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Optimizing pharmacotherapy in the elderly using modelling and simulation methods. Application to Bilastine.

Valentina Lo Re

PhD Thesis, 2021

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Doctoral Thesis

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A mia nonna

Table of content

Table of content

LIST OF TABLES

LIST OF FIGURES

Abbreviations & Acronyms

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Abbreviation	Definition
%	Percentage
ADME	Absorption, distribution, metabolism and elimination
AE	Adverse event
AIC	Akaike information criterion
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area Under the (plasma concentration versus time) Curve
BSA	Body surface area
BUN	Blood urea nitrogen
BW	Body weight
C, Conc.	Concentration
CFR	Code of Federal Regulations
CI	Confidence interval
CL	Clearance
Cmax	Maximum concentration
CNS	Central nervous system
Cp	Plasma concentration
CRF	Case report form
Css	Concentration at steady state
CLr	Renal clearance
CO	Cardiac output
CV	Coefficient of variation
CYP450	Cytochrome P450

D	Dose
E	Effect
Emax	Maximum effect
EC_{50}	Half maximal effective concentration
ECW	Extracellular body water
ECG	Electrocardiogram
e.g.	Exempli gratia (“for example”)
EDTA	Ethylenediamine tetraacetic acid
EMA	European Medicines Agency
etc.	etcetera
F	Bioavailability
FDA	Food and Drug Administration
FOCE	First Order Conditional Estimation
γ	Hill exponent
fu	Unbound Fraction
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
HPLC/MS-MS	High-performance liquid chromatography-tandem mass spectrometry
HR	Heart rate
ICF	Informed consent form
ICH	International Conference on Harmonization
IC_{50}	Inhibitory concentration that produces 50% of maximum response
ID	Individual
IND	Investigational New Drug Application
i.e.	Id est (“in other words” or “that is”)

IIV	Interindividual variability
IPRED	Individual prediction
IRB	Institutional Review Board
IRES	Individual residuals
i.v.	Intravenous
IWRES	Individual weighted residuals
k_{12}, k_{21}	Transfer rate constant between compartments
Ka	Absorption rate constant
Ke	elimination rate constant
kg	Kilogram
L	Liter
LLOQ	Lower limit of quantification
LD_{50}	Dose at which 50% of treated test animals are moribund or die
LDH	Lactate dehydrogenase
log	Decimal logarithm
LogP	Octanol/water partition coefficient
LW	Liver weight
m^2	square meter
MedDRA	Medical Dictionary for Regulatory Affairs
min	minute
mg	Milligram
mL	milliliter
MW	Molecular weight
M&S	Modelling and simulation
n	Number of individuals
NCA	Noncompartmental analysis

NCR	No carbon required
NDA	New Drug Application
NMEM, NONMEM	Non-linear mixed-effect modelling
ng	nanogram
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OATP	Organic anion-transporting polypeptide
OBJ	Objective function in NONMEM®
OTC	Over-the-counter
PAR	Perennial allergic rhinitis
PD	Pharmacodynamic(s)
PPB	Plasma Protein Binding
p	Significance coefficient
P-gp	P-glycoprotein
PBPK	Physiologically based pharmacokinetic modelling
PK	Pharmacokinetic(s)
pKa	Logarithmic acid dissociation constant
Pop	Population
PRED	Mean population prediction
POPPK	Population pharmacokinetics
Q	Intercompartmental (distributional) clearance
Q12h	Every 12 hours
QAU	Quality Assurance Unit
RES	Residual
RBC	Red blood cell

SC	Schwarz criterion
SAE	Serious adverse experience
SD	Standard deviation
SAR	Seasonal allergic rhinitis
ss	Steady state
SEE	Standard error of estimate
t	Time
Tlast	Time corresponding to the last quantifiable concentration (Clast)
$t_{½}$	Elimination half-life
T_{max}	Time at which C_{max} was observed
TBW	Total body water
WBC	White blood cell
UHPLC/MS-MS	Ultra-high performance liquid chromatography-tandem mass spectrometry
US FDA	United States Food and Drug Administration
V	Volume of distribution
Vc	Volume of distribution of the central compartment
Vp	Volume of the peripheral distribution compartment
VPC	Visual predictive check
vs.	versus
WRES	Weighted residuals
WRSS	Sum of squared weighted residuals
ε	Epsilon: residual value between predicted and observed concentration
η	Eta: residual value between predicted and observed parameter
µL	Microlitre
µM	Micromolar

ω	Omega: standard deviation of interindividual error
σ	Sigma: standard deviation of intraindividual error
θ	Theta: structural parameter
-2LL	-2 times the log of the likelihood

Background

Background

Bilastine is a selective, second-generation H_1 antihistamine approved worldwide for the treatment of allergic rhinoconjuntivitis (AR) and chronic urticaria (CU). It has been developed by FAES FARMA as tablets with a target dose of 20 mg once daily in adults (Sadaba et al. 2013).

Bilastine has a well-defined therapeutic window and favorable Pharmacokinetic (PK) properties and, it has demonstrated to be safe even at a supratherapeutic doses. It does not undergo significant hepatic metabolism and the majority of the drug is recovered after oral administration unchanged in faeces and urine (Sadaba et al. 2013). Moreover, it doesn't cause sedative effects or affect cognitive performance and it has a limited propensity for metabolism related drug-drug interactions (Martin K Church et al., 2019). All these features make bilastine a suitable option for geriatric patients with allergic disorders.

Even for a well-established drug such as bilastine, that has been also evaluated in a small group of subjects aged >65 years old (Sologuren et al., 2018), the uncertainties related to aging and the underlying mechanisms behind the differences observed with young adults are not fully understood. The evaluation of the impact of aging on the PK of drugs is essential to aid in the understanding of age-related changes and for evaluating the potential need for dosing adjustment in elderly.

The elderly represents the larger medicated segment of the population prescribed for the treatment of acute and chronic medical conditions such as various degrees of renal impairment. (Schlender et al., 2016) Despite the increasing size of the geriatric population and specific guidance on the elderly regarding medicinal products, this group of patient population is clinically understudied and remain under-represented in clinical research assessing therapeutic efficacy and safety of novel therapies. In fact, while specific dosing regimens and labelling recommendations based on clinical trial data are available for adults, they are frequently lacking for geriatrics. Evidence of dosing, efficacy, safety and tolerability in this population are insufficiently documented and the administration of the majority of drugs is supported only by extrapolation from the clinical experience in adults.

Elderly adults are a vulnerable population due to the physiological changes associated with age and the additional problems of polypharmacy and comorbidity diseases that may affect the PK and PD of drugs in this population. A number of studies have been performed in old adults in order to evaluate how physiological changes and diseases associated with aging may have an impact on PK and pharmacodynamics (PD). Despite this, only few studies have been published and the bulk of these are observational studies with their inherent potential biases.

The lack of data in elderly limits one's ability to understand the underlying mechanisms determining the blood profile of drugs in this special population. Pharmacometrics methods, not relying on

observations but on parameters and related covariates in the elderly could be beneficial to take the most out of the available data.

Overcoming these limitations could be achieved by combining quantitative approaches, such as Population Pharmacokinetic (PopPK) and more Physiologically-based approaches allowing to identifying gaps in scientific knowledge (Sinha et al., 2014; Stader et al., 2018).

In the case of bilastine, allometric extrapolation of its bicompartmental PK parameters in adults into pediatrics by using predictive biomarkers such as body weight or BSA has already been successfully used in children (Encinas et al., 2013; Vozmediano et al., 2019).

However, when a case to be studied is even more complicated, such as geriatrics, establishing dosing recommendation still remains to be solved. A reason why the need is yet unmet is mainly because of the substantial heterogeneity of the geriatric group derived from various aging rate/extent and high number of disease and comedications.

In fact, along with age-related gradual changes, the incidence for chronic diseases and co-morbidity increases are followed by chronic drug therapy. However, drug therapy in the elderly is much more challenging and complex than in younger adults especially due to this co-morbidity and the increasing number of drugs for the treatment of different conditions (polypharmacy). Comorbid conditions included diabetes, hypertension, high cholesterol level, coronary artery disease, cancer, and chronic kidney disease (CKD). Particularly, CKD is common in the elderly population. Data from literature suggest that all physiological renal changes age-related (decreased kidney size, decreased renal blood flow, decreased number of functional nephrons) lead to a decrease glomerular filtration rate and thus, to a reduced renal clearance, directly impacting the total clearance for a drug with exclusive renal clearance such as bilastine.

Due to all above the use of predictive pharmacokinetic (PK) and pharmacodynamics (PD) models including also changes in renal impairment of drugs in elderly patients are difficult to predict (Corsonello et al., 2010). A deeper knowledge about how physiological changes during aging may affect the absorption, distribution, metabolism, and elimination (ADME) of drugs is required.

Particularly, a thorough understanding of bilastine pharmacokinetics in the older person is essential for improved therapeutic outcomes, improved compliance, reduced morbidity and improved quality of life.

Introduction

Introduction

Active and healthy aging is one of the key goals shared by all European Countries. Thanks to the progress of medicine, the lengthening of life expectancy is neither a mirage nor a goal for only few people anymore. Population ageing is a global phenomenon. Demographics indicate an aging population with a longer life expectancy. The number and the proportion of older people in the world have both increased substantially in recent years in most countries, and the growth is projected to accelerate in the coming decades.

The global population aged 65 years or over numbered 703 million in 2019, and the number of older persons is expecting to double to 1.5 billion in 2050 (Figure 1). Globally, a person aged 65 years in 2015-2020 could expect to live, on average, an additional 17 years. By 2045-2050, that figure will have increased to 19 years. Between 2015-2020 and 2045-2050, life expectancy at age 65 is projected to increase in all countries (Nations et al., 2019).

The number and the proportion of older people in the world have both increased substantially in recent years in most countries, and the growth is projected to accelerate in the coming decades.

The global population aged 65 years or over numbered 703 million in 2019, and the number of older persons is expecting to double by 2050, when it is projected to reach nearly 1.5 billion (Figure 1) (Nations et al., 2019).

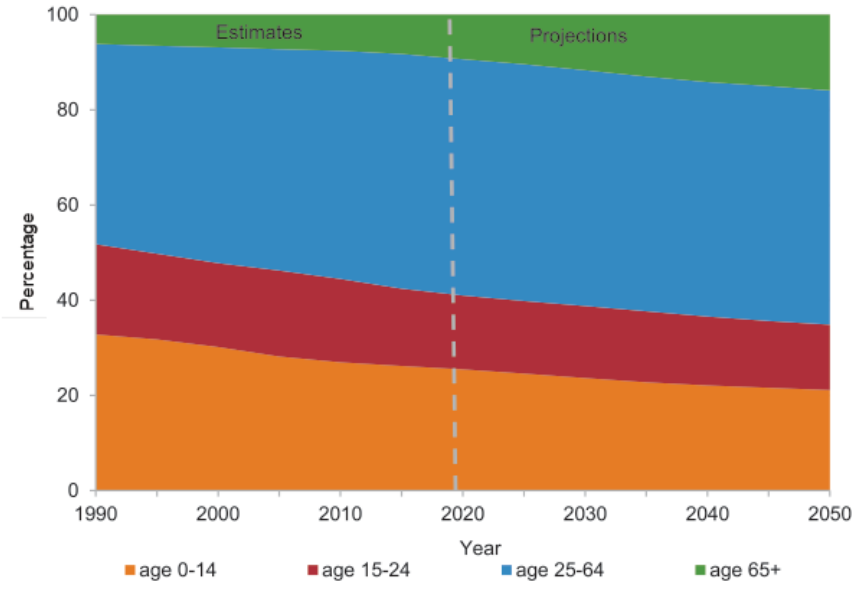


Figure 1. Global population by broad age groups, 1990-2050 (percentage)

Data Source: United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects: the 2019 Revision.

The interest in physiological changes in special population, such as geriatric patients, both on an organic and functional level, and the acquisition of data related to the aging process, markedly increased because of the increasing number of elderly people in the general population and its implications for health care. For example, the prevalence of allergic diseases such as allergic rhinoconjuntivitis and urticaria in elderly patients has been reported to be greater than 10% and includes the symptoms of sneezing, nasal discharge, stuffiness, and itching as a result of inflammation of the nasal mucosa, usually accompanied by allergic conjunctivitis.

The main pharmacological treatment of allergic rhinitis are oral antihistamines and intranasal corticosteroids. First generation antihistamines, although effective at controlling symptoms, demonstrate poor receptor selectivity and so, they are associated with a variety of adverse events (AEs) including sedation. On the contrary, second-generation H_1 receptor antagonists, such as bilastine, demonstrate more specific targeting of receptors and lesser capacity to cross the hematoencephalic barrier (less lipophilic) and so they are less likely to cause adverse CNS effects than the first H_1-antagonists in elderly (Todo Bom et al., 2009). Bilastine is a selective histamine H_1 receptor antagonist. During allergic response mast cells undergo degranulation which releases histamine and other substances. By binding to and preventing activation of the H_1 receptor, bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.

1. Pharmacological characteristics of bilastine

Bilastine (ATP CODE R06AX29), (2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl) ethyl) phenyl]-2-methylpropionic acid), (Figure 2) is a drug substance developed by FAES FARMA for the treatment of the symptoms of allergic rhinitis and chronic urticaria as tablets with a target dose of 20 mg once daily.

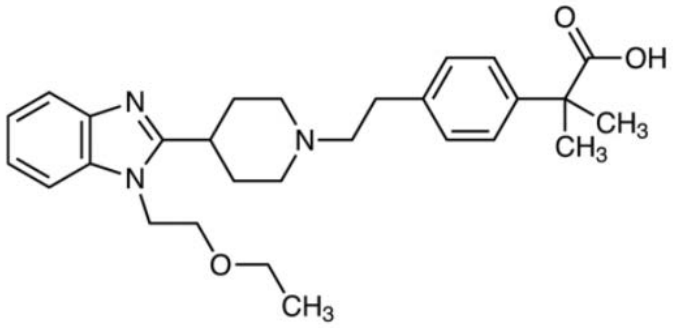


Figure 2. Chemical structure of bilastine (systematic name: 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl) ethyl) phenyl]-2-methylpropionic acid).

Histamine, an organic nitrogenous compound ((2-(1H-imidazol-4-yl) ethanamine, C5H9N3), is an important chemical mediator involved in the inflammatory response, as well as regulating physiological function in the gut and acting as a neurotransmitter for the brain, spinal cord, and uterus. It is metabolized from the precursor histidine and it is released into some synapses, and also into the blood stream where it acts as a hormone. Histamine is also known as a neuromodulator, since it regulates the release of other neurotransmitters, like acetylcholine, norepinephrine, and serotonin (Shahid et al., 2009).

Histamine is stored mainly preformed in cytoplasmic granules of mast cells and basophils which are bound to the anionic side-chains of the proteoglycans that make up the granule matrix. These cells are most abundant in the organs expressing allergic diseases: the skin, upper and lower respiratory tracts, and gastrointestinal tract, as well as the reproductive mucosa. After exposure, an allergen is presented to TH cells via antigen-presenting cells. Specific antibodies of the IgE type are produced by B cells and interact with receptors on the surface of basophils and mast cells leading to the release of histamine (Figure 3). The actions of histamine are mediated through four distinct G-protein-coupled receptors that transfer extracellular signals via G proteins. Most of the important histamine effects in allergic diseases are mediated through the H_1 receptor and include smooth muscle cell contraction in the gastrointestinal tract, increase in blood pressure, temperature, swelling, and bronchial constriction (González-Deolano et al., 2018; Shahid et al., 2009).

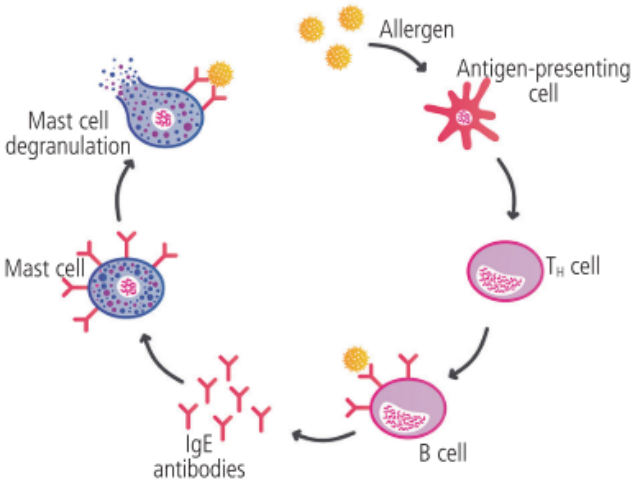


Figure 1. Schematic representation of the mechanism of release of histamine *(Adapted from González et al. 2018)*.

Bilastine is a high specific H_1 receptor inverse agonist that interfere with histamine action at H_1-receptors on sensory neurons and small blood vessels and stabilize the inactive form of H_1R, down-regulating the constitutive receptor activity. In vitro studies have shown that bilastine has a high specific affinity for the H_1-receptor, but it has no or very low affinity for 30 other tested receptors. Bilastine has a rapid onset of action and a long duration of action. It has been shown to have a long residence time at the H_1-receptor, resulting in prolonged receptor antagonism, with 60–70% antagonism evident 24 hours after dosing.

Moreover, bilastine, through the ubiquitous transcription factor nuclear factor-kB, also exerts anti-inflammatory activity by inhibiting the release of histamine, IL-4 and tumor necrosis factor (TNF)-α from human mast cells and granulocytes (Figure 4) (Balakrishnan, 2014; Simons et al., 2008).

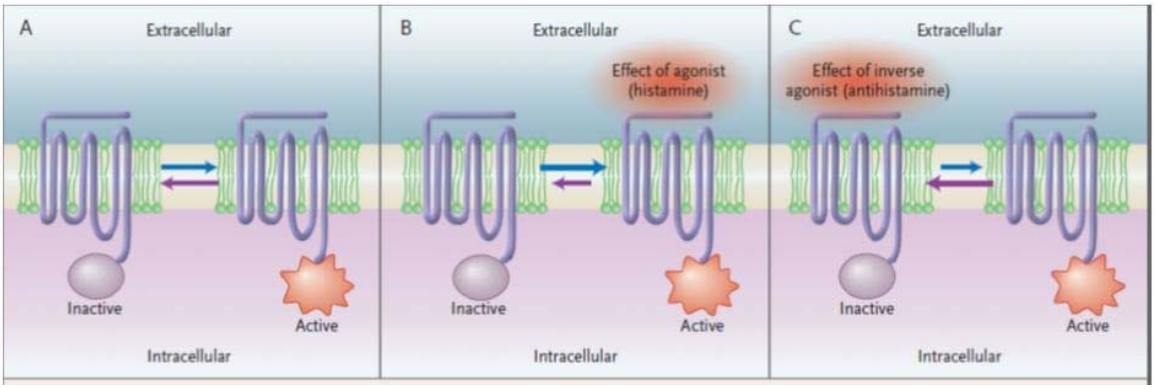


Figure 4. Mechanism of action of histamine and H_1-antihistamine acting as inverse agonist. A, the inactive state of the histamine H_1-receptor is in equilibrium with the active state. B, the agonist, histamine, has preferential affinity for the active state, stabilizes the receptor in this conformation, and shifts the equilibrium toward the active state. C, An H_1-antihistamine (inverse agonist) has preferential affinity for the inactive state, stabilizes the receptor in this conformation, and shifts the equilibrium toward the inactive state *(Adapted from Balakrishnan, 2014).*

1.1 *Main features of the preclinical development of bilastine*

Pharmacological studies showed bilastine to be highly selective for the H_1 receptor in both in vitro and *in vivo* studies. Bilastine was shown to dose-dependently inhibit ^{3}H-pyrilamine binding to H_1 receptors in the guinea pig cerebellum and similar findings were obtained in a human embryonic kidney cell line. Moreover, additional in vitro studies demonstrated that bilastine had no significant antagonist activity at a diverse range of other receptors: H_2, H_3, and H_4, N-type voltage-dependent calcium receptors, and M_1-M_3 muscarine receptors. (Jauregizar et al., 2009; Jàuregui et al., 2012)

Bilastine metabolism was defined using Caco-2 cells, with and without induction of the two main cytochromes involved in intestinal metabolism: CYP1A1 and CYP3A4. No metabolites were produced as a result of cellular activity, indicating an absence of intestinal metabolism. Additional in vitro studies in human microsomes and hepatocytes showed that bilastine is neither an inducer nor an inhibitor of CYP450 isoenzymes. Bilastine has also been showed to have anti-inflammatory properties after in vitro studies in human mast cells (HMC-1) and granulocytes by inhibiting interleukin-4 (IL-4) and tumor necrosis factor- α (TNF α). A summary of physicochemical characteristics of bilastine is presented in Table 1 (Bachert et al., 2010; Corcóstegui et al., 2005).

Table 1. Bilastine physicochemical characteristics

Parameter	Input Value	Source	Note
LogP	2.3	Experimental	DrugBank
Molecular Weight	463.622 (g/mol)	Experimental	DrugBank
pKa	8.78 (basic)/ 4.4 (acid)	Experimental	DrugBank
Solubility	0.00203 mg/mL	Experimental	DrugBank
LogP (computational logarithm of the partition coefficient between n-octanol and water)			

Bilastine shows no central nervous system penetration and has minimal sedative properties as expected for a drug with a total H1-receptor occupancy (H_1RO) in the brain for bilastine has been reported to be less than zero. Generally, second generation H_1 antihistaminic drugs, such as bilastine, are characterized by a more hydrophilic nature than first-generation ones. This aspect explains, at least in part, their non-sedating features. In fact, as suggested by several studies, hydrophilicity alone could not be sufficient to keep drugs from entering the brain. Based on in vitro studies, bilastine appears to be a substrate of the multidrug resistance protein 1 (MDR1) or P-glycoprotein (also referred to as P-gp), an active efflux transporter extensively distributed and expressed in the intestinal epithelium, in liver cells, in the cells of the proximal tubule of the kidney, and in the capillary endothelial cells composing the blood–brain barrier. In the blood–brain barrier it plays an important role in pumping out bilastine back into the capillaries (Figure 5) (Maria Luisa Lucero et al., 2012). The *in vitro* inhibitory effects of bilastine were also assessed on several human transporters revealing that bilastine is also a substrate for the intestinal drug transporter OATP1A2 which is colocalized with P-gp to the brush border domain of enterocytes (Glaeser et al., 2007).

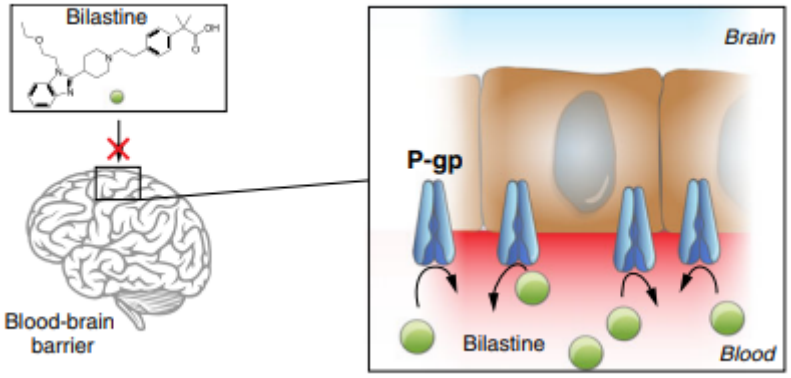


Figure 5. Mechanism of action of P-glycoprotein (P-gp) in preventing the uptake of bilastine into the brain (Adapted from Church et al. 2017).

In general, *in vivo* tests showed bilastine to have similar or greater potency than cetirizine where histamine was used to induce response. Particularly, the potency of bilastine where IgE was used to induce an allergic response was inferior to cetirizine. Potency of bilastine was greater than fexofenadine both in vitro and *in vivo*. Pharmacokinetic parameters of bilastine have been studied after single dose studies both by oral and IV routes in various species such as rats and dogs. The studied doses in dogs indicate bioavailability (calculated as unchanged bilastine) higher than 42% reaching sometimes to 69%. No pre-systemic metabolism *in vivo* has been observed in rats and dogs confirming the *in vitro* evidence. This, and the lack of hepatic metabolism, limits the potential for drug–drug interactions. Since bilastine does not undergo significant metabolism, no dosage adjustments are required in patients with renal or hepatic impairment or in elderly people (Lasseter et al., 2013). Preclinical PK data available are presented in Table 2 and Table 3.

Table 2. Animal study designs of bilastine in rats and dogs after single intravenous (i.v.) and oral (p.o.) administration

Study	Animals (N sex)	Administration route: dose (mg)
PK linearity (*in vivo*)	Rat (23M)	p.o: 5, 10, 20 and 40
PK (*in vivo*)	Rat (4M)	i.v.: 10
PK linearity (*in vivo*)	Dog (2F–2M)	p.o: 10, 20 and 50
PK (*in vivo*)	Dog (2F–2M)	i.v.: 10
F female, M male		***Extracted from Vozmediano et al., 2013***

Table 3. Compartmental oral (p.o.) and intravenous (i.v.) pharmacokinetic parameters of bilastine in rat and dog

Oral PK parameters	**Vc/F (L)**	**CL/F (L/h)**	**Vss/F (L)**	**V_p/F (L)**	**Q/F (L/h)**	**f_u**
Rat (n = 4; 0.25 kg)	0.40	1.24	0.50	0.10	0.02	0.16
Dog (n = 3; 16 kg)	10.71	14.50	20.71	10.00	0.58	0.42
Intravenous PK parameters	**Vc (L)**	**CL (L/h)**	**Vss (L)**	**Vp (L)**	**Q (L/h)**	**f_u**
Rat (n = 4; 0.25 kg)	0.16	0.50	0.20	0.04	0.008	n.a
Dog (n = 3; 16 kg)	5.36	7.25	10.4	5.04	0.290	n.a
Extracted from Vozmediano et al., 2013						

In vivo studies have investigated the toxicological profile of bilastine showing that no mortality or clinical signs of toxicity occurred after single oral doses of 2000 mg/kg in mice and 5000 mg/kg in rats. Repeat oral dose toxicity studies in dogs showed that a dose of 60 mg/kg/day during 28 days was well tolerated, while higher doses, up to 1500 mg/kg, showed frequent vomiting and diarrhoea, but no mortality. After 13 weeks administration of up to 1000 mg/kg no mortality was recorded, and only slight signs of toxicity were seen in the higher dose groups. A 52-week study in dogs with doses up to 800 mg/kg/day showed again no mortality at any dose and good tolerance up to 125 mg/kg. Batteries of mutagenicity tests have shown bilastine to be non-mutagenic. No fertility or reproductive toxicities were observed in rats exposed to bilastine up to 1000 mg/kg and in rabbits receiving bilastine up to 400 mg/kg/day. (María Luisa Lucero et al., 2012)

In safety pharmacology studies bilastine proved to have no cardiovascular effects in guinea pigs and in beagle dogs at doses expected to exceed the therapeutic dose in humans. Bilastine exerted no behavioral changes and no effects on motor activity in mice and rats. No anticonvulsant activity was observed, and no potentiation of the depressant effect was seen when bilastine was given to mice treated with ethanol or diazepam. Bilastine had no effect on the respiratory and gastrointestinal systems in rats or mice. Regarding pharmacological interactions, bilastine did not modify the hypoglycemic effect of insulin and only high doses were able to reduce the alloxan-induced hyperglycemia. Finally, no modification of coagulation parameters and no interaction with warfarin was observed.

1.2 *Main features of the clinical development of bilastine*

Sixteen Phase I studies, 7 Phase II and 5 Phase III clinical studies have been conducted with oral doses of bilastine. Overall, these studies have involved more than 450 healthy volunteers and more than 6.000 patients worldwide, both in single administration and in repeated doses. All these studies have been conducted complying ICH Good Clinical Practice (GCP) regulations and under conditions stated in the Declaration of Helsinki and have followed the current FDA and EMEA guidelines for the clinical development of medicinal products for the treatment of allergic rhinoconjuntivitis.

A PK/PD model based on data from 310 healthy volunteers (8429 bilastine plasma concentrations) has been established. According to this, bilastine PK follows a two-compartmental model with first order absorption and elimination. Several covariates, including demographic, biochemical data and vital signs (sex, age, height, weight, albumin, creatinine, GOT, BUN, GGT, bilirubin, alkaline phosphatase, and pulse) were studied and no relationship was found between them and the PK parameters (Jauregizar et al., 2009). The published Population PK/PD model parameter estimates of bilastine are presented in Table 4.Clinical studies design details from which analyzed bilastine plasma concentration-time data were available are reported in Table AI-1 in Annex I.

Table 4. Bilastine Population pharmacokinetic-pharmacodynamic population model developed with Phase 1 studies *(Adapted from Jauregizar et al. 2019)*

PK Parameter in healthy volunteers	Estimate θ (**SEE$_\theta$**)	**SEE% (SEE$_\theta$)**
CL (L/h)	18.1 (1.8)	29.0 (8.7)
Vc (L)	59.2 (2.2)	35.4 (9.6)
Q(L/h)	1.59 (3.9)	56.5 (10.3)
Vp (L)	30.2 (5.1)	73.1 (9.6)
Ka (h^{-1})	1,50 (3.2)	35.4 (16.9)
σ (%)	26.6 (6.1)	

Population pharmacokinetic-model fit to plasma concentration time data from all available Phase 1 studies. Population pharmacokinetic-parameter estimates (with relative standard errors[a] (SEE$_\theta$) expressed as %) and interindividual variability expressed as the percentage of the coefficient of variation of the SEE (SEE [%]). The relative standard error is the standard error divided by the parameter estimate.

CL= absolute total body clearance of the drug from plasma; Vc= absolute central compartment volume of distribution; Q= absolute intercompartmental clearance; Vp= absolute peripheral compartment volume of distribution; k_a= first-order absorption rate constant.

PD Parameter		**K_{in} [ng/mL/h]**	**K_{out} [h^{-1}]**	**IC_{50} [ng/mL]**
Wheal	Estimate[a] η [b]	0.44 (14.60) 29.36 (32.95)	1.09 (15.14) 14.04 (81.22)	5.15 (16.16) 55.95 (45.05)
Flare	Estimate[a] η [b]	11.10 (8.48) 24.02 (45.41)	1.03 (8.35) 26.98 (26.65)	1.25 (14.56) 65.65 (29.93)

Pharmacokinetic-pharmacodynamic population model fit of the wheal and flare effects
[a] Values are expressed as estimate (%SEE$_\theta$). The SEE$_\theta$ is the standard error divided by the parameter estimate.
[b] Values are expressed as %SEE.
η= interindividual variability; SEE= standard error; SEE$_\theta$= relative standard error of the pharmacodynamic parameter; IC_{50} = estimated concentration producing 50% inhibition; k_{in} = zero-order rate constant for production of response; k_{out} = first-order rate constant for loss of response.

Extracted from Jauregizar 2009

Bilastine is administered orally at a dose of 20 mg once daily in adults. Studies in man volunteers showed that the absorption of bilastine is fast, linear and proportional to the administered doses, achieving maximum plasma concentrations (C_{max}= 220 ng/mL) after 1–1.5 hours. Bilastine has a mean elimination half-life of approximately 12–14.5 hours and it is 84–90% bound to plasma proteins. Concurrent food intake and grapefruit juice reduce the bioavailability of bilastine that has been estimated to be around 61%. Based on its hydrophobicity and P-gp-mediated efflux, it would be expected a low absorption of the drug. A possible explanation for this is attributable to the other drug transporter of bilastine, the OATP1A2, that in contrast to the P-gp, enhances the absorption of bilastine (Figure 6) (M.K. Church et al., 2017).

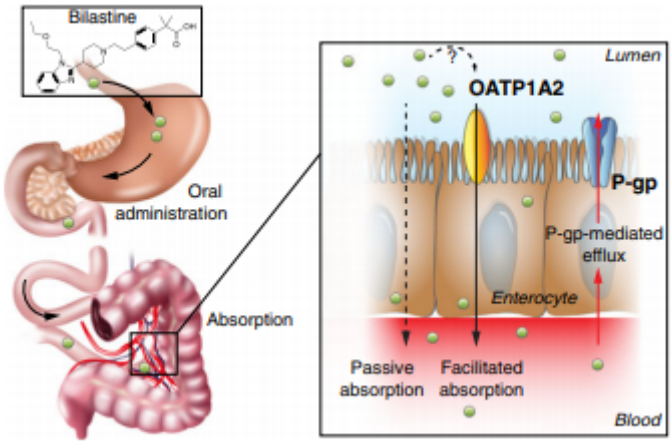


Figure 6. Mechanism of facilitated intestinal uptake of bilastine. P-glycoprotein (P-gp) facilitates the excretion of bilastine into the intestine while the Organic Anion Transporting Polypeptide 1A2 (OATP1A2) pomp out the bilastine into the blood *(Adapted from Church et al. 2017)*

Bilastine doesn't undergo significant metabolism and does not interact whit the CYP450 system, which limits its potential for drug-drug interactions. Bilastine is generally well tolerated, even when administered at above-standard doses with no related SAEs or clinically significant changes in vital signs, laboratory safety parameters or ECG findings. Cardiological safety was analyzed pooling ECG data from Phase I, Phase II and III studies and no significant influence on any ECG parameter was found after bilastine administration, suggesting a safe cardiological profile.

It is also important to note that no significant anticholinergic effect was found at any bilastine dose tested. Interaction studies of bilastine with erythromycin, ketoconazole and diltiazem demonstrated that the extent of exposure and accumulation of bilastine are significantly increased when coadministered with these CYP450 and P-glycoprotein inhibitors. Nevertheless, these concomitant medications appeared to be safe and well-tolerated. No positive interaction on heart rate was seen, and the QTc prolongations observed with combined treatments were equal to or lower than those with the CYP450/Pg-p inhibitors alone. The results suggest cardiological safety of bilastine when co-administered with a CYP450/Pg-P inhibitor.

A favorable safety profile was observed in clinical trials both in adults and in children. The potential effect of bilastine in the CNS was studied against hydroxyzine and placebo, showing that no differences were detected up to 40 mg bilastine and placebo in the psychomotor performance. A slight sedative effect was found with hydroxyzine and 80 mg bilastine, greater with hydroxyzine after the first dose administered than after repeated administration. The freedom from somnolence it is a fundamental feature of bilastine.

The clinical activity of bilastine has been investigated in 5 completed Phase II studies and 5 completed Phase III studies on symptoms of allergic rhinitis in subjects suffering from SAR and PAR, and on symptoms of CIU. During these studies, bilastine has been administered by oral route to approximately 2,300 patients and evaluated for efficacy and safety and 513 of them have received bilastine 20 mg daily for at least 1-year (long-term safety extension). Approximately 1,000 additional patients have received bilastine in ongoing studies. Bilastine 20 mg once daily has shown to be statistically superior to placebo and equally efficacious to comparators for reducing nasal and non-nasal symptoms of allergic rhinoconjuntivitis and for reducing symptoms of chronic idiopathic urticaria.

In a randomized, double blind, single dose, four period, crossover study conducted on healthy male volunteers who received 5 doses of bilastine, single dose of cetirizine and placebo showed that histamine-induced wheal was significantly reduced at 0.5 and 1 hour after bilastine administration but not thereafter. Regarding safety, no statistically significant differences among bilastine doses and placebo were found whereas statistically significant differences were found between bilastine and comparators regarding several AEs, mainly somnolence and fatigue. Neither deaths and nor SAEs related to bilastine were reported during the studies. (Sologuren A, Valiente R, Crean C, 2007)

No accumulation pattern was shown for bilastine after repeated dosing in a 14-day PK study of escalating daily doses from 10 to 100 mg.

The above summarized clinical studies have allowed to describe and conclude the following pharmacological characteristics (Table 5).

Table 5. PK, Safety and Efficacy characteristics of bilastine in adult

	Well Defined in Adult	***PK characteristics***
PK linear	✓	Linearity demonstrated in adults in the range 2.5-220 mg.
Drug does not accumulate	✓	No drug accumulation was observed with repeated bilastine administration
Rapid first order absorption	✓	Bilastine is rapidly absorbed after oral administration with a time to maximum plasma concentration of around 1.3 hours. No accumulation was observed. The mean value of bilastine oral bioavailability is 61%.
Food Effect	✓	Food significantly reduces the oral bioavailability of bilastine by 30%.
Protein binding	✓	At therapeutic doses bilastine is 84-90% bound to plasma proteins.
Drug is not metabolized	✓	Bilastine is not metabolized and is eliminated as unchanged in urine and faeces
Excreted by glomerular filtration	✓	Renal elimination represents a major contributor in the elimination of bilastine. biliary excretion is expected to be only marginally involved in the elimination of bilastine
Renal elimination proportional to the GFR	✓	Renal clearance is proportional to the patient's renal function.
Efficacy	✓	Efficacy demonstrated in the treatment of urticaria and allergic rhinoconjuntivitis
Safety and tolerability	✓	Lack of sedative potential, Lack of cardiovascular effects Freedom from cardiac toxicity

Only limited pharmacokinetic data are available in subjects older than 65 years with no apparent change in PK metrics across age groups when analyzed using non compartmental approaches (BILA 459-05 clinical trial). Moreover, the safety profile of bilastine in the elderly was assessed in a prospective, multicenter, observational, open-label, 3-month follow-up study in patients aged ≥65 years with allergic rhinoconjuntivitis and/or urticaria. Bilastine 20 mg showed a favorable safety profile with a low incidence of treatment-emergent AEs (TEAEs) in patients aged ≥65 years (Martin K. Church et al., 2020). Even for a well-established drug such as bilastine, the uncertainties related to aging and the underlying mechanisms behind the differences observed with young adults are not fully understood. The evaluation of the impact of aging on the PK of drugs is essential to aid in the understanding of age-related changes and for evaluating the potential need for dosing adjustment in elderly.

2 Pathophysiological changes in the elderly and influence of ADME processes

Aging is not a uniform process and the overall effect of age-related changes may be very variable within the geriatric population and even within an individual (Mangoni et al., 2004; Reeve et al., 2015). One of the most important changes, is the immunosenescence referring to physiological changes affecting the immune system. One of the mayor consequences include the atrophy of the thymus, the principal organ responsible for the process of the T-lymphocyte maturation. As the organ shrinks, the T cell areas are replaced with fatty tissue, in a process called involution. Naive T-cells decrease, although the total number of T cells does not undergo major changes, thanks to the increase of the subpopulation of memory T cells. Particularly, a decreased production of T-helper 2 cytokines could result in a decrease of IgE production, the immunoglobulin involved in the allergic response. The compromised immunological response triggers a proinflammatory state defined as ‘inflammaging’ characterized by an increase in levels of proinflammatory cytokines (IL-1b, IL-6, IL-8, and TNF-a) associated with both tissue neutrophilia and symptomatic bacterial infection/colonization in aged patients (Figure 7) (Garrido et al., 2019).

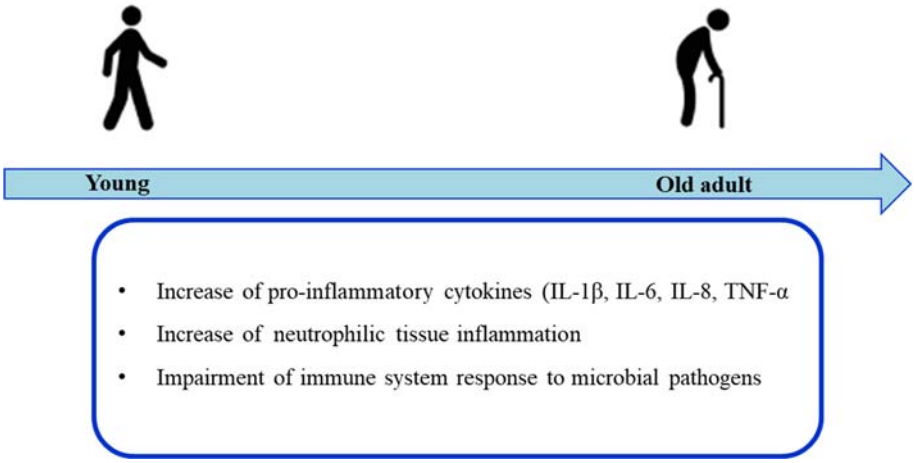


Figure 7. Schematic changes occurring during the immunosenescence in older people *(Adapted from Morse et al. 2019)*

However, the available epidemiological data are insufficient, and international cohort studies based on a large elderly population are lacking. Therefore, allergic rhinitis in elderly people is underdiagnosed and undertreated (Hilmer, 2008; Mukker et al., 2016).

Physiological aging also involves a progressive impairment in the functional reserve of multiple systems and organs. As a result, there is a failure in maintaining homeostasis under conditions of physiological stress that may affects different regulatory systems in different subjects. In fact, in elderly the maintenance of physiological parameters within certain pre-set limits or optimal conditions result impaired, taking more time than in young subjects (Massoud et al., 2017; ph, Liu, Orlu Gul, & Basit, 2016).

As a consequence of this reduced homeostatic ability, parameters such as body temperature, total blood volume, osmotic pressure, serum proteins and sugar blood level may result altered. Thus explaining at least partly the increased interindividual variability occurring as people get older (Reeve et al., 2015). Aging is largely determined by genetics, and influenced by life style (e.g. smoking), a wide range of environmental factors, such as diet, exercise and exposure to microorganisms (Massoud et al., 2017). Resulting changes affect the molecular, cellular and tissue level (Midlöv P., 2013; Singh et al., 2014). Particularly, changes at molecular level include damage to mitochondrial and nuclear DNA caused by increased oxidative stress, increased lipid peroxidation, telomere shortening, altered gene expression, and up regulation of cell apoptosis (Sera et al., 2012). With aging, critical physiological changes occur in the cardiovascular system, in pulmonary mechanics, in renal physiological features, gastrointestinal tract, and endocrine, immune and stress responses (Aalami et al., 2003; Massoud et al., 2017). As a consequence of these anatomic and physiological modifications, elderly people are more prone to experience infection diseases (Gavazzi et al., 2002).

