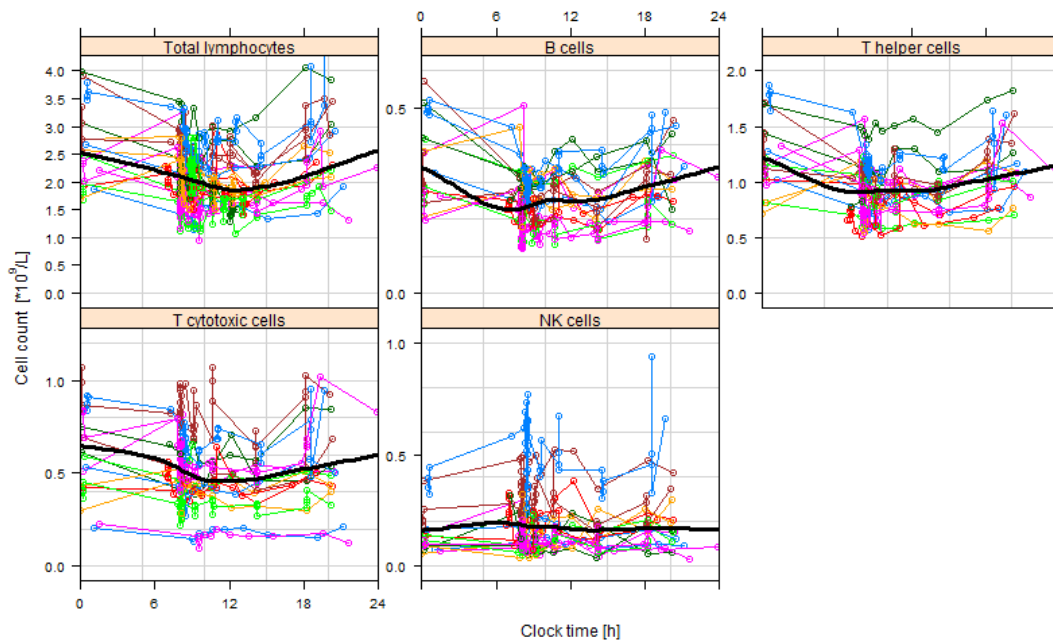
Figure S2 Comparison of IWRES vs. clock time for total lymphocyte count, B cells, T helper cells, T cytotoxic cells, and NK cells in placebo subjects for models with (right) and without (left) circadian rhythm model component.

43
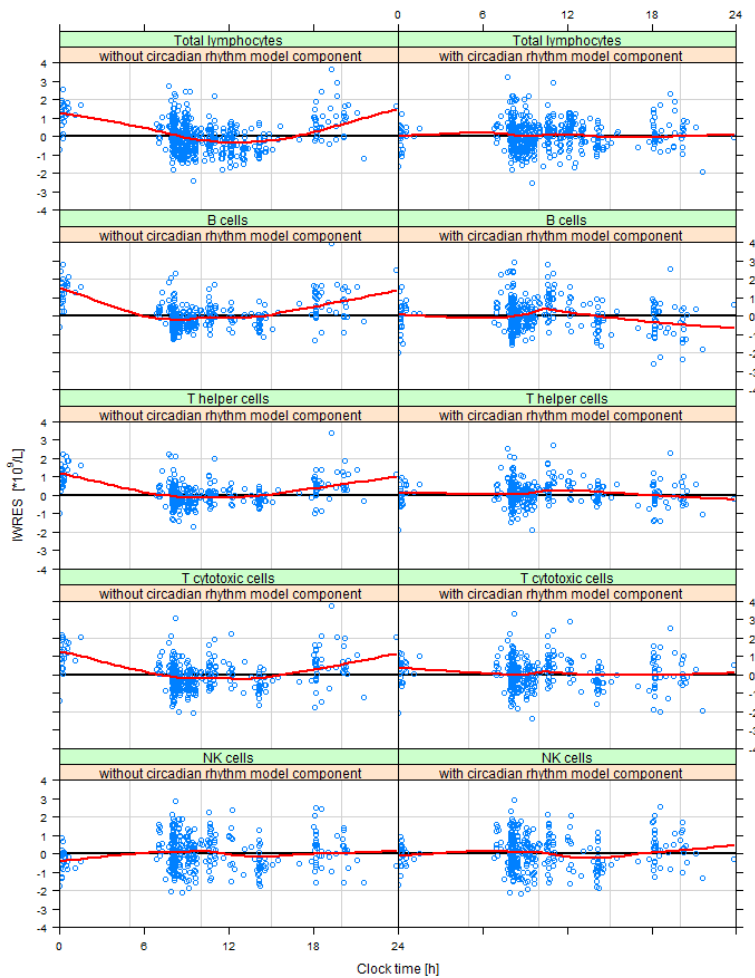
Figure S3 Goodness-of-fit diagnostic plots: IWRES vs. time (top) and vs. predicted concentrations (bottom) for total lymphocyte count, B cells, T helper cells, T cytotoxic cells, and NK cells. Colors indicate dose groups, brown lines are local regression fits (loess), IWRES individual-weighted residuals.
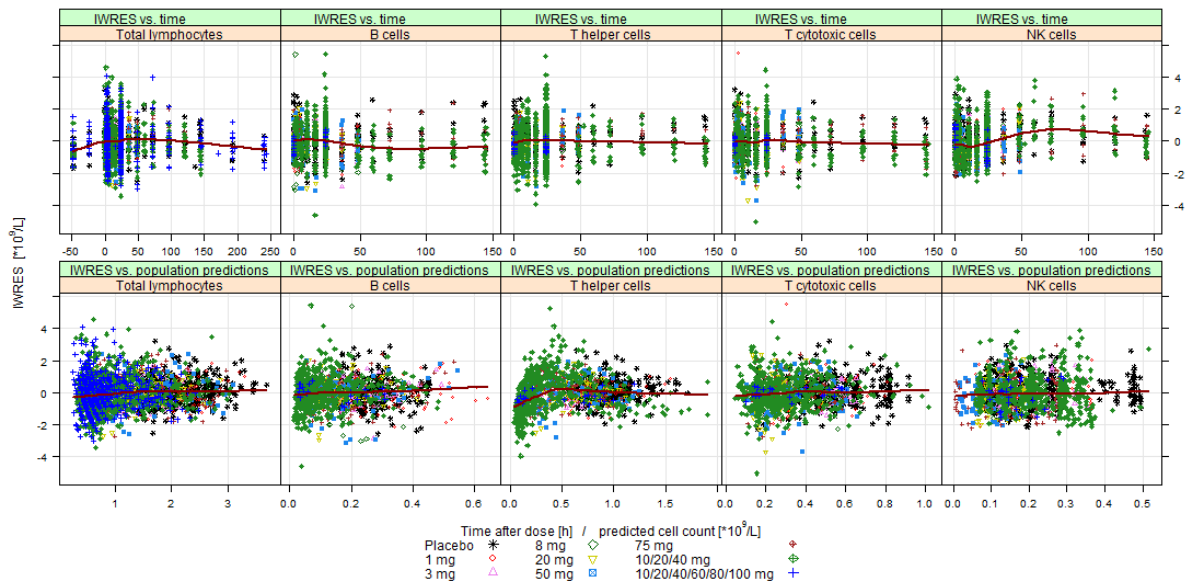
IWRES individual-weighted residuals

Figure S4 Concentration versus effect (cell count reduction) plots for total lymphocyte count, B cells, T helper cells, T cytotoxic cell, and NK cells.
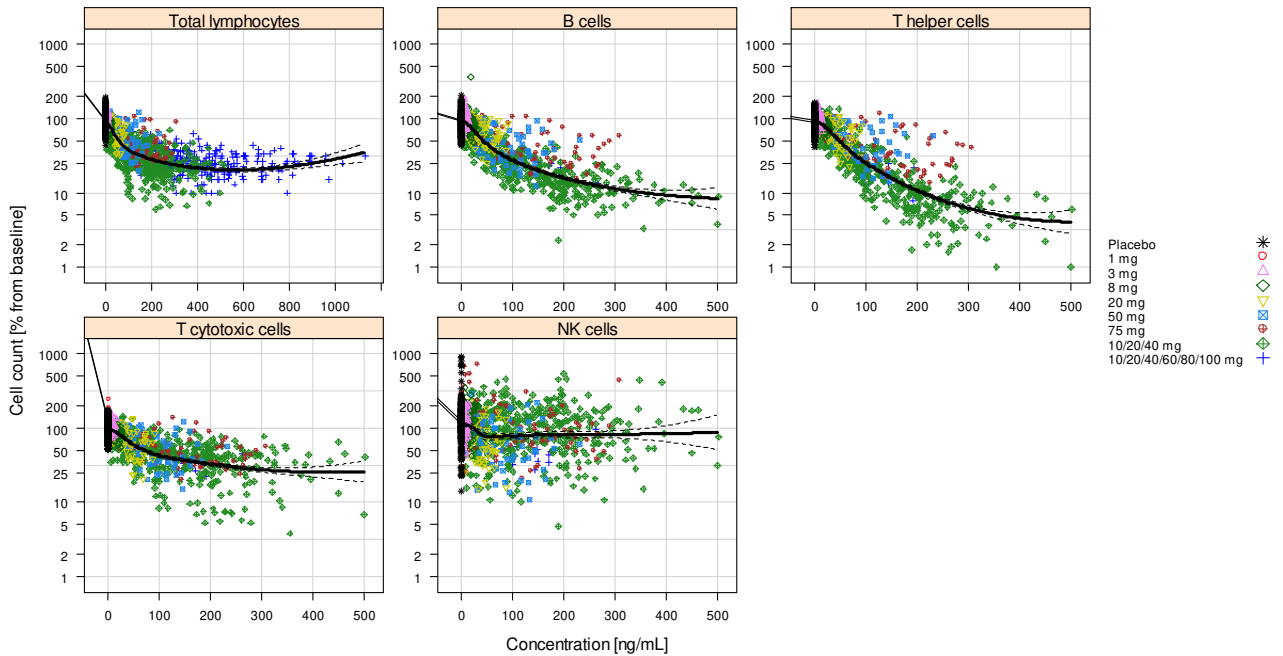
9.3 Supplementary material for publication IV: Modeling tolerance development for the effect on heart rate of the selective S1P$_1$ receptor modulator ponesimod