Understanding the effects of aging on most systems, including changes in digestive activity and frequent diseases associated, it is fundamental to generate knowledge regarding safety, efficacy, PK and PD of many drugs that may affect the response to treatments in elderly.

Moreover, when considering the impact of aging, a distinction between healthy older adults and frail older people should be made. The latter refers to subgroup of patients in which the coexistence of multiple diseases strongly related to aging exhibit them at a greater risk of adverse drug reactions (ADRs) and mortality than healthy elderly (Hubbard et al., 2013).

Therefore, it is important to define the "normal" changes that occur as age advances, those that are part of the physiological phenomenon of aging and unrelated to specific diseases.

Aging is associated with various physical and physiological impairments at different levels, such as decreased in the ability to swallow due to the loss of teeth, decreased saliva production and gingivitis (Brownie, 2006). As we age, it is frequent the presence of periodontal diseases, gingivitis and edentulism along with the condition of xerostomia (or dry mouth) due to a decreased salivary gland function. All this conditions, together with the impairment of the ability of detect the basic taste (sweet, bitter, sour, salty and umami) contribute to poor appetite, decreased energy intake and more in general to the condition of "anorexia of aging" that is considered a serious problem in elderly (Brownie, 2006; Hubbard et al., 2013; Turner DDS et al., 2007). This disorder is associated to various adverse health outcomes in elderly subjects such as malnutrition, sarcopenia (decrease in muscle mass) and physical frailty (Landi et al., 2016).

Malnutrition, in turn, is a contributing factor to, among them, infectious diseases, impairment of immune response and risk of respiratory and cardiac problems (Brownie, 2006).

Moreover, weakened nutritional state in turn may contribute to alter the body composition and anthropometric measurements affecting in this way the pharmacokinetics of drugs as a direct consequence, predispose the old adult to pathological weight loss essentially affecting the total body water and the lean body mass with a consequential increase in body fat (Mangoni et al., 2004; Rodrigues et al., 2012; Soenen et al., 2016). Another common problem in older age is the condition of dysphagia that seems caused by swallowing difficulties.

A gradual loss of blood vessel distensibility (90%) along with intimal hyperplasia and thickening and decrease in vascular compliance developed with aging. These changes represent a risk factor for silent coronary artery disease. Concerning the pulmonary system, it has been demonstrated a significant association between some of the changing over time body composition variables and the spirometric variable FEV1 (forced expiratory volume) and FCV (forced vital capacity) (Lowery et al., 2013).

It has been largely demonstrated that physiologic changes and diseases associated with aging have an impact on pharmacokinetics (PK) well as pharmacodynamics (PD) processes (Massoud et al., 2017;

Reeve et al., 2015). All age-related changes affecting PK can impact differently the phases of absorption, distribution, metabolism, and excretion (ADME), whereas PD results in an impairment effect of drug on its target site occurring at the receptor or signal-transduction level (McLachlan & Pont, 2012; Scott-Warren & Maguire, 2017). Table 6 outlines the main age relating changes affecting both pharmacokinetics and pharmacodynamics.

Table 6. Physiological changes in elderly that can affect pharmacokinetics and pharmacodynamics *(Adapted from Klotz at al. 2011)*

Pharmacokinetics		
Process	**Physiological changes in the elderly**	**Possible PK consequences**
ABSORPTION	• Increased gastric pH • Delayed gastric emptying • Decreased intestinal CYP450 • Decreased intestinal P-gp expression and activity	Rarely clinically significant
	• Reduced splanchnic blood flow • Decreased absorption surface of the small intestine • Decreased gastrointestinal motility	Reduced absorption of proteins, vitamins and some drugs
DISTRIBUTION	• Increased body fat • Decreased lean body mass • Decreased total body water	Increased Vd and t1/2 of liposoluble drugs (ex. Diazepam) Reduced Vd of water-soluble drugs (e.g., digoxin) Increased plasma concentration of hydrophilic drugs
	• Decreased serum albumin	Increased free fraction in plasma of a few highly protein-bound acidic drugs
	• Increased α1-acid glycoprotein	Not considered significant
	• Decreased P-gp expression and activity	Supratherapeutic plasma concentrations and drug toxicity
	• Increased α1-acid glycoprotein	Not considered significant
	• Decreased hepatic blood flow (20-50%) • Decreased hepatic mass (20-30%)	First-pass metabolism can be less effective. Phase I metabolism of some drugs might be slightly impaired. The function of CYP 3A4 is decreased with age.
ELIMINATION	• Decreased renal blood flow • Decreased glomerular filtration rate (15-40%)	Renal elimination of drugs can be impaired

Pharmacodynamics	
Physiological changes in the elderly	**Possible PD consequences**
• Alterations in receptor affinity • Alterations in receptor number • Enhanced or diminished post-receptor response • Central nervous system sensitivity • Enhanced receptor response • Reduced CNS dopamine • Increased EPS symptoms • Reduced serotonin receptor function • Enhanced sensitivity to antidepressants • Altered GABA-benzodiazepine receptor function	• Reduced CNS acetylcholine • Enhanced anti-cholinergic side effects • Sedation, confusion, psychosis, delirium • Urinary retention, constipation • Use of drugs with anticholinergic effects are associated with a decline in various measures of cognitive function, especially in the very old or those with pre-existent dementia • Increased sensitivity to benzodiazepine, alcohol, barbiturate

Pharmacokinetics describes how the drug is handled within the body, including the absorption after administration, distribution across the body, biotransformation, and elimination from the body (ADME). With advancing age, a variety of factors ranging from individual drug characteristics (e.g., lipophilicity, degree of protein binding), comorbid diseases and concomitantly taken drugs operate to alter the response of a patient to a drug. Over the recent years, the impact of the various age-related changes affecting the PK and the drug effect and thus, the drug's bioavailability, has been investigated in order to reach the safety use of drug therapy in elderly patients (Massoud et al., 2017).

The process of drug movement from intake (e.g., oral delivery systems) to its site of action is very complex. The active pharmaceutical ingredient (API) availability and its PK and/or pharmacodynamics (PD) are strictly dependent on the product bioavailability (i.e., the rate and extent of drug absorption), the drug characteristics (i.e., lipophilicity, degree of protein binding) and the physiological age-related changes. Accordingly, deviations between the dose and the observed response within or between individuals depends on the ability to metabolize, absorb, excrete, and transform medications.

The repercussion of these pathophysiological changes in the elderly are intimately dependent on the characteristics of the drug that is being evaluated. In the next sections, a general overview of the changes in the ADME processes in the elderly are described.

2.1 *Changes in Drug Absorption and Oral Bioavailability in the elderly*

The process of absorption refers to the movement of the drug from its site of administration to the bloodstream. The rate and extent of absorption depends on several factors including the site of administration, the formulation and the chemical properties of the drug. Understanding the effects of aging on the gastrointestinal system it is fundamental to evaluate the drug absorption process in elderly. When a drug follows the intravenously administration, the drug is injected directly into the bloodstream so it does not undergo the process of absorption and it seems not affected by aging (Hilmer, 2008). By contrast, administration by other routes take into account several parameters that affected the availability of the drug, due to incomplete absorption (Hilmer, 2008).

Oral bioavailability (F) refers to the fraction of an orally administered dose of drug that reaches the systemic circulation in an unchanged form, and graphically it is expressed as the area under the concentration-time curve (AUC). The absorption of drugs via the oral route depends on multiple factors including the physiology of the gastrointestinal tract (GIT), the drug movement across the biological membranes (which may be passive or active) and the drug factors (Massoud et al., 2017).

There's good evidence that gastric and intestinal physiology in geriatric subjects undergoes different morphological and functional changes that may contribute to affect drugs disposition with respect to their therapeutic efficacy and safety (Merchant et al., 2016).

Factors such as gastric pH, gastrointestinal motility, intestinal permeability, integrity of mucosa, gastrointestinal blood flow and last but not least patient–related factors may contribute to affect the bioavailability of drugs by regulating the dissolution, absorption and metabolism of orally ingested drugs (Massoud et al., 2017; Reeve et al., 2015).

Acid secretion by the stomach is an important non-immunological defense against ingested pathogens and is of particular importance for the process of dissolution and absorption (Soenen et al., 2016).

In fact, even small changes in GI pH profile could have a great impact on these processes leading to an alteration of the pharmacokinetics' drugs (Abuhelwa et al., 2016).

When compared to young adults, geriatric subjects experience major physiological changes that may impact drug absorption and bioavailability including, reduced gastric acid production (elevated gastric pH), reduced gastric motility, reduced splanchnic blood flow, reduced intestinal absorptive surface area and reduced function of intestinal drug metabolism and transporters (Sitar, 2007). How pH profile changes with aging is controversial.

A decline in the secretion of hydrochloric acid under basal conditions with aging (condition of achlorhydria) was reported by Hilmer et al. to lead to an increase of GI pH that may affect the extent of absorption for drugs whose solubility is pH dependent (Hilmer, 2008).

On the contrary, the data reviewed by Reeve et al. suggested that the condition of achlorhydria (characterized by consistent hyposecretion) is secondary to very frequent conditions such as, among them, gastric mucosal atrophy, then to factors directly related to the process of aging per se (Hilmer, 2008; Reeve et al., 2015).

Moreover, it has been suggested that the decrease gastric acidity may be a direct consequence of changes in the enzyme secreting cells and organs or hormonal and neural regulatory alterations (Mangoni et al., 2004). This together with other age-dependent factors (proton pump inhibitors, surgery, modification of intestinal flora and intestinal mucus) may predispose the small intestine to an enhanced risk of bacterial gastrointestinal infection (Gavazzi et al., 2002; Soenen et al., 2016).

Conversely, Merchant and other groups of research, reviewed available evidence on gastric pH in the elderly, reporting that apparently elderly subjects maintain the ability to acidify gastric contents compared to young subjects (Merchant et al., 2016; Shi et al., 2011).

Whether gastric emptying changes within aging it is still controversial. Different studies in aging subjects, alone or with young controls, reported no influence in gastric emptying within aging. Madsen et al. performed a study in 16 healthy volunteers of mean age 81 years to assess the propulsive effect of all main segments of the gastrointestinal tract, reporting no influence in gastric emptying within aging (Massoud et al., 2017; Soenen et al., 2015).

On the other side, some demonstrated that in geriatric patients, post-prandial peristalsis and gastric contractile force were reduced (Hunt et al., 2015; Ramsay et al., 2011). More recently researches showed that, although a modest slowing of gastric emptying may occur during aging, the overall rate of emptying remains within the range established for healthy volunteers compared to young subjects (Soenen et al., 2015). The rate of gastric emptying may have implications for the magnitude of the postprandial decline in blood pressure, a phenomenon known as postprandial hypotension. As reviewed by different authors, gastric emptying time resulted different between fluids and food but it is doubtful if there is an age-dependency. Geriatric patients suffer of frequent episodes of gastroesophageal reflux due to an impairment of the peristaltic contractions in the lower esophagus, along with an increase in esophageal acid exposure leading to an increased risk of drug-induced esophageal lesions (Merchant et al., 2016; Ramsay et al., 2011).

Even though the impact of aging on esophageal motility is not completely understood, it has been reported that old people sometimes experience a weakening of the muscles of the esophagus, which contracts less vigorously after swallowing (Bai et al., 2016; Stader et al., 2018). The latter lead to a significant decrease in the amplitude of peristaltic pressures but not duration and velocity (Besanko et al., 2014). Particularly, disturbances in function of the lower esophageal sphincter are more frequent even in healthy, asymptomatic, older adults, along with a slowing of colonic transit secondary due to the loss of local neurons (Corsonello et al., 2010). Colonic motility along with an impairment of the anorectal function have been correlated to GI disorders very common with aging such as dyspepsia, diarrhea and constipation (Shi et al., 2011; Soenen et al., 2016). A summary of the effects of ageing on the human gastrointestinal environment is presented in Table 7.

Even all these changes do not seem to significantly change the total absorption process, but represent a risk factor contributing to enhance drug-drug interactions (Massoud et al., 2017). Moreover, depending on the kind of medications, changes in gastric and intestinal motility could have a different potential effect. Poorly soluble drugs (such as carbamazepine) could have an increased of total absorption consequently to the increased transit time. Conversely, highly soluble drugs show a delayed in the absorption process leading to a reduced maximum concentration (Reeve et al., 2015). The bioavailability of some antiepileptic drugs, to give one example, resulted affected by the gastric pH and gastric emptying impairments (Corsonello et al., 2010). Nevertheless, generally the total extent absorption doesn't seem to undergo significant changes (area under the curve AUC), whereas the rate (lower peak concentration and longer time to peak) results altered (Currie et al., 2011; Reeve et al., 2015). However, the high interindividual variability, comorbidities and the increased consumption of medication in elderly may affect gastrointestinal motility in different way, avoiding

the possibility to provide unique, safe and effective drug treatment guidelines (Reeve et al., 2015; Shi et al., 2011).

Table 7. Effects of ageing on the human gastrointestinal environment

(Extracted from Merchant et al., 2016)	**HEALTHY ADULT (18-65y)**	**ELDERLY (65-83y)**
pH stomach	1.5	1.1-1.6
pH intestine (duodenum)	6.4	6.5
(Extracted from Burckart et al., 2016)	**HEALTHY ADULTS (mean 30y)**	**HEALTHY ELDERLY (mean 75 y)**
pH stomach	1-2.5	1.3 (1.1-1.6)
pH intestine(duodenum)	5-6.5	6.5
Total GI transit	69 ± 5 h	76 ± 6 h
Gastric emptying time	mean: 1.36 h (0.53-1.96 h) (liquid marker) mean: 2.01 h (1.03-2.73 h) (Solid marker)	mean: 1.37 h (1.07-1.85 h) (liquid marker) mean: 2.07 h (1.32-3.03 h) (solid marker)
Small intestinal transit time	mean: 3.95 h (1. 52-6.6 h) (liquid marker) mean:3.63h (2.4-5.89 h) (solid marker)	mean: 4.95 h (2.21-8 h) (liquid marker) mean: 4.77 h (2.53-8.13 h) (solid marker)
Colon	mean: 39 h	mean: 66 h

Due to intestinal atrophy, there seems to be a decrease of the surface area for drug absorption which, together with the reduced concentration gradient caused by the poorer splanchnic blood flow may delay absorption rate for some drugs. As we age, the small intestine does not seem to undergo major structural changes. Nevertheless, a progressive thinning of the wall and the presence of irregular and rarefied villi have been described, along with a decrease of the intestinal plasma membrane calcium pump proteins (Corsonello et al., 2010). This condition seems to slow the absorption of substrates that are actively transported, such as (vitamin B12, iron and calcium) and it may affect drugs that are transport through active mechanism (Duraković et al., 2013). Passive intestinal transport of drugs such as penicillin, diazepam and metronidazole remains largely unaltered with aging (Reeve et al., 2015). Few exceptions include digoxin, ciprofloxacin and indomethacin whose process of absorption seem to be slowed down (Massoud et al., 2017). Furthermore, a decline in Vitamin D with age has been reported. The duodenal expression of the TRPV6 (calcium channel transient receptor-subfamily V- member 6) vitamin D-dependent proteins reduced leading to the lowering of calcium absorption,

especially evident in postmenopausal women where a decline in vitamin-D receptors has also been reported.

2.2 *Changes in Drug Distribution in the elderly*

After a drug enters the systemic circulation, either directly via intravenous or after oral administration, it is distributed to the body's tissues.

The distribution of drugs in the older person can vary due to different factors, such as changes in critical organ perfusion and changes in body composition. As the body ages, cardiac output is often reduced and peripheral vascular resistance increases, leading to a decrease in total systemic perfusion of the vital organs, including the kidneys and liver. This reduction in perfusion can decrease the body's ability to distribute, metabolize and excrete drugs. Moreover, drug distribution is dependent upon body composition (Reeve et al., 2015).Thus, estimation of the body composition can be of great significance. Unfortunately, little is known about age-related changes in body composition in elderly adults. Generally, even if several methods are available to estimate body composition, it's easier to obtain information in younger that in the elderly age groups. In addition, the majority of the reports in the literature on the body composition of healthy elderly adults are generally based on the determination of only one variable, besides total body potassium or total body water, or body density measurements. The assessment of body composition in the elderly could change depending on the techniques that are used to detect them. The bioelectrical impedance analysis (BIA) is a very common non-invasive, rapid technique that can be easily applied to assess body composition in population studies. In fact, Bia systems are calibrated for the population under study by developing prediction formulae based on criterion methods from resistance, stature and other easily acquired variables. It has been found to well predict variables such as TBW, ECW and ICW in elderly patients, even in critical illness conditions (Powers et al., 2009). Nevertheless, there is a paucity of data on differences between methods for the assessment of body composition in elderly subjects and nowadays, there is not a universal formula.

Aging is associated with sarcopenia, defined as a reduction in muscle mass and function, a decrease in bone mass and an increase in body fat. Most studies have reported an overall increase in body fatness ranging from 20-40% as people grow older. Apparently, FM increases up to 60-70 years, after which appears to stabilize (Schlender et al., 2016). Particularly, the pattern of body fat distribution within the body resulted altered with aging and it's characterized by a reduction in subcutaneous fat and an increase in visceral and intramuscular fat (Byambasukh et al., 2015; Rossi et al., 2008). In addition, a progressive reduction in fat-free mass (FFM), body cell mass as well as in muscle mass has been also reported (Dey et al., 2003; Kyle et al., 2001). The loss of skeletal muscle mass, the so called "sarcopenia", has been associated with weakness, disability and morbidity. Generally, the

amount and distribution of body fat, muscle, and water compartments play a critical roles influencing physical functional status, nutritional and endocrine status, quality of life, and comorbidity in elderly people, such as Diabetes type II and heart disease (Yamada et al., 2009). It has also been claimed that the increase body fat likely leads to an increase in proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α.

Adipose tissue enhances the volume of distribution for highly lipophilic drugs, prolonging their elimination half-lives because of the re-diffusion of the drug from the fatty tissue. Drugs such as diazepam, chlordiazepoxide, lignocaine fall into this category and a continue use of them may result into accumulation. On the other hand, for hydrophilic drugs, that are mainly water-soluble, the V decreases leading to higher plasma concentration levels in elderly. Decreased V has been observed for drugs such as Digoxin, ethanol, cimetidine and H2 antagonists (Massoud et al., 2017). Nevertheless, for most drugs this change is unlikely clinically important (Shi et al., 2011).

Mean values for total body water (TBW) are reported to range from about 38 to 46 L in white men and 26 to 33 L in white women (Chumlea et al., 2001). Total body water consists of two compartments: ICW, intracellular water and ECW, extracellular water. ICW comprises the fluid in muscle and organ cell, whereas ECW amount to plasma, interstitial fluid and connective tissue fluids (Powers et al., 2009). A decrease of total body water (TBW) by 10-15%, due to a change of the ratio of intercellular to intracellular water, has been reported (Corsonello et al., 2010; Currie et al., 2011). Other studies evaluating the hydration state and body water distribution in healthy elderly subjects (mean age 75) reported that TBW represents mainly the 72% of FFM both in males and in females, whereas the ECW the 47% of TBW (Sergi et al., 2004). TBW results affected by different conditions or diseases frequently geriatric subjects. As a consequence of these age-related changes, the volume of distribution (V) resulted affected (Massoud et al., 2017). The apparent Volume of distribution (V) represents an important theoretical volume of fluid into which the total drug administered would have to be diluted to produce the concentration in plasma. It provides a reference for the plasma concentration expected for a given dose and it is defined as the drug concentration in the body divided by its concentration in the blood (Lee Goldman, 2017).

In addition to changes in body composition, altered concentrations of plasma proteins may affect the distribution of highly protein-bound drugs. Drugs can circulate in two forms, that is to say bound or unbound to plasma proteins, and only unbound (free) drug has a pharmacologic effect. Thus, alterations in drugs-binding proteins may determine a higher pharmacological activity of drugs due to an increase of serum level of unbound (free) drug (fu) that is also more available for metabolism and elimination. This is mainly true for very highly bound drugs with a small V (Reeve et al., 2015;

Shi et al., 2011). The major plasma-binding proteins are albumin, lipoprotein and α1-acid glycoprotein that can influence the pharmacokinetics and pharmacodynamics properties of drugs.

Many factors could affect plasma proteins levels such as renal disease, hepatic disease, trauma, stress, surgery, pregnancy. Plasma proteins levels have been reported to change with aging (Reeve et al., 2015). A number of studies have been performed in older patients to evaluate the variations of both albumin and α1-acid glycoprotein serum levels. Albumin have different important physiological functions including regulation of colloidal osmotic pressure, transportation of a wide variety of compounds, among them, fatty acids (FA), hormones, bile acids. Moreover, albumin binds a vast array of drugs, mainly acidic drugs thus, reducing the free concentration of compounds, as well as limiting their biologic activity, distribution, and rate of clearance. Albumin seems to decrease of 10-15% in older adults, likely because of the increased elimination via the kidneys. A further decrease in albumin concentration could be enhanced by chronic conditions including arthritis, cancer, and Crohn's disease (Reeve et al., 2015). Reeve et al in its review list other studies conducted on drugs highly bound to albumin, such as benzodiazepines, or warfarin whose unbound fraction resulted decreased. Nevertheless, it was highlighted that even though a statistically significant reduction in albumin serum concentration was found in older subjects compared to younger compared groups, there was no correlation between the amount of unbound fraction and protein binding (Reeve et al., 2015). Several studies have reported a decline of albumin with aging. Albumin seems to decrease of 10-15% in older adults, likely because of the increased elimination via the kidneys (Cooper et al., 1989; Salive et al., 1992; Veering et al., 1990). Stader et al. reported a decline in albumin concentration of about 1.5% in each age decade and proposed a descriptive equation able to predict age-dependent albumin levels (Stader et al., 2018).

Bilastine has also been demonstrated to be a substrate of another important binding proteins: the P-glycoprotein (P-gp). P-gp is a cell transmembrane protein expressed mainly in enterocytes of small intestines, but also, in lymphocytes, liver, kidneys and blood-brain barrier. It is a critical efflux transporter for many medications, actively transporting them back to the intestinal tract, contributing to decrease their absorption (Reeve et al., 2015). As reported by Gabardi et al., a vast array of drugs, including immunosuppressive drugs, calcium-channel blockers and corticosteroids, are substrates of the P-gp. An increase of lymphatic and hepatic P-gp expression had been observed with age, whereas P-gp content of the kidneys declined in an age-dependent manner (Gabardi et al., 2015).

A decline of the cerebrovascular P-gp protein has also been observed and has been related to the accumulation of toxic substances in the brain that may lead to a higher risk of neurodegenerative pathology frequently occurring in geriatric patients (Corsonello et al., 2010; Massoud et al., 2017).

In practice, even if there is good evidence of age-related changes in serum proteins concentrations, it seems that these changes do not exert a significant clinical effect on the total exposure to the drug. This is mainly due to an increased availability of the free drugs at the elimination organs that balance the potential higher peak plasma concentration (Mangoni et al., 2004; Shi et al., 2011).

2.3 *Changes in Drug Metabolism in the elderly*

In general, drug metabolism occurs mainly in the liver with minor contributions from the kidney, lungs, skin and intestine, resulting in a conversion of the active drug into a less active or inactive substance (Reeve et al., 2015).

With aging, the liver undergoes functional and phonotypical changes. Hepatocytes' number has been reported to decrease along with the hepatic volume and the liver blood flow whose reduction has been estimated to be of 20-30% and of 30-50%, respectively (Massoud et al., 2017).

Hepatic metabolism takes part in the biotransformation of the vast majority of drugs to more polar metabolites by several cytochrome P450 (CYP)-dependent phase I reactions (oxidation, reduction and hydroxylation) and/or phase II pathways (conjugation with glucuronate, sulphate or acetate) (Hilmer, 2008; Reeve et al., 2015; Shi et al., 2011). Drug metabolism is dependent on liver blood flow, protein binding, volume of distribution and drug transfer into hepatocytes. As reported by Reeve et al., with aging, both hepatic blood flow and liver size seem to decrease by 20-50 % (Reeve et al., 2015). The drug clearance by the liver depends on the rate of elimination of a drug from the body and the plasma concentration [*CL (l/h) = Rate of elimination (mg/h)/ Cp (mg/l)*].

Therefore, the reduction of hepatic blood flow affects the rate at which a drug is entering the hepatocytes and thus their capacity to metabolize the drug. For drugs with decreased hepatic metabolism, it has been reported a clearance decreases of 30 to 40%. Nevertheless, in the case of bilastine, since it is not affected by hepatic metabolism, the previously reported changes do not affect the drug clearance. First-pass metabolism (metabolism, typically hepatic, that occurs before a drug reaches systemic circulation) is also affected by aging, resulting in increased bioavailability of orally administered drugs (Hilmer, 2008). There's good evidence that advancing age is associated with a decrease in first-pass metabolism probably due to a reduction in liver mass and in hepatic blood flow (Reeve et al., 2015; Shi et al., 2011). This change likely affects drugs undergoing extensively first-pass metabolism. Moreover, bioavailability of prodrugs like ACE inhibitors (codeine, enalapril, perindopril), that need first-pass metabolism to be activated, may be reduced or slowed down (Massoud et al., 2017).

2.4 *Changes in Drug Elimination in the elderly*

Drug elimination mainly takes place in the kidney by glomerular filtration, tubular excretion or by both ways. Aging is accompanied by various renal changes, among them, the reduced glomerular filtration rate (GRF), reduced tubular function and renal blood flow (Shi et al., 2011).

The main challenge in evaluating the GFR's percentage of decrease with age consists in identifying the normal references values in the elderly (over 65 years of age). In fact, even though a physiological GFR decline with aging has been suggested and confirmed by many studies, defining general reference values remain still controversial. There are some doubt on the possible onset of GFR decline (Hilmer, 2008; Reeve et al., 2015). Renal mass and volume decrease of 15-30% leading to a reduction in nephrons (Delanaye et al., 2012). The number of glomeruli decreases, and the mass of the juxtamedullary nephrons falls resulting in a decreased in the filtration area of the glomerular basement membrane and decreased permeability. With aging, glomerular filtration rate (GFR) has been reported to reduced 1% per year of life (Delanaye et al., 2012). The GRF represents the volume of glomerular filtrate produced per unit time by all nephrons and it is about 120 mL/min in young healthy people. With aging it has been estimated that the GFR reduced by 15-40% (Massoud et al., 2017).

Moreover, various diseases like hypertension, vascular diseases and diabetes may contribute to the decline in renal function. Despite the decline in glomerular filtration rate, there is no concomitant increase in plasma creatinine because of age-related loss of muscle mass. Therefore, creatinine is not a reliable indicator of glomerular filtration rate in the elderly subject. Creatinine clearance results decreased; however, the age-related decrease varies substantially from person to person (Massoud et al., 2017).

3 Changes affecting pharmacodynamics in elderly

Pharmacodynamic changes (response to drug dosing) with age are less studied and known compared to pharmacokinetic changes. In fact, to measure drug response at site of action especially when the mechanism of action is not known it is not easy. Moreover, pharmacodynamic response depends on receptor number and affinity, signal transduction mechanisms, cellular responses, and homeostatic mechanisms along with inter-individual variability (Hilmer, 2008).

Pharmacodynamic changes may occur at variety of sites in the body using various drug-receptor interfaces and through number of mechanisms. Human body is a complex system and it is difficult to investigate abnormality with a good precision. It is possible to conduct in vitro and animal experiments to differentiate and address various scientific issues between receptors and/or post receptor changes (second messenger mechanisms); however, extrapolation from animal data to human data further complicates the situation. In general, pharmacodynamic response declines with

age and may be explained by number of factors such as changes in receptor number and affinity, changes in CNS and changes in reflux responses (Abdulkader et al., 2017).

Generally, age causes change in receptor density, affinity, and the ability to activate second messengers in signal cascades impacting the pharmacodynamics response in elderly population. As observed by different authors, cholinergic dysfunction and memory loss in aged rats due to decreased number of muscarinic acetylcholine receptors with aging. Moreover, a decrease in number of μ opioid receptors, as well as, a decrease in opioid peptide content is reported. Specific drugs binding to these receptors lead to increased impotence, hypodipsia, anorexia like behavioral changes in elderly population.

Age causes diminished calcium responsiveness and changes in calcium mobilization, which is required for different functions including secretion, neurotransmission, muscle contraction, and cell division. Thus, diminished calcium responsiveness could affect all these processes requiring calcium. The sensitivity of CNS acting drugs get altered with age, e.g., benzodiazepines, tricyclic antidepressants, barbiturates, opiates etc (Mangoni et al., 2004).

Significant reduction in the half maximal effective concentration (EC_{50}) was observed in elderly population due to increased sensitivity of midazolam in older patients. Besides age, blood supply to the brain may get compromised by atherosclerotic narrowing of vertebral and carotid systems in elderly population leading to neuronal loss and altered drug sensitivity. Sensitivity to anticoagulant drugs also increases with age. Although there were no significant age dependent pharmacokinetic differences reported in case of warfarin, increased effect, and risk of bleeding is reported in elderly subjects when same dose of warfarin is administered to elderly and young adults likely due to increased intrinsic sensitivity of warfarin with age. Therefore, lower initial and standard doses are recommended in elderly patients (Massoud et al., 2017). Similarly, increased sensitivity to anticoagulant effects of dabigatran was observed in elderly patients, and lower doses of dabigatran are recommended in patients 80 years of age or above. Elderly population is less sensitive to baroreceptor reflex and responsiveness (Sharma et al., 1998; Turnheim, 2004). Because of these changes, they are more prone to postural hypotension and bradycardia when they take nitroglycerin, diuretics, phenothiazines, and peripheral α-blockers. It is suggested that these symptoms are due to increased vascular smooth muscle action of nitrates. In conclusion the pharmacodynamics changes occurring with age have to be considered in development and prescription. This might not only relate to the prescribed dose but also to the risk-benefit assessment of specific drugs for older patients due to the declining homeostasis, increasing vulnerability and adverse drug reactions severity. For example, the increased risk for hypotension with antihypertensive drugs or the increased sensitivity

for CNS drugs increases the risk for falls, which are a major factor for mobility loss (Midlöv P., 2013).

4. Modelling and Simulation as a tool to define and optimize dosing in geriatrics

Pharmacometrics, defined as the science that quantifies drug, disease and trial information to aid efficient drug development and/or regulatory decisions, provide a powerful tool to characterize, understand, and predict a drug's pharmacokinetic and pharmacodynamic behavior. It helps in quantify uncertainty of information about that behavior, and allow to rationalize data-driven decision so that the right dose can be given to the right patient to maximize drug efficacy and reduce drug toxicity (Ette EI, 2007).

The physiological and biochemical differences between young adults and old adults determine differences in the pharmacokinetics and pharmacodynamics of a drug. Understanding the mechanisms involved in the PK and PD pharmacokinetic and pharmacodynamic behavior of a drug is essential to properly characterize its dose-effect relationship. The relationship between the administered dose and the drug concentration in blood or plasma is known as pharmacokinetics (PK, what the body does to the drug), whereas the relationship between drug concentration and observed response is defined as pharmacodynamics (PD, what the drug does to the body).

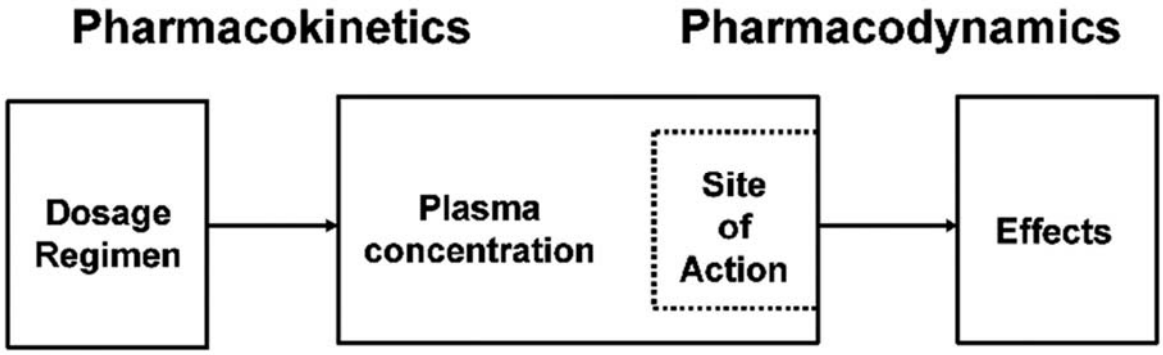


Figure 8. Schematic illustration of dose-effect relationship through pharmacokinetics and pharmacodynamics. The hypothetic effect compartment bridges the pharmacokinetic with the pharmacodynamic model *(Adapted from Rowland and Tozer 2nd Ed.)***.**

Figure 8 represents in a schematic form the processes taking place between drug administration and response, including PK, distribution to the biophase and the different steps between target engagement and measured response. As soon as a drug enters the body, the response variable will display a certain profile over time that is influenced by the PK properties of the drug.

PK can be expressed in different ways: (1) characterization of the time course of the concentration of the active compound(s) [drug and/or metabolite(s)], generally in plasma, or (2) what the body does to the drug. Proper PK practice involves a modelling exercise with the aim to get precise PK

parameters that accurately represent and quantify the main physiological process responsible for the fate of the drug in the body. One major PK goal is gaining understanding and resolving the time course and factors determining drug access to target sites. This is known as "biophase distribution".

Pharmacodynamics (PD) refers to the relationship between drug concentration, generally measured in plasma (Cp), at the site of action (receptor) and the observed pharmacologic response. This drug–receptor interaction initiates a cascade of events resulting in a pharmacodynamic response or effect.

The functional exposure–response relationships could be expressed in the form of a linear or loglinear relationship, an Emax model, a Hill equation, or an indirect response model. When any of these models are estimated, then the relationship between dose and the PD outcome can be predicted by linking dose–PK/PD–biomarker. An outcomes model translates some surrogate endpoint or biomarker (QTc, blood pressure, international normalized ratio, etc.) into a clinical endpoint such as cure or no cure, improved versus worsened, survival, time to event, disease progression, or wellness score.

The two main pharmacodynamic parameters of a drug are the maximum effect (E_{max}) and the concentration producing 50% of the maximum effect (C_{50}). The C_{50} is also known as EC_{50}.

Reversible pharmacodynamic effects of drugs can be broadly classified as direct and indirect responses. In direct PD model the drug is directly responsible for the pharmacodynamic response being measured. One example of a direct PD model is the pharmacodynamic response to moxifloxacin. As moxifloxacin concentrations increase, the QT interval also increases. Thus, the PD measure (QT interval) is directly related to the drug (moxifloxacin) concentration (Deshpande et al., 2010).

The indirect PD model is slightly different in that the drug does not directly affect the pharmacodynamic response. Instead, the drug affects a precursor which then influences the pharmacodynamic measure. In this model, the precursor is converted into or secreted as the response variable or mediator which, in turn, is then removed from the system. Drugs with indirect actions can produce their effects by acting on one or more of the indicated steps shown in this model. The agent can cause inhibition or stimulation of the synthesis or secretion of the response variable or of its removal, or of processes leading to the production of precursor. An indirect response model was developed to describe the pharmacodynamic relationships between flare or wheal areas and bilastine plasma concentrations (Figure 9). Bilastine was shown to dose-dependently inhibit ^{3}H-pyrilamine binding to H_1 receptors in the guinea pig cerebellum and similar findings were obtained in a human embryonic kidney cell line. Moreover, additional *in vitro* studies demonstrated that bilastine had no significant antagonist activity at a diverse range of other receptors: H_2, H_3, and H_4, N-type voltage-

dependent calcium receptors, and M_1-M_3 muscarine receptors (Jauregizar et al., 2009; Jàuregui et al., 2012).

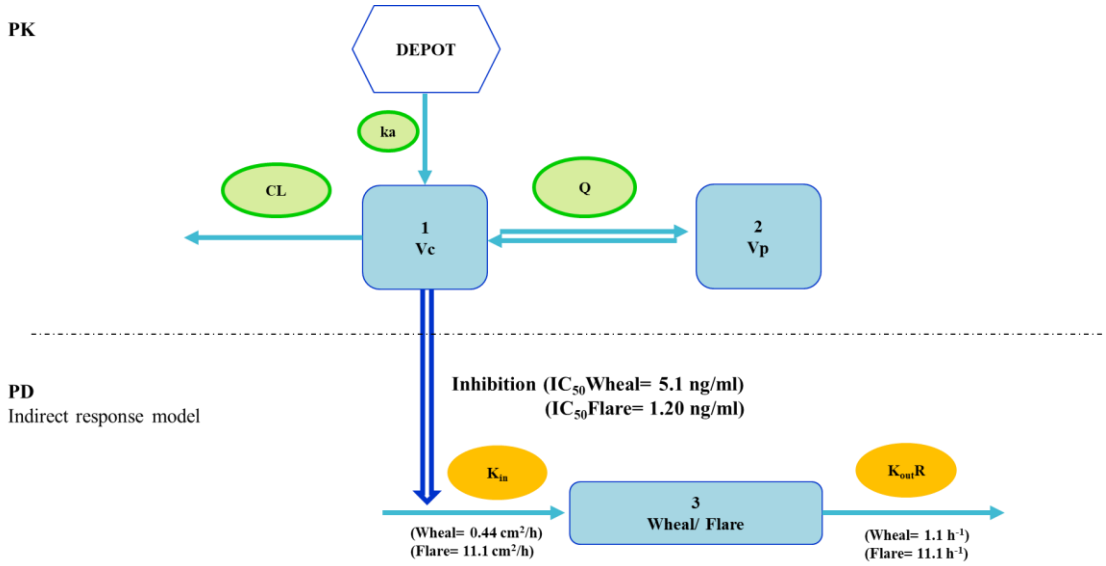


Figure 9. Schematic representation of the indirect response model proposed for bilastine in adults from Phase 1 studies. kin= zero-order rate constant for production of response, kout= first-order rate constant for loss of response; R= response *(Adapted from Jauregizar et al., 2009)*

4.1 *Methods of PK or PK/PD Data Analysis*

The major types of PK data analyses are non-compartmental, compartmental, and physiological.

4.1.1 *Noncompartmental analysis (NCA)*

Noncompartmental analysis (NCA) is used to identify certain pharmacokinetic parameters without deciding on a particular compartmental structure. Analysis is rapid and simple. By the way, NCA methods require intensive pharmacokinetic sampling in patients, and also have limited usefulness when it comes to predict or simulate what will happen upon drug administration in other situations e.g., dose adjustment. The results are purely descriptive and non-predictive unless the function selected is linked to physical phenomena (e.g. 1st order kinetics) (Bulitta et al., 2014).

Nevertheless, noncompartmental analysis (NCA) is able to rapidly provide information about drug exposure, through variables such as the area under the plasma concentration curve (AUC) and the moment curve (AUMC) of a drug concentration-time graph. and perhaps the drug's associated pharmacokinetic parameters, such as, elimination half-life, T_{max}, C_{max}, clearance given that it solely depends on AUC and the administered dose, thus representing a model independent parameter that is not modified regardless of the type of model employed in its calculation (Gabrielsson et al., 2012).

However, this kind of analysis does not allow obtaining parameters and, consequently, it is not possible to predict or simulate what will happen upon drug administration in different situations.

4.1.2 *Compartmental PK Analysis*

Compartmental modelling describes and predicts the concentration-time curve based on the movements of the drug between compartments (kinetic or physiological model). They are based on a system of ordinary differential equations that can be used to predict the concentration at any time.

The pharmacokinetic characteristics can be quantitatively expressed by its parameters, such as the elimination rate constant (denoted as *K*), half-life ($t_{1/2}$), apparent volume of distribution (*Vd*) and total clearance rate (*CL*).

Vd is defined as the relation between dose and initial concentration (Vd= Dose/Co). Volume of distribution indicates the extent of drug distribution that depends on both body composition and physicochemical properties of the drug (chemical structure, molecular weight, lipophilicity, dissociation constant, etc.). For example, a large volume of distribution usually indicates that the drug distributes extensively into body tissues and fluids. Conversely, a small volume of distribution often indicates limited drug distribution (Ahmed, 2015).

CL, in a first order process, is the relation between elimination rate (or distribution rate, for intercompartmental clearances) and concentration in blood or plasma. Clearance is a parameter independent of the complexity of the kinetic model (mono-bi or tricompartmental) and is calculated simply as Dose / AUC, thus demonstrating its relevance for dose extrapolation or prediction. Systemic clearance makes reference to the global elimination of the drug from the body, which can be integrated from data describing the different processes involved (in vitro studies on metabolism, excretion in urine and/or feces, etc.). After infusion or multiple dosing, CL is related to the steady state concentration (Css) or average Css (dose= infusion rate / CL), so this parameter can be used to predict the dose regimen (maintenance dose = target Css x CL). Both CL and Vd are primary parameters and are directly related to the physiological processes of the organism giving information on where the drug is distributed and the organs which eliminate it.

Other PK parameters, commonly employed in dose adjustment, are the elimination half-life ($t_{1/2}$) and the elimination rate constant (K_{el}), mixed parameter depending on both CL and Vd (K_{el}= CL/ Vd).

Nevertheless, the development of the model may be difficult. The simplest PK compartmental model is the one compartmental PK model with IV bolus administration and first-order elimination. The most complex PK models rely on the use of physiological information to ease development and validation. Compartmental models are mathematical descriptions typically used for biological organisms; in these types of modelling, an organism is considered as subdivided into communicating parts called compartments (mono-, bi-, tri-, and multi-compartmental models) (Rescigno, 2010).

Each compartment is characterized by a variable with homogeneous chemical-physical properties. The distinguishing feature of this model is that it addresses phenomena dynamic, e.g., those

phenomena that have a time-dependent variability. So, between a compartment and the other there are exchange of matter and typically this is accompanied by a temporal variation of the concentration in each single compartment. The purpose of applying this model is to know how the concentration C of a drug varies over time in the various parts of the body (modelled with a number of compartments). Moreover, the compartmental kinetic parameters defining the dose-concentration relationship may be interpreted from a physiological (and even physio pathological) perspective thus rendering extremely valuable information. This allows their extrapolation from e.g., healthy adults to other special populations such as patients, elderly or children based on a semi-physiologic approach like the one presented in this work. For example, the volume of distribution, which establishes a link between the administered dose and the reached or targeted initial concentration, is related to physiological volumes (blood volume, total body water, etc.) given that it ultimately depends on both body composition and physicochemical properties of the drug (chemical structure, molecular weight, lipophilicity, dissociation constant, etc.). Clearance is a first order process (i.e., the relationship between rate and concentration) that may be expressed as the ratio between the administered dose and the resulting area under the plasma concentration curve, thus demonstrating its relevance for dose extrapolation or prediction. Systemic clearance refers to the global elimination of the drug from the body, which can be integrated from data describing the different processes involved (in vitro studies on metabolism, excretion in urine and/or feces, etc.). Both elimination half-life and elimination rate constant are mixed parameters derived from other primary parameters, their physiological interpretation being consequently more difficult.

The one-compartment open model is the simplest way to describe the process of drug distribution and elimination in the body. This model assumes that the drug can enter or leave the body (i.e., the model is “open”), and the entire body acts like a single, uniform compartment.

For those drugs showing a bi- or tri-exponential decay in their pharmacokinetic profiles, it is necessary to characterize additional parameters, such as the intercompartmental distributional clearance(s) and the volume of distribution of the peripheral compartment(s).

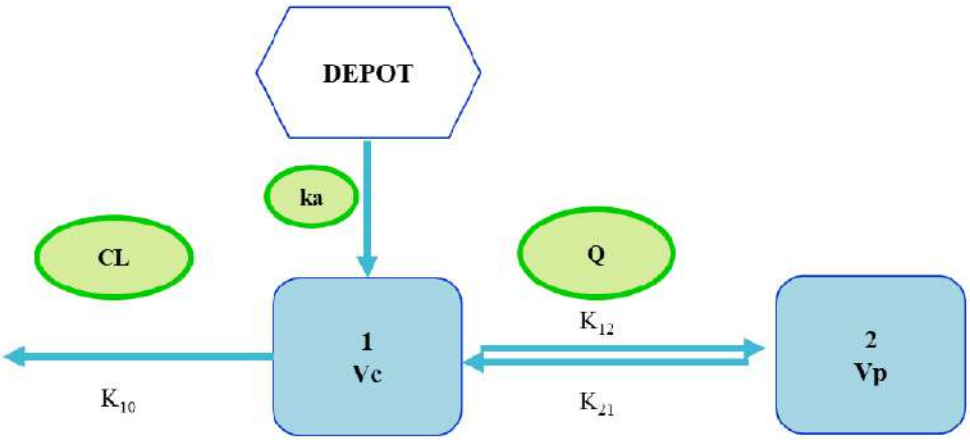


Figure 1. Schematic representation of a bicompartmental pharmacokinetic model. CL systemic clearance, Vc and Vp volumes of distribution of the central and peripheral compartments, k transfer rate constants between the central and the peripheral compartments.

Pharmacokinetic of bilastine is described by a two-compartment model that considers the body divided into central and peripheral compartment (Figure 1). The central compartment (compartment 1) consists of blood and the highly perfused organs and tissues such as heart, brain, lungs, liver, and kidney where the distribution of the drug is practically instantaneous. The peripheral compartment (compartment 2) includes those organs that are less well-perfused such as adipose and skeletal muscle, and therefore the administered drug will equilibrate more slowly in these organs.

4.1.2.1 *Population Pharmacokinetic Modelling (PopPK Analysis)*

The most widely used approach in PK analysis is the population analysis, in which all data from all subjects in the study are included simultaneously, but the individuality is preserved. Each patient contributes to characterize the rest of the population and contributes to generate the typical (and individual) profiles. Population modelling represents a tool to identify and describe relationship between a subject's physiologic characteristics and observed drug exposure or response providing a complete quantitative characterization of the PK profile of a drug in the target population (Li, 2020). Population models are comprised of several components: structural models, stochastic models, and covariate models. Structural models are functions that describe the time course of a measured response and can be represented as algebraic or differential equations. Stochastic models describe the variability or a random effect in the observed data, and covariate models describe the influence of factors such as demographics or disease on the individual time course of the response (Upton, 2012, 2013). The most widely used software packages that can be used to develop population PK and PK/PD models are NONMEM and MONOLIX.

4.1.3 *Physiologically-Based Pharmacokinetic (PBPK) Modelling*

The physiologically based pharmacokinetic (PBPK) model is a compartmental model but differs from classical pharmacokinetic models in that the compartments represent actual tissue and organ spaces and their volumes are the physical volumes of those organs and tissues.

Physiologically based pharmacokinetic (PBPK) models have increasingly been employed in children, pregnant women and health-impaired individuals showing a value power and applicability for the prediction of drug disposition in special condition or in special population (Bjorkman, 2005a; Encinas et al., 2013; Vozmediano et al., 2019).

Physiologically-based pharmacokinetic (PBPK) modelling is a computational approach that simulates the absorption, distribution, metabolism and elimination (ADME) of chemical substances in the bodies of organisms. PBPK models consist of systems of differential mass balance equations representing biological tissues and fluids as well as physiological processes.

In PBPK models, parameters are assigned using physiological measurements (blood flow, organ sizes) and resolved by direct analysis of plasma concentrations and tissue transport, binding, and metabolic properties. They have great value in translating preclinical data to man, anticipating changes of physicochemical properties of drugs, and assessing the impact of altered physiology such as occurs in geriatrics.

In fact, these methods can be optimize by the inclusion of physiology related process, thus, optimizing their predictive performance allowing to describe the pharmacokinetics of drug in different populations and to distinguish between age- and disease-related physiological alterations and to evaluate and confirm dosing recommendations in specific populations (Sinha et al., 2014; Stader et al., 2018). Two main different approaches can be employed in the developing of PBPK models. the "top down" approach is mainly based on observed experimental data. A limitation of this approach is that the empirical data obtained are only relevant to the range of the input data. The 'bottom-up', on the other hand, represents a more mechanistic approach integrating a large number of chemical-specific data, physiological or anatomical parameters as well as pharmacokinetic processes. It allowed to describe the relationship between variables based on causative underlying principles (Utembe et al., 2020). Top-down and bottom-up approaches could be combined in a 'middle-out'-approach allowing the utilization of available *in vivo* information as well as the determination of unknown or uncertain parameters. Generally, predictive models may be classified as deterministic or probabilistic (stochastic) depending on the nature of the input variables. Fixed values of the input variables, while probabilistic models can take into account the uncertainty and variability in one or more of the input parameters.

4.1.4 *Semi-physiologically based modelling approach*

Semi-physiologically based modelling represents a mechanistic option that can be used for predicting or extrapolating drug disposition across aging and differs from an empirical model that is based on direct observation, measurement, and extensive data records. On the contrary, a mechanistic model is based on an understanding of the behavior of a system's components.

A distinguishing feature of mechanistic models over machine learning models is their ability to assume that a complex system can be understood by examining the workings of its individual parts and the manner in which they are coupled. This typically involves constructing mathematical formulations of causal mechanism and using analytical tools to validate these hypotheses with observed data.

The term 'semi-physiologic' refers to the fact that this approach meets in the middle of a standard population PK/PD analysis and a full physiologically based pharmacokinetic analysis because both drug specific and system specific information is combined or integrated into a simple model. Indeed, this method is based on the physiological connection to PK/PD characteristics and model parameters for each age population, which means that key principles from physiology is taken into account in combination with the non-linear mixed-effect modelling approach for estimation of population PK/PD parameters across the geriatric population.

All the described methods of analysis can be employed for PK/PD prediction with advantages or disadvantages depending on the type of data available. The semi mechanistic approach proposed in this work integrated the available drug knowledge concerning disposition in adults as well as the impact of the physiological changes on the PK of bilastine followed by the application of modelling and simulation techniques.

Objectives

Objectives

This research was designed to acquire a deeper knowledge on how physiological changes during aging may affect the pharmacokinetics (PK) (absorption, distribution, biotransformation and elimination (ADME)) and pharmacodynamics (PD) (the biochemical and physiological effects of a drugs at their site of action).

The general objective of this project has been to establish if the use of predictive PK/PD models that include changes in the ADME processes as a function of age, already applied to pediatrics, will be also predictive in the geriatric population.

The antihistamine drug bilastine has been employed as a probe drug, on the one hand due to the wide knowledge of the research team on this drug, and on the other hand as a PK/PD data package, although limited, was available in geriatric patients to inform the model building and to validate the predictions.

The following concrete objectives were proposed:

- To investigate the differences between young and old adults and identify the potential physiological factors that can affect the PK and PD of bilastine
- To construct a metadatabase based on a structured literature search for aging Caucasians including information of anatomical, (patho)physiological, and biological system parameters with the associated variability both in adult and elderly subjects, and also separating the data by gender. Adult data will be used as the starting point for model development and scaling equations (developed with public domain data) will be included in the different ADME processes
- To elaborate pharmacometric strategies that allow integrating all the acquired knowledge into a predictive model in geriatrics that allows to establish adequate/optimal dosing recommendations for this vulnerable population
- To analyze all collected data for the generation of mathematical models as a tool to identify and describe relationships between subject's physiologic characteristics as a function of aging and observed drug exposure or response that could be used to obtain the required information for proper dosing in the elderly
- To develop and compare different approaches using mathematical relationships that are capable of defining the changes that occur with age in each of the ADME processes involved in a drug's PK linked with the PD in order to establish the dose-response relationship in the elderly

The present doctoral thesis project comprises 3 sections as briefly described below:

Section 1: Influence of Age in the Pharmacokinetics and Pharmacodynamics of Bilastine in Healthy Volunteers: Development of a combined adult and geriatric model

The data from study BILA-495-05 including PK and PD data from young and elderly healthy subjects aged 18-80 years has been assessed. The differences between young and old adults have been explored using PK/PD modelling and simulation. The PK/PD model structure already identified as suitable for young adults receiving bilastine (Jauregizar et al, 2009) and the flare surrogate indirect effect was applied to the data from study BILA-495-05 and the effects of age and gender were evaluated both on the pharmacokinetic profile of bilastine and on the inhibitory effect of bilastine on histamine-induced effect. The knowledge gathered during this step has been used to identify and to extend the understanding of the physiological pathways playing a major role in the drug's PK/PD behavior and the changes induced by aging.

Section 2: Development and evaluation of a Senescence predictive PK model in the geriatric population

Public domain data has been used to develop a metadatabase that is able to serve as a basis for establishment of mathematical relationships that are capable of defining the changes that occur with age in each of the ADME processes involved in bilastine's PK. A predictive PK model, (Senescence model) was then developed by using the young adult-based model and including scaling equations obtained from the metadata base exercise. The scaling equations accounted for changes on the absorption, distribution and elimination processes with aging as well as individual subject's demographics that could help individualize the predictions. The PK Senescence model based on PK scaling as it is considered a surrogate of efficacy and similar between adults and geriatric subjects as concluded in Section 1. Moreover, in order to validate the Senescence model predictions, PK data from study BILA 459-05 were used to develop a PopPK model (Geriatric PopPK model). The geriatric PopPK model was developed with data from N=16 elderly subjects from study BILA 459-05 using non-linear mixed effects and standard procedures for population analysis. The geriatric model was also used to calculate the individual absorption constant for each subject as this parameter could not be efficiently scaled with the metadata base information.

Section 3: Application of the Senescence model to patients with various degrees of renal insufficiency

Finally, in order to evaluate the repercussion in the PK of bilastine in elderly patients with comorbidities such as renal impairment, the final validated Senescence model was used to simulate the PK profiles in elderly patients with different degrees of renal impairment and the predictions validated with data from a dedicated study (BILA 2808/RI). Individual demographics and GFR

related conditions of each patient were used to simulate the PK profile and the predictions were contrasted with the observations for each of the different severity groups (normal, mild, moderate and severe renal impairment).

Section 1

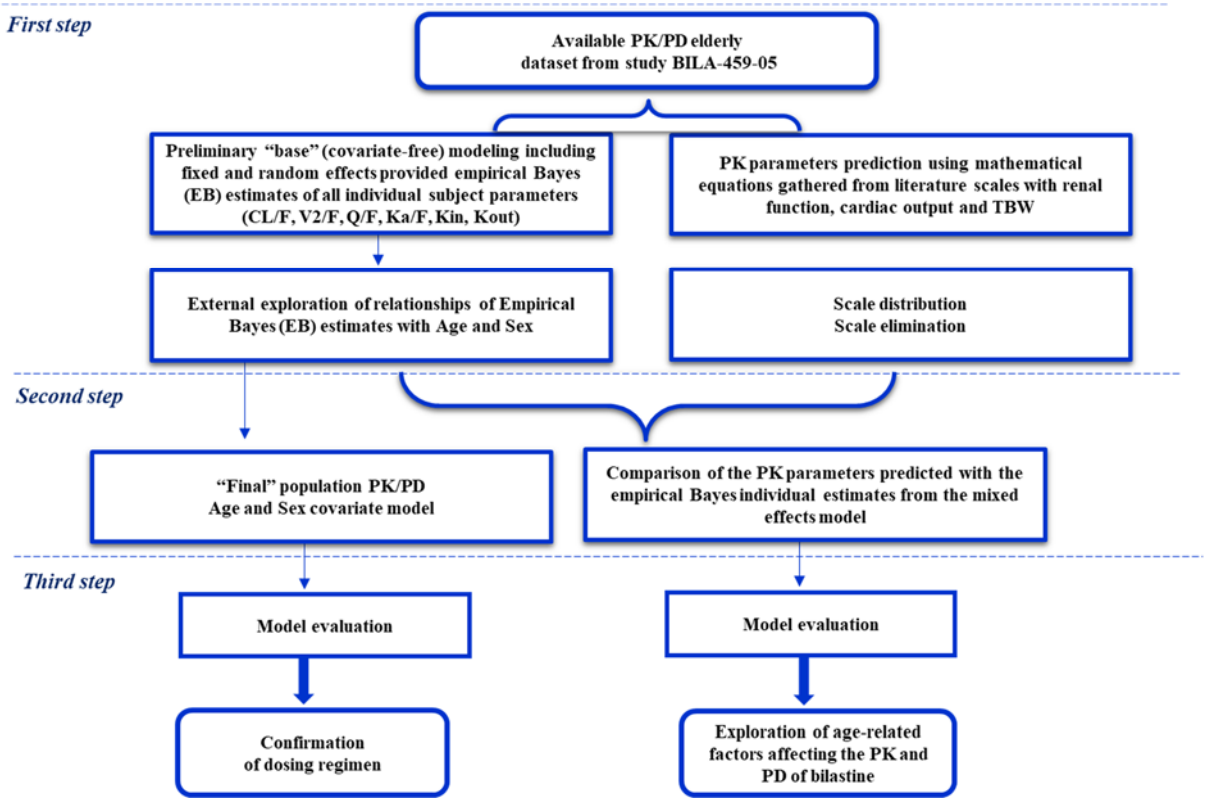
Part of the results presented in this Section were presented at **the XI Jornadas de Modelización y Simulación en Biomedicina (ModelBio 2019)** (conference organized in Alicante 21/23 September 2019). The research work has been presented as oral presentation titled "Population analysis aimed to evaluate the influence of aging on the pharmacokinetics (PK) and pharmacodynamics (PD) of bilastine in healthy volunteers" (Lo Re V, Lukas JC, Encinas E, Campo C, Labeaga L, Rodríguez M). The abstract is attached to the present document as Abstract ModelBio 2019.

SECTION 1. Influence of Age in the Pharmacokinetics and Pharmacodynamics of Bilastine in Healthy Volunteers: Development of a combined adult and geriatric model

The aim of this first section of the research project was to examine the existing differences between adults and geriatrics in the pharmacokinetic processes and the pharmacodynamics of bilastine along with the identification of the physiological and ADME related factors that play a major role in bilastine PK and PD. The impact of age on the PK and PD of bilastine was explored via population modelling in healthy subjects aged 18-80 years with simultaneous assessment of both PK and PD as inhibition effect of cutaneous reaction (flare). Data from a combined dataset of 32 healthy subjects aged 18-80 years from the Phase 1 trial BILA 459-05 were provided by the pharmaceutical company FAES Farma S.A.

- **1. Methodology Section 1**

The overall strategy followed to achieved the goal of this section of the research project is summarized in P1 Figure 1.



P1 Figure 1. Overall strategy used to achieve the goal of this section of research project

The first step consists in exploring the possible effect of age and sex for bilastine using PK and PD observations from trial BILA 459-05. A preliminary "base" model was developed by using NONMEM and its Bayesian POSTHOC method in order to extract individual trial subject PK parameters estimates that were used to explore potential correlations between parameters and age and

sex as covariates. After the identification of the parameters revealing a dependence upon age and sex, a "final" covariate population PK/PD model was developed. In parallel, the PK parameters estimates via this model-based approach were contrasted with the same PK parameters predicted using mathematical equations gathered from an extensive bibliographic search and taking into account age-relating factor likely affect the PK parameters identified.

- **1.1** ***Subjects enrolled in Phase I study and Study Design***

Study BILA 459-05 was a phase I, single-dose, single-center, open-label, parallel-group comparison study. A total of 32 healthy males and females' volunteers (16 young and 16 elderly subjects), either aged between 18 and 35 (inclusive) or aged 65 or older, were enrolled in the study and included in 4 groups according to their age and gender (P1-Table 1). This study was performed in strict compliance with ICHP-GCP Guidelines, the Declaration of Helsinki (18th World Medical Assembly, 1964) and its last revision (Fortaleza, October 2013), as well as local laws and regulations of the countries where the study was performed. The Independent Ethics Committees of each participating center reviewed and approved the protocol, informed consent document signed by each individual patient, prior to recruitment of the patients. The PK and PD of a 20 mg oral dose of bilastine in healthy young and elderly male and female subjects were evaluated.

P1-Table 1. Summary of study design

Dose= 20 mg		
	Age Range (Years)	**No. of Subjects**
Young Male	18–35	N = 8
Young Female	18–35	N = 8
Elderly Male	≥ 65	N = 8
Elderly Female	≥ 65	N = 8

The demographic characteristics of the study population is summarized in P1 Table ***2*** below.

P1 Table 2 Demographic characteristic of the study population

		Female	**Male**	**Overall**
Race	Caucasian	16	16	32
Age	Mean	45	47	46
	S.D.	23	24	24
	Minimum	19	18	18
	Maximum	77	83	83
Weight (kg)	Mean	65.6	75.3	70.5
	S.D.	10.9	6.1	10.0
	Minimum	48.4	63.4	48.4
	Maximum	85.1	83.9	85.1
Height (cm)	Mean	164	175	169
	S.D.	6	6	8
	Minimum	150	160	150
	Maximum	173	183	183

- **1.1.2 *Inclusion Criteria***

The following criteria were met by all subjects considered for study BILA 459-05:

- Skin prick tests with normal saline and with histamine (1 mg/mL in normal saline) were to be performed on the back of the subject. To be eligible for study participation, subjects were not to have had a wheal reaction of > 2 mm in diameter to saline and/or a wheal reaction of < 3 mm in diameter to 1 mg/mL histamine prick test (where the diameter was measured as the maximum distance from one side of the wheal to the other).
- Subjects (males and females) were aged between 18 and 35 years (inclusive) or aged 65 years or older.
- Subjects had no clinically significant abnormal findings on the physical examination, ECG, medical history, or clinical laboratory results during screening.
- Subjects had a negative screen for HIV and hepatitis B and C.
- Subjects had a negative urine screen for alcohol and drugs of abuse.
- Subjects gave voluntary consent to participate in this study.
- Subjects were non-smokers (3 months minimum).
- Female subjects were surgically sterile, at least 2 years postmenopausal, or agreed to utilize 1 of the following forms of barrier contraception from screening through completion of the study: condom with spermicide or diaphragm with spermicide.
- Female subjects aged between 18 and 35 inclusive had a negative serum pregnancy test.

- **1.1.3 *Exclusion Criteria***

Subjects were excluded from the study if there was evidence of any of the following criteria at screening or at any time during the study.

- Subjects did not have a history of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, pulmonary, immunologic, psychiatric, or cardiovascular disease or any other condition, which, in the opinion of the Investigator, would jeopardize the safety of the subject or impact the validity of the study results.
- Subjects did not have a history of fever, asthma, eczema, urticaria, or allergies of any origin, or history of allergic or adverse response to antihistamine drugs.
- Subjects had not participated in a previous clinical trial within 90 days prior to study initiation.
- Subjects did not donate blood within 90 days prior to study initiation.
- Subjects did not donate plasma within 90 days prior to study initiation.
- Subjects did not have an abnormal diet or substantial changes in eating habits within 30 days prior to study initiation.

- Subjects did not have treatment with any known enzyme-altering agents (barbiturates, phenothiazines, cimetidine, etc.) within 2 months prior to or during the study.
- Subjects did not use steroid preparations within 6 weeks prior to or during the study.
- Subjects did not have skin that was considered by the Investigator to be inappropriate for the study (e.g., presence of moles, tattoos, or excess hair).
- Subjects did not have a > 2 mm wheal reaction to saline and/or < 3 mm wheal reaction to 1 mg/mL HPT at screening.
- Subjects did not use any prescription medication within 14 days prior to or during the study.
- Subjects did not use any over-the-counter (OTC) medication within 7 days prior to or during the study.

- **1.1.4 *Treatment***

In each group, each subject received a single oral dose of 20 mg Bilastine administered with 240 mL tap water, in the morning at Hour 0 (9-9:30 am). The subjects were admitted to the study unit in the evening at least 10 hours prior to the scheduled dose. They remained in the unit until completion of the 48-hour post dose blood sample collection (Day 3). A meal or snack was served the evening of check-in on Day -1. All subjects were then required to fast for at least 10 hours prior to dosing. Water was allowed ad libitum during the study, except for 1-hour predose until 2 hours postdose. All subjects continued to fast through at least 4 hours following drug administration, at which time a standard clinic menu and meal schedule was followed. A flow chart showing pharmacokinetic, pharmacodynamic, and safety measures is reported in P1-Table 3.

P1-Table 3. Pharmacokinetic, Pharmacodynamic, and Safety Measurements

EVENT	Screening	Day –1	Day 1	Day 2	Day 3	Post-Study Follow-up
Bilastine Dosing			X			
Blood sampling (PK)*			X	X	X	
Skin Prick Test (saline)	X					
Histamine Prick Test	X		X	X		
Adverse events			X	X	X	X
***PK blood samples drawn predose (Hour 0) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, 36, and 48 hours postdose**						

- **1.1.5** ***Blood Sampling Schedule for PK analysis:***

Blood samples (7 mL) were drawn in green top/sodium heparin Vacutainer tubes at the following times: predose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, 36, and 48 hours postdose.
During the study there were a total of 16 blood samples collected per subject for study drug analysis. As a result, the subjects had 112 mL of blood collected during the study for study drug analysis. In addition, 15 mL of blood was collected at each of the following time points for clinical laboratory evaluation: screening, predose, 48 hours postdose, and at the post-study follow-up. Plasma samples were separated by centrifugation (approximately 2500 rpm x 15 minutes at 4°C). Within approximately 60 minutes of collection, samples were split into 2 aliquots and stored in clearly labelled containers (5 mL cryogenic vials, polypropylene, round bottom, self-standing) in a freezer set at or below -20°C until shipped for assay. A minimum of 1 mL of plasma was required per aliquot. Samples may have been held prior to processing and storing at room temperature under fluorescent lighting. The sample storage containers were properly labelled.

- **1.1.6** ***Effect-time Sampling Schedule for PD analysis:***

Histamine prick tests (100 mg/mL histamine in normal saline) were performed on the back of the subject at the following time points: predose, and at 1.5, 4, 8, 12, and 24 hours postdose. At each time point, 2 prick tests (both with 100 mg/mL histamine in normal saline) were performed on matching sites on opposing sides of the spine. Skin wheal and flare areas were measured 10 minutes after the prick tests. Each acetate was labelled with the subject's initials and identification number, group, time point, and the actual date and time (i.e., when the skin was 'caught' not when the acetate was put on and marked). Calculation of the wheal and flare areas was performed manually. An average area of the 2 sites for each time point was calculated and used for analysis.

- **1.1.7** ***Effect-time Analysis***

The following effect related metrics were calculated for the wheal and flare surfaces collected at each time point for the skin prick test. At each time point, wheal and flare surface inhibitions were computed as follows:

- Wheal inhib(t) = [W0-Wt)/W0] *100
- Flare inhib(t) = [(F0-Ft)/F0] *100

where W0 (F0) are wheal (flare) surfaces in cm2 at time = 0 (predose, baseline) per group.
Wt(Ft) are wheal (flare) surfaces in cm2 at time = 1.5, 4, 8, 12, and 24 hours postdose per group.

Wheal and flare surfaces and inhibitions were examined descriptively (number of observations available, the mean, median, minimum, maximum, Q1-Q3 range and the standard deviation) at each time point.

- **1.2 *Population PK and PK/PD modelling***

Plasma concentration and effect data were analyzed to obtain the PK and PD parameters and the impact of age as described below. A non-linear mixed effects method as implemented in the package NONMEM® (version 7.3, Icon Plc, Dublin, Ireland) was used in two ways:

The first was to apply NONMEM and its Bayesian POSTHOC method in preliminary "base" modeling whose primary goal was to extract individual trial subject estimates for all semi-physiological PK parameters. For that purpose, this model was "saturated" with random effects on all parameters. The macroscopic PK parameters obtained via this model-based approach were used both for exploration of potential correlations between parameters and covariates and then for comparison with allometric predictions of the same parameters made in a separate step. The following application of NONMEM was development of a "final" covariate population PK/PD model that was formal in the sense that all parameters including random effects and covariate coefficients were expected to be significant (standard errors of the estimate %) so that the asymptotic influence of Age (primarily then also Sex) on any future patient was concluded.

In earlier development, extensive knowledge had been gathered on the PK and PD characteristics of bilastine (Jauregizar et al., 2009). Bilastine PK is linear across 10 mg to over 220 mg and shows no concentration- dependent saturation or induction. Bilastine has no metabolism. It shows bicompartmental PK with 1st order absorption after oral dosing with a bioavailability after oral dosing of approximately 61% (Sádaba et al., 2013). .

Preliminary "base" (covariate-free) modeling including fixed and random effects provided empirical Bayes (EB) estimates of all individual subject parameters (CL/F, Vc/F, Q/F, Ka/F, Kin, Kout). These parameters were used to perform external exploration of relationships with Age and guide formal covariate model development. The EB individual subject parameter estimates for CL/F, Vc/F and Q/F were eventually also used to contrast with physiological scaling predictions.

In "final", asymptotic population PK/PD analysis, the goal was to explore the relationship of Age primarily and secondarily of Sex with the largest number of individual estimates of PK and PD model parameters i.e., with the largest number of random effects possible but without compromising formal convergence criteria. Estimation of standard errors and that these were within significance was a key requirement. This covariate model development was guided directly by preliminary correlations with parameters and physiological reasoning rather than purely pharmaco-statistical methods.

A mixed effects (population) PK/PD model was built using the extended Age range results from BILA 459-05 for flare inhibition using NONMEM and applying the pre-established PK structure for bilastine (Jauregizar et al., 2009). The bilastine bicompartmental PK structure with first order absorption was linked to an inhibitory indirect effect PK/PD model and estimated simultaneously (rather than fixing the PK from separate model runs). Base and covariate model development steps, but for Age and Sex only, was as standard for such development and as described elsewhere (Jàuregui et al., 2012; Sádaba Díaz De Rada et al., 2011; Vozmediano et al., 2014, 2017, 2019). Covariate model structures were implemented in NONMEM as follows,

$$Par_{TV} = THETA(.) \cdot (1 + THETA(..) \cdot \frac{Age}{55}) \qquad \textbf{Eq. 1.}$$

Where Par_{TV} is the modeled parameter typical population value THETA(.) is the intercept in the relationship of the PK parameter with the covariate, and THETA(..) is the slope of the relationship. Covariate "Age" was scaled by the median (55 years) of the BILA 459-05 population. In the factored relationship as tested, THETA(..) is estimated as the average fraction of change due to the covariate that can be easily viewed as a percentage.

1.3 *Physiology driven scale allometry for bilastine*

Physiology - based allometric relationships from core literature were used to predict the absolute (bioavailability = 100% equivalent) macroscopic ADME processes as reflected in compartmental PK semi-physiological model parameters, systemic clearance (CL), steady state (total volume, Vss), central volumes of distribution (Vc, Vss) and inter-compartmental clearance (Q). The parameter predictions were finally scaled by the known bioavailability of bilastine (61%). The first order absorption rate, Ka, was evaluable only within population modeling of actual data. Of note however that general knowledge and understanding of intrinsic body function relations are still evolving and may be treatment and disease specific.

Allometric data and relations from the public domain formed the basis for first deriving underlying physiological functions (glomerular filtration rate, GFR; unbound fraction of bilastine, fu; total body water, TBW) as functions of age and possibly other body characteristics. These were then scaled across age from young to older adults and, if needed, by known bilastine relations of PK parameters to physiological variables and finally corrected by the known bilastine bioavailability of F = 0.6, in order to approximate the macroscopic PK parameters of interest clearance (CL/F, Q/F, Vc/F, Vss/F) and their gradients of change, if any, across age. Then, alterations in key semi-physiological parameters (CL/F, Vc/F, Q/F) with age as seen in the "base" NONMEM model estimates were contrasted directly with their corresponding allometric predictions to aid in emerging knowledge regarding underlying intrinsic age-related processes.

Systemic clearance (CL)

Total clearance CL was predicted via scaling of GFR (related to maturation of the kidney volume), fu, and renal clearance (CLr) in young and elderly. Scaling across age groups and resolving for elderly was performed as follows.

$$CLr_{ger} = \frac{GFR_{ger} \cdot fu_{ger}}{GFR_{young} \cdot fu_{young}} \cdot CLr_{young} \qquad \textbf{Eq. 2.}$$

For variables corresponding to elderly ("ger") and young adults ("young"). The GFR across all ages and a function itself of Body Surface Area (BSA) was calculated as follows (Jauregizar et al., 2009),

$$GFR = e^{-0.00709 \cdot AGE + 0.5 \cdot BSA + 4.2} \qquad \textbf{Eq. 3.}$$

The starting clearance index was taken as CL/CLr = 2 (Sabada et al, 2013; Vozmediano et al, 2013, 2017). The binding of bilastine to plasma proteins is 84%–90% with albumin being the main binding protein (Encinas et al., 2013; Karafoulidou et al., 2009).

The unbound fraction was allometrically scaled as follows (Stader et al., 2018),

$$fu_{ger} = \frac{fu_{young}}{fu_{young} + (1 - fu) \cdot Sp} \qquad \textbf{Eq. 4.}$$

Where, Sp is the human serum albumin (HSA) plasma protein scaling factor as Sp_{HSA} (18 – 50 yr) = 1; Sp_{HSA} (50-69y) = 0.931; Sp_{HSA} (70-100y) = 0.866.

Steady state and central volume of distribution (Vss, Vc)

For Vc, TBW was used as a 1:1 correlate for steady state volume of distribution (Vss ~ Vc + Vp) as observed earlier for bilastine in extrapolation to pediatrics (Jàuregui et al., 2012). The relation was for Vp = 0.65*Vss (McNamara et al., 2019).

TBW was calculated as follows,

$$TBW_{women} = -2.097 + 0.1069 \cdot Height + 0.2466 \cdot Weight \qquad \textbf{Eq. 5.}$$

$$TBW_{men} = 2.447 - 0.09516 \cdot Age + 0.1074 \cdot Height + 0.336 \cdot Weight \qquad \textbf{Eq. 6.}$$

Inter-compartmental clearance (Q)

For Q the cardiac output (CO) was used in direct allometry as CO/(Q)_men = 221 and CO/(Q)_women = 200. The relation proposed by (Vozmediano et al., 2014, 2019) was used for CO calculation as follows,

$$CO\ (L/h) = 159 \cdot BSA - 1.56 \cdot AGE + 114 \qquad \textbf{Eq. 7.}$$

All calculated parameters were finally adjusted for the bioavailability of bilastine, F = 60%, thus converted into their "apparent" in-vivo post-oral absorption equivalents (CL/F, Vc/F, Q/F).

1.4 Softwares used in the analyses

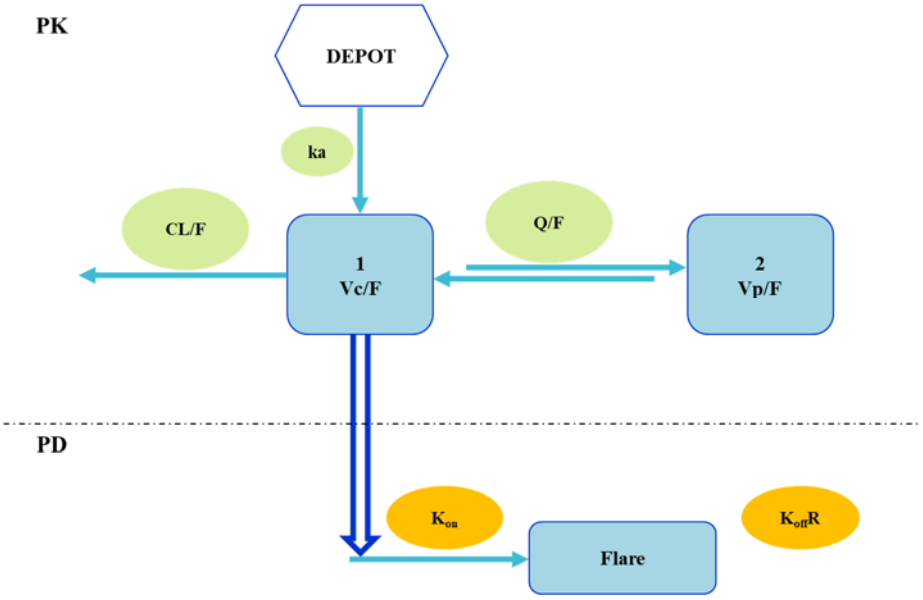
The modeling and simulation were carried out with the software package NONMEM® (version VII, Icon Plc, Dublin, Ireland). Data exploration, statistical testing external to NONMEM® and graphics were performed using S-PLUS® (version 8.2, TIBCO Software Inc, Palo Alto, CA, USA).

- **2. Results Section 1**

Eventually, N=32 subjects were available for analysis in BILA-495-05 split equally in men and women of ages 18 to 83 years (median = 55 years). The population practically consisted of two age groups, younger (mean = 23 yr; range = 18 – 31 yr) and older (mean = 69; range = 65–83 years).

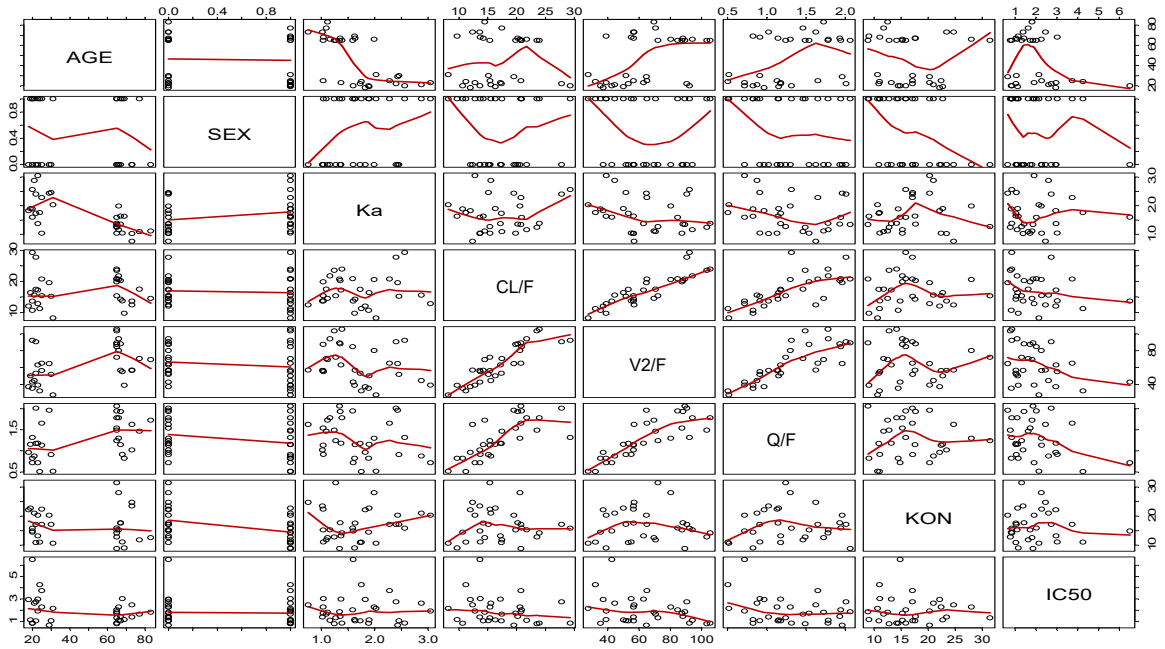
2.1 *Population PK/PD modeling*

A schematic of the PK and PD model representation of bilastine kinetics and dynamics is depicted in P1 Figure 2 below.



P1 Figure 2. PK/PD compartmental model representation of bilastine and the flare effect. The kinetics show a bicompartmental structure and an indirect effect type model is applied for flare also relating the dependence of key PK parameters with physiological variables. (Oral dose is administered in "Depot"; Ka = 1st order absorption rate; CL/F = Total apparent systemic clearance of bilastine; Vc/F =Apparent central volume of distribution; Q/F = Inter-compartmental clearance; Vp/F = Apparent peripheral compartmental distribution volume; Kon = 0th order rate of onset of the flare effect; Koff = 1st order rate of flare decay; R = The flare response)

The "base" – no covariate – mixed effects method for bilastine PK/PD from the BILA 459-05 and the flare surrogate effect was used to extract individual subject EB parameter estimates. The primary predictors, Age and Sex, were explored versus Empirical Bayes (EB) estimates in a correlation matrix with PK/PD parameters for flare as shown in P1 Figure 3.



P1 Figure 3. Dependence of "base" model PK/PD parameters on Age and Sex. EB parameter estimates versus covariates are shown with a lowness fit (red line). Ka = First order absorption rate; IC_{50} = Inhibitory bilastine concentration at 50% inhibition for the flare effect (SEX: 1 = Women; 0 = Men)

Age appears to corelate with Ka, Vc/F and Q/F and there may be a weak relation between Sex, Ka and Kon. Since Q/F and Vc/F are strongly corelated themselves subsequent population model testing of Age was performed on Ka and Vc/F and Sex on Ka. Due to the physiological relevance of the IC_{50}, Age was also tested on that parameter. Similarly, Sex was tested on Vc/F as physiologically a difference may be expected.

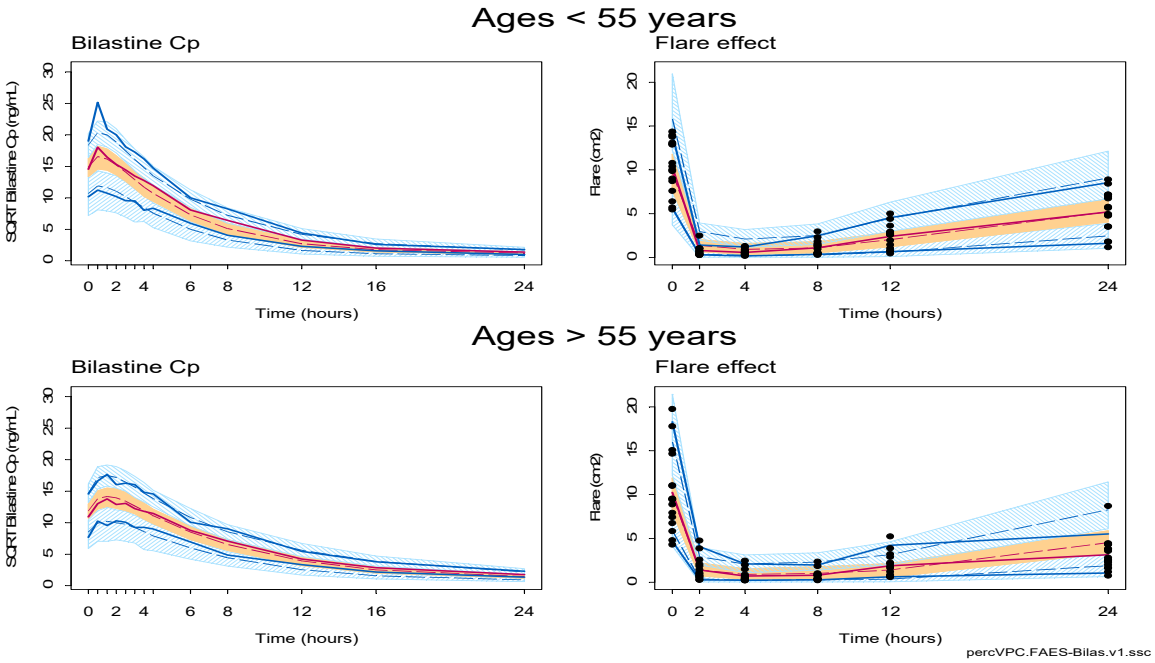
Subsequent to preliminary covariate exploration external to model runs, the most relevant relations with Age and Sex were implemented in NONMEM and models tested sequentially according to population model development criteria. Covariate coefficients with (proportional) standard error estimates of < 50% were eliminated. P1 Table 4 lists the final model PK/PD parameter estimates with Age and Sex as covariates estimated using the BILA 459-05 observations.