PK model parameter estimates

The parameters of the population PK model described in (34) and used to estimate individual PK parameters for each of the subjects included in the PK/PD analysis are provided in Table S1. The individual parameters were added to the data set to explore the PK/PD relationship. This sequential approach was used as PK are assumed to be more accurately assessed compared to heart rate.

Table S1 Population parameter estimates of the PK model of ponesimod (34) (105).

Parameter	Description	Estimates (%RSE)
T_{lag} (h)	Absorption lag time with tablet C + fasted	0.40 (6)
T_{lag} (h)	T_{lag} with capsule C	0.59 (5)
T_{lag} (h)	T_{lag} with tablet C + food	0.64 (13)
Tk_0 (h)	Duration of the zero-order absorption process	0.58 (5)
Fr (unitless)	Fraction absorbed via zero order	0.15 (8)
k_a (1/h)	Absorption rate constant	0.93 (7)
V_c/F (L)	Apparent central volume of distribution	165 (2)
Body weight on V_c	Covariate effect of body weight on V_c	0.85 (4)
V_p/F (L)	Apparent peripheral volume of distribution	107 (4)
V_p/F (L)	V_p for a subject of race Black	67 (11)
Body weight on V_p	Covariate effect of body weight on V_p	0.69 (21)
Q/F (L/h)	Apparent inter-compartmental flow	21 (11)
CL/F (L/h)	Apparent clearance	6.64 (1)
CL/F (L/h)	CL for a subject of race Black	5.65 (4)
Body weight on CL	Covariate effect of body weight on CL	0.42 (10)
Inter-individual variability (% CV)		
IIV T_{lag}		43 (8)
IIV Tk_0		56 (8)
IIV Fr		62 (10)
IIV k_a		61 (6)
IIV V_c/F		22 (6)
IIV V_p/F		29 (9)
IIV Q/F		10 (244)
IIV CL/F		26 (3)
Residual error terms		
a	Additive error	0.006 (28)
b	Proportional error	0.21 (1)

%RSE relative standard error, %CV coefficient of variation (percentage), IIV inter-individual variability

Subject characteristics

An overview of subject characteristics included in the PK/PD analysis is shown in Table S2.

Table S2 Subject characteristics by study.

Study (code)	Age, y Median (min, max)	Body weight, kg Median (min, max)	Height, cm Median (min, max)	BMI, kg/m^2 Median (min, max)	HR baseline[c], bpm Median (min, max)	Sex m/f	Race
1	35 (22, 48)	80 (64, 98)	182 (167, 190)	25 (20, 28)	60 (43, 84)	48/0	White: 48
2	30 (17, 58)	75 (45, 98)	173 (153, 190)	24 (18, 30)	64 (58, 86)	23/24	White: 47
3	27 (18, 53)	70 (64, 96)	178 (167, 185)	24 (20, 28)	64 (61, 73)	12/0	White: 6 Black: 5 Other: 1
4	50 (33, 60)	63 (51, 77)	163 (155, 176)	23 (20, 31)	62 (52, 73)	0/23	White:17 Asian: 2 Black: 2 Other: 2
5	39 (19, 65)	75 (46, 98)	173 (149, 195)	26 (20, 30)	66 (52, 85)	17/13	White: 10 Black: 20
6	25 (18, 59)	69 (49, 96)	170 (157, 189)	24 (19, 28)	56 (47, 78)	7/7	White: 9 Asian: 2 Other: 3
7	26 (22, 52)	73 (56, 97)	179 (154, 196)	23 (20, 27)	53 (43, 66)	10/6	White: 15 Asian: 1
8	34 (20, 45)	79 (58, 101)	173 (153, 191)	26 (20, 30)	63 (51, 90)	48/10	White: 30 Asian: 2 Black: 22 Other: 4
9	31 (18, 57)	72 (55, 92)	173 (151, 187)	25 (20, 30)	65 (52, 92)	15/17	White: 29 Asian: 1 Black: 2
Total	34 (17, 65)	75 (45, 101)	174 (149, 196)	25 (18, 31)	62 (43, 92)	180 (64%)/ 100 (36%)	White: 211 (75%) Asian: 8 (3%)[d] Black: 51 (18%) Other: 10 (4%)[d]