P1 Table 4. Final population PK model for flare inhibition. Parameter descriptions are as in the schematic. (SEE = Standard error of the (model) estimates; SEE% = A coefficient of variation presentation of the SEE)

Parameter	Estimate	SEE	SEE%
Ka (hr^{-1})	1.95	0.326	16.7
CL/F (L/hr)	17	0.439	2.6
Vc/F (L)	41.9	2.56	6.1
Q/F (L/hr)	1.13	0.0891	7.9
Vp/F (L)	25.2	3.13	12.4
Kon (Flare-cm^2/hr)	15.5	2.87	18.5
Koff (1/hr)	1.61	0.343	21.3
IC_{50} (ng/mL)	1.88	0.155	8.2
Age.p. Ka	-0.247	0.132	53.4
Age.p. Vc	-0.582	0.116	19.9
Sex.p. Vc	-0.181	0.029	16
ω_CL	13%	10%	47%
ω_Kon	33%	21%	40%
σ_prop_Bilas	34%	9%	7%
σ_add_Flare	1.3 cm^2/hr	0.45 cm^2/hr	14%

The shrinkage of the random components (omega) was 14%, 5.2% for CL/F and Kon, respectively, within the low range for the systemic and PD parameters. The coefficients of variation (SEE%) of all effects, fixed and random variances were also all below 50% the level of putative significance of the estimates. Age had an impact on the absorption rate, Ka, that was near significance (SEE of 54%). Age and Sex significantly affected the volume of distribution. Vc/F was increased by 58% from younger to elderly on average. For example, the expected Vc/F for male subjects of 20 and 80 years (using 41.9 L as the intercept, 0.582 as the slope and recalling that Age is scaled by the median of 55 years) would be 51 L and 77 L, respectively (similar to TBW). Women are expected to have an 18% reduction compared to that of men on average at both Age extremes.

Covariates Age and / or Sex were not significantly related to clearance - crucial for steady state exposure - or any PD parameter (Kon and IC_{50}) for flare inhibition. The VPC of the PK and PK/PD parts of the model are shown in P1 Figure 4 divided by age groups.

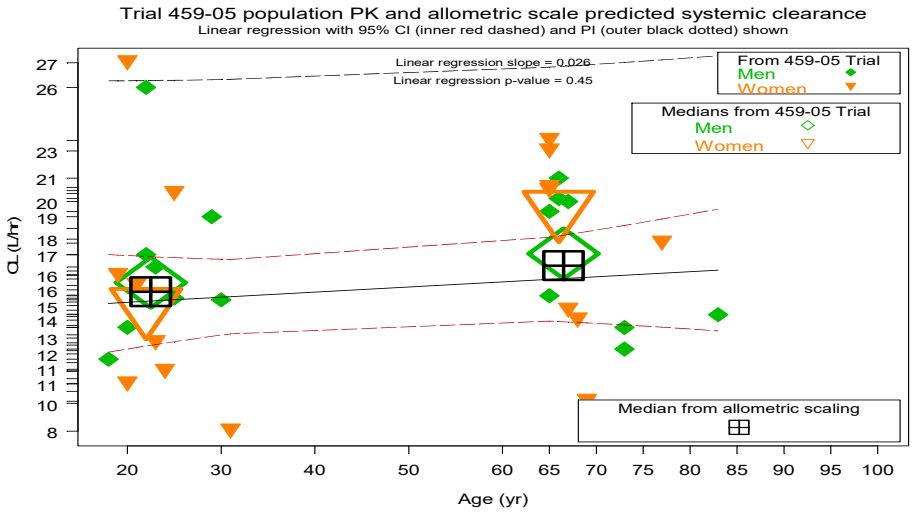


P1 Figure 4. Visual predictive check for bilastine and plasma split around the age median. Plasma on the left panels and flare effect on the right panels. Upper row for Ages < 55 and lower row for Ages > 55. Blue shaded areas are the 95% confidence intervals of the 5th and 95th percentile of the predictions and their mean (dashed lines) and the orange area the 95% confidence interval of the mean (red dashed line) prediction. The solid lines are the corresponding quantiles of the observations, the latter also shown for the flare effect as black dots. Both the abscissa and ordinate axes are square root transformed for visual clarity

The simulation test qualifies the compartmental model for bilastine.

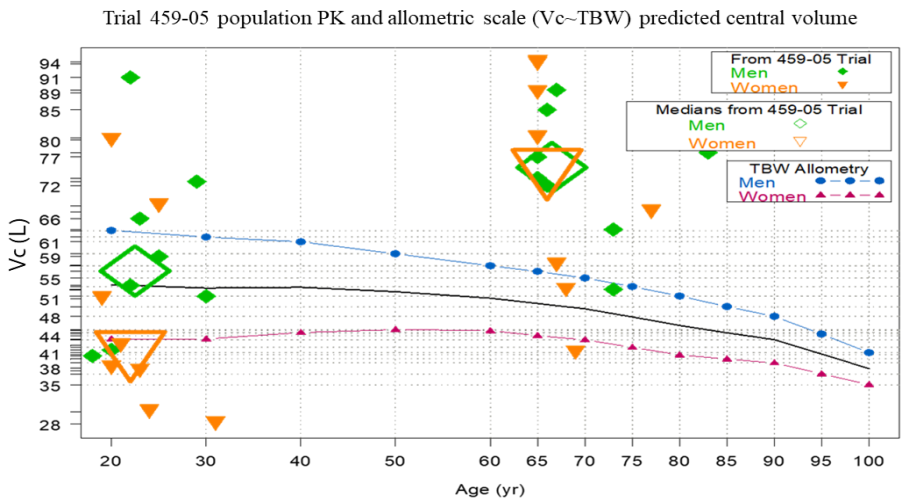
2.2 *Physiological exploration*

The allometric scales (Equations 2 - 7) were used to calculate the average expected renal clearance by Age and Sex given physiological characteristics of bilastine. Then, the CL/CLr ratio was used to obtain values for systemic clearance as shown in P1 Figure 5 with overlay of the empirical Bayes predictions for CL/F from the base PK/PD model for bilastine.



P1 Figure 1. Age dependence of total systemic apparent clearance, CL/F, of bilastine as predicted by physiology-based scaling (crossed squares) and contrasted with empirical Bayes individual parameter estimates from the PK/PD model for flare (large open symbols). A regression for CL/F versus Age is also shown (continuous line is mean and dashed inner and outer lines are confidence and predictive 95% intervals, respectively)

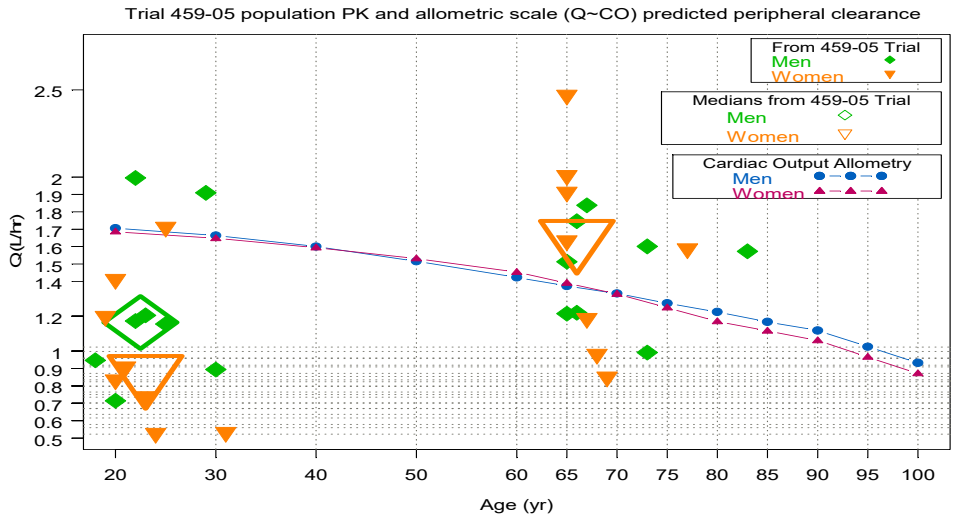
The physiology and model predictions overlap perfectly. A linear regression model was applied on the individual parameter estimates. The regression of clearance across Age had a non-significant slope. The median observations (for men) were within the confidence interval of the regression. The allometric prediction (independent to the regression) fell on the line at the two Age extremes. The whole, indicates both the lack of impact of Age on bilastine systemic (total) clearance and that physiologically, bilastine behaves as expected for renal clearance drugs showing also the expected equilibrium protein binding primarily related to albumin. The typically expected decline of clearance with Age is not observed with bilastine. The relationship predicted for apparent (bioavailability adjusted) central volume of distribution (Vc/F) based on TBW (P1 Figure 6). The individual parameter estimates are from the covariate free, base, model (uncorrelated parameters). The final model estimates have the same mean position (large symbols) but individual estimates are corelated with the predictor (not shown).



P1 Figure 2. Age dependence of apparent central volume of distribution for bilastine (Vc/F) as predicted by physiological-based scaling (continuous lines) and contrasted with empirical Bayes (EB) individual parameter estimates from the base – no covariate – PK/PD model (solid symbols). The medians of the EB parameters are also shown (large open symbols)

The standard allometric model predicts a drop in distribution volume as it is linked directly to total body water. Although, an empirical adjustment of the TBW relation is needed to bridge with the standard allometric relation into bilastine a physiological explanation also exists. Bilastine has bi-compartmental distribution also in deep tissue and partially body fat. The changing ratio of total body water across Age and Sex then accounts for the observed differences versus the water-based approach. TBW (higher for men at young ages) decreases with Age in relation to body fat for men and less for women with a trend to equalize with Age. Increase in the relative proportion of body fat with Age leads to an increase in apparent distribution volume for bilastine.

The cardiac output method (Equation 5) was used to predict apparent inter-compartmental clearance (Q/F) based on generic physiology and assuming no change in bioavailability, F, with age. The prediction was contrasted with the individual subject model predicted parameters (P1 Figure 7). Note that the apparent difference observed in the BE estimates was not significant at a population parameter level.



P1 Figure 7. Age dependence of inter-compartmental clearance of bilastine as predicted by physiological (cardiac output, CO) based scaling (straight lines) and contrasted with empirical Bayes individual parameter estimates from the final PK/PD model (solid symbols for individual data and open large symbols for medians)

The inter-compartmental clearance is increased with age most likely for the same reasons as with Vc, i.e., related to the changing water to fat ratio in body tissues. For both Vc and Q, in the case of a drug like bilastine the general formulations (shown as continuous lines in the figure) based on more hydrophilic compounds suggests the existence of a more complex relation to underlying physiology.

- **3. Discussion Section 1**

Changes in body composition occurring during the process of aging, also across sexes, may impact differentially the post-absorption kinetics of drugs also depending on their lipo / hydrophilicity. Apart from potential renal excretion changes, an overall increase in body fat with age ranges from 20-40% up to 70 years, stable thereafter (Vozmediano et al., 2017). A decrease of total body water (TBW) by 10-15% also occurs, due to a change of the ratio of intracellular to extracellular water as intracellular water is reduced (Stader et al., 2018). Fat distribution is also altered with aging, characterized by a reduction in subcutaneous fat and an increase in visceral and intramuscular fat (Schlender et al., 2016) Bilastine, is a lipophilic compound with 1st order absorption, exclusively renal excretion and showing linear kinetics across dose and dose repetition (Jàuregui et al., 2012; Vozmediano et al., 2017). Although extensively studied in both adults and pediatrics (Vozmediano et al., 2014, 2017) also across renal impairment and drug-drug interaction situations via a series of clinical trials and population PK and PK/PD analyses its behaviour in older ages had not be explicitly studied. In order to close this gap also regarding the question of dose adjustment in elderly adults, a dedicated trial, BILAS-495-05 was conducted comprising adults of 18 to 83 years of age split equally between the two sexes and treated with a single p.o. dose of 20 mg bilastine. It was an observational PK and PD

assessment study. The bilastine effect finally modelled in this analysis was reduction of a skin flare after challenge and a single 20 mg dose of bilastine.

The objective of the present analysis of BILA-495-05 was two tiered. First it was to identify ageing related alterations in key systemic compartmental PK parameters (CL/F, Vc/F, Q/F), a macroscopic view of systemic processes, and PD parameters (assuming the flare effect a surrogate for all bilastine PD) via population PK/PD Age and Sex covariate modelling. Second, it was to explore sub-macroscopic physiological processes directly routed to the compartmental PK parameters through application and comparison with public domain allometric scales from GFR, fu, TBW and CO.

A population PK/PD model was applied, using the established bicompartmental structure for bilastine PK and an indirect effect system for the flare reduction effect. In *ad-hoc* covariate modelling, Age and Sex were tested on PK and PD parameters guided by exploration of empirical bayes (EB), POSTHOC parameter estimates. First order, linear relationships across the complete covariate range were implemented in NONMEM.

The population of BILA 495-05 actually composed two groups clustered by Age, 18 to 31 and 61 to 83 years old. An increase of 58% in the central distribution volume, Vc/F, was seen in EB parameters in the elder group and a reduction of 18% related to Sex (less for women). Trends existed with Sex also in the onset rate for flare but were not significant. Importantly, there was no differentiation in systemic clearance. The implication is important as it implies that at steady state, where exposure is only relying on clearance, there is no change expected across the range 18 to 83 years for bilastine at 20 mg single daily p.o. dosing.

The underlying physiology for clearance, was represented by a sequence of scales starting with unbound fraction specific for bilastine across younger to elderly, then the GFR across age, then renal clearance and finally total clearance. This independent prediction coincided absolutely with the population EB estimates and showed no differentiation with age.

In principle, changes in volume of distribution relate to changes in the lipid to water partition ratio (Jàuregui et al., 2012; Vozmediano et al., 2017). Predictions in children of bilastine disposition based on allometric scaling had shown a relation between distribution volumes (Vc/F, Vss/F) and TBW (Vozmediano et al., 2014, 2017). The physiology for Vc/F was assumed proportional to TBW. TBW, related to Vss/F, ranges from 38 to 46 L in adult men and 26 to 33 L in women (Shi et al., 2008) which corelates with the parameter estimate magnitudes (Vss/F = Vc/F + Vp/F).

The inter-compartmental clearance, Q, was assumed proportional to cardiac output and scaling factors previously established for men and women were used for scaling (Vozmediano et al., 2014, 2019).

However, the public domain allometric scales for these two parameters, based on the disposition of the “average”, rather hydrophilic, metabolized drug - neither being the case for bilastine - failed for

both Vc/F (Vss/F) and Q/F. The culprit appears to be a re-distribution of intra/ extra cellular water and the water / fat ratio in central tissues. Body fat increases while total body water as well as lean body mass decreases with age. Then, the distribution volume and inter-compartmental clearances of lipophilic compounds such as bilastine may be expected to show a relatively minor increase compared to the younger adult groups. Altered concentrations of plasma proteins may affect the distribution of highly protein-bound drugs.

Regarding implications for efficacy or safety, a post-authorization safety study (PASS) with either allergic rhinoconjuntivitis and/or urticaria in elderly patients was performed (FAE-BIL-2012-01). The known favorable efficacy and safety profile of Bilastine 20 mg was confirmed with low incidence of AEs in patients aged ≥ 65 years also in agreement with the present PK/PD analysis of BILA 05-495. As per the existing summary of product characteristics (SmPC), no efficacy differences had been observed so far for the elderly population in the overall clinical development program of bilastine and no dose adjustment is required for elderly or renally impaired patients.

In conclusion, alterations of the PK or PD parameters with Age or Sex for bilastine from the age spanning BILA 495-05 study were not crucial for dose adjustment. An increase of Vc with Age alone had no clinical significance and did not lead to a dose adjustment requirement. However, due to the observed confounding among covariates, further investigation with a physiologically based PK (PBPK) model will be undertaken to better elucidate the underlying bilastine PK and the aging related modifications that could impact the dose under combined circumstances (i.e elderly and renal impairment, elderly and DDIs, etc).

Section 2

Part of the results presented in this Section were published in the following paper:
Chaejin Kim, Valentina Lo Re, Monica Rodriguez, John C Lukas, Nerea Leal, Cristina Campo, Aintzane García-Bea, Elena Suarez, Stephan Schmidt, Valvanera Vozmediano "**Application of a dual mechanistic approach to support bilastine dose selection for older adults"** which is attached to the present document as manuscript I.

Part of the results presented in this Section were presented at the **XIII Jornadas Modelización y Simulación en Biomedicina** (ModelBio 2020) (online conference organized by the Universidad de Vitoria-Gasteiz on 25/26 November 2020). The research work has been presented as oral presentation titled "Application of a dual PBPK model-based approach across the age population of adults using bilastine as a probe drug" (Lo Re V, Chaejin Kim, Lukas JC, Campo C, Garcia A, Stephan Schmidt, Valvanera Vozmediano, Suarez E, Rodríguez M). The abstract is attached to the present document as Abstract ModelBio 2020.

Part of the results presented in this Section were presented at the **11th American Conference on Pharmacometrics (ACoP11)**, (online meeting organized by the International Society of Pharmacometrics (ISoP) on November 9 – 13, 2020). The research work has been presented as a poster presentation and was awarded as one of the four best presentations for the ACoP11 Trainee Communication Challenge sponsored by the Journal of Pharmacokinetics and Pharmacodynamics. The poster and the corresponding abstract titled "Application of a dual PBPK model-based approach across the age population of adults using bilastine as a probe drug" (Chaejin Kim1, Valentina Lo Re2, Monica Rodriguez2, Lukas JC2, Campo C3, Garcia A3, Stephan Schmidt1, Valvanera Vozmediano) are attached to the present document as ACoP11.

SECTION 2. Development and evaluation of a Senescence predictive PK model in the geriatric population.

This section of the Research project aimed to better understand the relationship of the age-related factors identified in Section 1 affecting the PK of bilastine.

A structured literature search (described in the Introduction, section 1.3) was performed in order to develop a population metadatabase spanning several decades of literature review for aging Caucasians considering anatomical, physiological and biological system parameters with the associated variability both in in adult and elderly subjects was constructed (Table AII-2-Table AII-3 in Annex II).

A qualitative and quantitative description of the changes described in ADME processes (absorption, distribution, metabolism and elimination) of drugs and their relationship with age was made.

The constructed database has been used to develop a predictive model for elderly (Senescence model). The data collected were analyzed and the descriptive equations developed for the most relevant parameters predictive of the PK of bilastine.

The semi physiologic approach proposed in this research project integrated the available drug knowledge concerning disposition in adults as well as the impact of the physiological changes on the PK of bilastine followed by the application of modelling and simulation techniques. The goal was to elucidate the underlying mechanisms affecting the PK/PD of bilastine combining a compartmental model structure together with principles of the physiology that apply to distribution and elimination processes in order to determine the impact of key physiological parameters (i.e., free-fraction in plasma, total body water composition, enzyme activity and glomerular filtration rate) on the PK and PD parameters of bilastine in the geriatric population.

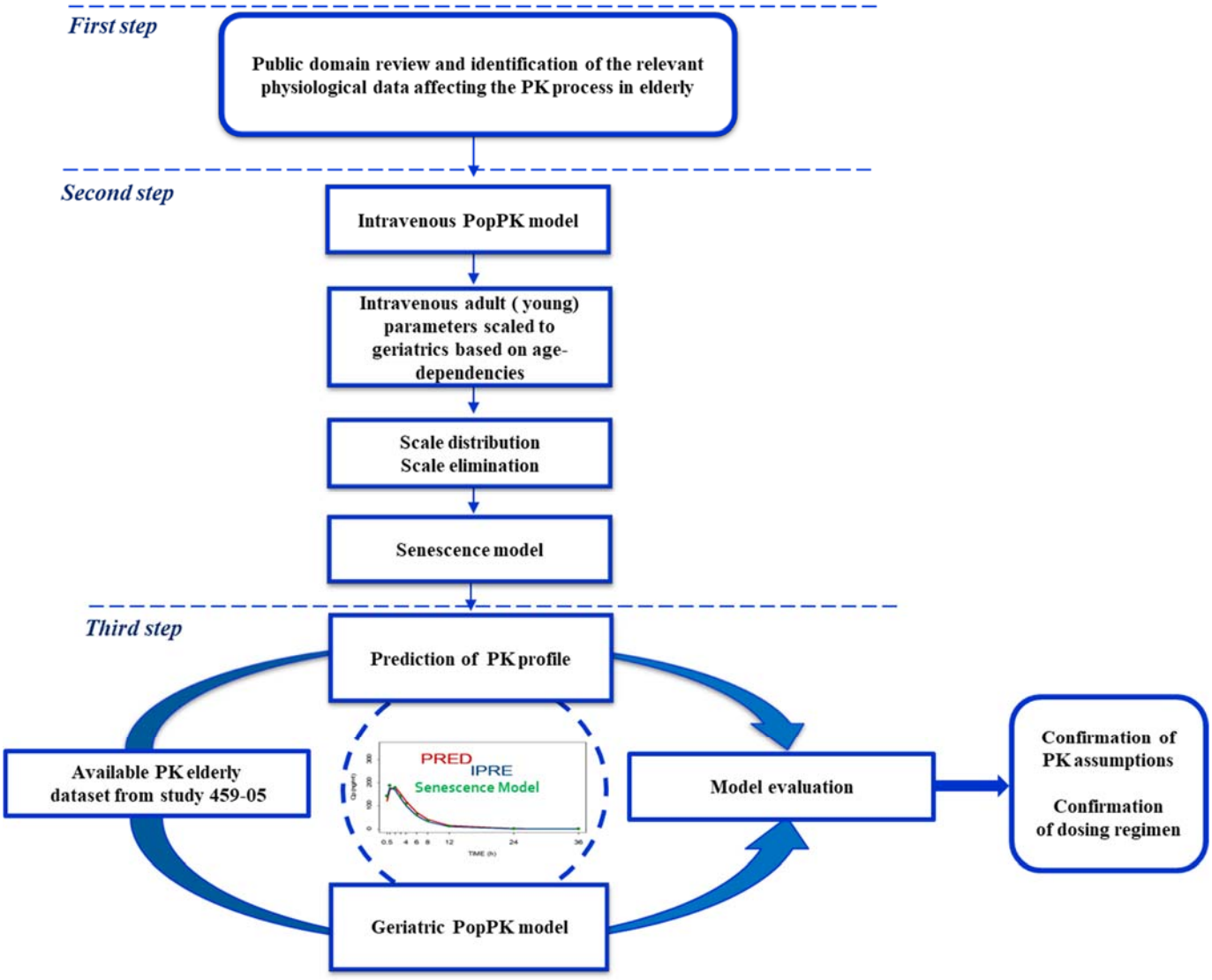
Elderly PK data available from Study BILA 459-05 have been used to evaluate the Senescence model's predictive performance.

1 Methodology Section 2

The overall strategy followed for the Senescence model development and validation is summarized in P1 Figure 1. The first step in developing the Senescence model, the learning stage, consists on the identification of the relevant factors that can contribute to changes in the PK between adult and geriatric patients and the development of several databases that gather and summarize this information. The information obtained during this first step has been used to identify and to extend the understanding of the physiological pathways playing a major role in bilastine's PK/PD behavior. After the completion of the learning stage, the acquired knowledge has been applied to develop a

preliminary semi-physiological model able to predict the PK in elderly subjects. Adult data have been used as the starting point for model development.

Subsequent steps were followed during model development to find the appropriate scaling equations (developed with public domain information) to incorporate into the different ADME processes responsible for the bilastine's PK that allow scaling of relevant PK parameters in the different geriatric age groups. PK data is available in a limited number of geriatrics for the antihistamine bilastine (Study BILA 459-05 described in Table AI-2 in Annex I). This data was used to evaluate the Senescence model's predictive performance from adults to geriatrics and further optimize the model, if needed.



P2 Figure 1. Overall extrapolation and validation strategy used during Senescence model development

1.1 *First step: Learning stage*

Public domain review and identification of data for ontogenic database construction with the relevant physiological data affecting the PK and PD processes in elderly for subsequent modelling activities.

The extensive bibliographic search was carried out on multidisciplinary research platforms, such as BD MEDLINE, PubMed, Google Scholar and other medical and scientific search engines. The terms *aged, elderly, old, age-dependent, aging, ageing, frail elderly or geriatric* were used in combination with terms related to parameters of interest.

All collated data were carefully analyzed. No restrictions were applied regarding the language or the publication year of the article. Abstracts were screened, and studies included if the study population were Caucasians, at least age had been reported in addition to the parameter of interest, and subjects were healthy or their disease/organ function was deemed unlikely to affect the parameter of interest.

Particularly, the metadatabase includes information of different physiological parameters such as Total body water (TBW), Fat free mass (FFM), Body Fat mass (FAT) that change with aging.

The database also includes changes in renal excretory function related to the glomerular filtration rate (GFR), creatinine clearance, hepatic system as well as changes in the major plasma-binding proteins such as albumin, lipoprotein and P-glycoprotein (P-gp) that can influence the pharmacokinetic and pharmacodynamics properties of drugs in the geriatric population. Moreover, drugs commonly used in the geriatric population were identified such as the most common disorders frequent in later life and included in the database.

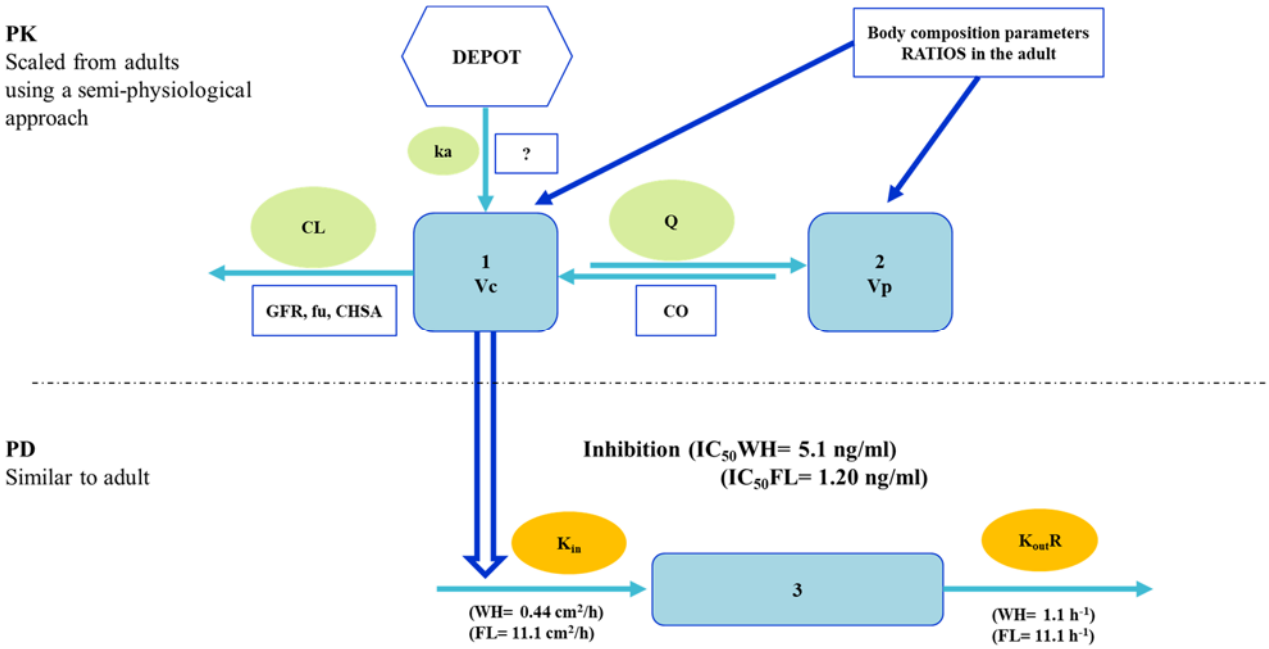
Drug-related problems (DRPs), including adverse drug reactions (ADRs), and drug-drug interactions (DDi) that can adversely impact elderly people were also examined. Comparative publications of pharmacokinetics between adults and older adults were identified and the results retrieved from each database compared. A preliminary exploratory data analysis was assessed with the drug data, relating the PK data with age. The information obtained during this first "learning stage" was used to identify and to extend the understanding of the physiological pathways playing a major role in the drug's PK/PD behavior.

1.2 *Second step: Senescence Model development*

The senescence model consists of a combination of the selected equations most suitable for each systemic PK parameter and best describing their change as a function of age, in this case, concretely for bilastine. Several equations were developed per ADME process and tested for suitability.

In addition, the PK parameters that define the adult behavior of bilastine described by a two-compartmental kinetics with 1st order absorption (P2 Figure 2) (Jauregizar et al., 2009) was also considered for scaling together with the PK of the drug after intravenous (IV) administration. The PK

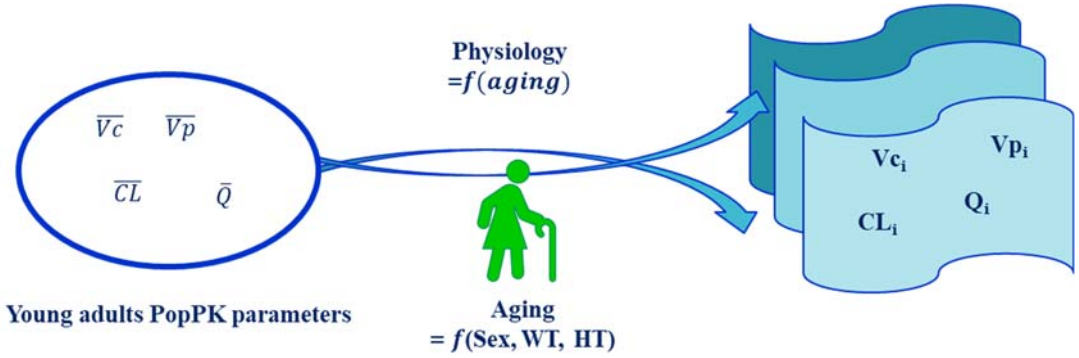
senescence model was then linked to the PD, which was showed to be similar to adults in section 1 of this research project.



P2 Figure 2. Pharmacokinetic/ pharmacodynamic semi-physiological approach developed for bilastine in elderly according to the well described PK/PD model of bilastine *(Adapted from Vozmediano et al. 2017)*

PK= Pharmacokinetics; PD= Pharmacodynamics; CL= systemic clearance; Vc= Central volume of drug distribution-compartment no.1, Q= Inter-compartmental clearance; Vp= Peripheral volume of distribution-compartment no.2; GFR= Glomerular Filtration rate; fu= unbound fraction; CO= cardiac output; IC_{50}= inhibitory concentration where 50% of the maximum inhibition factor is attained; WH= Wheal allergic response effect; FL= Flare allergic response effect; Kin= Allergic response induction (in compartment no.3) secondary to H_1 stimulation; "Inhibition"= bilastine blockage of H_1 stimulation.

The resulting scaling equations have been incorporated into the different ADME processes responsible for bilastine's PK and used to scale PK parameters in elderly and simulate their PK profile with the sole input of gender, age and weight. The scaling semi-physiological approach used is described in P2 Figure 3.



P2 Figure 3. Semi-physiological approach used for the Senescence model development where $\overline{Vc}$, $\overline{Vp}$, $\overline{Q}$, and $\overline{CL}$ are respectively the mean population central compartment Volume of distribution, peripheral compartment Volume of distribution, Intercompartmental clearance and apparent total body clearance for a typical adult subject reported in the literature. PK assumptions considering physiological and demographic parameters that change with age were made in order to scale the PK parameters of bilastine in elderly from adults

1.2.1 *Clinical PK study data (study BILA-2909/BA)*

Adult intravenous PK parameters were used as a starting point for scaling into geriatrics. These PK parameters after IV administration were obtained by developing a PopPK model (Intravenous PopPK model) from a dedicated bioavailability study (BILA-2909/BA) using non-linear mixed effects and standard procedures for population analysis. BILA-2909/BA was designed to investigate bilastine oral bioavailability in humans. It was a randomized, open label, single dose, single center, two-arm crossover-controlled trial under fasting condition. Six male and six female subjects aged between 18 to 24 years (mean 20.8 years) participated in the trial receiving 20 mg single dose of the PO tablet (Bilaxten^{TM} FAES FARMA, Bilbao, Spain) and 10mg of bilastine IV single dose over 5 min. The wash out period between the two treatments was of at least 14 days, and the sequence of the treatments was determined by randomization in balanced manner (Table AI-2 in Annex I-Study code BILA-2909/BA, Sadaba et al, 2013). Intravenous PK parameters have been reported to provide a robust and accurate resource allowing to improve the understanding of the relationship between fundamental chemical characteristics and drug disposition (Vozmediano et al., 2017). The methodology followed and the assumptions considered to scale each PK process is summarized below.

Clearance

Renal clearance for bilastine is governed by renal glomerular filtration and accounts for the majority of the total clearance, apart from the percentage actively secreted to the faeces via P-gp (67% of the drug is collected in faeces and the biliary route is not responsible this value). Therefore, CLr was scaled as function of the age-related changes described for the glomerular filtration rate (GFR) and in the unbound fraction (fu) (Eq 1 to Eq 4 P2-Table 3). In order to calculate the oral total CL, the ratio

between the intravenous total clearance (CLiv) and the intravenous renal clearance in adults (CLiv/CLr iv) was considered (Eq.4 P2-Table 3).

Inter-compartmental clearance (Q)

It is generally well established that organ blood flow influences the distribution of drugs in the body. (Jauregizar et al., 2009). Age-related changes in blood flow distribution were accounted for as changes in cardiac output (CO), an important index of cardiac performance, that been reported to decrease with aging (Lombardo et al., 2018). Cardiac output is recognized as an important marker of intercompartmental clearance. The CO/Qiv ratio calculated for adult was used to scale the Q in elderly assuming this ratio is maintained across aging.

CO data for elderlies and reference adult aged 30-50 years were calculated using the equations obtained from Stadler et al. (Eq. 5 P2-Table 3). The BSA was calculated according to DuBois and DuBois (Eq. 6). (Bjorkman, 2005b; Henthorn et al., 1992; Starke et al., 2008). The Qiv for adult was taken from the developed PopPK model of the intravenous data in young adults. (P2 Table 2) CO and body surface area values used for scaling are described in detail in Table AII-2 and Table AII-3 of Annex II. Then, the CO/Q ratio calculated (Eq.8 P2-Table 3) for an adult reference aged 30-50 years was used to scale the Q in geriatrics (Katory, 1979; Schlender et al., 2016).

Volume of distribution

Changes in body composition during the process of aging may affect differently the distribution of drugs according to the lipophilicity or hydrophilicity of the drug compound. Considering that bilastine follows a two-compartmental PK, central and peripheral volumes were scaled.

TBW and TBF values were used to estimate the steady state intravenous volume of distribution (Vss abs) that, for a drug which confers multicompartment characteristics, represents the sum of intravenous Vc (Vc abs) and intravenous Vp (Vp abs). Total Body water volumes for elderly males and females were estimated using Watson equations taking into account anthropometric parameters such as weight and height, sex and age (Eq.9 P2-Table 3, Eq.10 P2-Table 3) (DuBois et al., 1915). Additionally, TBF was also considered and estimated using the equations described by Stader et al. (Eq. 11 P2-Table 3) (Van Sassenbroeck, 2002; Vozmediano et al., 2017).

1.3 *Third step: Validation of the Senescence Model predictions using the Geriatric PopPK model and simulations*

In order to qualify the predictions of the parameters scaled using the senescence equations and also estimate the absorption constant, not scaled, a population PK model (Geriatric PopPK model) was also developed with data from geriatrics of Clinical trial BILA 459-05 (Table AI-1 in Annex I).

Study BILA 459-05 was a phase I, single-dose, single-center, open-label, parallel-group comparison study. (Table AI-2 in Annex I) This study was conducted in accordance with the latest version of the World Medical Association Declaration of Helsinki (see protocol) and ICH Guideline for Good Clinical Practice (GCP). A total of 32 healthy males and females' volunteers (16 young and 16 elderly subjects) aged 18-80 years were enrolled according to age and gender and received by mouth (p.o.) a single, 20 mg dose of bilastine following a fast of 8 to 10 hours. Blood samples for pharmacokinetic analysis were collected through the 48-hour post dose interval. From each patient, time of dose, total dose, number of doses, sampling times and bilastine concentrations were available.

Only the sixteen (n=16) healthy males and females subjects aged 65 or older were taken into account for this modelling exercise. The population model was then applied to calculate the Bayes individual PK parameters of the population used in the senescence predictions. Comparisons are made at an individual and mean level.

Individual estimates of parameters η's (e.g., η_{jCL}) and therefore individual parameter values (for example CLj) and the individual concentration profiles (Cp_{ij}) were obtained with NONMEM VII using the FOCE option in the $ESTIMATION record. CLj is then an empirical Bayes estimate of the jth individual's clearance based on the population parameters and observed concentrations. The resulting Bayesian predictions were compared with the scaled individual PK parameters in the same population with the Senescence model.

1.4 *Softwares used in the analyses*

For the Senescence model, the Intravenous PopPK model and the Geriatric PopPK model, modeling and simulation were carried out with the software package NONMEM® (version VII, Icon Plc, Dublin, Ireland). Data exploration, statistical testing external to NONMEM® and graphics were performed using S-PLUS® (version 8.2, TIBCO Software Inc, Palo Alto, CA, USA).

2 Results Section 2

The individual demographic characteristics from Study BILA 459-05 were used as a starting point to create a virtual elderly population that will be used in the development and validation of the senescence model. In this way, the relevant information extracted from the physiological databases created was assigned to each subject considering their age, gender, weight and height. The identified metrics relevant for scaling of each PK parameter are summarized in P2 Table 1 together with the characteristics of the patient population used in this analysis. Reference adult body composition parameters are reported in Table AII-4 in Annex II.

P2 Table 1. Demographic data descriptions of the elderly subject's group and data source

Available from study BILA 459-05					Relevant Metrics Extracted from Medatabase						
ID	**Gender**	**Age (Years)**	**Weight (kg)**	**Height (cm)**	**BSA (m^2)**	**fu**	**CSHA (g/L)**	**TBF (kg)**	**TBW (L)**	**GFR (L/h)**	**CO (L/h)**
17	male	73	68.7	177	1.85	0.1362	42.52	12.59	37.87	4.28	294.05
18	male	67	79.9	174	1.95	0.1350	42.95	21.89	41.86	4.80	319.04
19	male	66	73.2	179	1.91	0.1348	43.02	14.53	40.24	4.88	315.48
20	male	65	63.4	168	1.72	0.1346	43.09	14.03	35.85	4.96	286.13
21	male	83	73.9	167	1.83	0.1382	41.82	21.73	37.63	3.44	275.19
22	male	73	83.7	170	1.95	0.1362	42.52	26.72	42.16	4.28	310.57
23	male	66	77.0	160	1.80	0.1348	43.02	27.76	39.48	4.88	297.79
24	male	65	80.5	173	1.94	0.1346	43.09	22.86	42.14	4.96	321.85
25	female	69	48.4	150	1.41	0.1354	42.81	20.01	25.87	4.01	231.00
26	female	65	82.1	169	1.93	0.1346	43.09	32.29	36.21	4.29	319.20
27	female	65	64.4	161	1.68	0.1346	43.09	24.73	30.99	4.29	279.59
28	female	67	78.0	164	1.85	0.1350	42.95	32.30	34.67	4.14	303.02
29	female	77	70.2	160	1.73	0.1370	42.24	29.24	32.32	3.37	269.58
30	female	65	85.1	168	1.95	0.1346	43.09	34.89	36.85	4.29	322.58
31	female	65	72.4	161	1.76	0.1346	43.09	30.17	32.97	4.29	293.21
32	female	68	68.0	163	1.73	0.1352	42.88	26.06	32.10	4.08	283.61

fu Unbound fraction, GFR Glomerular filtration rare, CHSA Albumin molar concentration, TBW Total body water, TBF Total body fat, BSA Body surface area, CO Cardiac Output

Reference Adult body composition parameter (adult of reference was considered aged 30-50 years):

CO male (L/h)=352.11, CO female (L/h)=318.19 (Watson et al., 1980); GFR male (L/h) = 113.03, GFR female (L/h) = 99.66 (Stader et al., 2018); fu adult = 0.13 (Stader et al., 2018); CHSA (g/L) = 44.86 (Schlender et al., 2016)

2.1 *Application of scaling equations: Senescence predictions in elderly patients from study BILA 459-05*

The population PK model using intravenous data after administration of 10 mg of bilastine to young adults was developed in NONMEM (FOCE method–2 compartments with first order absorption) using data available from BILA-2909/BA study (Table AI-2 in Annex I). PK parameters in elderly were scaled considering these intravenous adult PK parameters of reference obtained and detailed in P2 Table 2 before correcting them by the bioavailability.

P2 Table 2. Adult Population pharmacokinetic model fit to plasma concentration-time data from study BILA 2909/BA. Population pharmacokinetic-parameter estimates with relative standard error of the (model) estimates (SEE) expressed as % and interindividual variability expressed as a percentage of the coefficient of variation of the SEE (SEE %)

Intravenous PopPK model			
Absolute Parameter	**Estimate θ**	**SEE_θ**	**SEE%**
Vc (L)	37.6	2.25	6
Vp (L)	17.2	3.57	20.8
CL (L/h)	15.5	0.86	5.5
Q (L/h)	1.01	0.19	18.8
ωCL	13%	9%	49%
ωVc	11%	10%	77%
σ%	49%	11%	5%

[a] The relative standard error is the standard error divided by the parameter estimate.
CL= absolute total body clearance of the drug from plasma; Q= absolute intercompartmental clearance; Vc= absolute central compartment volume of distribution; Vp= absolute peripheral compartment volume of distribution.

The equations applied to each PK process to scale the absolute parameters from adults into geriatrics are summarized in P2 Table 3.

P2 Table 3. Semi-Physiological Scaled Equations Used in the Extrapolation of Bilastine PK Parameters to Elderly

Parameter	Equation and/or Reference	PK Parameter related	Equation to scale absolute PK in elderly
CSHA (g/L)	$CHSA = -0.0709\ x\ Age + 47.7$ **Eq.1**	**Fraction unbound (fu)**	$fu\ ger = \frac{1}{1+\frac{CHSAger\ x\ (1-fuad)}{CHSAad\ x\ fuad}}$ **Eq.2**
GFR (mL /min)	$GFR(ml/min/100g\ kidney) = 26.6 \times \left(1 - \frac{0.9 \times (Age(yr) - 30)^{1.5}}{TA_{50}{}^{1.5} + (Age(yr) - 30)^{1.5}}\right)$ *Where* TA_{50}*:54(male), 59(female)* The GFR equation and relevant Age and sex dependent kidney weight is obtained from Schlender et al. 2016	**Renal Clearance (L/h) (CLr)**	$CLr_{ger}iv = \frac{GFRger\ x\ fu_{ger}}{GFRad\ x\ fu\_ad}\ x\ CLr_{ad}iv$ **Eq.3** $ratio\ \frac{CL\ iv}{CLr\ iv}$ **Eq.4**
CO (L/h) **BSA (m^2)**	$CO = 159\ x\ BSA - 1.56\ x\ Age + 114$ **Eq.5** $BSA = 0.007184\ x\ weight\ (kg)^{0.725}\ x\ 59\ x\ height(cm)^{0.425}$ **Eq.6**	**Intercompartmental Clearance (L/h) (Q)**	$ratio\ \frac{COad}{Q\ iv}\ male = 348.62$ **Eq.7** $ratio\ \frac{CO\ ad}{Q\ iv}\ female = 315.04$ **Eq. 8**
TBW (L) **TBF (Kg)**	$TBW_{male} = 2.447 - 0.09516\ x\ Age(yr) + 0.1074\ x\ height(cm) + 0.3362\ x\ weight(kg)$ **Eq.9** $TBW_{female} = -2.097 + 0.1069\ x\ height\ (cm) + 0.2466\ x\ weight\ (kg)$ **Eq.10** $TBF\ (kg) = 0.68\ x\ weight\ (kg) - 0.56\ x\ height(cm) + 6.1\ x\ Sex + 65$ $male = 0, female = 1$ **Eq.11**	**Volume of distribution (L) (Vss, Vc and Vp)**	$Vss \cong TBW + TBF$ **Eq.12** $Vc\ iv = 0,65\ x\ Vss\ iv$ **Eq.13** $Vp\ iv = Vss\ iv - Vc\ iv$ **Eq.14**
		Absorption rate constant (h^{-1}) (Ka)	The constant of absorption (Ka) was estimated with the Geriatric PopPK model

Abbreviations are: fu Unbound fraction, GFR Glomerular filtration rare, CHSA Albumin molar concentration, TBW Total body water, TBF Total body fat, BSA Body surface area, CO Cardiac Output, V Volume of distribution, ss Steady state, c Central, p Peripheral, F Bioavailability, CL Clearance, r Renal, ad Adult, fu Unbound fraction, GFR Glomerular filtration rare, CHSA Albumin molar concentration, Cp Plasma Concentration, CO Cardiac Output, Q Intercompartmental Clearance, K_a Absorption rate constant

The PK assumptions used to scale the PK parameters for elderly are detailed for each ADME process. PK is assumed to be linear and proportional in the dose range studied.

Intravenous renal clearance (CLr iv) obtained from the iv administration of the drug (10 mg) and the quantification of renal clearance in young adults, 8.27 L/h (Jauregizar et al., 2009) was scaled to geriatrics by using an adaptation of the equation from Edginton et al.(Eq.3 P2-Table 3) (Stader et al., 2018)

$$\boldsymbol{CLr_{ger}iv = \frac{GFRger \times fu_{ger}}{GFRad \times fu\_ad} \times CLr\_ad\_iv} \qquad \textbf{Eq.3 P2-Table 3}$$

Where:

- fu_{ger} unbound fraction of bilastine in geriatrics (as described below)
- fu_{ad} unbound fraction of bilastine described as 0.13 in adults (Sadaba et al., 2013a)
- GFR ger was obtained from the generated databases available in Table AII-2 and Table AII-3 in Annex II
- GFR ad glomerular filtration rates in adults were also extracted from the corresponding database
- CLr_{adult} described as 8.27 L/h was obtained from a dedicated bioavailability study (Edginton et al., 2006)

The aging of Glomerular Filtration Rate (GFR) data were taken from PKSIM of Schlender et al. (Jauregizar et al., 2009) according to the selected geriatric ages ranges (5 year intervals), of both sexes.

The fu was calculated as a function of the molar albumin concentration (CHSA) and the following criteria have been assumed:

- Albumin is the main protein to which bilastine binds to
- The affinity for the protein is not affected by aging
- Binding of bilastine to albumin is not a saturable process.

Albumin concentration was calculated using Stader et al.2018 (Sadaba et al., 2013a) equation (Eq.1 P2-Table 3) described below:

$$\boldsymbol{CHSA\left(\frac{g}{L}\right) = -0.0709 \times Age + 47.7} \qquad \textbf{Eq.1 P2-Table 3}$$

The following equation from McNamara et al. (Eq.2 P2-Table 3) for the calculation of the fraction unbound was rearranged for geriatrics (Schlender et al., 2016):

$$fu_{ger} = \frac{1}{1+\frac{CHSAger \times (1-fuad)}{CHSAad \times fuad}} \qquad \textbf{Eq.2 P2-Table 3}$$

Once the renal clearance has been scaled, plasma clearance was estimated. For this, the relationship between the percentage of drug excreted in urine and that corresponding to other routes (faeces) is considered. This ratio is 1.87.

For Inter-compartmental clearance (Q), the relationship with cardiac output was considered calculating the corresponding values for a reference adult and also per geriatric age range. The equation describe in methods (Eq.5 P2 Table 3) was used and the values obtained are shown in P2 Table 1 Then, CO/Qiv ratio was calculated for an adult aged 30-50 years (male and female) and it was then applied to back calculate the Qiv for each corresponding Cardiac output value in the elderly patients per age range (P2 Table 4).

As detailed in P2 Table 3, the steady state intravenous volume of distribution (Vss iv) was considered to be similar to the physiological total body water (TBW) volume and to the total body fat (TBF) (Eq.12 P2-Table 3). The value represents the global distribution of the drug in elderlies.
The relationship of intravenous Vc to intravenous Vss in adult (0.65) (Eq. 13 P2-Table 3) was assumed to be maintained across aging and used to scale Vc iv in elderly. Then peripheral volume (Vp) was calculated from the relation Vss = Vc + Vp (Eq.14 P2-Table 3).
P2 Table 4 and P2 Table 5 show respectively the individual, and the mean of the absolute PK parameters of bilastine calculated in elderlies. These parameters are scaled from the adult intravenous parameters using the equations summarized in P2 Table 3.

P2 Table 1. Individual absolute PK parameters of Bilastine estimated in Elderly using the Senescence model

Individual Senescence Predictions							
ID	**Age (Years)**	**Vss abs (L)**	**Vc abs (L)**	**Vp abs (L)**	**CLr abs (L/h)**	**CL abs (L/h)**	**Q abs (L/h)**
17	73	50.47	32.80	17.66	5.47	10.25	0.84
18	67	63.75	41.44	22.31	6.09	11.40	0.92
19	66	54.77	35.60	19.17	6.17	11.57	0.90
20	65	49.89	32.43	17.46	6.26	11.73	0.82
21	83	59.36	38.58	20.78	4.46	8.36	0.79
22	73	68.88	44.77	24.11	5.47	10.25	0.89
23	66	67.24	43.70	23.53	6.17	11.57	0.85
24	65	65.00	42.25	22.75	6.26	11.73	0.92
25	69	45.89	29.83	16.06	5.78	10.82	0.73
26	65	68.50	44.53	23.98	6.14	11.50	1.01
27	65	55.73	36.22	19.50	6.14	11.50	0.89
28	67	66.97	43.53	23.44	5.95	11.16	0.96
29	77	61.55	40.01	21.54	4.91	9.20	0.86
30	65	71.74	46.63	25.11	6.14	11.50	1.02
31	65	63.14	41.04	22.10	6.14	11.50	0.93
32	68	58.16	37.80	20.35	5.86	10.99	0.90

Abbreviations are: V Volume of distribution, abs absolute, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, Q Intercompartmental Clearance.

P2 Table 2. Mean PK Absolute Parameters of Bilastine predicted in Elderly using the Senescence equations

Mean Senescence Predictions	
Parameter	**Predicted**
Vss abs (L)	60.69
Vc abs (L)	39.45
Vp abs (L)	21.24
CLr abs (L/h)	5.84
CL abs (L/h)	10.94
Q abs (L/h)	0.89

Abbreviations are: V Volume of distribution, abs absolute, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, Cp Plasma Concentration, Q Intercompartmental Clearance.

However, considering that bilastine is administered orally, all individual parameters are corrected by the bioavailability (Stader et al., 2018) with a value of 61%. and the resulting individual and average PK parameters are shown in P2 Table 6 and P2 Table 7, respectively.

P2 Table 3. Bioavailability corrected Individual PK Parameters of Bilastine predicted in Elderly using the Senescence equations

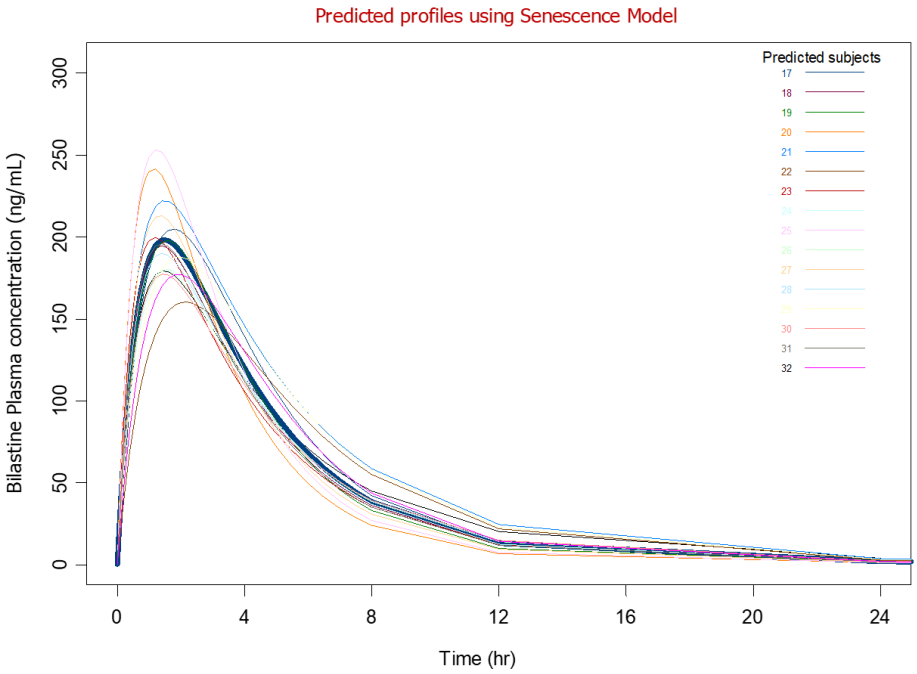
Senescence Individual Predictions						
ID	**Gender**	**Age (Years)**	**CL/F (L/h)**	**Vc/F (L)**	**Vp/F (L)**	**Q/F (L/h)**
17	male	73	16.81	53.78	28.96	1.38
18	male	67	18.70	67.94	36.58	1.50
19	male	66	18.96	58.37	31.43	1.48
20	male	65	19.24	53.16	28.62	1.35
21	male	83	13.70	63.25	34.06	1.29
22	male	73	16.81	73.39	39.52	1.46
23	male	66	18.96	71.64	38.58	1.40
24	male	65	19.24	69.26	37.30	1.51
25	female	69	17.74	48.89	26.33	1.20
26	female	65	18.86	72.99	39.30	1.66
27	female	65	18.86	59.38	31.97	1.45
28	female	67	18.29	71.36	38.43	1.58
29	female	77	15.08	65.59	35.32	1.40
30	female	65	18.86	76.44	41.16	1.68
31	female	65	18.86	67.28	36.23	1.53
32	female	68	18.02	61.97	33.37	1.48

Abbreviations are: V Volume of distribution, abs absolute, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, Q Intercompartmental Clearance.

P2 Table 4. Bioavailability corrected Mean PK Parameters of Bilastine predicted in Elderly using the Senescence equations

Senescence Predictions	
Parameter	**Predicted**
Vc/F (L)	64.67
Vp/F (L)	34.82
CL/F (L/h)	17.97
Q/F (L/h)	1.46
CV (%) Vc	12.59
CV (%) Vp	12.59
CV (%) CL	8.91
CV (%) Q	8.51
Abbreviations are: V Volume of distribution, F Bioavailability, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, Cp Plasma Concentration, Q Intercompartmental Clearance CV Coefficient of variation.	

The resulting scaled individual PK parameters were used to simulate the time evolution of bilastine plasma levels after an oral dose of 20 mg for each subject of study BILA 459-05 (P2 Figure 4) and compared with the Bayesian estimates and mean parameters from the Geriatric PopPK model developed.



P2 Figure 1. Individual (colored lines) and mean (blue thick line) elderly predicted (BILA 459-05) using the Senescence model after an oral administration of 20 mg of bilastine

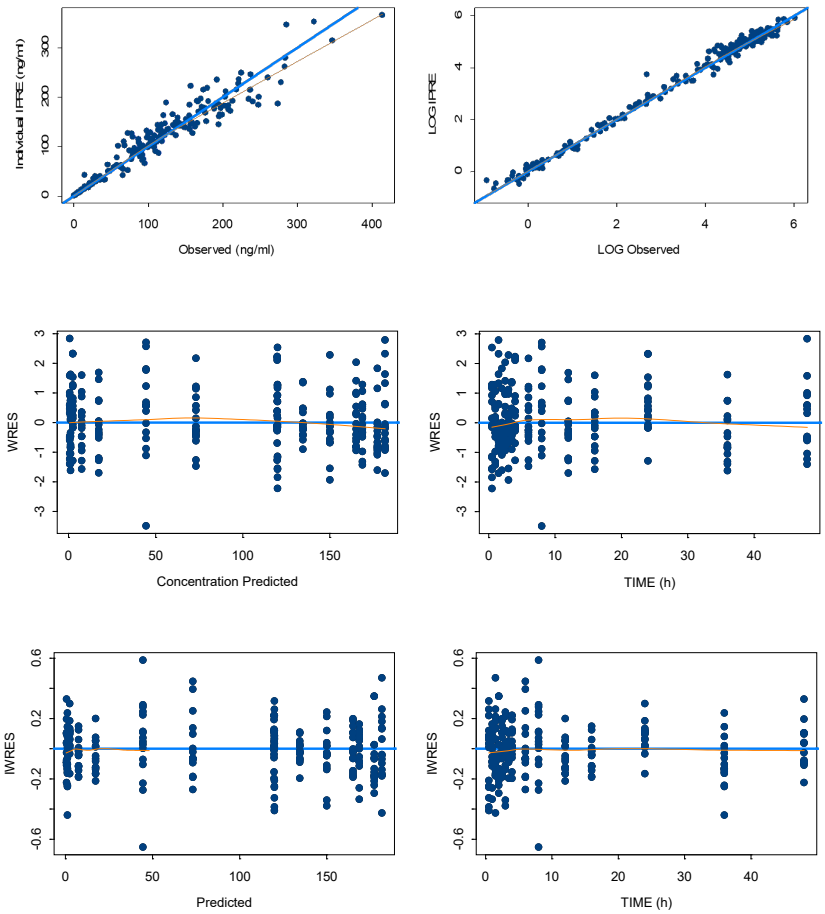
2.2 *Geriatric PopPK model and qualification of the scaling process*

The PK model based on study BILA 459-05 (only elderly patients, Table AII-2 in II) best fitting plasma bilastine concentrations was a two-compartment model parameterized in terms of clearance (CL), volumes of distribution of the central and peripheral compartments (V_c and V_p), Intercompartmental clearance (Q) and K_a (Model ADVAN8 TRANS4). In all analysis, the interindividual variability (IIV) was model as exponential (e.g., for CL) {$CL_j = CL * \exp(\eta_{jCL})$} where μjCL denotes the difference between the estimated parameter (CL_J) of individual j and the typical value (CL) in the population. The IIV was modelled the same way for the other parameters. The η's are zero mean random variables with variance ω^2 (ω^2_{CL}). The ω^2's are the diagonal elements of the inter-individual (IIV) variance-covariance matrix, Ω.

Goodness of fit of different models to the data was evaluated using different criteria such as change in objective function value (OFV), condition number, visual inspection of different diagnostic plots,

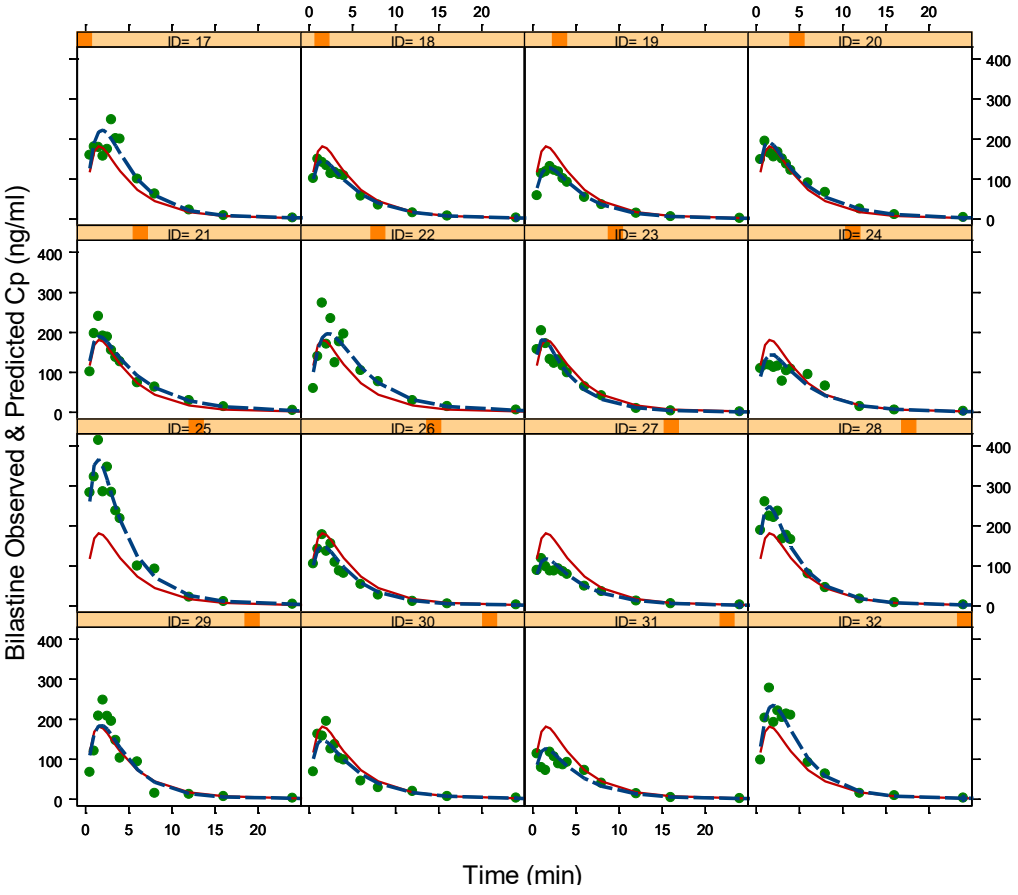
precision of the parameter estimates, and decreases in IIV and residual variance. At all stages of model development, diagnostic plots were examined to assess model adequacy and possible lack of fit. In addition, plots of observations versus individual and populations predictions, weighted individual residuals versus individual predictions and time (IWRES) were evaluated.

The standard diagnostic plots assessing the goodness of fit for the base structural PK model are provided in P2 Figure 5. In the upper panels, PRED and IPRED are plotted against the observations, both in linear and log scales. In all cases, the predicted concentrations are fairly evenly distributed about the line of identity, thus indicating the appropriateness of the structural model selected and the lack of major bias. The lower panels show a uniform and random distribution of the residuals about the zero line when scatter plotted against population-predicted concentration or time. As expected, plot of residuals (RES) against PRED or time shows the cone shape typical of heteroscedastic (proportional) residual error model, which is corrected by using the weighted residuals (WRES) and individual weighted residuals (IWRES) instead.



P2 Figure 2. Standard diagnostic plots of the Geriatric PopPK model developed in NONMEM® 7 (N=16)

Fit of individual data according to the base structural PK model is provided in P2 Figure 6 which represent concentrations on a linear scale. Red and blue lines respectively correspond to the mean population prediction (PRED) and individual prediction (IPRED) provided by the model, whereas green dots represent the real observations.



P2 Figure 3. Fit of individual concentration-time data by the base structural model developed for bilastine in geriatric volunteers

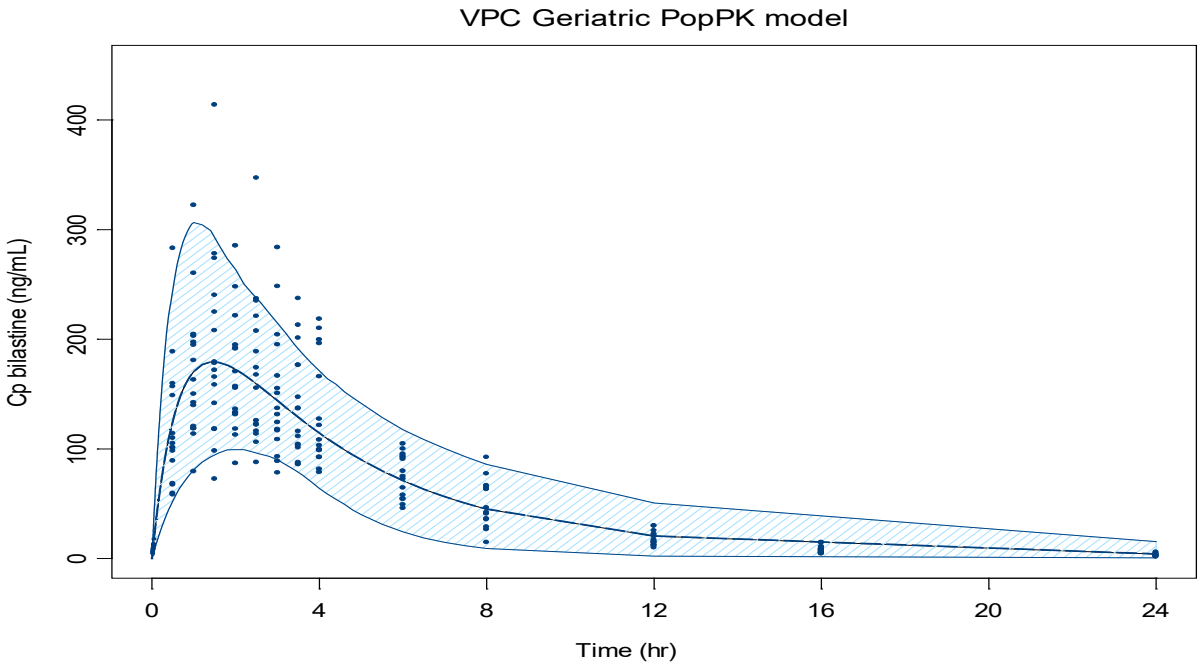
Overall, it can be concluded that model fits are characterized by a satisfactory degree of precision, which means that the quantitative value of the PK parameters was correctly estimated by the compartmental model. Based on P2 Figures 5-6 above, it can be overall concluded that the base structural model adequately describes the experimental data. The final Geriatric PopPK model parameters estimated for the geriatric dataset from study BILA 459-05 (N= 16) are presented in P2 Table 8 below.

P2 Table 5. Geriatric Population pharmacokinetic model fit to plasma concentration-time data from study BILA 459-05. Population pharmacokinetic-parameter estimates with relative standard errors (ESθ) and interindividual variability expressed as a percentage of the coefficient of variation (SEE (%))

Geriatric PopPK model		
Parameter	**Estimate**	**SEE (%)**
Vc/F (L)	73.3	8.73
Vp/F (L)	33.8	17.88
CL/F (L/h)	17.6	6.60
Q/F (L/h)	1.49	10.82
Ka (h^{-1})	1.28	9.43
ωKa	32.6	35.41
ωCL	27.6	25.59
ωVc	33.9	27.81
ωQ	32.1	29.75
ωVp	43.9	41.94
σ%	18.7	18.69

***$SEE_θ$= standard error of estimates as % calculated via bootstrap (N=341)**
Bootstrap mean parameter values Vc/F (L)=73.8; Vp/F (L)=34.6; CL/F (L/h) =17.7; Ka (h^{-1}) = 1.32; Q/F (L/h) =1.50

Abbreviations are: CL= clearance; K_a= first-order absorption rate constant; Q= intercompartmental clearance; Vc= central compartment volume of distribution; Vp= peripheral compartment volume of distribution; F= bioavailability; ω= interindividual variability; σ: residual unexplained variability



P2 Figure 7. Visual predictive check for the geriatric PopPK model in the elderly subset population (BILA 459-05)

P2 Figure 7 shows the Visual Predictive Check performed to validate the Geriatric PopPK model where the blue line and the blue area represent the mean and 95% confidence interval of the model predictions.

2.3 *External validation of the Senescence model*

P2 Table 9 shows the comparison between the individual parameters (Bayesian) of elderly subjects from study BILA 459-05 obtained with the Geriatric PopPK model and the individual predictions made in the same subjects using the Senescence equations described in P2 Table 3.

P2 Table 6. Comparison between the Bayesian parameters of elderly subjects from study BILA 459-05 obtained with the Geriatric PopPK model and the individual predictions made in the same subjects using the using the RI Senescence model

ID	CL/F L/h) *Senescence*	CL/F (L/h) *PopPK*	Vc/F (L) *Senescence*	Vc/F (L) PopPK	Vp F (L) Senescence	Vp/F (L) PopPK	Q/F (L/h) Senescence	Q/F (L/h) PopPK	Ka* (h^{-1}) PopPK
17	16.81	13.85	53.78	52.16	28.96	24.65	1.38	1.22	0.88
18	18.70	20.61	67.94	94.93	36.58	45.97	1.50	1.80	1.42
19	18.96	22.88	58.37	98.88	31.43	58.27	1.48	2.28	1.05
20	19.24	15.47	53.16	76.53	28.62	28.53	1.35	1.16	1.66
21	13.70	14.14	63.25	76.46	34.06	35.59	1.29	1.26	1.46
22	16.81	12.51	73.39	61.00	39.52	48.99	1.46	1.65	0.79
23	18.96	20.75	71.64	78.04	38.58	23.90	1.40	1.27	1.89
24	19.24	20.30	69.26	91.61	37.30	41.09	1.51	1.70	1.16
25	17.74	9.76	48.89	36.59	26.33	15.94	1.20	0.81	1.46
26	18.86	21.65	72.99	88.05	39.30	52.89	1.66	2.14	1.31
27	18.86	25.56	59.38	118.23	31.97	63.91	1.45	2.37	1.39
28	18.29	14.23	71.36	54.11	38.43	22.89	1.58	1.13	1.47
29	15.08	17.68	65.59	62.19	35.32	35.40	1.40	1.71	0.93
30	18.86	20.62	76.44	93.95	41.16	38.67	1.68	1.61	1.42
31	18.86	24.70	67.28	110.13	36.23	45.63	1.53	1.91	1.43
32	18.02	13.69	61.97	46.54	33.37	19.47	1.48	1.10	0.81
Mean	**17.94**	**18.02**	**64.67**	**77.46**	**34.82**	**37.61**	**1.46**	**1.57**	**1.28**
*** Ka (h^{-1}) (not scaled) was estimate using the Geriatric PopPK Model and used to inform the Senescence model**									

Moreover, the percentage error for each parameter and subject were calculated and the average predictions error was less than 10% for every scaled parameter and it is shown in P2 Table 10.

P2 Table 7. Evaluation of predictive accuracy of Senescence model vs Geriatric PopPK model. The standardized error is shown

ID	Percentage of error CL/F	Percentage of error Q/F	Percentage of error Vc/F	Percentage of error Vp/F
Mean	-6%	**0%**	9%	-6%
$\%error = \frac{(estimated\ Geritraic\ PopPK\ model - predicted\ Senescence\ model)}{estimated\ Geritraic\ PopPK\ model} x\ 100$				

The senescence scaled parameters were considered adequate and simulations were performed both at an individual and average level. The absorption constant was not scaled and therefore was considered to be equal to the one calculated in adults.

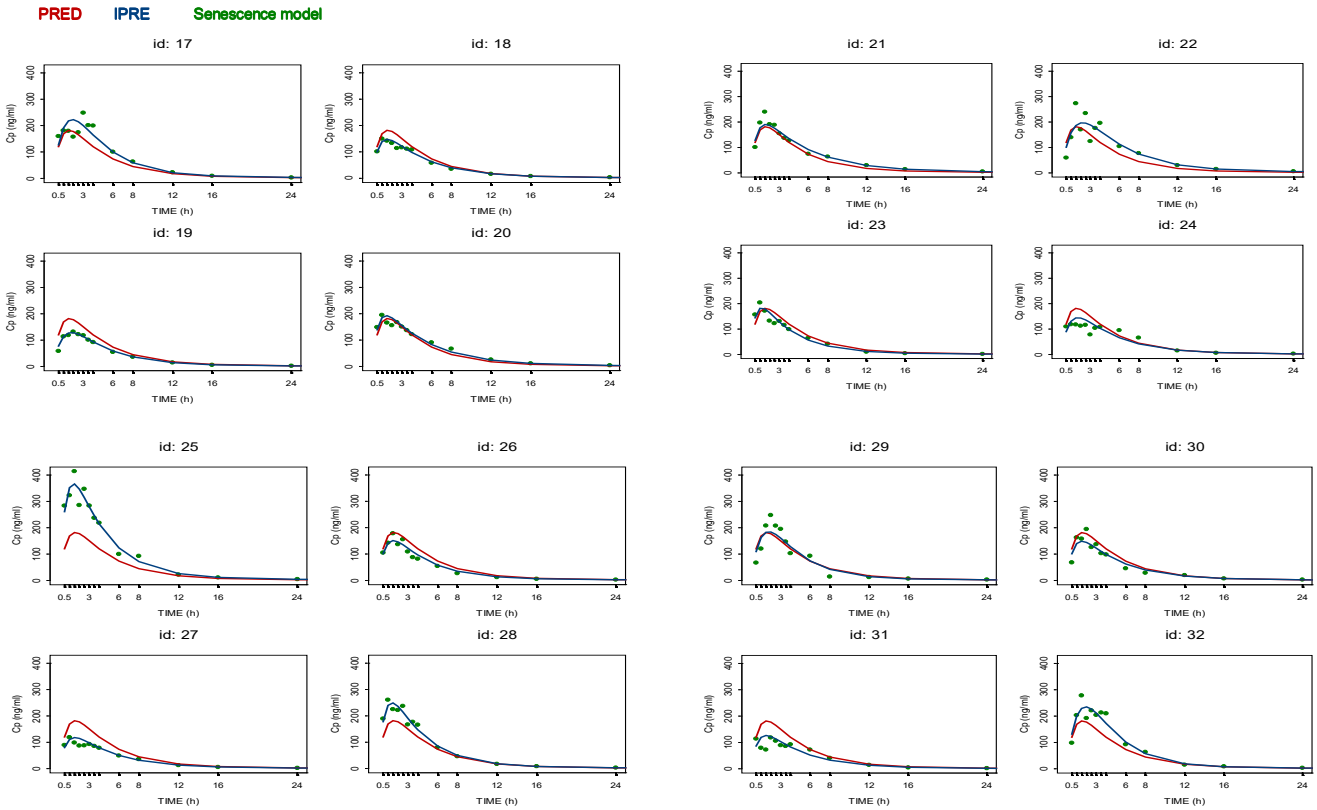
PK parameters, both scaled and modelled from the elderly data, were used to calculate the rate and extent of absorption (P2 Table 11).

P2 Table 8. Rate and extent of absorption obtained using the geriatric PopPK model and the PK parameters obtained in geriatrics by applying the senescence equations

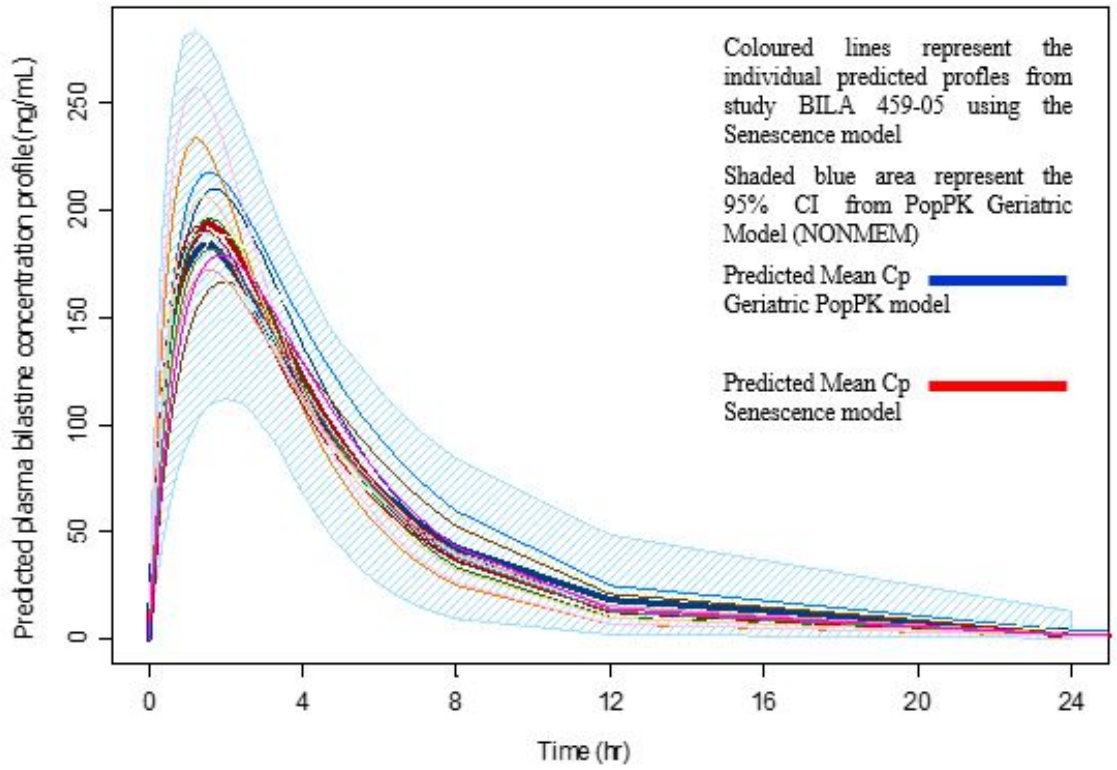
Parameter	C_{max} (ng/mL)	$AUC_{[last]}$ (ng*h/mL)
Senescence predictions	197.85	1111
Geriatric PopPK model	176.02	1129.40
Abbreviations are: C_{max} maximal concentration, AUC area under the curve		

Both C_{max} and $AUC_{[last]}$ were accurately predicted using the PK parameters scaled by means of the senescence equations.

P2 Figure 8 shows a comparison between the time evolution of bilastine plasma levels after an oral dose of 20 mg simulated using both PK scaled parameters using the senescence model and the Bayesian estimates parameters from the Geriatric PopPK model. Red and blue lines respectively correspond to the mean population prediction (PRED) and individual prediction (IPRED) provided by the Geriatric PopPK model, whereas green dots represent the predictions obtained with the Senescence model.



P2 Figure 8. Fit of individual concentration-time data by the base structural model developed for bilastine in geriatric volunteers



P2 Figure 9. Visual Predictive check (VPC) reporting the PK interval (mean, 95%, blue area) in the elderly population (BILA 459-05) using the Geriatric PopPK model versus the individual elderly predicted (BILA 459-05) using the Senescence model after an oral administration of 20 mg of bilastine

Finally, a good match between profiles between the PK interval (mean, 95%) in the elderly population (BILA 459-05) using the Geriatric PopPK and the individual profiles predicted using the Senescence model was observed, confirming the adequacy of the equations selected for prediction of each ADME process in elderlies (P2 Figure 9).

3 Discussion Section 2

Until now, the impact of aging related changes in physiological variables and their repercussion in the dosing recommendations has not been extensively studied. In pediatrics, for example in general dosing recommendations are still made by body weight assuming this already integrates all maturation processes and their influence in PK of drugs. In elderly patients, dose is usually decreased empirically by applying factors that do not account for the specific characteristics of the drug or the elderly subset under consideration. The physiological variables that are influenced mostly by age are TBW, TBF, GFR, etc.

In this sense, PK parameters such as volume of distribution and clearance can be widely affected by the process of aging. Concretely, bilastine follows a two compartmental behavior, expected by its high lipophilicity and therefore changes in the peripheral and central volumes are expected to occur with again. The Senescence approach has elucidated the importance of key age-dependent variables such as cardiac output, total body water, total body fat and glomerular filtration rate on bilastine disposition that, when taken into account, have allowed to obtain accurate predictions of plasma bilastine concentrations in elderly.

Similarly, Vozmediano et al (2017) already applied this physiologically driven scaling from adults into children with an optimal outcome. For bilastine volume of distribution showed a relationship with the physiological total body water (TBW) in rats and dogs. This relationship was assumed to be maintained in humans and therefore, used to assess the prediction of the volume of distribution of bilastine in children.(McNamara et al., 2002). Taking this into consideration, and also considering that in elderlies changes in body composition include not only a decrease of total body water (TBW), estimated as 10-15%, but also changes in body fat composition (increase of 20-40%), both variables were considered.

During aging, most studies have reported an overall increase in body fat ranging from 20-40% as people grow older. Apparently, fat mass (FM) increases up to 60-70 years, after which it appears to stabilize (Sadaba et al., 2013b). Particularly, the pattern of body fat distribution within the body results altered with aging. As body fat increases, total body water as well as lean body mass decreases. The starting clearance index for adult ($Cltot_{iv}/CLr_{iv}$), and the bioavailability was considered to be 61% relative to the IV route. These data seem to be apparently in contradiction with the physicochemical and pharmacokinetic properties of bilastine. If it is acknowledged that bilastine is mainly excreted renally (and more precisely through filtration, Clr~GFR) and considering that no metabolism (systemic or presystemic) is present and that the biliary secretion is negligible (less than 5% or total dose), the total CL should be in line with the renal clearance and not almost two-fold different. This argument also applies in explaining the relatively low value of F after oral

administration for a drug with high solubility and permeability properties. However, bilastine is a substrate of P-gp, which favors its non-distribution to SNC but affects the elimination processes also. In this sense, bilastine has been recovered in faeces after oral administration in a proportion of 67%. After IV administration the amount in faeces is also expected to be 40% as the % of drug in urine was found to be around 60% versus 40% after oral administration. This behavior that has also been reported for other P-gp substrates leads to the situation where 100% BA cannot be considered for the IV route as is usually accepted for most drugs.

However, the % of secretion to faeces can also be dependent or different depending on the demonstration route and this means that the 40% value observed after IV cannot be directly applied to the oral route. A possible explanation for this is attributable to the intrinsic bioavailability of bilastine (F) depending on transporters (P-gp) in the GI tract can affect drug bioavailability even after intravenous administration of the drug that, by definition, it is considered to be 100%.

Particularly, (P-gp), a critical efflux transporter for many medications, such as bilastine, actively transporting them back to the intestinal tract, contributing to decrease their absorption (Vozmediano et al., 2014, 2019). This active secretion to faeces, via P-gp cannot be considered a systemic clearance component in the sense of its definition as volume of plasma eliminated per unit of time. However, if the relationship between the dose and the drug disposition considered, the effect of P-gp even intravenous administration is included as part of the global CL value, explaining the relationship observed between CLt and CLr, not equal to unity after iv administration.

No aging effect on known bilastine bioavailability (F) was considered since active transporters in the intestine (P-gp and OATP) were showed to be no significantly altered in elderly subjects. (Schlender et al., 2016). Particularly, P-gp' function seems to be well preserved in patients of advanced age (Brenner et al., 2004).

The fact that the senescence approach could predict accurately the individual PK as well as the average AUC in elderly patients, allows to validate the assumptions that were considered when scaling each parameter and their relationship with ageing and physiology.

These results highly support the utility of the physiological scaling approach in relating the PK parameters to physiological processes underlying the aging process and in obtaining the required information for proper dosing in the elderly.

The development of a physiological based approach, allows to understand mechanisms related with the PK processes of a drug. In this case, it has been very useful to understand and quantify underlying mechanism that account for clearance, bioavailability, and distribution of the drug. Moreover, the changes in physiological variables as a function of aging has been directly applied to predict the PK properties and this allows to conclude that the properties of the drug are well known and described.

The validation of the individual predictions based only on the age and gender of the subject confirms that the pathways related to all ADME, processes and their relationship with aging are well defined and quantitatively established and can be therefore use to predict the behavior of similar drugs in elderly patients. Moreover, comorbidities and comedication based simulations could also be applied using a similar approach.