Min minimum, max maximum, y years, BMI body mass index, HR heart rate, bpm beats per minute, m male, f female

[c] defined as the last HR measurement prior to first study drug administration, mean if multiple measurements were taken

[d] category comprises <5% of the data and therefore not considered in the covariate analysis

Clock time at treatment start

Treatments were initiated between 07:00 and 11:00 AM with the majority between 08:00 and 09:00 AM. Figure S1 shows the distribution of clock time when treatments were started. Overall, only a few subjects started treatment before 08:00 AM and after 09:00 AM.

Figure S1 Distribution of clock time at treatment start.
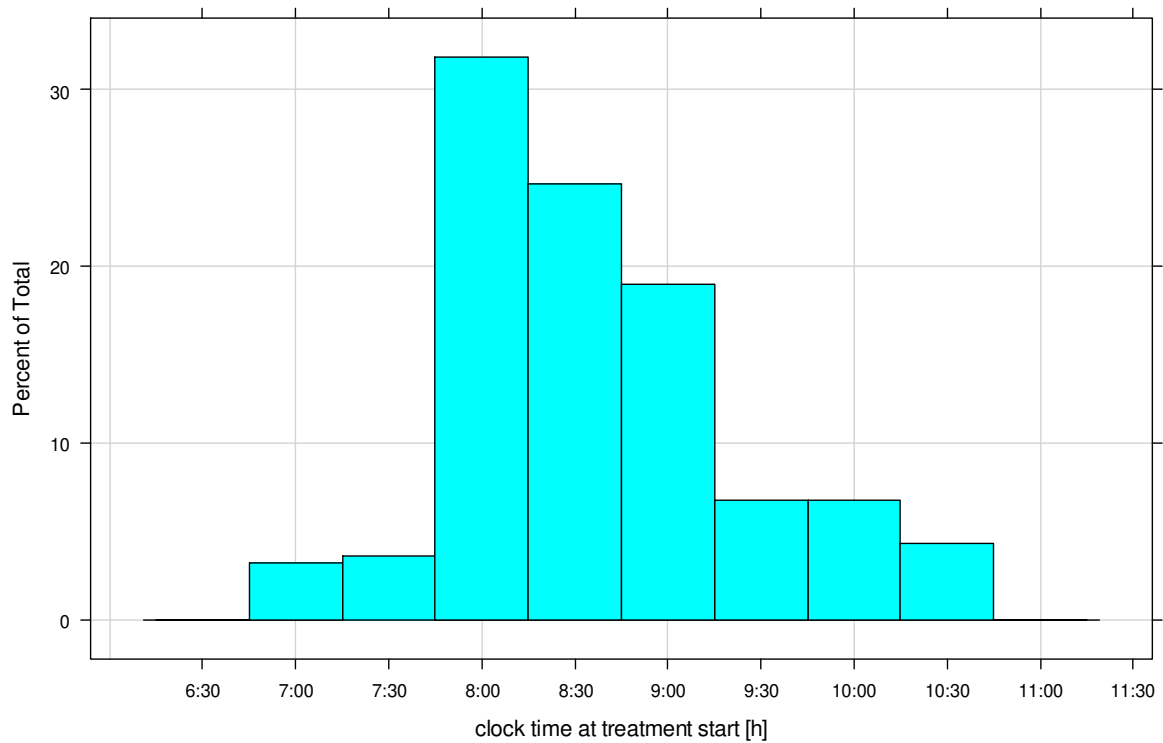
Observed first-dose effect on heart rate

Observed heart rate decreases (percent change from baseline) at the time of maximum decrease (1.5-2.5 h) following first dosing, stratified by dose are shown in Figure S2. The mean maximum decrease following administration of 75 mg was approximately 40%. Thus, the estimated I_{max} (44.9 %) is in line with the observed data. As initial doses of >75 mg were not clinically tested for safety reasons, there remains some uncertainty about the 'true' I_{max}. However, the maximum decrease between initial doses of 50 and 75 mg is similar and might have reached a plateau, i.e., I_{max}.