This project was carried out as part of an international project ("Desarrollo de una plataforma para optimizar la dosificacion de los regimens farmacologicos en adulto mayores ") started on 2017 as a collaboration with the Center for Pharmacometrics and Systems Pharmacology-University of Florida (USA) and Dynakin, S.L. (Derio, Basque Country). In parallel, the general analysis in the elderly population and the predictions already made were combined with Physiologically based PK approaches with GastroPlus performed by the University of Florida. The bottom up/ top-down approach allowed to fine tune the predictions by applying additional physiological based factors that have been identified, by using PBPK models to influence the PK of the drug.

Specifically, this work has been included in the publication *"Application of a dual mechanistic approach to support bilastine dose selection for older adults"* published on Pharmacometrics & Systems Pharmacology (CPT:PSP) Journal.

Section 3

Part of the results presented in this Section were presented at **the XII Jornadas de Modelización y Simulación en Biomedicina (ModelBio 2019)** (conference organized by the University of Salamanca on 21/23 September 2019). The research work has been presented as oral presentation titled "Population Pharmacokinetic Analysis of Bilastine in subjects with various degrees of renal insufficiency: prediction in elderly populations" (Lo Re V, Rodríguez M, Lukas JC, Encinas E, Campo C, Garcia A, Suarez E). The abstract is attached to the present document as Abstract ModelBio 2019.

SECTION 3. Application of the Senescence model to patients with various degrees of renal insufficiency

Section 3 of the Research project was aimed to evaluate the repercussion in the PK of bilastine in elderly patients with renal impairment through the application of the developed Senescence model.

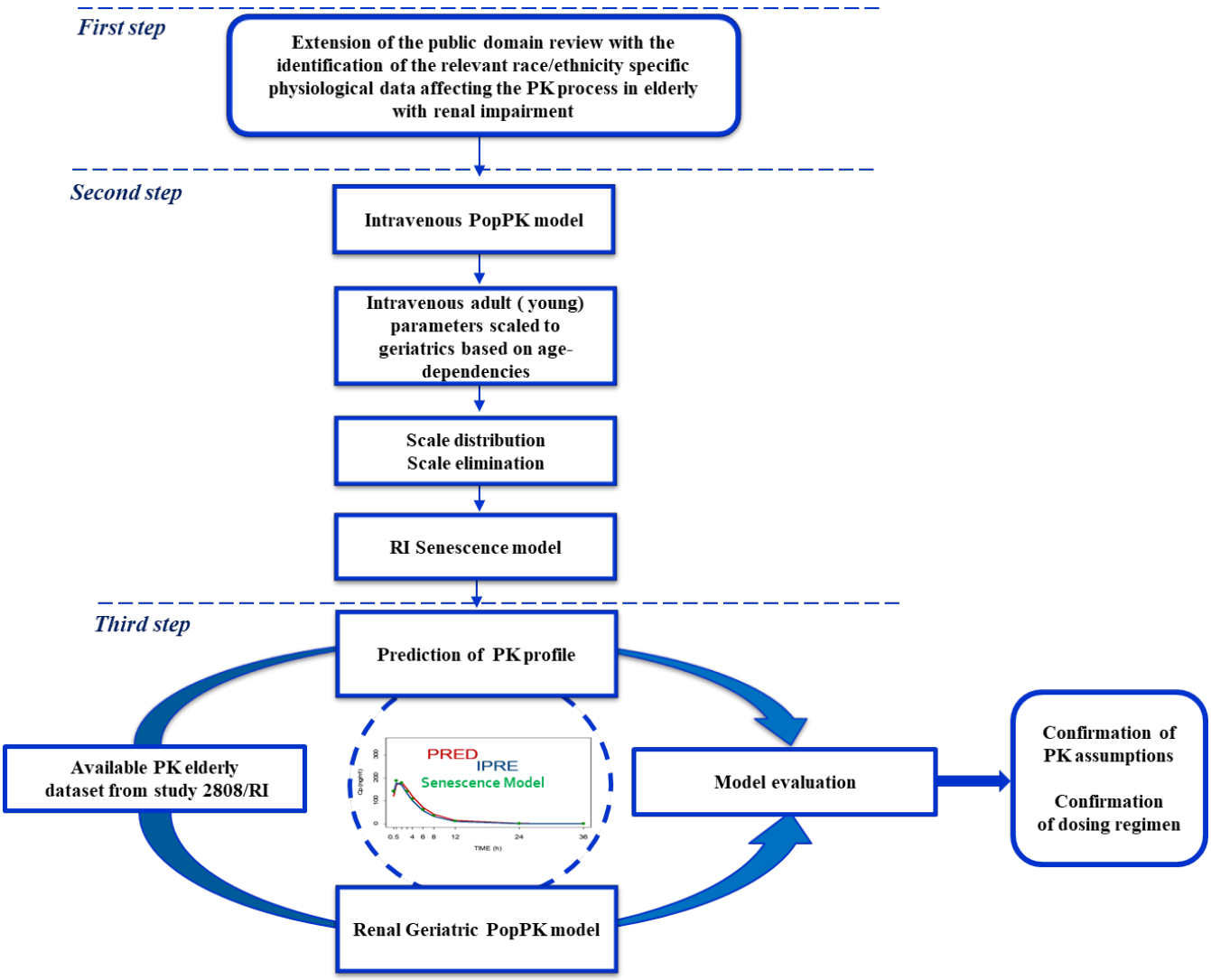
Available PK data were provided by FAES FARMA from study BILA 2808/RI including elderly patients with impaired renal function. The Senescence model was used to simulate the PK in these elderly patients while the accuracy of the individual predictions was evaluated by using a population modelling approach (Renal Geriatric PopPK model) developed for oral bilastine using the same available data.

1 Methodology Section 3

The overall strategy followed for the Senescence model application (hereafter referred to as Renal Impairment (RI) Senescence model) and validation in scaling PK parameters in elderlies with renal impairment is summarized in P3 Figure 1.

The first step, learning stage, consists of the extending of the previously developed metadatabase including race/ethnicity-specific physiological changes of systems associated with aging.

After the completion of the learning stage, the acquired knowledge has been applied to the already developed Senescence model described in Section 2 of this manuscript and the new references values were incorporated into the equations that allowed scaling of relevant PK parameters in the different geriatric's groups of study BILA 2808/RI (P3 Table 3).



P3 Figure 1. Overall extrapolation and validation strategy used during Senescence model development in elderly with various degree of renal impairment

1.1 *First step: Learning stage*

Extension of the public domain review with the identification of the relevant race/ethnicity specific physiological data affecting the PK process in elderly with renal impairment.

As discussed in the previous sections, there are few studies describing the PK of drugs in the elderly population. Moreover, the available studies are performed in a selected group of healthy older subjects that do not involve coexisting pathologies affecting the pharmacokinetics of the drugs under study, such as renal impairment.

Data from literature suggest that all physiological renal changes age-related (decreased kidney size, decreased renal blood flow, decreased number of functional nephrons) lead to a decrease glomerular filtration rate and thus, to a reduced renal clearance, directly impacting the total clearance for a drug with exclusive renal clearance such as bilastine. A progressive decrease in glomerular filtration rate (GFR) and renal blood flow (RBF), with wide variability among individuals in healthy elderly people is very common. (Stader et al., 2018; Thompson et al., 2009; Veering et al., 1990) It is well established that senescence or normal physiologic aging is associated with structural changes in the kidney, whose function is a major determinant of health in the elderly (Brenner et al., 2004).

Generally, the fall in GFR is due to reductions in the glomerular capillary plasma flow rate, and the glomerular capillary ultrafiltration coefficient. In addition, a primary reduction in afferent arteriolar resistance is associated with an increase in glomerular capillary hydraulic pressure. These hemodynamic changes occur in concert with structural changes, including loss of renal mass estimated to be about 20-25%, increased fibrosis, tubular atrophy and arteriosclerosis. The formation of tubular diverticuli, atrophy, fat degeneration, among others, lead to functional alterations making the aging kidney more vulnerable to acute kidney injury, including normotensive ischemic nephropathy, as well as progressive chronic kidney disease (CKD) (Denic et al., 2016; Drenth-van Maanen et al., 2013). Evaluating glomerular filtration rate (GFR) in the elderly is key to the diagnosis and management of CKD, which is highly prevalent in this population. Accurate GFR assessment is particularly important for drug dose adaptation (Musso et al., 2015). In addition, age-related changes in cardiovascular hemodynamics, such as reduced cardiac output and systemic hypertension, are likely to play a role in reducing renal perfusion and filtration. Moreover, increases in cellular oxidative stress that accompany aging result in endothelial cell dysfunction and changes in vasoactive mediators resulting in increased atherosclerosis, hypertension and glomerulosclerosis (Weinstein et al., 2010).

On the other hand, GFR is race and population dependent. Changes in GFR and the development and incidence of renal impairment differ between black and white subjects. As reported by Peralta et al. 2011, compared with whites, blacks show a higher rate of kidney function decline than whites (0.31

mL /min per 1.73 m^2/yr faster on average, P= 0.001). Moreover, generally black population have a higher risk for chronic kidney disease (CKD), increased progression of disease and a higher incidence of end-stage kidney disease (ESDR). Particularly, the prevalence of ESRD has been reported to be 3.7 times greater in blacks than in whites (Tarver-Carr et al., 2002).

Based on these considerations, the previously described metadatabase in Section 2, providing a basis for establishing a predictive Senescence model for the Caucasian healthy elderly age range, needed extension. The study BILA 2808 was carried out in The United States with a highly heterogenous population with a different GFR range than the European references. This means also that the predictive model had to be adjusted to the values observed in the clinical study and therefore the scaling equations took into consideration the physiological values form databases in American population.

1.2 *Second step: Renal Impairment Senescence model application*

1.2.1 *Clinical PK study data (study BILA-2909/BA)*

Adult intravenous PK parameters obtained from the dedicated bioavailability study (Study code BILA 2909/BA) described in Table AI-2 in Annex I were used as a starting point for scaling into geriatrics with renal impairment.

1.2.2 *Clinical PK study data (study BILA 2808/RI)*

Study BILA 2808/RI was an open-label, single-dose, parallel-group study in healthy subjects and in subjects with various degrees of renal insufficiency. As reported by Lasseter et al.2013, the study was conducted to determine the pharmacokinetics and tolerance of bilastine in patients with varying degrees of renal impairment showing that the renal route is the main excretory route for bilastine in plasma and bilastine clearance is proportional to renal function. In addition, it has been showed that patients with renal impairment have increased C_{max} and AUC. Nevertheless, in the study patients with severe renal failure, C_{max} values did not reach concentrations higher than those that could potentially cause adverse effects.

Specific physiological information, including the GFR, was available from the group of elderly subjects with different degrees of renal impairment treated with bilastine (study BILA 2808/RI, N=32). Study BILA 2808/RI (Table AII-2 in Annex I) enrolled a total of 24 male and female subjects with a range age between 65 and 72, with 6 per group (healthy, mild, moderate, and severe renal impairment) and receiving by mouth (p.o.) a single, 20 mg dose of bilastine following a fast of 8 to 10 hours. Subjects were assigned to these groups according to renal function as determined by iothalamate clearance. P3 Table 1 summarizes the mean values of glomerular filtration rates (GFR) from the individuals which was used for their classification at screening in each of the studied groups.

P3 Table 1. Glomerular filtration rate mean values for the different group of patients

Glomerular filtration rate (GFR)	
Group 1 (Healthy)	>80 mL/min/1.73m^2
Group 2 (Mild RI)	50-80 mL/min/1.73m^2
Group 3 (Moderate RI)	30-50 mL/min/1.73m^2
Group 4 (Severe RI)	≤30 mL/min/1.73m^2

Therefore, their individual demographic characteristics were used as a starting point to create a virtual elderly population used in the application and validation of the RI Senescence Model in this impaired situation. In this way, the relevant information extracted from the physiological databases constructed was assigned to each subject considering their age, gender, race, weight and height. The identified characteristics relevant for scaling of each PK parameter using the senescence equations are summarized in P3 Table 2 together with the details of the patient population used in this analysis.

P3 Table 2. Demographic data description of the elderly subject groups and data source

Available from study BILA 2808/RI								Relevant Metrics Extracted from Medatabase		
ID	**Age (Years)**	**Gender**	**Race**	**Height (cm)**	**Weight (kg)**	**GFR (L/h/1.73m^2)**	**fu**	**TBW (L)**	**TBF (kg)**	**CO (L/h)**
Group 1										
40	67	male	white	167.6	71.40	6.36	0.084	44.80	26.69	256.44
43	66	female	white	160.0	73.90	5.64	0.069	31.40	41.73	364.42
45	65	male	black	165.1	71.00	6.84	0.069	46.40	27.20	331.29
47	67	male	white	163.0	78.60	5.88	0.069	44.80	32.52	263.81
51	62	male	white	170.0	71.90	7.20	0.109	44.80	25.19	245.40
52	67	male	white	168.0	78.60	7.68	0.101	44.80	30.02	307.24
Group 2										
2	79	female	white	162.6	85.80	3.84	0.068	31.40	48.82	315.34
3	71	male	white	171.0	56.80	3.72	0.084	44.80	18.99	274.85
4	72	male	white	180.0	67.80	4.02	0.074	44.80	21.23	293.25
6	62	male	white	167.6	74.80	4.62	0.093	44.80	27.55	272.39
9	72	male	white	157.5	65.40	3.60	0.075	44.80	28.88	353.38
10	71	male	white	157.5	68.20	3.18	0.060	44.80	30.25	279.76
Group 3										
13	69	male	black	182.9	98.40	2.70	0.103	46.40	32.57	355.83
17	70	male	white	167.6	92.10	1.80	0.096	44.80	37.45	317.79
23	80	female	black	161.0	69.50	2.88	0.115	34.10	41.05	300.62
33	73	male	white	162.6	67.10	2.16	0.102	44.80	27.65	353.38
36	68	male	white	167.6	72.50	2.28	0.141	44.80	27.40	292.03
46	68	male	black	180.3	76.00	2.04	0.098	46.40	24.07	357.06
Group 4										
5	77	male	white	159.0	59.80	1.68	0.089	44.80	25.92	258.90
21	61	male	white	170.2	80.50	1.26	0.149	44.80	29.06	249.08
22	71	male	white	165.0	83.70	0.66	0.114	44.80	34.69	303.07
24	77	female	white	152.0	55.80	1.62	0.161	31.40	36.96	332.11
26	66	female	black	154.9	57.50	0.90	0.188	34.10	34.92	348.47

ad adult; GFR Glomerular Filtration Rate; TBW Total Body Water; TBF Total Body Fat; CO Cardiac Output, fu Fraction unbound
Reference Adult body composition parameter: (adult of reference were considered aged 30-50 years)
CO adult male/female=288 L/h (Light et al., 1993a); GFR L/h/1.73m2 adult male white=6.30, GFR adult female white = 6.54, adult male black= 6.22, adult female black= 5.72 (Schlender et al., 2016); fu adult = 0.13 (Jauregizar et al., 2009);

1.2.1.1 *PK Parameters scaled in the geriatric RI population using the RI Senescence Model equations and patient characteristics*

Clearance (CL)

As described in the Part 2 of this research, bilastine is almost exclusively eliminated via renal excretion. It does not undergo significant hepatic metabolism and is excreted unchanged in either the faeces via P-gp (67%) or urine (33%), as showed in a mass balance study (Sologuren A et al.2009)
Therefore, CLr was scaled as function of the age-related changes described for the glomerular filtration rate (GFR) and in the unbound fraction (fu) (Eq. 1 to 4 P3 Table 3).
In this study, individual Glomerular filtration rate (GFR) data of subjects of the study have been used to scale CLr. Due to the different ethnicity of the subjects of the study (83.3%) of Hispanic or Latino ethnicity and 4 (16.7%) not of Hispanic or Latino ethnicity) the GFR data of adult of reference were taken from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 (Schlender et al., 2016) according to the race/ethnicity, sex and age.
Moreover, individual fraction unbound (fu) data were available from an "in vitro" study of plasma protein binding of the drug (study code: FF-0019) with plasma from renal impaired patients of study BILA 2808/RI. Study FF-0019 was performed with the main purposes of establishment the linearity of bilastine in plasma protein binding and obtain the fu data from subjects with normal renal function and with different degrees of renal impairment.

Inter-compartmental clearance (Q)

Individual Cardiac output (CO) values from study BILA 2808/RI were calculated considering that CO is the product of the heart rate (HR), or the number of heart beats per minute (bpm), and the stroke volume (SV), which is the volume of blood pumped from the ventricle per beat (CO= HR × SV, Eq.3 P3 Table 3) (Bruss et al., 2019). Individual Heart rate (HR) parameters were available from the study BILA 2808/RI. As reported by Giles et al., the mean stroke volume (SV) estimated for old subjects aged 65+ is 81.8 mL independently of the renal dysfunction of the groups. (Giles N. Cattermole et al., 2017) Other authors reported no correlation between the cardiac index (CI) and the renal function. (Wilfried Mullens, MD et al., 2016) CO output value of adult male/female was considered as 288 L/h (Light et al., 1993b).

Volume of distribution (Vd)

In line with what has been described in Part 2, the sum of Volumes of central compartment (Vc) and Volumes of peripheral compartment (Vp) (Vss) was assumed to resemble to TBW and TBF (Eq 6 P3 Table 3). The relationship of intravenous Vc to intravenous Vss in adult (0.65) (Eq. 7 P3 Table 3)

was assumed to be maintained across aging independently of race and used to scale Vc iv in elderly. Then peripheral volume (Vp) was calculated from the relation Vss = Vc + Vp (Eq.8 P3 Table 3).
Total Body water volumes for elderly white and black males and females were estimated using data from Chumlea et. al. 2001. Additionally, TBF was also considered and estimated using the equations described by Jackson et al. in the 2002 Heritage medical study. Taking into accounts the relationship between sex, age and race on body fat measurements both in white and black Americans.

1.3 *Third step: Validation of the RI Senescence Model predictions using the Renal Geriatric PopPK model and simulations*

In order to qualify the individual predictions of the parameters scaled using the senescence equations a population PK model (Renal Geriatric PopPK model) was developed with PK data available from the same Clinical trial BILA 2808/RI (Table AII-2 in Annex II).
Population modeling and simulations were carried out using nonlinear mixed-effects methods implemented in the NONMEM population PK modeling package (version 6.2, Icon Plc, Dublin, Ireland). The first-order conditional estimation (FOCE) procedure was used throughout and covariate modelling of GFR as a continuous indicator of renal insufficiency within the population runs was included. Four different steps were used to develop the model: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model validation.
The adequacy of the developed structural models was evaluated using both statistical and graphical methods. Model diagnosis and covariate elimination were based on the following criteria: (a) Objective function change: According to standard statistical theory the change in the NONMEM objective function is approximately chi-square (χ^2) distributed and can be used for model comparison with degrees of freedom equal to the difference in number of parameters. (b) The precision (standard error) for the estimates of the estimated parameters. These estimates are assumed significant if their 95% confidence interval (CI 95%) does not include zero. (c) graphical exploration of residuals.
A base model for the full patient (all GFR levels) population was first developed. Then, the four RI patient subgroups were modeled as a categorical covariate.
Covariate model development followed three steps: (i) Forward inclusion sequence of separate terms (fixed effects) for each RI subgroup separately on both the CL/F and Vc/F parameters (ii) Backward removal at the $p<0.005$ after removal of a single covariate term. Terms that were significant from step (ii) were kept and constituted the final covariate model. When a removed subgroup term was not significant, the same fixed effect of the next most significant subgroup was used (so two or more subgroups could have the same shift in CL or Vc versus the Healthy).

We assumed that all patient subgroups arose from compartmental PK parameter distributions of similar (inter-individual variability) variance (this was also needed due to the small number of patients in each subgroup N=6).

Interindividual variability (random effects) in the PK parameters was based on the assumption of a log-normal distribution. The value of intersubject variability in each parameter was taken as the square root of the variance, expressed as coefficient of variation expressed together with its SEE (SEE%).

Residual (unexplained) variability in bilastine plasma concentrations, representing a composite of model misspecification, variability in the analytical method, intraindividual variability, digression between the actual and nominated sampling times, and dosing times, as well as other undefined factors, was modelled using a proportional error. The precision of each parameter was calculated by dividing the estimated standard error of each parameter by the value of the parameter estimate and expressed as a percentage.

The final optimal categorical covariate PK model was used to derive the empirical Bayes PK parameters for each individual subjects of the study (e.g., CL/F for each subject in each subgroup). These estimates were used for comparisons to the senescence predictions. Comparisons were made at an individual and mean level.

1.4 *Softwares used in the analyses*

For the Renal Geriatric PopPK model, modeling and simulation were carried out with the software package NONMEM® (version 6.2, Icon Plc, Dublin, Ireland). The first-order conditional estimation method with random effects interaction was used throughout the analysis. RI Senescence model simulations were performed using the software package NONMEM®. Data handling, generation of plots used to visualize and evaluate the model were performed using S-Plus version 6.2.1 (Insightful software, Seattle, WA).

2 Results Section 3

2.1 *Application of scaling equations: Senescence predictions in elderly patients from study BILA 2808/RI with renal impairment*

Adult IV PK parameters obtained from the dedicated bioavailability study (Study code BILA 2864, Sadaba et al, 2013) described in Table AI-2 in Annex I were used as a starting point for scaling into geriatrics with renal impairment. The equations applied to each PK process to scale the intravenous parameters from adults into geriatrics, are outlined below along with the new reference parameters (P3 Table 3).

P3 Table 3. Senescence scaled Equations used in the extrapolation of bilastine PK parameters adapted to predict elderly renal impaired patients from study BILA 2808/RI

Parameter	Equation and/or Reference	PK Parameter related	Equation to scale absolute PK in elderly with renal impairment
CSHA (g/L)	Available values from study FF-0019	**Fraction unbound (fu)**	Available values from study FF-0019
GFR (mL/min)	Available from study BILA 2808/RI	**Renal Clearance (L/h) (CLr)**	$CLr_{ger} = \frac{GFRger \times fu_{ger}}{GFRad \times fu\_ad} \; x \; CLr_{ad}$ Eq.1 $ratio \; \frac{CLiv}{CLr}$ Eq.2
CO (L/h)	$CO = HR \times SV$ **Eq.3** **HR** Available from study BILA 2808/RI **SV** Estimated as 81.8 mL for old subjects aged 65+ independently of the renal impairment	**Intercompartmental Clearance (L/h) (Q)**	$ratio \; \frac{CO \; male/female}{Qiv} = 285.15$ **Eq.4**
TBW (L) **TBF (Kg)**	$TBW(L)_{\; black \; male/female}$ Available from Metadatabase $TBF(kg)$ (1.39 x BMI) + (0.16 x Age) – (10.34 x gender) – 9 $male = 1, female = 0$ **Eq.5**	**Volume of distribution (L) (Vss, Vc and Vp)**	$Vss \cong TBW + TBF$ **Eq.6** $Vc = 0,65 \; x \; Vss \; iv$ **Eq.7** $Vp = Vss \; iv - Vc \; iv$ **Eq.8**
		Absorption rate constant (h^{-1}) (ka)	The constant of absorption (ka) was fix to the Renal Geriatric PopPK model

Abbreviations are: V Volume of distribution, ss Steady state, c Central, p Peripheral, F Bioavailability, CL Clearance, r Renal, ad Adult, fu Unbound fraction, GFR Glomerular filtration rare, CHSA Albumin molar concentration, Cp Plasma Concentration, CO Cardiac Output, Q Intercompartmental Clearance, k_a Absorption rate constant

The PK assumptions used to scale the PK parameters into elderly are detailed for each process. It is important to point out that a patient (ID number 28) corresponding to the severe group, showed a value of total renal CL similar to the one observed in the healthy population and for this reason this subject has been excluded from the analysis performed using the RI Senescence model.
The PK parameters were calculated by scaling using the adapted RI Senescence Model equations to predict renal impaired patients. The individual characteristics for each patient were used.

Clearance

Individual GFR data of subjects of study BILA 2808/RI together with the values of fu are detailed in P3 Table 2. Adult of reference was considered aged (30-50). Reference values for adult of Schlender et al. 2016 (Schlender et al., 2016) according to the selected geriatric sex and the race/ethnicity were used and are reported in P3 Table 4. In order to calculate the oral total CL, the ratio between the intravenous total clearance (CLiv) and the intravenous renal clearance in adults (CLiv/CLr) obtained from study BILA 2909/BA was considered.

Intercompartmental Clearance

Intercompartmental clearance was extrapolated as a proportion of the cardiac output (CO) by age. As reported in the Method Part, CO values were calculated considering data available from study (Heart rate-HR) and data available from literature (mean stroke volume-SV). Steps for the calculation of the CO values using Eq.3 P3 Table 3 are shown in P3 Table 4 below. Then, the CO/Q ratio calculated (Eq.4 P3 Table 3) for an adult reference aged 30-50 years was used to scale the Q in geriatrics (Van Sassenbroeck, 2002; Vozmediano et al., 2017). The Qiv for adult was taken from the developed PopPK model of the intravenous data in young adults.

P3 Table 4. Scaled values of intravenous intercompartmental clearance for each patient from study BILA 2808/RI

ID	HR (b*min)	SV mean (mL)	CO (mL/min)	CO (L/h)	Q abs (L/h)
Group 1					
40	52.25	81.80	4274.05	256.44	0.90
43	74.25	81.80	6073.65	364.42	1.28
45	67.50	81.80	5521.50	331.29	1.16
47	53.75	81.80	4396.75	263.81	0.93
51	50.00	81.80	4090.00	245.40	0.86
52	62.60	81.80	5120.68	307.24	1.08
Group 2					
2	64.25	81.80	5255.65	315.34	1.11
3	56.00	81.80	4580.80	274.85	0.96
4	59.75	81.80	4887.55	293.25	1.03
6	55.50	81.80	4539.90	272.39	0.96
9	72.00	81.80	5889.60	353.38	1.24
10	57.00	81.80	4662.60	279.76	0.98
Group 3					
13	72.50	81.80	5930.50	355.83	1.25
17	64.75	81.80	5296.55	317.79	1.11
23	61.25	81.80	5010.25	300.62	1.05
33	72.00	81.80	5889.60	353.38	1.24
36	59.50	81.80	4867.10	292.03	1.02
46	72.75	81.80	5950.95	357.06	1.25
Group 4					
5	52.75	81.80	4314.95	258.90	0.91
21	50.75	81.80	4151.35	249.08	0.87
22	61.75	81.80	5051.15	303.07	1.06
24	67.67	81.80	5535.13	332.11	1.16
26	71.00	81.80	5807.80	348.47	1.22

ratio (CO/ Qiv) adult 285.15 L/h, CO adult male/female=288 L/h (Messerli et al., 1979), Qiv 1.01

Abbreviations are: b*min beats per minute, Q Intercompartmental Clearance, abs absolute, iv intravenous

P3 Table 5 and P3 Table 6 show respectively the individual and the mean intravenous PK parameters estimated in geriatric population with different degrees of renal impairment (study BILA 2808/RI) using the Senescence model.

P3-Table 5. Individual absolute PK parameters of bilastine estimated in renal impaired elderly patients using the Senescence equations

RI Individual Senescence Predictions							
ID	**Age (Years)**	**Vss abs (L)**	**Vc abs (L)**	**Vp abs (L)**	**CLr abs (L/h)**	**CL abs (L/h)**	**Q abs (L/h)**
Group 1							
40	**67**	71.49	46.47	25.02	5.38	18.29	0.90
43	**66**	73.13	47.54	25.60	3.79	12.90	1.28
45	**65**	73.60	47.84	25.76	4.85	16.48	1.16
47	**67**	77.32	50.26	27.06	4.13	14.03	0.93
51	**62**	69.99	45.49	24.50	7.91	26.91	0.86
52	**67**	74.82	48.63	26.19	7.82	26.57	1.08
Group 2							
2	**79**	80.22	52.14	28.08	2.55	8.68	1.11
3	**71**	63.79	41.46	22.33	3.17	10.77	0.96
4	**72**	66.03	42.92	23.11	2.98	10.15	1.03
6	**62**	72.35	47.03	25.32	4.34	14.77	0.96
9	**72**	73.68	47.89	25.79	2.74	9.30	1.24
10	**71**	75.05	48.78	26.27	1.93	6.58	0.98
Group 3							
13	**69**	78.97	51.33	27.64	2.83	9.64	1.25
17	**70**	82.25	53.46	28.79	1.74	5.90	1.11
23	**80**	75.15	48.85	26.30	3.68	12.52	1.05
33	**73**	72.45	47.09	25.36	2.22	7.56	1.24
36	**68**	72.20	46.93	25.27	3.24	11.02	1.02
46	**68**	70.47	45.80	24.66	2.04	6.95	1.25
Group 4							
5	**77**	70.72	45.97	24.75	1.51	5.12	0.91
21	**61**	73.86	48.01	25.85	1.90	6.45	0.87
22	**71**	79.49	51.67	27.82	0.76	2.58	1.06
24	**77**	68.36	44.43	23.93	2.53	8.61	1.16
26	**66**	69.02	44.86	24.16	1.89	6.41	1.22
Abbreviations are: V Volume of distribution, abs absolute, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, Q Intercompartmental Clearance							

P3 Table 6 Mean absolute PK parameters of bilastine predicted in elderly with renal insufficiency using the Senescence equations

Mean RI Senescence Predictions				
Parameter	**Predicted Group 1**	**Predicted Group 2**	**Predicted Group 3**	**Predicted Group 4**
Vc abs (L)	47.71	46.70	48.91	46.99
Vp abs (L)	25.69	25.15	26.34	25.30
CL abs (L/h)	19.20	10.04	8.93	5.83
Q abs (L/h)	1.03	1.05	1.16	1.05
Abbreviations are: V Volume of distribution, abs absolute, c Central, p Peripheral, CL Clearance, Q Intercompartmental Clearance				

Considering that bilastine is administered orally, all individual parameters were corrected by the known bioavailability (61%) (Sadaba et al., 2013b) and the resulting individual and average PK parameters are shown in P3 Table 7 and P3 Table 8, respectively.

P3 Table 7 Bioavailability corrected Individual PK Parameters of bilastine predicted in Renal impaired Elderly patients using the Senescence equations

ID	Age (Years)	Vc /F (L)	Vp/F (L)	CL/F (L/h)	Q/F (L/h)
Group 1					
40	**67**	76.17	41.02	29.99	1.47
43	**66**	77.93	41.96	21.15	2.10
45	**65**	78.43	42.23	27.02	1.90
47	**67**	82.39	44.37	22.99	1.52
51	**62**	74.58	40.16	44.11	1.41
52	**67**	79.73	42.93	43.56	1.77
Group 2					
2	**79**	85.48	46.03	14.24	1.81
3	**71**	67.97	36.60	17.66	1.58
4	**72**	70.36	37.89	16.64	1.69
6	**62**	77.10	41.51	24.22	1.57
9	**72**	78.51	42.27	15.25	2.03
10	**71**	79.97	43.06	10.78	1.61
Group 3					
13	**69**	84.14	45.31	15.80	2.05
17	**70**	87.65	47.19	9.68	1.83
23	**80**	80.08	43.12	20.52	1.73
33	**73**	77.20	41.57	12.39	2.03

36	**68**	76.94	41.43	18.06	1.68
46	**68**	75.09	40.43	11.39	2.05
Group 4					
5	**77**	75.36	40.58	8.40	1.49
21	**61**	78.71	42.38	10.57	1.43
22	**71**	84.71	45.61	4.23	1.74
24	**77**	72.84	39.22	14.11	1.91
26	**66**	73.55	39.60	10.51	2.00

Abbreviations are: V Volume of distribution, c Central, p Peripheral, F Bioavailability, CL Clearance, Q Intercompartmental Clearance.

P3 Table 8 Bioavailability corrected Mean PK Parameters of bilastine predicted in Renal impaired Elderly patients by group using the Senescence equations

RI Senescence Predictions				
Parameter	**Predicted Group 1**	**Predicted Group 2**	**Predicted Group 3**	**Predicted Group 4**
Vc/F (L)	78.20	76.56	80.18	77.03
Vp/F (L)	42.11	41.23	43.17	41.48
CL/F (L/h)	31.47	16.46	14.64	9.56
Q/F (L/h)	1.69	1.71	1.89	1.72

Abbreviations are: V Volume of distribution, c Central, p Peripheral, F Bioavailability, CL Clearance, Q Intercompartmental Clearance

The resulting scaled individual PK parameters were used to simulate the time evolution of bilastine plasma levels after an oral dose of 20 mg considering the fixed ka (2.16 h-1) and Tlag (0.209 h). The resulting scaled PK parameters were compared with the Bayesian estimates and mean parameters from the Geriatric PopPK model developed.

2.2 *Renal Geriatric PopPK model and qualification of the scaling process*

From each patient of study BILA 2808/RI, time of dose, total dose, number of doses, sampling times and bilastine concentrations were available, as well as urinary excretion data. Time zero was considered the time of the first bilastine dose and sampling time was calculated relative to that expressed in hours. Total dose was expressed in mg and concentrations in ng/mL. Drug concentrations below the limits of quantification were excluded from the database.

The analysis considered the following premises:

- linearity across doses and time and across concentrations for the binding as well as the lack of important accumulation after repeated dosing
- no change in the intrinsic pharmacodynamics of bilastine related to renal impairment
- the effect of concomitant medication on changes in the non-renal excretion in relation with renal function will not be addressed

2.1 *Renal Geriatric PopPK model development*

Population pharmacokinetic base model

A base population PK model was developed in NONMEM for the entire GFR volunteer population based on the structure of the earlier bilastine models. P3 Table 9 lists the parameters estimates for the base model. In the table the standard error of the estimate (SEE) is also expressed as coefficient of variation of the SEE (SEE%) of the parameter estimate calculated as:

$$\text{SEE\%} = \frac{SEE_{Par}}{TV_{Par}} \qquad \textbf{Eq. 9}$$

Where "Par" is any of the PK parameters, SEE is standard error and TV is the typical value parameter estimate ("Estimate" in the P3 Table 9 and P3 Table 10).

P3 Table 9. Population PK model parameters from BILA 2808/RI in patients with renal impairment. Base model

Base model Renal Geriatric PopPK model			
Parameter	**Estimate**	**SEE**	**SEE%**
CL/F (L/h)	19.6	1.82	9.3%
Vc/F (L)	79.7	6.85	8.6%
Q/F (L/h)	3.32	0.366	11.0%
Vp/F (L)	53.6	7.31	13.6%
Ka (h^{-1})	2.44	0.557	22.8%
Tlag (h)	0.213	0.0108	5.1%
ω CL	0.162	0.0391	24%
ω Vc	0.179	0.0539	30%
ω Vp	0.225	0.0839	37%
ω ka	0.876	0.317	36%
σ	0.0621	0.00943	15%

Abbreviations are: CL= apparent total body clearance of the drug from plasma; k_a= first-order absorption rate constant; Q= apparent intercompartmental clearance; Vc= central compartment volume of distribution; Vp= peripheral compartment volume of distribution.

All parameters are well estimated (SEE% below 50%).

Population pharmacokinetic final model

Categorical covariate structures were introduced in the model for the GFR groups on CL/F and Vc/F as shown in equation 9.

$$Par = TVPar \cdot (1 + \mathrm{cov}1.Yes \cdot THx1) \cdot (1 + \mathrm{cov}2.Yes \cdot THx2) \cdot (1 + \mathrm{cov}3.Yes \cdot THx3) \quad \textbf{Eq. 10}$$

Where "Par" and "TVPar" are individual and typical population estimates for either CL/F or Vc/F, "cov (1,2,3). Yes" are binary index variables taking the value of 1 in case of Mild, Moderate and Severe GFR groups respectively, and 0 otherwise. THx (1,2,3) are NONMEM fixed effect parameters to be estimated.

The structure was then reduced sequentially (i.e., setting THx to zero) and then combinations of the form e.g., THx2=THx3, were also tested. The final model structure parameter estimates are listed in P3 Table 10. A large drop in the NONMEM objective function was associated to the covariate introduction (from 1396 in the base model to 1372 in the final model).

P3 Table 10 Population PK model parameters from BILA 2808/RI in patients with renal impairment. Covariate model

Categorical covariate model Renal Geriatric PopPK model			
Parameter	**Estimate**	**SEE**	**SEE%**
CL/F (L/h)	31.5	3.44	10.9%
Vc/F (L)	91.9	9.23	10.0%
Q/F (L/h)	3.84	0.466	12.1%
Vp/F (L)	64.6	6.7	10.4%
Ka (h^{-1})	2.16	0.477	22.1%
Tlag (h)	0.209	0.0122	5.8%
CL Mild/Norm	-0.318	0.0695	-21.9%
CL MoSe/Norm	-0.490	0.0741	-15.1%
Vc MMS/Norm	-0.415	0.0848	-20.4%
ω CL	0.0764	0.0338	44%
ω Vc	0.090	0.0413	46%
ω Vp	0.187	0.0803	43%
ω ka	0.745	0.297	40%
σ	0.115	0.0143	12%

Abbreviations are: V Volume of distribution, c Central, p Peripheral, F Bioavailability, CL Clearance, Q Intercompartmental Clearance CL= apparent total body clearance of the drug from plasma; k_a= first-order absorption rate constant; Q= apparent intercompartmental clearance; Vc= central compartment volume of distribution; Vp= peripheral compartment volume of distribution.

CL Normal and Vc Normal are CL/F and Vc/F for GFR > 80 mg/mL CL Mild/Norm is the ratio by which the CL_Normal is reduced for the Mild GFR situation. These ratios can also be viewed as percentage reductions. CL_MoSe/Norm is the ratio for both Moderate and Severe ("MoSe") that were barely distinguishable in the model and were considered equal. Similarly, for V2 a common ratio "jump" addresses all other GFR related insufficiency in "MMS" (Mild, Moderate, Severe).

The CL/F and Vc/F for each GFR group can be estimated as follows for example for the clearance of the GFR Mild group. Mean values for total clearance calculated with the use of this equation are shown in P3 Table 11.

$$CL_{MILD} = 31.5 \cdot (1 - 0.318) \qquad \textbf{Eq. 11}$$

Population parameter estimates for the final GFR subpopulation model. Parameters for CL/F are identifiable for all subgroups. But only the Moderate GFR have a difference in Vc/F.

P3 Table 11 Mean values corresponding to total plasma clearance in the different renal impairment groups

Total Plasma Clearance	Group 1 Healthy	Group 2 Mild	Group 3 Moderate	Group 4 Severe
CL/F (L/h)	**31.5**	**21.48**	**15.3**	**16.06**
Abbreviations are: CL Clearance, F Bioavailability				

2.2 *Renal Clearance calculation*

As urinary excretion data were also available from study BILA-2808/RI, individual renal clearance (CLr) was calculated using Eq.10 below and the mean values are summarized in P3 Table 12.

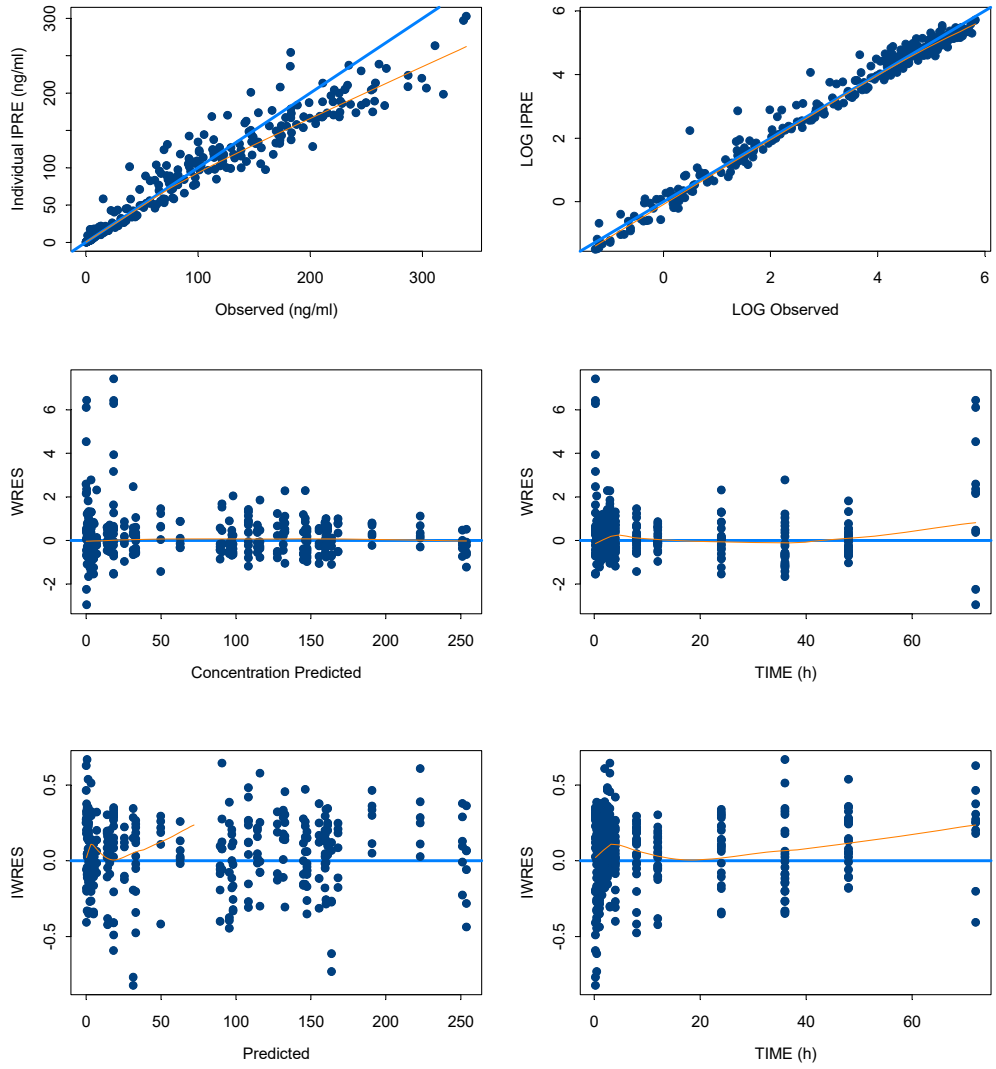
$$CL_r = \frac{amount\ of\ drug\ accumulated\ in\ urine}{AUC_{plasma}} \qquad \textbf{Eq. 12}$$

P3 Table 12 Mean and associated standard deviation (SD) values corresponding to calculated renal clearance in the different groups

Renal Clearance	Group 1 Healthy	Group 2 Mild RI	Group 3 Moderate RI	Group 4 S evere RI
CLr (L/h)	8.80	6.95	5.71	4.06
SD	2.24	1.28	2.27	3.79
Abbreviations are: CLr Renal Clearance, SD Standard Deviation				

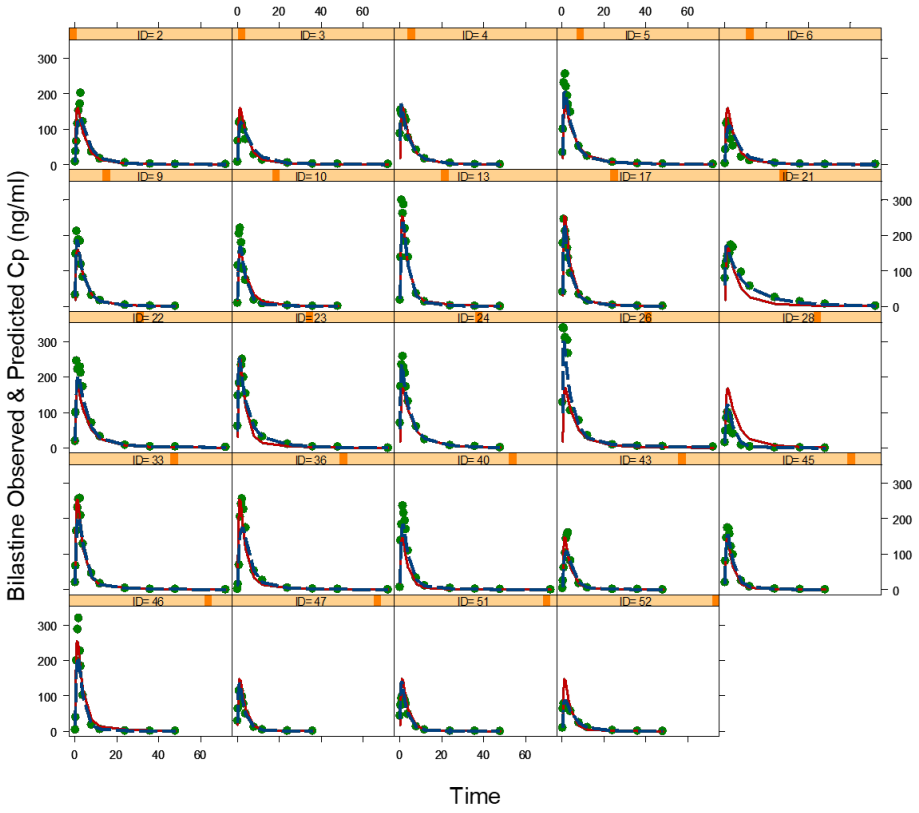
2.3 *Diagnostic plot for final CL, V – GFR categorical covariate model*

The diagnostic plots assessing the goodness of fit for the final PK model are provided in P3 Figure 2. In the upper panels, PRED and IPRED are plotted against the observations, both in linear and log scales. In all cases, the predicted concentrations are fairly evenly distributed about the line of identity, thus indicating the appropriateness of the structural model selected and the lack of major bias. The lower panels show a uniform and random distribution of the residuals about the zero line when scatter plotted against population-predicted concentration or time. As expected, plot of residuals (RES) against PRED or time shows the cone shape typical of heteroscedastic (proportional) residual error model, which is corrected by using the weighted residuals (WRES) and individual weighted residuals (IWRES) instead.



P3 Figure 2. Diagnostic plots of the final model developed

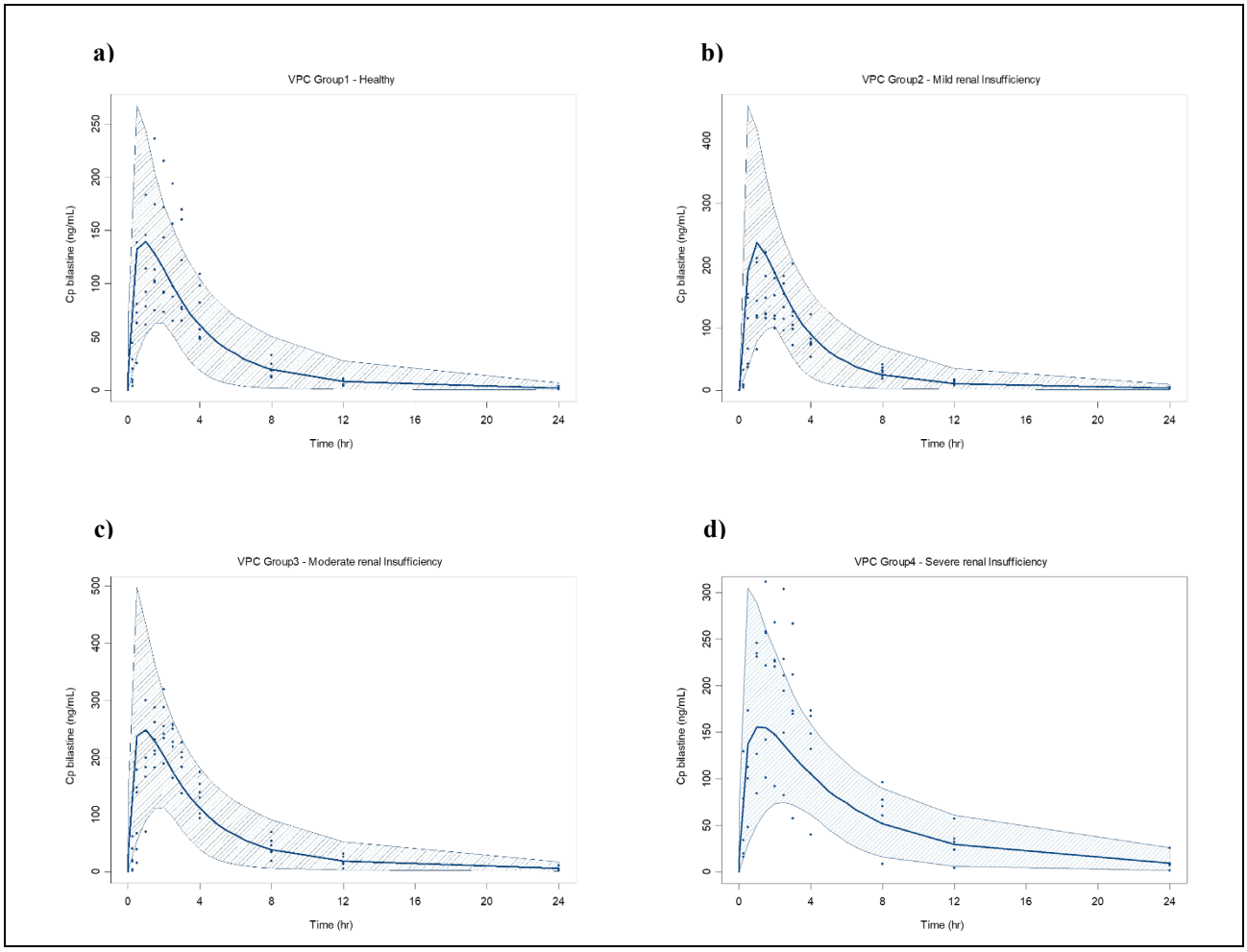
Fit of individual data according to the base structural PK model is provided in P3 Figure 3 which represent concentrations on a linear scale. Red and blue lines respectively correspond to the mean population prediction (PRED) and individual prediction (IPRED) provided by the model, whereas green dots represent the real observations.



P3 Figure 3. Fit of individual concentration-time data by the final model developed for bilastine in geriatric volunteers' groups

Overall, it can be concluded that model fits are characterized by a satisfactory degree of precision, which means that the quantitative value of the PK parameters was correctly estimated by the categorical covariate model. Based on P3 Figure 4 above, it can be overall concluded that the categorical covariate model adequately describes the experimental data.

A Visual Predictive Check performed for each group to validate the Renal Geriatric PopPK model is shown in P3 Figure 4 (a, d).



P3 Figure 4. Predicted and observed concentration-time profiles of bilastine after a single oral dose of 20mg in the elderly population of study BILA 2808/RI by the different renal impairment groups (a Healthy, b Mild, c Moderate, d Severe). The blue line and the blue area represent the mean and 95% confidence interval of the model predictions

2.4 *External Validation of the Renal Impairment Senescence model*

The individual parameters (Bayesian) of elderly subjects from study BILA 2808/RI were obtained using the Renal Geriatric Model and they were used to compare with the individual predictions made in the same subjects using the RI Senescence equations P3 Table 13.

P3 Table 13 Comparison between the Bayesian Parameters of elderly subjects from study BILA 2808/RI obtained with the Renal Geriatric PopPK model and the individual predictions made in the same subjects using the RI Senescence model

ID	Vc/F (L) *RI Senescence*	Vc/F (L) *R PopPK*	Vp/F (L) *RI Senescence*	Vp/F (L) *R PopPK*	CL/F (L/h) *RI Senescence*	CL/F (L/h) *R PopPK*	Q/F (L/h) *RI Senescence*	Q/F (L/h) *R PopPK*
Group 1								
40	76.17	70.40	41.02	64.60	29.99	21.10	1.47	3.36
43	77.93	80.20	41.96	48.20	21.15	33.40	2.10	3.36
45	78.43	83.20	42.23	36.90	27.02	26.90	1.90	3.36
47	82.39	108.00	44.37	43.10	22.99	38.30	1.52	3.36
51	74.58	111.00	40.16	66.80	44.11	36.90	1.41	3.36
52	79.73	171.00	42.93	46.40	43.56	35.50	1.77	3.36
Group 2								
2	85.48	83.70	46.03	77.80	14.24	19.60	1.81	3.36
3	67.97	124.00	36.60	107.00	17.66	19.90	1.58	3.36
4	70.36	104.00	37.89	31.70	16.64	22.10	1.69	3.36
6	77.10	126.00	41.51	127.00	24.22	21.10	1.57	3.36
9	78.51	82.20	42.27	41.00	15.25	20.70	2.03	3.36
10	79.97	74.60	43.06	59.90	10.78	25.80	1.61	3.36
Group 3								
13	84.14	47.20	45.31	28.00	15.80	18.20	2.05	3.36
17	87.65	65.20	47.19	35.40	9.68	19.40	1.83	3.36
23	80.08	65.40	43.12	43.30	20.52	12.40	1.73	3.36
33	77.20	54.60	41.57	50.90	12.39	17.10	2.03	3.36
36	76.94	51.60	41.43	46.50	18.06	16.10	1.68	3.36
46	75.09	43.10	40.43	44.00	11.39	22.60	2.05	3.36
Group 4								
5	75.36	73.90	40.58	81.60	8.40	14.90	1.49	3.36
21	78.71	108.00	42.38	35.40	10.57	10.30	1.43	3.36
22	84.71	70.20	45.61	79.20	4.23	12.90	1.74	3.36
24	72.84	71.10	39.22	38.90	14.11	14.20	1.91	3.36
26	73.55	57.00	39.60	98.00	10.51	10.30	2.00	3.36

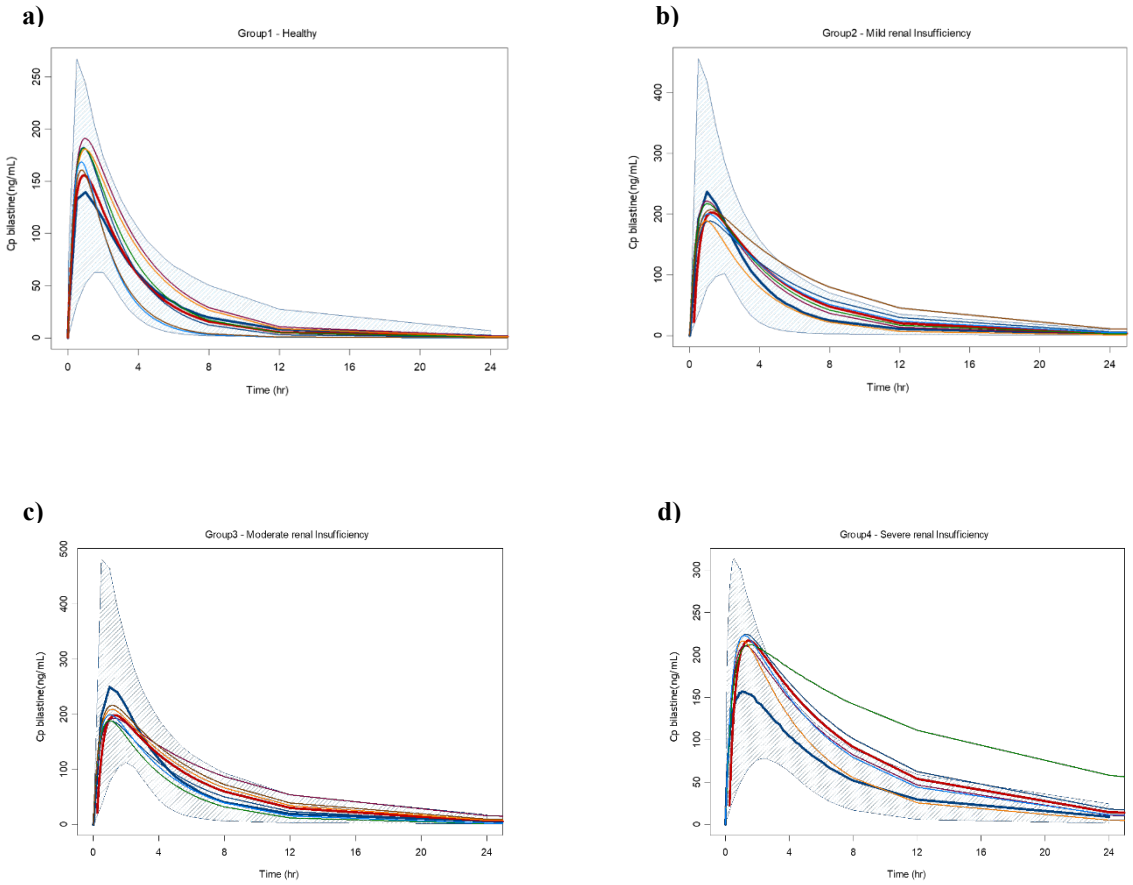
ka (h^{-1}) (not scaled) was estimated using the Renal Geriatric PopPK Model (2.42 h^{-1}) and used to inform the RI Senescence model; RI Senescence Renal Impairment Senescence Model, R PopPK Renal Geriatric PopPK model

PK parameters, both scaled using the RI Senescence Model and modelled using the Renal Geriatric PopPK model, were used to calculate the mean $AUC_{[last]}$ values as well as the C_{max} per group (P3 Table 14).

P3 Table 14. Rate and extent of absorption obtained using the Renal Geriatric PopPK model and the PK parameters obtained in geriatrics groups by applying the senescence equations

Renal Geriatric PopPK model	C_{max} (ng/mL)	$AUC_{[last]}$ (ng·h/mL)	RI Senescence model	C_{max} (ng/mL)	$AUC_{[last]}$ (ng·h/mL)
Group 1 Healthy	191.26	635.53	**Group 1 Healthy**	147.00	624.67
Group 2Mild	221.50	1214.70	**Group 2Mild**	160.00	928.79
Group 3 Moderate	216.37	1366.12	**Group 3 Moderate**	254.00	1134.22
Group 4 Severe	224.00	2091.17	**Group 4 Severe**	168.00	1597.44
Abbreviations are: AUC area under the curve, C_{max} maximum concentration					

Finally, the individual predictions obtained with the RI Senescence Model in elderly population by group were compared with the 95% confidence interval (CI) of the Renal Geriatric PopPK model by group (P3 Figure 5 a, d).



P3 Figure 5. Visual Predictive check (VPC) reporting the PK interval (mean, 95%) in the elderly population (BILA 2808/RI) using the Renal Geriatric PopPK model versus the individual elderly predicted (BILA 2808/RI) using the RI Senescence Model after an oral administration of 20 mg of bilastine in the four pathological groups (a Healthy, b Mild, c Moderate, d Severe)

In Group 1 AUC was accurately scaled to healthy elderly individuals by the RI Senescence Model (predicted using RI Senescence Model: predicted using Renal Geriatric PopPK Model ratio of 1.02). In Group 2 (P3 Figure 5 b and P3 Table 14) the C_{max} and the AUC were overpredicted by the SI Senescence Model (38% and 31%, respectively). In Group 3 the predicted Cmax and AUC were in accordance with the estimated with the Renal Geriatric Model (predicted using RI Senescence Model: predicted using Renal Geriatric PopPK Model ratio of 0.85 and 1.20, respectively). RI Senescence predictions in Group 4 (P3 Figure 6 d) show a 7% of outliers. The C_{max} and AUC were overpredicted by 33% and 31% respectively.

3 Discussion Section 3

In this third part of the research project, the Senescence Model already developed was used as a base to adapt the equations to the pathophysiological situation studied here, including equations to consider the different degrees of renal impairment in geriatrics. Specifically, the model was used to simulate the PK profile in elderly patients with impaired renal function. The accuracy of individual predictions was evaluated with the use of a population model developed with data from a group of elderly subjects (65 to 72 years) with different degrees of renal impairment treated with oral bilastine. Patients enrolled in the clinical study (n=24) were assigned to 4 groups according to their renal function (glomerular filtration rate) as determined by administration of iothalamate clearance. Glomerular filtration rate (GFR) is an important variable to categorize renal impairment, and usually it is tested as covariate in PK studies and therefore should be measured as accurately as possible. There are several methods to calculate the degree of renal impairment, but caution should be taken in the elderly population some of these methods are not correctly validated and they can lead to erroneous categorization of the population.

It is generally accepted that impaired kidney function is a condition highly prevalent in the elderly and it is associated with increased risks of all-cause mortality, cardiovascular disease, and progression to chronic kidney disease (CKD). However, in the elderly we need to take into account that age related changes lead to a physiological decrease in the GFR and that the available formulas used to estimate the GFR in this group have not been validated. GFR can be calculated indirectly by the clearance of endogenous filtration markers, such as serum creatinine (CLCr) or, recently, cystatin C (CLCys) (Drenth-van Maanen et al., 2013).

The CLCr is frequently used to evaluate the GFR in clinical practice through a urine collection within 24 hours. However, serum creatinine is a poor screening test for renal failure in elderly patients, leading to marked under investigation and under recognition of renal failure in this population. In fact, lower than normal muscle mass and decreased dietary protein intake, both situations that are somewhat common in the elderly, can result in falsely depressed serum creatinine levels.

On the other hand, Cystatin C has been shown to be a better predictor of cardiovascular events and death than creatinine, particularly among persons with eGFR >60 ml/min per 1.73 m2 and in elderly (Galteau et al., 2001).

The GFR estimate is most commonly derived using 1 of 3 equations (the Cockcroft-Gault- CG, the modification of diet in renal disease-MDRD, or the Chronic Kidney Disease Epidemiology Collaboration-CKD-EPI) taking into consideration the classification of CKD according to the KDOQI guidelines (Gill et al., 2007; Lamb et al., 2005).

The MDRD and CKD-EPI equations estimate the GFR directly, whereas the Cockcroft-Gault equation estimates creatinine clearance (CrCl).

The Modification of Diet in Renal Disease (MDRD) equation appears to underestimate GFR in populations with higher levels of GFR, such as patients with type 1 diabetes without microalbuminuria and people undergoing kidney transplant donor evaluation. Moreover, the use of the MDRD equation in healthy people (without CKD) can underestimate the GFR (healthy people have more muscle mass and a higher protein ingestion and, therefore, generate more creatinine than people with CKD). As a result, an "healthy" elderly could be categorize as affected by CKD by obtaining a reduced GFR when using the MDRD equation systematically (Levey et al., 2007; Omuse et al., 2017).

The CKD-EPI equation is more accurate than the MDRD study equation across a wide range of characteristics, including age, sex, race, body mass index, and presence or absence of diabetes or history of organ transplantation.

Taking into consideration the above-mentioned information related to the GFR estimation and also that GFR is race and population dependent , in the present study the GFR values of reference used in the RI Senescence Model were taken from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH - Leverkusen, Germany) of Schlender et al. 2016 (Schlender et al., 2016) according to the **race/ethnicity, sex and age.**

On the other hand, as urinary excretion data were also available from study BILA-2808/RI, individual renal clearance (CLr) was used to calculate the rate between total plasma CL and CLr and to explain the role of kidney function in bilastine elimination. The calculated ratio remains constant through the groups 1–4, indicating that the renal is the main rout of bilastine elimination despite the drug have been showed to be secreted in faeces in different clinical studies. The latter mechanism can be explained by the fact that bilastine is a substrate of P-gp that actively effluxes the drug from the gut epithelial cells back into the intestinal lumen and therefore accounts for part of the drug collected in faeces.

In study BILA-2808/RI, GFR was directly measured in patients using iothalamate method and the GFE values were used in the final Renal Geriatric PopPK model using the PK data and renal function data in this study.

The individual parameters (Bayesian) of elderly subjects from study BILA-2808/RI obtained using a population modeling approach, were used for comparison with the individual predictions made in the same subjects using the RI Senescence equations and this served as an internal validation of the predictive capacity of the Senescence model developed. The systemic PK of bilastine is defined by

four parameters: central and peripheric volume of distribution, intercompartmental clearance, and plasma clearance. The value of AUC or drug exposure mainly depends on plasma clearance and furthermore, in drugs mainly eliminated by the renal route, is totally related to renal function. In case of the drug is administered orally, such as bilastine, the value of bioavailability should be taken into consideration also. In this context it is highly recommended to use data obtained by i.v. route in order to enable a correct or not confounded physiological interpretation of the PK parameters. In this sense, the four i.v. parameters obtained from the bioavailability study BILA 2909/BA were used as a starting point for scaling into geriatrics with renal impairment as a basis of the Senescence model.

In the Renal Geriatric PopPK model, the GFR was demonstrated to be an impactful covariate affecting plasma clearance in all groups. However, only the moderate renal impairment group showed a difference in the central volume of distribution. Consequently, as mentioned before, the mean AUC values obtained using both Renal Geriatric PopPK model and RI Senescence Model were higher as the degree of renal insufficiency progressed compared with the healthy group. However only slight differences were observed in C_{max}. This can be explained considering that C_{max} depends on the volume of distribution which is independent of plasma clearance and does not affect the AUC value. The volume of distribution is related to TBW and TBF and these values are usually altered in the elderly as mentioned before in this research project (the average decline of TBW is reported to be about 10-15%, while the TBF increase of 20-40%).

For the TBW and TBF estimation, considering that in this specific case the population of the study BILA-2808/RI was characterized by different ethnic groups (83.3% were of Hispanic or Latino ethnicity and 16.7% not of Hispanic or Latino ethnicity), biological differences in the body composition of blacks and whites relative to parameters such as fat-free body mass (water, mineral, and protein), fat patterning, and body dimensions and proportions were considered.

As reported by Prothro et al.1995 (JW et al., 1995) in its cross-sectional study blacks have a greater bone mineral density and body protein content than do whites, resulting in a greater fat-free body density. Additionally, there are racial differences in the distribution of subcutaneous fat and the length of the limbs relative to the trunk (JW et al., 1995).

The fat-free body (FFB) can be further subdivided into protein, mineral components and total body water (TBW) that makes up the largest portion. As reported by Chumlea et al. 2001, while in white subjects mean values for total body water (TBW) are reported to range from about to 36 to 46 L with smaller values occurring at older ages in both sexes, blacks have larger TBW means ranging from 51 to 32 L. The differences reported for the TBW values between black and white adult/elderly subjects are attributable to the increased prevalence of obesity among blacks being the TBW positively associated to TBF (Chumlea et al., 2001).

The prediction of TBW volume in renal disease is critical in order to prescribe and monitor treatment. In fact, TBW reflects urea distribution (V) and is used in calculating the dose of dialysis (or assessing its performance) in the determination of Kt/V, where "K" is urea clearance and "t" is the duration of dialysis. In routine clinical practice, V is commonly predicted in peritoneal dialysis from the anthropometric equations for TBW developed by Watson et al. 1980 (Chumlea et al., 2001; Watson et al., 1980).

Nevertheless, the same Watson equations that have been previously applied to estimate TBW values in the elderly population analyzed does not apply to the population of elderly of interest. In fact, these equations were developed only for white subjects and does not consider possible biological differences due to race/ethnicity.

Based on that, Chumlea et al. developed and validated sex/race specific equations able to predict how total body water (TBW) changes across aging and depending on race (white and black). So, data from Chumlea et. al. 2001 of Total Body water volumes for elderly white and black males and females were used to estimate the TBW in subjects of study 2808/RI (Chumlea et al., 2001, 2002).

Additionally, racial differences in the distribution of subcutaneous fat have been reported. Particularly, blacks tend to have less subcutaneous fat in the extremities than in the trunk but tend to carry relatively more fat on the back and lateral portions of their bodies, whereas whites have greater amounts of subcutaneous fat on the front of their bodies (Wagner et al., 2000).

Black Americans have a high prevalence of obesity or obesity-related diseases. A genetic component leading to this condition has been identified in the LEP gene coding for the leptin, a hormone mainly produced by adipocytes. Level of leptin is positively correlated with fat mass, leptin signals to the hypothalamus to lower the appetite, exerting its anorexigenic functions.

It has been shown that obese people have unusually high levels of leptin causing a condition called "leptin resistance'. In this condition the brain does not respond to leptin, so, obese people keep eating despite adequate (or excessive) fat stores. (Izquierdo et al., 2019) Research shows a racial difference in leptin concentrations whose concentration has been demonstrated significantly higher in black than withes, suggesting a role for genetic factors in differences in body composition and obesity prevalence between the two races, particularly highlightable in black and white women. (Wagner et al., 2000)

Total body fat (TBF) was estimated using the equations described by Jackson et al. in the 2002 Heritage medical study. In the Heritage study, the relationship between sex, age and race on body fat measurements both in white and black Americans were examined and a formula for predicting body fat percentage from BMI, age and gender was proposed. This study found a slight difference in body fat percentage in men, depending on race. However, black women are characterized by an increase in the percentage of body fat when compared with white woman (Jackson et al., 2002).

It is important to mention that in the present study elderly subjects were healthy and their age varied from 65-72 and therefore no differences in TBW and TBF were observed. Therefore, only slight changes were observed in volume of distribution values.

The RI Senescence model's predictive performance was satisfactory evaluated using a population modelling approach (Renal Geriatric PopPK model), thus indicating that the main assumptions concerning processes determining the age-dependent pharmacokinetics of bilastine in renal impairment are overall well described. Concretely, in Group 1 (P3 Figure 5 a) a good match between profiles predicted using the RI Senescence Model (0% outliers) and the CI obtained from Renal Geriatric PopPK model was observed confirming the adequacy of the equations selected for prediction of each ADME process in this elderlies' group. AUC was accurately scaled to healthy elderly individuals by the RI Senescence Model (predicted using RI Senescence Model: predicted using Renal Geriatric PopPK Model ratio of 1.02).

The evaluation of the predictions in subjects in groups 2–4 need to take into account that these groups were receiving concomitant medications for the treatment of their renal impairment and related symptoms. As reported by Lasseter et al., at least one subject in groups 2–4 received drugs for diabetes mellitus and half of the subjects in groups 3 and 4 received antihypertensive drugs. Moreover, the incidence of other pathologies was increased in groups 2 to 4.

The concomitant pathologies affecting subjects from groups 2 to 4 lead to physiological changes apart from those solely caused by the renal impairment condition which were the only ones taken into consideration by the RI Senescence Model. Therefore, the interpretation of the results of the predictions for these groups must also consider the above-mentioned reasons together with the limited number of patients diagnosed with only Renal Insufficiency. Nevertheless, the predictions could be considered adequate given that percentage of outliers are less than 10% (2%, 3% and 7% respectively in Group 2, 3 and 4). In the case of bilastine, despite of the clear trend of increasing area under the plasma concentration–time curve (AUC) in groups 1–4, the overall exposure in all the groups was within safety margins that corresponded to the mean estimated exposure and 95 % CI of a single 80 mg oral dose of bilastine (AUC_{∞} of 4225.6 ng*h/mL, 95 % CI 3,174.7–6278.4). Considering the wide therapeutic margin of bilastine, dose adjustments were not needed for these patients.

In conclusion, the final validated predictive model could represent a valuable tool to explore the impact of different pathophysiological situations on bilastine disposition. Several virtual scenarios could be simulated mainly focusing on comorbidities present in aged patients such as, hepatic impairment and cardiac arrest, between others, or could be applied to simulate plasma profiles at different ages using calculated parameters. These simulations will allow to foresee if the combined

effect of aging and comorbidities will influence drug dosing strategies considering the specific therapeutic margin of different drugs commonly employed in geriatrics.

Conclusions

- A predictive model providing PK/PD parameters for bilastine from young to old adults was developed by means of a semi-physiologic approach. The impact of age on the PK and PD of bilastine was explored via population modelling in healthy subjects aged 18-80 years with simultaneous assessment of both PK and PD as inhibition effect of cutaneous reaction (flare). Physiological factors such as total body water (TBW), cardiac output (CO), glomerular filtration rate (GFR) were identified to likely affect the central volume of distribution (Vc/F), the intercompartmental clearance (Q/F) and the total clearance (CL) of bilastine as a first step for Subsequent modelling approaches.
- A population Medatabase spanning several decades of literature review was developed for aging Caucasians considering anatomical, physiological, and biological system parameters underlying the PK of drugs and concretely for bilastine. The constructed database provided an essential source of data for parameterization of a predictive model in elderly (Senescence model) consisting of scaling equations based on physiology that were incorporated into the different ADME processes responsible for the bilastine's PK in order to predict the drug's performance in the different geriatric age groups.
- The semi physiologic approach proposed in this research project integrates the available drug PK knowledge in adults as well as the impact of the physiological changes in elderly affecting the PK of bilastine followed by the application of modelling and simulation techniques. The underlying mechanisms affecting the PK of bilastine were elucidated by combining a compartmental model structure together with principles of physiology related to distribution and elimination processes in order to determine the impact of key physiological parameters (i.e., free-fraction in plasma, total body water composition, and glomerular filtration rate) that were predictive of PK behavior of bilastine in adults and also in the geriatric population.
    a. The Senescence model's predictive performance was satisfactory evaluated using elderly PK data available from Study BILA 459-05, thus indicating that the main assumptions concerning the age-dependent processes determining the pharmacokinetics of bilastine, are overall well described.
    b. Given that the proposed Senescence model structure was built on a physiological basis, it was used as a tool to explore the impact of hypothetical pathophysiological situations on bilastine PK. Concretely, the already developed Senescence model was adapted to predict PK in elderly patients with renal impairment. Overall, the predictive model was properly evaluated also in

this population using data from Study BILA 2808/RI including elderly patients with impaired renal function.

References

Aalami, O. O., Fang, T. D., Song, H. M., & Nacamuli, R. P. (2003). Physiological features of aging persons. *Archives of Surgery*, *138*(10): 1068–1076. https://doi.org/10.1001/archsurg.138.10.1068

Abdulkader, R. C. R. M., Burdmann, E. A., & Lu, M. (2017). Aging and decreased glomerular filtration rate : An elderly population-based study. *Plos One*, 12(12): e0189935. https://doi.org/10.1371/journal.pone.019935

Abuhelwa, A. Y., Foster, D. J. R., & Upton, R. N. (2016). A Quantitative Review and Meta-Models of the Variability and Factors Affecting Oral Drug Absorption-Part I: Gastrointestinal pH. *The AAPS Journal*, 18(5): 1309–1321. https://doi.org/10.1208/s12248-016-9952-8

Ahmed, T. A. (2015). Pharmacokinetics of Drugs Following IV Bolus, IV Infusion, and Oral Administration. *Ahmed TA (ed) Basic Pharmacokinetic Concepts and Some Clinical Applications (1st edn), IntechOpen* 54–98. https://doi.org/10.5772/61573

Bachert, C., Kuna, P., & Zuberbier, T. (2010). Bilastine in allergic rhinoconjunctivitis and urticaria. *Allergy: European Journal of Allergy and Clinical Immunology*, 65(93): 1–13. https://doi.org/10.1111/j.1398-9995.2010.02404.x

Bai, J. P. F., Burckart, G. J., & Mulberg, A. E. (2016). Literature Review of Gastrointestinal Physiology in the Elderly, in Pediatric Patients, and in Patients with Gastrointestinal Diseases. *Journal of Pharmaceutical Sciences*, 105(2): 476–483. https://doi.org/10.1002/jps.24696

Balakrishnan, R. (2014). Antihistamines as Allergy Drugs. Blockbuster Drugs, 4(6): 236–240. https://doi.org/10.1093/oso/9780199737680.003.0007

Besanko, L. K., Burgstad, C. M., Cock, C., Heddle, R., Fraser, A., & Fraser, R. J. L. (2014). Changes in esophageal and lower esophageal sphincter motility with healthy aging. *Journal of Gastrointestinal and Liver Diseases*, 23(3): 243–248. https://doi.org/10.1543/jgld.2014.1121

Bjorkman, S. (2005a). Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs. *British Journal of Clinical Pharmacology*, 59(6): 691–704. https://doi.org/10.1111/j.1365-2125.2004.02225.x

Brenner, S. S., & Klotz, U. (2004). P-glycoprotein function in the elderly. *European Journal of Clinical Pharmacology*, 60(2): 97–102. https://doi.org/10.1007/s00228-004-0733-4

Brownie, S. (2006). Why are elderly individuals at risk of nutritional deficiency? *International Journal of Nursing Practice*, 12(2): 110–118. https://doi.org/10.1111/j.1440-172X.2006.00557.x

Bruss, Z. S., & Raja, A. (2019). Physiology, Stroke Volume. *Treasure Island (FL): StatPearls Publishing*. http://www.ncbi.nlm.nih.gov/pubmed/31613466

Byambasukh, O., Buukuu, G., Chinbat, O., Tungalag, D., & Tsedendamba, D. (2015). Body Composition Characteristics and Anthropometric Measurements of Older Mongolian Adults. *Cent Asian J Med* 1(1):

33–40. http://www.cajms.mn

Chumlea, W. C., Guo, S. S., Kuczmarski, R. J., Flegal, K. M., Johnson, C. L., Heymsfield, S. B., Lukaski, H. C., Friedl, K., & Hubbard, V. S. (2002). Body composition estimates from NHANES III bioelectrical impedance data. *International Journal of Obesity*, 26(12): 1596–1609. https://doi.org/10.1038/sj.ijo.0802167

Chumlea, W. C., Guo, S. S., Zeller, C. M., Reo, N. V, Baumgartner, R. N., Garry, P. J., Wang, J., Pierson, R. N., Heymsfield, S. B., & Siervogel, R. M. (2001). Total body water reference values and prediction equations for adults. *Kidney International*, 59(6): 2250–2258. https://doi.org/10.1046/j.1523-1755.2001.00741.x

Church, M.K., & Labeaga, L. (2017). Bilastine: a new H 1 -antihistamine with an optimal profile for updosing in urticaria. *Journal of the European Academy of Dermatology and Venereology*, 31(9): 1447–1452. https://doi.org/10.1111/jdv.14305

Church, Martin K., Tiongco-Recto, M., Ridolo, E., & Novák, Z. (2020). Bilastine: a lifetime companion for the treatment of allergies. *Current Medical Research and Opinion*, 36(3): 445–454. https://doi.org/10.1080/03007995.2019.1681134

Cooper, J. K., & Gardner, C. (1989). Effect of Aging on Serum Albumin. *Journal of the American Geriatrics Society*, 37(11): 1039–1042. https://doi.org/10.1111/j.1532-5415.1989.tb06917.x

Corcóstegui, R., Labeaga, L., Innerárity, A., Berisa, A., & Orjales, A. (2005). Preclinical pharmacology of bilastine, a new selective histamine H 1 receptor antagonist: Receptor selectivity and in Vitro antihistaminic activity. *Drugs in R and D*, 6(6): 371–384. https://doi.org/10.2165/00126839-200506060-00005

Corsonello, A., Pedone, C., & Incalzi, R. A. (2010). Age-related pharmacokinetic and pharmacodynamic changes and related risk of adverse drug reactions. *Current Medicinal Chemistry*, 17(6): 571–584. http://www.ncbi.nlm.nih.gov/pubmed/20015034

Currie, G. M., Wheat, J. M., & Kiat, H. (2011). Pharmacokinetic considerations for digoxin in older people.*The Open Cardiovascular Medicine Journal*, 5: 130–135. https://doi.org/10.2174/1874192401105010130

Delanaye, P., Schaeffner, E., Ebert, N., Cavalier, E., Mariat, C., Krzesinski, J. M., & Moranne, O. (2012). Normal reference values for glomerular filtration rate: What do we really know? *Nephrology Dialysis Transplantation*, 27(7): 2664–2672. https://doi.org/10.1093/ndt/gfs265

Denic, A., Glassock, R. J., & Rule, A. D. (2016). Structural and Functional Changes With the Aging Kidney. *Advances in Chronic Kidney Disease* 23(1): 19–28. https://doi.org/10.1053/j.ackd.2015.08.004

Deshpande, D., Srivastava, S., Meek, C., Leff, R., Hall, G. S., & Gumbo, T. (2010). Moxifloxacin pharmacokinetics/pharmacodynamics and optimal dose and susceptibility breakpoint identification for treatment of disseminated mycobacterium avium infection. *Antimicrobial Agents and Chemotherapy*,

54(6): 2534–2539. https://doi.org/10.1128/AAC.01761-09

Dey, D. K., Bosaeus, I., Lissner, L., & Steen, B. (2003). Body composition estimated by bioelectrical impedance in the Swedish elderly. Development of population-based prediction equation and reference values of fat-free mass and body fat for 70- and 75-y olds. *European Journal of Clinical Nutrition*, 57(8): 909–916. https://doi.org/10.1038/sj.ejcn.1601625

Drenth-van Maanen, A. C., Jansen, P. A. F., Proost, J. H., Egberts, T. C. G., van Zuilen, A. D., van der Stap, D., & van Marum, R. J. (2013). Renal function assessment in older adults. *British Journal of Clinical Pharmacology*, 76(4): 616–623. https://doi.org/10.1111/bcp.12199

DuBois, D., & DuBois, E. F. (1915). Fifth paper the measurement of the surface area of man. *Archives of Internal Medicine,* 15(5): 868–881. https://doi.org/10.1001/archinte.1915.00070240077005

Duraković, Z., & Vitezić, D. (2013). Pharmacodynamics and pharmacokinetics in the elderly. *Periodicum Biologorum,* 115(4): 517–520.

Edginton, A. N., Schmitt, W., & Willmann, S. (2006). Development and evaluation of a generic physiologically based pharmacokinetic model for children. *Clinical Pharmacokinetics,* 45(10): 1013–1034. https://doi.org/10.2165/00003088-200645100-00005

Encinas, E., Calvo, R., Lukas, J. C., Vozmediano, V., Rodriguez, M., & Suarez, E. (2013). A predictive pharmacokinetic/pharmacodynamic model of fentanyl for analgesia/sedation in neonates based on a semi-physiologic approach. *Pediatric Drugs*, 15(3): 247–257. https://doi.org/10.1007/s40272-013-00291

Ette EI, W. P. (2007). Pharmacometrics: The Science of Quantitative Pharmacology. New York, NY: Wiley-Interscience; Huang, X. H., & Li, J. (2007). Pharmacometrics: The Science of Quantitative Pharmacology. *American Journal of Pharmaceutical Education*, 71(4): 75. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1959219/

Gabardi, S., Tullius, S. G., & Krenzienc, F. (2015). Understanding alterations in drug handling with aging: A focus on the pharmacokinetics of maintenance immunosuppressants in the elderly. *Organ Transplantation*, 20(4): 424–430. https://doi.org/10.1097/mot.0000000000000220

Gabrielsson, J. (2006). Pharmacokinetic & pharmacodynamic data analysis :Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applicatio*ns. Swedish Pharmaceutical Press (4th ed.)*, 11–223.

Galteau, M., Guyon, M., Gueguen, R., Siest, G., & Clinique, D. B. (2001). Determination of Serum Cystatin C : Biological Variation and Reference Values. *Clin Chem Lab Med,* 39(9), 850–857. doi: 10.1515/CCLM.2001.141.