Figure S2 First-dose effect of ponesimod on heart rate based on observed data: relative change in heart rate from baseline at 1.5-2.5 h post-dose following fist-dose administration stratified by dose.
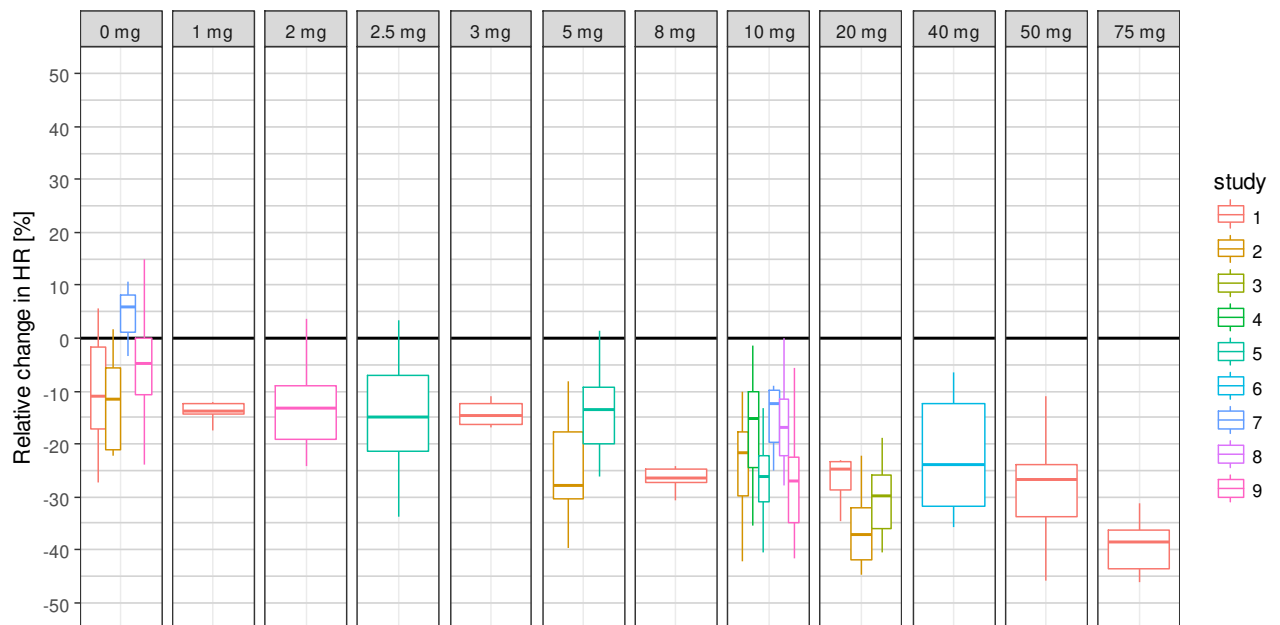
Model qualification – goodness-of-fit plots

Goodness-of-fit (GOF) diagnostic plots are shown in Figures S3 to S6. Observed and model-predicted concentrations (Figure S3) correspond well as indicated by the random and uniform scattering of the data points around the line of identity, the diagonal. Low concentrations are partly under-predicted by the model on the population level. On the individual level however, this under-prediction disappears. The data that appear to be under-predicted on the population level mainly arise from Phase 2 clinical studies in which time after (last) dose is not always accurately recorded (e.g., patients being asked for the time of last drug intake). Different error models, i.e., proportional, additive, and combined error models, were assessed and did not further improve the model fit. Thus, this observation might be attributable to inter-individual and inter-study variability rather than model misspecification. This hypothesis is supported by the unusually large variability in the low concentration range. Residual plots displayed in Figure S4 do not suggest model misspecification.

Figure S3 Goodness-of-fit diagnostic plots: Observed vs. population- (left) and individual-predicted (right) ponesimod concentrations.
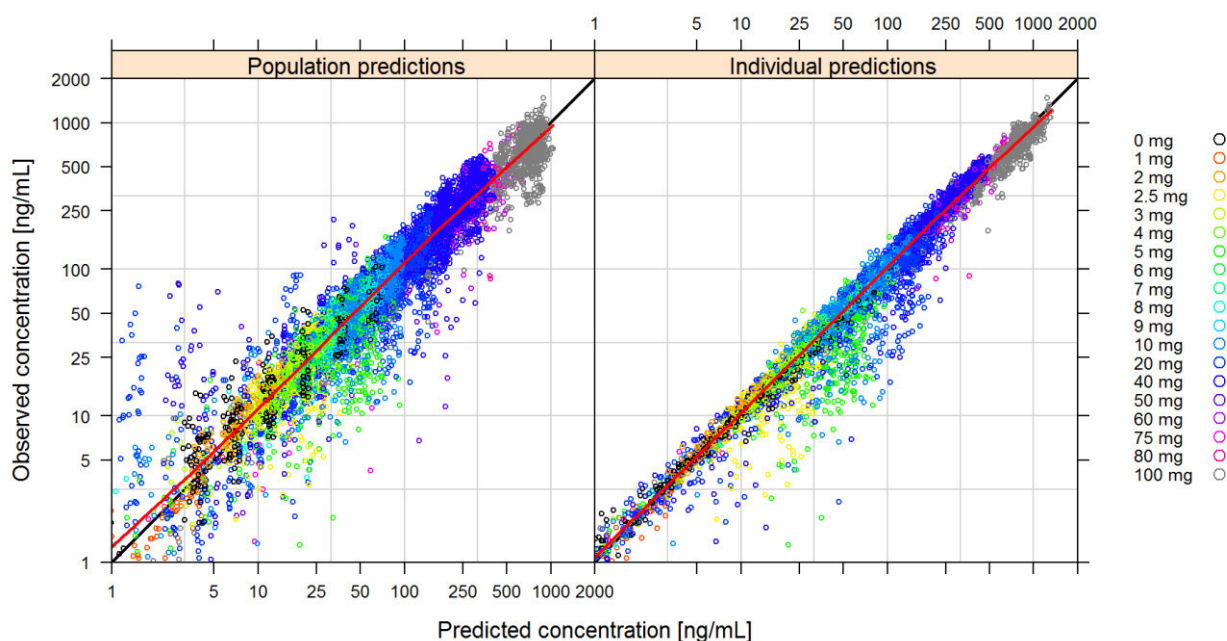
Colors indicate doses, solid black lines are lines of unity, solid red lines are local regression fits (loess).

Figure S4 Goodness-of-fit diagnostic plots: PWRES (left) and IWRES (right) vs. population/individual predictions (a), vs. time (b), and vs. time after dose (c).
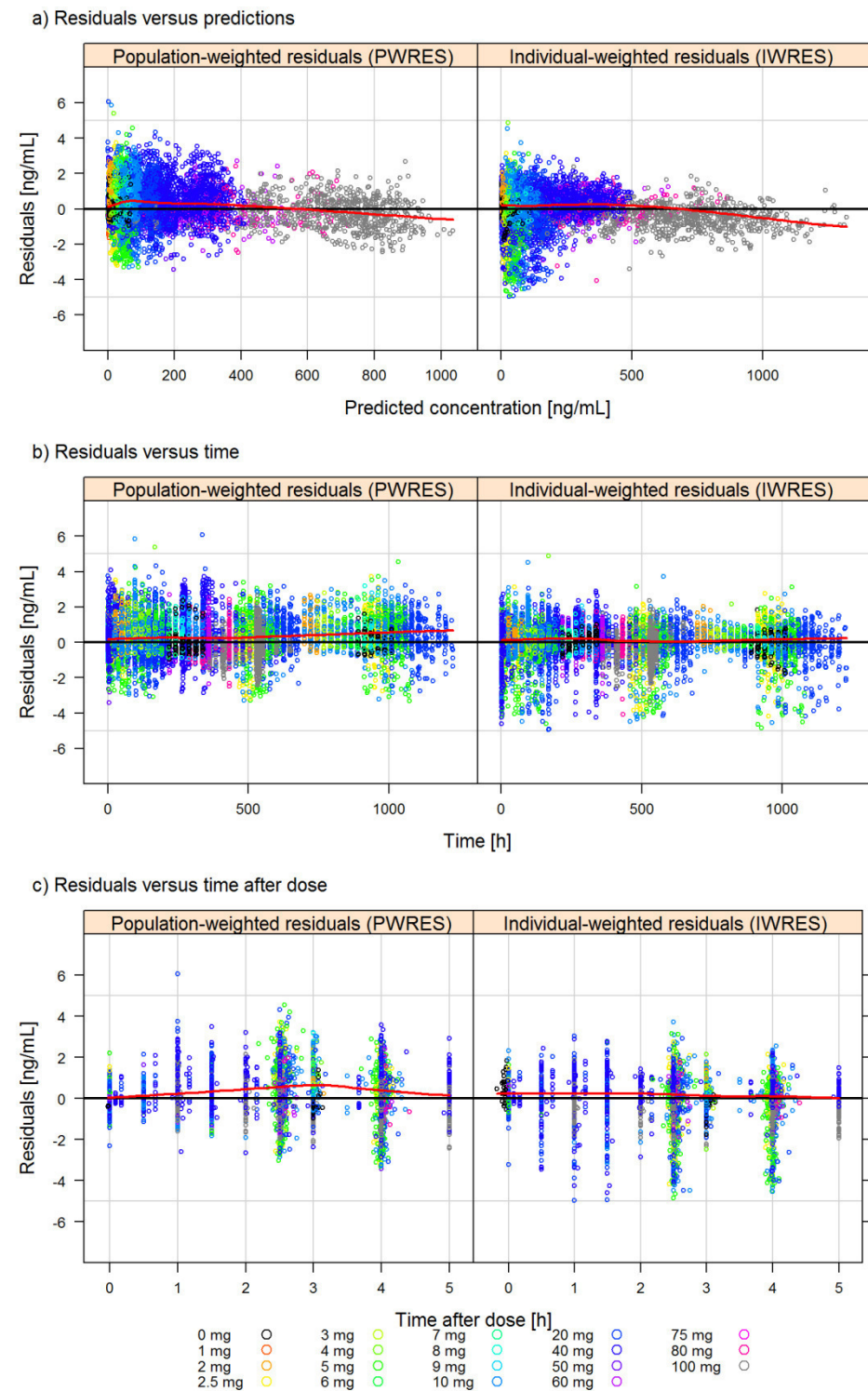
Colors indicate doses, red lines are local regression fits (loess), PWRES population-weighted residuals, IWRES individual-weighted residuals.

GOF plots comparing observed to model-predicted heart rate values are displayed in Figure S5. The slight under-prediction of low heart rate values on the population level disappears when accounting for inter-individual variably. Overall, the alignment between observations and model predictions were considered adequate. Residuals do not change with the magnitude of predicted values, time, and time after dose indicating proper selection of the structural model (Figure S6).

Figure S5 Goodness-of-fit diagnostic plots: Observed vs. population- (left) and individual-predicted (right) heart rate.
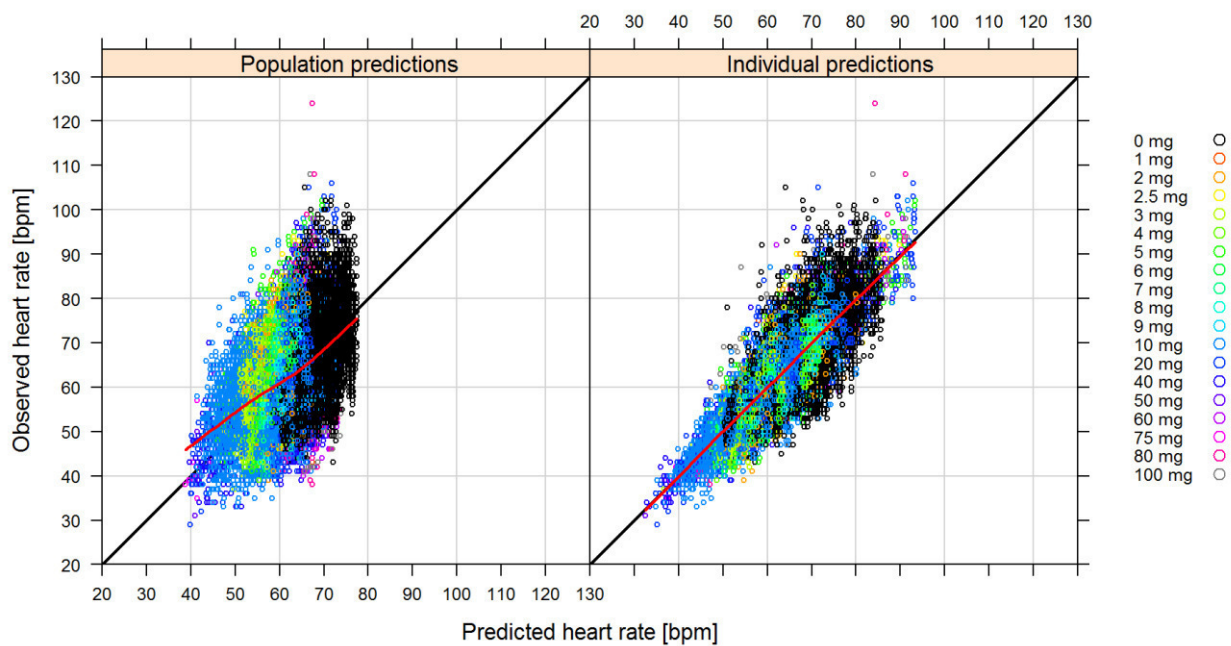
Colors indicate doses, solid black lines are lines of unity, solid red lines are local regression fits (loess).

Figure S6 Goodness-of-fit diagnostic plots: PWRES (left) and IWRES (right) vs. population/individual predictions (a), vs. time (b), and vs. time after dose (c).
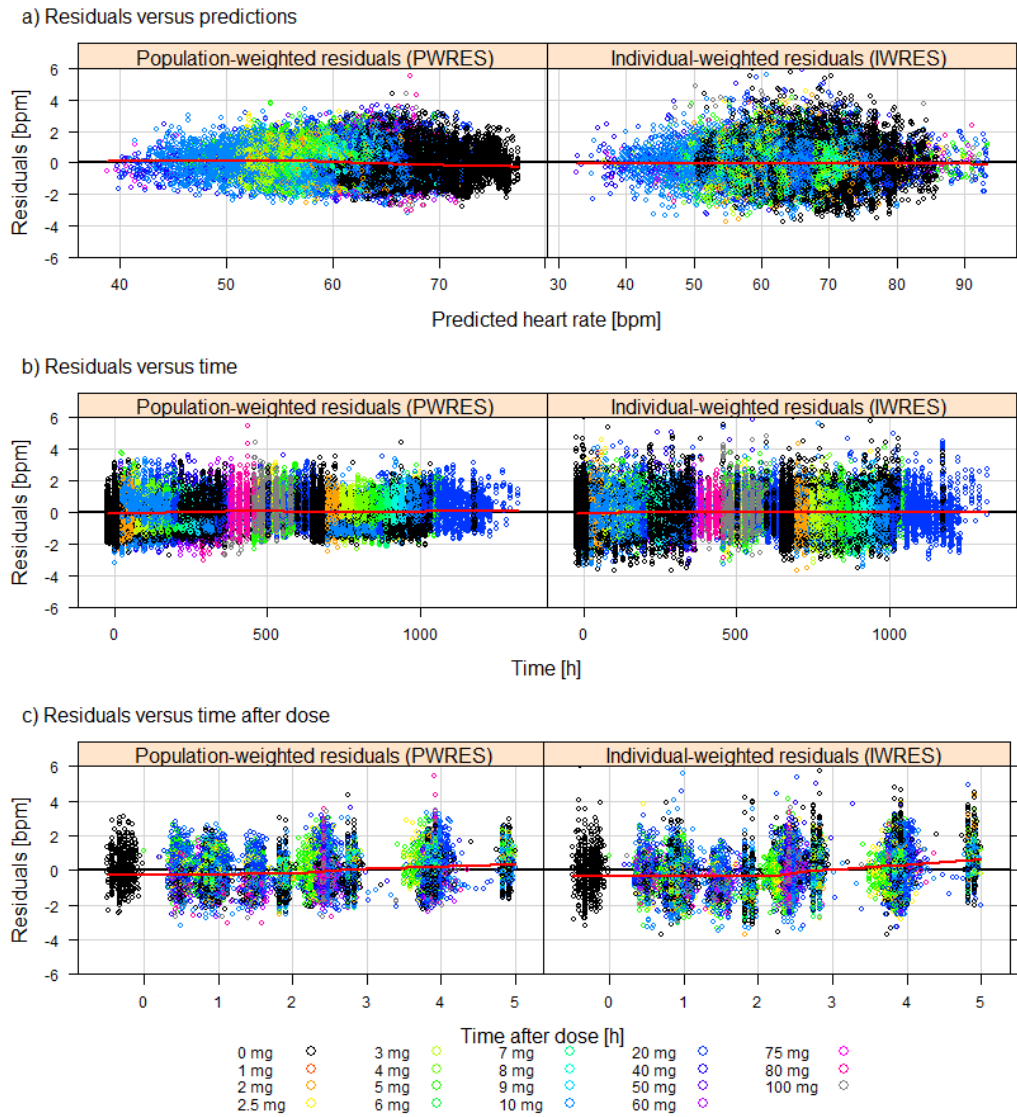
Colors indicate doses, red lines are local regression fits (loess), PWRES population-weighted residuals, IWRES individual-weighted residuals.

Model qualification – prediction-corrected visual predictive check

The prediction-corrected visual predictive check compares the observed to the model-predicted 5th, 50th, and 95th percentiles. The medians of the observed data are predicted with reasonable accuracy (Figure S7). The variability of low and high heart rate values is slightly over-predicted by the model as indicated by the green lines that are partially outside the prediction interval. However, this was not considered problematic for the following reasons.

The over-prediction is only marginal at the absolute level, i.e., 2-3 bpm. Considering the high natural fluctuation of heart rate this was considered acceptable.

The visual appearance of the graph depends on the binning of the data, i.e., the choice of interval numbers and limits. Due to the different designs of studies included in the analysis and the large number of measurements, choosing proper binning settings was challenging. The binning was optimized for good assessment of the first-dose effect that was key in predicting the incidence of bradycardia on treatment initiation.

The aim of the analysis was to simulate and compare different dosing and up-titration regimens. Here, if the slight over-prediction leads to a higher incidence of predicted bradycardia, this would be the case for all simulated regimens while the identification of the best up-titration scheme would still yield the same result.

Figure S7 Prediction-corrected visual predictive check comparing the empirical 5th, 50th, and 95th percentiles (green lines) with the simulated 5% (blue), 50% (red) and 95% (blue) prediction intervals.

10. Acknowledgments