Garrido, M. J., & Trocóniz, I. F. (2019). Pharmacokinetic and Pharmacodynamic Modelling in Anaesthesia. *Personalized Anaesthesia: Targeting Physiological Systems for Optimal Effect,* 14–28. https://doi.org/10.1017/9781316443217.002

Gavazzi, G., & Krause, K. H. (2002). Ageing and infection. *Lancet Infectious Diseases*, 2(11): 659–666. https://doi.org/10.1016/S1473-3099(02)00437-1

Giles N. Cattermole, P. Y. Mia Leung, & Grace Y. L. Ho. (2017). The normal ranges of cardiovascular parameters measured using the ultrasonic cardiac output monitor. *Physiological Reports*, 5(6): e13195 https://doi: 10.14814/phy2.13195

Gill, J., Malyuk, R., Djurdjev, O., & Levin, A. (2007). Use of GFR equations to adjust drug doses in an elderly multi-ethnic group a cautionary tale. *Nephrol Dial Transplant*., 22(10): 2894-9. https://doi.org /10.1093/ndt/gfm289

Glaeser, H., Bailey, D. G., Dresser, G. K., Gregor, J. C., Schwarz, U. I., McGrath, J. S., Jolicoeur, E., Lee, W., Leake, B. F., Tirona, R. G., & Kim, R. B. (2007). Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clinical Pharmacology and Therapeutics*, 81(3): 362–370. https://doi.org/10.1038/sj.clpt.6100056

González-Deolano, D., & Álvarez-Twose, I. (2018). Mast cells as key players in allergy and inflammation. *Journal of Investigational Allergology and Clinical Immunology*, 28(6): 365-378. https://doi.org /10.18176/jiaci.0327

Grasela, T. H., & Sheiner, L. B. (1991). Pharmacostatistical modeling for observational data. *Journal of Pharmacokinetics and Biopharmaceutics*, 19(3): S25–S36. https://doi.org/10.1007/BF01371006

Henthorn, T. K., Krejcie, T. C., & Avram, M. J. (1992). The relationship between alfentanil distribution kinetics and cardiac output. *Clinical Pharmacology & Therapeutics*, 52(2): 190–196. https://doi.org/ 10.1038/clpt.1992.129

Hilmer, S. N. (2008). ADME-tox issues for the elderly. Expert Opinion on *Drug Metabolism & Toxicology*, 4(10): 1321–1331. https://doi.org/10.1517/17425255.4.10.1321

Hubbard, R. E., O'Mahony, M. S., & Woodhouse, K. W. (2013). Medication prescribing in frail older people. *European Journal of Clinical Pharmacology*, 69(3): 319–326. https://doi.org/10.1007/s00228-012-13872

Hunt, R. H., Camilleri, M., Crowe, S. E., El-Omar, E. M., Fox, J. G., Kuipers, E. J., Malfertheiner, P., McColl, K. E. L., Pritchard, D. M., Rugge, M., Sonnenberg, A., Sugano, K., & Tack, J. (2015) The stomach in health and disease. *Gut*, 64(10): 1650–1668. https://doi.org/10.1136/gutjnl-2014-307595

Izquierdo, A. G., Crujeiras, A. B., Casanueva, F. F., & Carreira, M. C. (2019). Leptin, obesity, and leptin resistance: where are we 25 years later? *Nutrients* 11(11): 2704. https://doi.org/10.3390/nu11112704

Jackson, A. S., Stanforth, P. R., Gagnon, J., Rankinen, T., Leon, A. S., Rao, D. C., Skinner, J. S., Bouchard, C., & Wilmore, J. H. (2002). The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *International Journal of Obesity*, 26(6): 789–796. https://doi. org/10.1038/sj.ijo.0802006

Jauregizar, N., Fuente, L. D. La, Lucero, M. L., Sologuren, A., Leal, N., & Rodríguez, M. (2009). Pharmacokinetic-pharmacodynamic modelling of the antihistaminic (H 1) Effect of Bilastine. *Clinical Pharmacokinetics*, 48(8): 543–554. https://doi.org/10.2165/11317180-000000000-00000

Jàuregui, I., García-Lirio, E., Soriano, A. M., Gamboa, P. M., & Antépara, I. (2012). An overview of the novel H1-antihistamine bilastine in allergic rhinitis and urticaria. *Expert Review of Clinical Immunology*, 8(1): 33–41. https://doi.org/10.1586/eci.11.87

JW, P., & CA, R. (1995). Body measurements of black and white elderly persons with emphasis on body composition. *Gerontology*, 41(1). https://doi.org/10.1159/000213659

Karafoulidou, A., Suarez, E., Anastasopoulou, I., Katsarou, O., Kouramba, A., Kotsi, P., Zografidis, A., & Lukas, J. C. (2009). Population pharmacokinetics of recombinant factor VIII:C (ReFacto®) in adult HIV-negative and HIV-positive haemophilia patients. *European Journal of Clinical Pharmacology*, 65(11): 1121–1130. https://doi.org/10.1007/s00228-009-0699-3

Katory, R. (1979). Normal and Body Cardiac Size Output in Relation to Age RYo KATORI The First Department of Internal Medicine , Kinki University S chool of Medicine , Osaka , *The Tohoku journal of experimental medicin*e, 128(4): 377-87. https:// doi: 10.1620/tjem.128.377

Kyle, U., Genton, L., Slosman, D., & Pichard, C. (2001). Fat-free and fat mass percentiles in 5225 healthy subjects aged 15-98 years. *Nutrition*, 17(7-8): 534-41. doi: 10.1016/s0899-9007(01)00555-x.

Lamb, E. J., Tomson, C. R. V., & Roderick, P. J. (2005). Estimating kidney function in adults using formulae. *Annals of Clinical Biochemistry*, 42(5): 321–345. https://doi.org/10.1258/0004563054889936

Landi, F., Calvani, R., Tosato, M., Martone, A., Ortolani, E., Savera, G., Sisto, A., & Marzetti, E. (2016). Anorexia of Aging: Risk Factors, Consequences, and Potential Treatments. *Nutrients*, 8(2): 69. https://doi.org/10.3390/nu8020069

Lasseter, K. C., Sologuren, A., La Noce, A., & Dilzer, S. C. (2013). Evaluation of the single-dose pharmacokinetics of bilastine in subjects with various degrees of renal insufficiency. *Clinical Drug Investigation*, 33(9): 665–673. https://doi.org/10.1007/s40261-013-0110-0

Lee Goldman, A. S. (2017). Goldman-Cecil Medicina Interna - Lee Goldman, Andrew Schafer (24th ed.)

Levey, A. S., Coresh, J., Greene, T., Marsh, J., Stevens, L. A., Kusek, J. W., & Van Lente, F. (2007). Expressing the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values. *Clinical Chemistry*, 53(4): 766–772. https://doi.org/10.1373/clinchem.2006.077180

Li, X. (2020). Application of population, physiologically based, and semi-physiological pharmacokinetic modeling to assess sources of pharmacokinetic variability in individual drugs. Universitäts- und Landesbibliothek Bonn. https://doi.org/10.1002/jcph.1619

Light, K. C., Turner, J. R., Hinderliter, A. L., & Sherwood, A. (1993a). Race and gender comparisons: I.

Hemodynamic responses to a series of stressors. *Health Psychology*, 12(5): 354–365. https://doi.org/10.1037//0278-6133.12.5.354

Lombardo, F., Berellini, G., & Obach, R. S. (2018). Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 1352 drug compounds. *Drug Metabolism and Disposition*, 46(11): 1466–1477. https://doi.org/10.1124/dmd.118.082966

Lowery, E. M., Brubaker, A. L., Kuhlmann, E., & Kovacs, E. J. (2013). The aging lung. *Clinical Interventions in Aging*, 8: 1489–1496. https://doi.org/10.2147/CIA.S51152

Lucero, María Luisa, Arteche, J. K., Sommer, E. W., & Casadesus, A. (2012). Preclinical toxicity profile of oral bilastine. *Drug and Chemical Toxicology*, 35(1): 25–33. https://doi.org/10.3109/01480545.2012.682652

Lucero, M.L., Gonzalo, A., Ganza, A., Leal, N., Soengas, I., Ioja, E., Gedey, S., Jahic, M., & Bednarczyk, D. (2012). Interactions of bilastine, a new oral H 1 antihistamine, with human transporter systems. *Drug and Chemical Toxicology*, 35(1): 8–17. https://doi.org/10.3109/01480545.2012.682653

Mangoni, A. A., & Jackson, S. H. D. (2004). Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications, 2004. *British Journal of Clinical Pharmacology*, 57(1): 6–14. https://doi.org/10.1046/J.1365-2125.2003.02007.X

Massoud, L., Agha, H. Al, & Taleb, M. (2017). Pharmacokinetic and Pharmacodynamic Changes in Elderly People. *World Journal Of Pharmaceutical And Medical Research*, 3(11): 14–23.

McLachlan, A. J., & Pont, L. G. (2012). Drug metabolism in older people - A key consideration in achieving optimal outcomes with medicines. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 67 A(2): 175–180. https://doi.org/10.1093/gerona/glr118

McNamara, P. J., & Alcorn, J. (2002). Protein binding predictions in infants. *AAPS PharmSci*, 4(1): 19–26. https://doi.org/10.1208/ps040104

McNamara, P. J., & Meiman, D. (2019). Predicting Drug Binding to Human Serum Albumin and Alpha One Acid Glycoprotein in Diseased and Age Patient Populations. *Journal of Pharmaceutical Sciences*, 108(8): 2737-2747. https://doi.org/10.1016/j.xphs.2019.03.018

Merchant, H. A., Liu, F., Orlu Gul, M., & Basit, A. W. (2016). Age-mediated changes in the gastrointestinal tract. *International Journal of Pharmaceutics*, 512(2), 382–395. https://doi.org/10.1016/j.ijpharm.2016.04.024

Messerli, F. H., DeCarvalho, J. G. R., Christie, B., Frohlich, E. D., Katory, R., Mullens, W., Nijst, P., Cattermole, G. N., (1979). Cardiac Output and Renal Dysfunction Definitely More Than Impaired Flow. *Stroke*, 67(5): 354–365. https://doi.org/10.1016/j.jacc.2016.03.537

Michael E. Burton, & Wilkins, L. W. &. (2006). *Applied Pharmacokinetics & Pharmacodynamics: Principles*

of Therapeutic Drug . 4th ed. Philadelphia: Lippincott Williams & Wilkins 70(6): 148.

Midlöv P. (2013). Pharmacokinetics and pharmacodynamics in the elderly.OA Elderly Medicine. Midlöv P. Pharmacokinetics and Pharmacodynamics in the Elderly. *OA Elderly Medicine*;1(1): 1. http://www.oapublishinglondon.com/article/621#

Mukker, J. K., Singh, R. S. P., & Derendorf, H. (2016). Pharmacokinetic and pharmacodynamic considerations in elderly population. *AAPS Advances in the Pharmaceutical Sciences Series*, 26: 139–151. https://doi.org/10.1007/978-3-319-43099-7_10

Musso, C. G., Belloso, W. H., Scibona, P., Bellizzi, V., & Macías Núñez, J. F. (2015). Impact of renal aging on drug therapy. *Postgraduate Medicine*, 127(6): 623–629. https://doi.org/10.1080/00325481.2015.1063957

Nations, U., of Economic, D., Affairs, S., & Division, P. (2019). *World Population Ageing 2019*: Highlights. Department of Economic and Social Affairs, Population Division (2019)

O'Connor, A., & O'Moráin, C. (2014). Digestive function of the stomach. *Digestive Diseases*, 32(3): 186–191. https://doi.org/10.1159/000357848

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Kanyua, A., Kagotho, E., Amayo, A., Ojwang, P., & Erasmus, R. (2017). Comparison of equations for estimating glomerular filtration rate in screening for chronic kidney disease in asymptomatic black Africans: A cross sectional study. *BMC Nephrology*, 18(1): 369. https://doi.org/10.1186/s12882-017-0788-y

PL, B. (2005). Covariate detection in population pharmacokinetics using partially linear mixed effects models. *Pharmaceutical Research*, 22(4): 541–549. https://doi.org/10.1007/S11095-005-2492-Z

Post, T. M., Freijer, J. I., Ploeger, B. A., & Danhof, M. (2008). Extensions to the Visual Predictive Check to facilitate model performance evaluation. *Journal of Pharmacokinetics and Pharmacodynamics*, 35(2): 185–202. https://doi.org/10.1007/s10928-007-9081-1

Powers, J. S., Choi, L., Bitting, R., Gupta, N., & Buchowski, M. (2009). Rapid measurement of total body water to facilitate clinical decision making in hospitalized elderly patients. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 64(6): 664–669. https://doi.org/10.1093/gerona/glp018

Ramsay, P. T., & Carr, A. (2011). Gastric acid and digestive physiology. *Surgical Clinics of North America* 91(5): 977–982. https://doi.org/10.1016/j.suc.2011.06.010

Reeve, E., Wiese, M. D., & Mangoni, A. A. (2015). Alterations in drug disposition in older adults. *Expert Opinion on Drug Metabolism & Toxicology*, 11(4): 491–508. https://doi.org/10.1517/17425255.2015.1004310

Rescigno, A. (2010). Compartmental Analysis and its Manifold Applications to Pharmacokinetics. *The AAPS*

Journal, 12(1): 61–72. https://doi.org/10.1208/s12248-009-9160-x

Rodrigues, A., Pereira, P. C., Vicente, A. F., A. Brito, J., Bernardo, M. A., & F. Mesquit, M. (2012). Food Intake, Body Mass Index and Body Fat Mass in Elderly. *Asian Journal of Clinical Nutrition*, 4(3): 107–115. https://doi.org/10.3923/ajcn.2012.107.115

Rossi, A., Fantin, F., Di Francesco, V., Guariento, S., Giuliano, K., Fontana, G., Micciolo, R., Solerte, S. B., Bosello, O., & Zamboni, M. (2008). Body composition and pulmonary function in the elderly: A 7-year longitudinal study. International *Journal of Obesity*, 32(9): 1423–1430. https://doi.org/10.1038/ijo.2008.103

Sadaba, B., Azanza, J. R., Gomez-Guiu, A., & Rodil, R. (2013b). Critical appraisal of bilastine for the treatment of allergic rhinoconjunctivitis and urticaria. *Therapeutics and Clinical Risk Management*, 9(1): 197–205. https://doi.org/10.2147/TCRM.S16079

Sádaba, B., Gómez-Guiu, A., Azanza, J. R., Ortega, I., & Valiente, R. (2013). Oral availability of bilastine. *Clinical Drug Investigation*, 33(5): 375–381. https://doi.org/10.1007/s40261-013-0076-y

Sádaba Díaz De Rada, B., Azanza Perea, J. R., & Gomez-Guiu Hormigos, A. (2011). Bilastine for the relief of allergy symptoms. *Drugs of Today* 47(4): 251–262). https://doi.org/10.1358/dot.2011.47.4.1587029

Salive, M. E., Cornoni-Huntley, J., Phillips. (1992). Serum albumin in older persons: Relationship with age and health status. *Journal of Clinical Epidemiology*, 45(3): 213–221. https://doi.org/10.1016/0895-4356(92)90081-W

Schlender, J. F., Meyer, M., Thelen, K., Krauss, M., Willmann, S., Eissing, T., & Jaehde, U. (2016). Development of a Whole-Body Physiologically Based Pharmacokinetic Approach to Assess the Pharmacokinetics of Drugs in Elderly Individuals. *Clinical Pharmacokinetics*, 55(12): 1573–1589. https://doi.org/10.1007/s40262-016-0422-3

Scott-Warren, V., & Maguire, S. (2017). Physiology of ageing. *Anaesthesia and Intensive Care Medicine*, 18(1): 52–54. https://doi.org/10.1016/j.mpaic.2016.10.015

Sera, L. C., & McPherson, M. L. (2012). Pharmacokinetics and pharmacodynamic changes associated with aging and implications for drug therapy. *Clinics in Geriatric Medicine*, 28(2): 273–286. https://doi.org/10.1016/j.cger.2012.01.007

Sergi, G., Lupoli, L., Volpato, S., Bertani, R., (2004). Body fluid distribution in elderly subjects with congestive heart failure. *Annals of Clinical and Laboratory Science*, 34(4): 416–422.

Shahid, M., Tripathi, T., Sobia, F., Moin, S., Siddiqui, M., & Khan, R. A. (2009). Histamine, histamine receptors, and their role in immunomodulation: An updated systematic review. *Open Immunology Journal*, 2(1): 9–41. https://doi.org/10.2174/1874226200902010009

Sharma, A., & Jusko, W. J. (1998). Characteristics of indirect pharmacodynamic models and applications to

clinical drug responses. *British Journal of Clinical Pharmacology*, 45(3): 229–239. https://doi.org/10.1046/j.1365-2125.1998.00676.x

Shi, S., & Klotz, U. (2011). Age-Related Changes in Pharmacokinetics. *Current Drug Metabolism*, 12(7): 601–610. https://doi.org/10.2174/138920011796504527

Shi, S., Mörike, K., & Klotz, U. (2008). The clinical implications of ageing for rational drug therapy. *European Journal of Clinical Pharmacology*, 64(2): 183–199. https://doi.org/10.1007/s00228-007-0422-1

Simons, F. E. R., & Simons, K. J. (2008). H1 Antihistamines. *World Allergy Organization Journal*, 1(9), 145–155. https://doi.org/10.1097/WOX.0b013e318186fb3a

Singh, S., & Bajorek., B. (2014). Defining 'elderly' in clinical practice guidelines for pharmacotherapy. *Pharmacy Practice*, 12(4). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4282767/

Sinha, V., Zhao, P., Huang, S. M., & Zineh, I. (2014). Physiologically Based Pharmacokinetic Modeling: From Regulatory Science to Regulatory Policy. *Clinical Pharmacology & Therapeutics*, 95(5): 478–480. https://doi.org/10.1038/clpt.2014.46

Soenen, S., Rayner, C. K., Horowitz, M., & Jones, K. L. (2015). Gastric Emptying in the Elderly. *Clinics in Geriatric Medicine* 31 (3): 339–353. https://doi.org/10.1016/j.cger.2015.04.003

Soenen, S., Rayner, C. K., Jones, K. L., & Horowitz, M. (2016). The ageing gastrointestinal tract. Current Opinion. *Clinical Nutrition and Metabolic Care*, 19(1): 12–18. https://doi.org/10.1097/MCO.0000000000000238

Sologuren A, Lucero M, Valiente R, Charles H, M. S. (2009). Human mass balance with [14C]-bilastine following oral administration to healthy volunteers. 9th Congress of the European Association for Clinical Pharmacology and Therapeutics; July 12–15, 2009, Edinburgh, UK. *Basic Clin Pharmacol Toxicol*.;105(1):106–107.

Sologuren A, Valiente R, Crean C, M. D. (2007). Relationship of dose to inhibition of wheal and flare for 5 doses of bilastine and iomg cetirizine. *Presented at: 36th Annual Meeting of the American-College-of-Clinical-Pharmacology*(47).

Sologuren, A., Viñas, R., Cordón, E., Riesgo, S. E., del Mar Forés, M., Senán, M. R., Fernández, S., Labeaga, L., & Ruiz-Miján, M. (2018). Open-label safety assessment of bilastine in elderly patients with allergic rhinoconjunctivitis and/or urticaria. *Allergy and Asthma Proceedings*, 39(4): 299–304. https://doi.org/10.2500/aap.2018.39.4136

Stader, F., Siccardi, M., Battegay, M., Kinvig, H., Penny, M. A., & Marzolini, C. (2018). Repository Describing an Aging Population to Inform Physiologically Based Pharmacokinetic Models Considering Anatomical, Physiological, and Biological Age-Dependent Changes. *Clinical Pharmacokinetics*, 58(1):1-19. https://doi.org/10.1007/s40262-018-0709-7

Starke, K., Board, E., Duckles, S., Eichelbaum, M.,(2008). *Modern Anesthetics* 182(182): 379-408. https://doi.org/10.1007/978-3-540-74806-9

Tarver-Carr, M. E., Powe, N. R., Eberhardt, M. S., Laveist, T. A., Kington, R. S., Coresh, J., & Brancati, F. L. (2002). Excess risk of chronic kidney disease among African-American versus white subjects in the United States: A population-based study of potential explanatory factors. *Journal of the American Society of Nephrology*, 13(9): 2363–2370. https://doi.org/10.1097/01.ASN.0000026493.18542.6A

Thompson, C. M., Johns, D. O., Sonawane, B., Barton, H. A., Hattis, D., Tardif, R., & Krishnan, K. (2009). Database for physiologically based pharmacokinetic (PBPK) modeling: Physiological data for healthy and health-impaired elderly. *Journal of Toxicology and Environmental Health*, 12(1): 1–24. https://doi.org/10.1080/10937400802545060

Todo Bom, A., & Mota Pinto, A. (2009). Allergic respiratory diseases in the elderly. *Respiratory Medicine*, 103(11): 1614–1622. https://doi.org/10.1016/j.rmed.2009.06.003

Turner DDS, M. D., Ship DMD, J. A., & Rcs, F. (2007). Dry Mouth and Its Effects on the Oral Health of Elderly People. *Journal of the American Dental Association*, 138(9): 15–20. https://doi.org/10.14219/jada.archive.2007.0358

Turnheim, K. (2004). Drug therapy in the elderly. *Experimental Gerontology*, 39(11–12): 1731–1738. https://doi.org/10.1016/j.exger.2004.05.011

Upton, R. N. (2012). Basic Concepts in Population Modeling , Simulation , and Model-Based Drug Development. *CPT Pharmacometrics Syst Pharmacol*. 1(9):e6. doi: 10.1038/psp.2012.4.

Upton, R. N. (2013). Basic Concepts in Population Modeling , Simulation , and Model-Based Drug Development - Part 2 : Introduction to Pharmacokinetic Modeling Methods. *CPT Pharmacometrics Syst Pharmacol.* 17;2(4): e38. doi: 10.1038/psp.2013.14.

Upton, R. N. (2014). Basic Concepts in Population Modeling , Simulation , and Model-Based Drug Development : Part 3 - Introduction to Pharmacodynamic Modeling Methods. *CPT Pharmacometrics Syst Pharmacol.* 3(1): e88. https://doi.org/10.1038/psp.2013.71

Utembe, W., Clewell, H. J., Sanabria, N., Doganis, P., & Gulumian, M. (2020). Current approaches and techniques in physiologically based pharmacokinetic (PBPK) modelling of nanomaterials. *Nanomaterials*, 10(7): 1–32. https://doi.org/10.3390/nano10071267

Van Sassenbroeck. (2002). Influence of Hypovolemia on the Pharmacokinetics and Electroencephalographic Effect of γ-Hydroxybutyrate in the Rat. *Anesthesiology*, 97(5): 1218–1226. https://doi.org/10.1097/00000542-200211000-00027

Veering, B., Burm, A., Souverijn, J., Serree, J., & Spierdijk, J. (1990). The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein. *British Journal of Clinical Pharmacology*, 29(2): 201–206. https://doi.org/10.1111/j.1365-2125.1990.tb03620.x

Vozmediano, V., Lukas, J. C., Encinas, E., Schmidt, S., Sologuren, A., Valiente, R., Labeaga, L., Campo, C., & Rodriguez, M. (2019). Model-informed pediatric development applied to bilastine: Analysis of the clinical PK data and confirmation of the dose selected for the target population. *European Journal of Pharmaceutical Sciences*, 128: 180–192. https://doi.org/10.1016/j.ejps.2018.11.016

Vozmediano, V., Ortega, I., Lukas, J. C., Gonzalo, A., Rodriguez, M., & Lucero, M. L. (2014). Integration of preclinical and clinical knowledge to predict intravenous PK in human: Bilastine case study. *European Journal of Drug Metabolism and Pharmacokinetics*, 39(1): 33–41. https://doi.org/10.1007/s13318-013-0131-3

Vozmediano, V., Sologuren, A., Lukas, J. C., Leal, N., & Rodriguez, M. (2017). Model Informed Pediatric Development Applied to Bilastine: Ontogenic PK Model Development, Dose Selection for First Time in Children and PK Study Design. *Pharmaceutical Research*, 34(12): 2720–2734. https://doi.org/10.1007/s11095-017-2248-6

Wagner, D. R., & Heyward, V. H. (2000). Measures of body composition in blacks and whites: a comparative review. *The American Journal of Clinical Nutrition*, 71(6):1392–1402. https://doi.org/10.1093/ajcn/71.6.1392

Watson, P. E., Watson, I. D., & Batt. (1980).Total body water volumes for adult males and females estimated from simple anthropometric measurements. *American Journal of Clinical Nutrition*, 33(1): 27-39. doi: 10.1093/ajcn/33.1.27.

Weinstein, J. R., & Anderson, S. (2010). The Aging Kidney: Physiological Changes. *Advances in Chronic Kidney Disease* 17(4): 302–307. https://doi.org/10.1053/j.ackd.2010.05.002

Wilfried Mullens, MD, P., & Petra Nijst, M. (2016). Cardiac Output and Renal Dysfunction. *Journal of the American College of Cardiology*, 67(19): 2209-2212. doi: 10.1016/j.jacc.2016.03.537.

Yamada, Y., Yokoyama, K., Oda, S., Masuo, Y., Hashii, Y., Ando, S., Okayama, Y. (2009). Proximal electrode placement improves the estimation of body composition in obese and lean elderly during segmental bioelectrical impedance analysis. *European Journal of Applied Physiology*, 107(2): 135–144. https://doi.org/10.1007/s00421-009-1106-6

Annex I

Annex I. Clinical study data used in the research project (complementary to methodology Section I, Section II and III).

Table AI-1. Summary of Phase I clinical studies from which the PK/PD model of bilastine originate (modified from Jauregizar 2009 with permission)

Study	Code	Description	Dosing regimen	No. of healthy adult subjects
1	**459-01**	Double-blind, ascending, single-dose study to evaluate the safety, tolerability and pharmacokinetics of BIL	SOD: 5, 10, 50 and 100 mg	36
2	**459-02**	Pharmacokinetic study to assess the single-dose bioavailability of BIL under fed and fasted conditions	SOD: 20 mg	12
3	**459-03**	Randomized, multiple-dose study to evaluate the safety and tolerability and pharmacokinetics of BIL at escalating doses	MOD: 10, 20, 50 and 100 mg/day for 14 days	36
4	**459-04**	Randomized, single-dose, placebo-controlled, four-period crossover study to evaluate the safety and tolerability, pharmacokinetics and antihistaminic activity of BIL at five dose levels compared with cetirizine	SOD: 5, 10, 20 and 50 mg	21
5	**459-05**	Open-label study to assess the effects of age and gender on the pharmacokinetics and pharmacodynamics of BIL	SOD: 20 mg	32
6	**CIM/02/100/01**	Randomized, double-blind, crossover, placebo- and positive standard-controlled, single-centre clinical trial for evaluation of CNS effects of BIL at different doses after single and repeat oral administration	MOD: 20, 40 and 80 mg/day for 7 days	20
7	**459-06**	Pharmacokinetic and safety study evaluating the potential interaction of erythromycin and BIL under steady-state conditions	SOD: 20 mg 24	24
8	**459-07**	Pharmacokinetic and safety study evaluating the potential interaction of ketoconazole and BIL under steady-state conditions	SOD: 20 mg 24	24
9	**459-08**	Randomized, double-blind, placebo-controlled, sequential group study to evaluate the safety, tolerability and pharmacokinetics of single, ascending doses of BIL and of multiple doses of BIL	SOD: 120, 160, 200 and 220 mg MOD: 140 and 220 mg/day for 7 days	54
10	**459-09**	Randomized, multiple-dose, double-blind, five-way crossover study of the ECG effects of bilastine	MOD: 20 and 100 mg/day for 4 days	30
11	**459-10**	Randomized, open-label, two-way crossover study to evaluate the effect of grapefruit juice on the single-dose pharmacokinetics of BIL	SOD: 20 mg	11
12	**459-11**	Randomized, open-label, two-way crossover study to evaluate the effect of diltiazem on the single-dose pharmacokinetics of BIL	SOD: 20 mg	11

CNS= central nervous system; ECG= electrocardiographic; MOD= multiple oral doses; SOD= single oral dose.

Table AI-2. Clinical studies used to develop and evaluate the Senescence and the RI Senescence model

All the clinical data used in the research project were part of bilastine clinical development and were approved by the corresponding Institutional Review Board (IRB), and conducted in accordance with the principles of the Declaration of Helsinki of 1975 (as revised in 1983).

Study	**BILA-2909/BA**	
Title of study	Study to Assess Oral Bioavailability of Bilastine (Estudio de Biodisponibilidad Oral de Bilastina)	
Study Phase	Phase 1	
Condition or disease	Healthy volunteers	
Objective of the study	The purpose of this study is to assess the absolute bioavailability of an oral bilastine formulation (test drug) compared to the intravenous administration of an IV bilastine formulation (control drug) in healthy volunteers.	**Dose= 20 mg P.O.** **Dose= 10 mg IV**
Trial design	This was a single centre, open label, cross-over, randomised, controlled, single dose study. The primary endpoint is the determination of plasma concentrations versus time (17 samples per subject at various time intervals after dosing) in order to assess the oral bioavailability of bilastine in healthy volunteers. The primary pharmacokinetic variable estimated were: the area under the plasma concentration versus time curve from time zero to infinity ($AUC_{0-\infty}$). Additionally, the following pharmacokinetic variables have been assessed: C_{max}, AUC_{0-t}, t_{max}, Ae, Clr, $t_{1/2}$. Additional objectives: To describe the safety and tolerability of a single administration of oral and intravenous bilastine in healthy volunteers.	
No. of subjects	Twelve healthy volunteers will be included. Each volunteer will take in random order one single dose of 20 mg oral bilastine and 10 mg IV bilastine with a minimum washout period of 14 days between them.	
A population PK model (Intravenous PopPK model) using data from young adults from study BILA-2909/BA. The obtained adult intravenous PK parameters have been used as a starting point for scaling into geriatrics.		
Study	**BILA 459-05**	
Title of study	An Open Label Study to assess the effects of age and gender on the Pharmacokinetic profile and pharmacodynamics of bilastine in healthy volunteers	
Study Phase	Phase 1	
Condition or disease	Healthy volunteers	
Objective of the study	The primary objectives of the study were as follows: • To assess the effects of age and gender on the pharmacokinetic profile of bilastine in healthy volunteers. • To assess the effects of age and gender on the inhibitory effect of bilastine on histamine-induced wheal and flares.	**Dose= 20 mg P.O.**
Trial design	This was a single center, open-label, single-dose, parallel group study design. The safety, tolerability, pharmacokinetics, and pharmacodynamics of a 20 mg oral dose of bilastine were evaluated	

No. of subjects	A total of 32 subjects were enrolled in the study. They all completed the study and were included in the statistical, pharmacokinetic, and pharmacodynamic analyses.	

Sixteen (16) elderly subjects of a total of 32 subjects (young adult and elderly) enrolled in the study were considered for the development of the Geriatric PopPK model. The purpose of developing the Geriatric PopPK model was to estimate a first order absorption constant to inform the Senescence model but also to use it as a reference. Moreover, the Bayesian PK parameters were estimated and used to validate the individual predictions with the Senescence model.

Study	**BILA 2808/RI** **Reported by Lasseter et al. 2013**	
Title of study	Evaluation of the Single-dose Pharmacokinetics of Bilastine in Subjects with Various Degrees of Renal Insufficiency.	
Study Phase	Phase I	
Objective of the study	The objective of this study is to determine the pharmacokinetics, safety, and tolerability of bilastine (20 mg) in healthy volunteers and subjects with renal insufficiency (6/group)	
Condition or disease	Healthy volunteers and subjects with renal insufficiency	
Trial design	This was a single center, open-label, single-dose, parallel study design. The safety, tolerability, pharmacokinetics, and pharmacodynamics of a 20 mg oral dose of bilastine were evaluated	**Dose= 20 mg P.O.**
No. of subjects	Twenty-four male and female subjects with a mean age of 68.30 were included; four groups of 6 subjects each were enrolled in the following treatment groups: **Group 1/ Normal:** (GFR) >80 mL/min/1.73 m^2 **Group 2/ Mild renal impairment:** (GFR) 50-80 mL/min /1.73 m^2 **Group 3/ Moderate renal impairment:** (GFR) 30-<50 mL/min/1.73 m^2 **Group 4/ Severe renal impairment:** (GFR) <30 mL/min/1.73 m^2	

Data from study 2808/RI were used to develop a population PK model (Renal Geriatric PopPK model).
The objective was to estimate the Bayesian PK parameters along with the absorption constant used to validate the individual predictions with the RI Senescence model.

Annex II

Annex II. Metadatabase developed for aging subjects considering anatomical, physiological, and biological system parameters required to inform the physiologically based pharmacokinetic model (complementary to methodology Section II and III).

- **Metadatabase for aging healthy Caucasians (complementary to section II)**

Table AII-1. Summary of equation and/or references used to derive the physiological or PK scaled parameters

Parameter	Equation and/or Reference	Parameter related
Height (cm)	Obtained from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 (Schlender et al., 2016)	**BSA (m^2) TBW (L) TBF (Kg)**
Weight (Kg)	Obtained from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 (Schlender et al., 2016)	**BSA (m^2) TBW (L) TBF (Kg)**
BSA (m2)	$BSA\ (m^2) = 0.007184\ x\ weight\ (kg)^{0.725}\ x\ 59\ x\ height(cm)^{0.425}$ DuBois & DuBois, 1915	**CO (L/h)**
TBW (L)	$\text{TBWmale (L)} = 2.447 - 0.09516 \text{ x Age(yr)} + 0.1074 \text{ x height(cm)} + 0.3362 \text{ x weight(kg)}$ $\text{TBWfemale (L)} = -2.097 + 0.1069 \text{ x height (cm)} + 0.2466 \text{ x weight (kg)}$ Watson et al., 1980	**Vss, Vc and Vp-iv (L)**
TBF (Kg)	$\text{TBF (kg)} = 0.68 \text{ x weight (kg)} - 0.56 \text{ x height(cm)} + 6.1 \text{ x Sex} + 65$ $male = 0, \quad female = 1$ Stader et al., 2018	**Vss, Vc and Vp-iv (L)**
$CO_{ger/ad}$ (L/h)	$CO\ (L/h) = 159\ x\ BSA - 1.56\ x\ Age + 114$ Stader et al., 2018	**Q iv (L/h)**
GFR_{ger} (mL/min)	Obtained from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 (Schlender et al., 2016)	**CLr iv (L/h)**
GFR_{ad} (mL/min)	Obtained from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 according to race/sex. Adult of reference were considered aged 30-50 years	**CLr iv (L/h)**
$CSHA_{ger/ad}$ (g/L)	$\text{CHSA (g/L)} = -0.0709 \text{ x Age} + 47.7$ Stader et al., 2018	**fu**
Abbreviations are: V Volume of distribution, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, fu Unbound fraction, GFR Glomerular filtration rare, CHSA Albumin molar concentration, TBW Total body water, TBF Total body fat, BSA Body surface area, CO Cardiac Output, Q Intercompartmental Clearance		

Table AII- 2. Demographic data descriptions of the virtual Caucasian male subjects

Caucasian male								
Age (Years)	**Height (cm)**	**Weight (kg)**	**GFR (mL/min)**	**BSA (m^2)**	**TBW (L)**	**FAT (kg)**	**CO (L/h)**	**CHSA (g/L)**
30	176.00	73.00	116.62	1.889	43.15	16.08	367.58	45.57
35	175.55	73.69	118.69	1.893	42.87	16.80	360.43	45.22
40	175.11	74.37	118.39	1.897	42.59	17.51	353.25	44.86
45	174.51	73.69	111.30	1.885	41.84	17.38	343.53	44.51
50	173.91	73.00	104.10	1.873	41.09	17.25	333.79	44.16
55	172.56	72.48	96.51	1.857	40.31	17.65	323.41	43.80
60	171.21	71.96	89.42	1.841	39.53	18.06	313.04	43.45
65	169.29	71.54	82.60	1.821	38.73	18.84	302.14	43.09
66	168.90	71.46	81.35	1.817	38.57	19.01	299.96	43.02
67	168.52	71.38	80.08	1.813	38.41	19.17	297.79	42.95
68	168.14	71.29	78.85	1.809	38.25	19.32	295.60	42.88
69	167.75	71.21	77.61	1.805	38.09	19.48	293.42	42.81
70	167.37	71.13	76.41	1.802	37.93	19.64	291.25	42.74
71	167.18	70.83	74.72	1.797	37.71	19.54	288.94	42.67
72	166.99	70.53	73	1.792	37.50	19.45	286.63	42.60
73	166.80	70.23	71.37	1.787	37.29	19.35	284.32	42.52
74	166.61	69.93	69.73	1.783	37.08	19.25	282.01	42.45
75	166.43	69.63	68.15	1.778	36.86	19.15	279.71	42.38
76	166.24	69.33	66.62	1.773	36.65	19.05	277.40	42.31
77	166.05	69.03	65.08	1.769	36.44	18.95	275.09	42.24
78	165.86	68.73	63.59	1.764	36.23	18.85	272.78	42.17
79	165.67	68.43	62.12	1.759	36.01	18.76	270.46	42.10
80	165.48	68.13	60.71	1.754	35.80	18.66	268.15	42.03
81	165.31	67.80	59.54	1.749	35.58	18.53	265.81	41.96
82	165.14	67.47	58.41	1.745	35.36	18.40	263.46	41.89
83	164.97	67.15	57.34	1.740	35.14	18.28	261.14	41.82
84	164.08	66.82	56.26	1.729	34.84	18.55	257.92	41.74

85	164.63	66.49	55.2	1.730	34.70	18.02	256.45	41.67
86	164.46	66.16	54.16	1.725	34.48	17.89	254.10	41.60
87	164.29	65.84	53.16	1.720	34.26	17.77	251.77	41.53
88	164.12	65.51	52.17	1.715	34.04	17.64	249.42	41.46
89	163.94	65.18	51.19	1.710	33.82	17.52	247.06	41.39
90	163.77	64.85	50.24	1.705	33.60	17.39	244.71	41.32
91	163.15	63.93	49.54	1.690	33.13	17.11	240.77	41.25
92	162.54	63.01	48.88	1.675	32.66	16.82	236.83	41.18
93	161.92	62.09	48.22	1.660	32.20	16.55	232.88	41.11
94	161.30	61.16	47.57	1.645	31.73	16.26	228.91	41.04
95	160.68	60.24	46.94	1.630	31.26	15.98	224.94	40.96
96	160.06	59.32	46.32	1.615	30.79	15.70	220.97	40.89
97	159.45	58.39	45.71	1.599	30.32	15.41	216.99	40.82
98	158.83	57.47	45.1	1.584	29.85	15.13	213.00	40.75
99	158.21	56.55	44.52	1.569	29.39	14.86	209.01	40.68
100	157.59	55.63	43.95	1.554	28.92	14.58	205.02	40.61

Abbreviations are: GFR Glomerular filtration rate, BSA Body surface area, TBW Total body water, TBF Total body fat, CO Cardiac Output CHSA Albumin molar concentration

Table AII- 3. Demographic data descriptions of the virtual Caucasian female subjects

Caucasian female								
Age (Years)	**Height (cm)**	**Weight (kg)**	**GFR (mL/min)**	**BSA (m^2)**	**TBW (L)**	**FAT (kg)**	**CO (L/h)**	**CHSA (g/L)**
30	163.00	60.00	107.35	1.644	30.12	20.62	328.60	45.57
35	161.93	61.65	104.89	1.655	30.42	22.34	322.58	45.22
40	160.87	63.29	100.70	1.666	30.71	24.05	316.47	44.86
45	160.42	64.26	95.88	1.673	30.90	24.96	309.84	44.51
50	159.97	65.23	90.79	1.680	31.09	25.87	303.20	44.16
55	159.16	65.39	84.07	1.676	31.04	26.44	294.69	43.80
60	158.35	65.54	77.65	1.671	30.99	26.99	286.17	43.45
65	156.85	63.86	71.45	1.642	30.42	26.69	273.64	43.09
66	156.55	63.52	70.25	1.636	30.30	26.63	271.13	43.02
67	156.25	63.18	69.07	1.630	30.19	26.56	268.62	42.95
68	155.95	62.84	67.93	1.624	30.07	26.50	266.10	42.88
69	155.65	62.51	66.81	1.618	29.96	26.44	263.61	42.81
70	155.36	62.17	65.69	1.612	29.84	26.37	261.10	42.74
71	154.99	61.56	64.26	1.602	29.65	26.17	258.03	42.67
72	154.62	60.96	62.87	1.593	29.46	25.97	254.97	42.60
73	154.25	60.35	61.46	1.583	29.27	25.76	251.90	42.52
74	153.88	59.75	60.08	1.574	29.09	25.56	248.83	42.45
75	153.51	59.14	58.76	1.564	28.90	25.35	245.75	42.38
76	153.14	58.53	57.46	1.555	28.71	25.14	242.66	42.31
77	152.77	57.93	56.15	1.545	28.52	24.94	239.59	42.24
78	152.40	57.32	54.90	1.536	28.33	24.73	236.50	42.17
79	152.03	56.72	53.65	1.526	28.14	24.53	233.42	42.10
80	151.66	56.11	52.43	1.516	27.95	24.33	230.32	42.03
81	151.49	55.87	51.59	1.513	27.87	24.26	228.13	41.96
82	151.32	55.63	50.79	1.509	27.80	24.19	225.93	41.89
83	151.15	55.39	49.97	1.505	27.72	24.12	223.74	41.82
84	150.98	55.15	49.19	1.501	27.64	24.05	221.54	41.74

85	150.81	54.91	48.42	1.497	27.57	23.99	219.35	41.67
86	150.64	54.67	47.66	1.493	27.49	23.92	217.15	41.60
87	150.47	54.43	46.92	1.488	27.41	23.85	214.95	41.53
88	150.30	54.19	46.18	1.484	27.33	23.78	212.75	41.46
89	150.13	53.95	45.46	1.480	27.26	23.71	210.56	41.39
90	149.97	53.71	44.77	1.477	27.18	23.64	208.37	41.32
91	149.76	52.86	44.11	1.465	26.95	23.18	204.99	41.25
92	149.55	52.01	43.48	1.454	26.72	22.72	201.59	41.18
93	149.35	51.16	42.85	1.442	26.48	22.25	198.20	41.11
94	149.14	50.30	42.24	1.430	26.25	21.79	194.76	41.04
95	148.93	49.45	41.65	1.418	26.02	21.33	191.33	40.96
96	148.73	48.60	41.05	1.407	25.79	20.86	187.89	40.89
97	148.52	47.75	40.48	1.395	25.55	20.40	184.43	40.82
98	148.31	46.89	39.90	1.383	25.32	19.93	180.94	40.75
99	148.11	46.04	39.33	1.370	25.09	19.47	177.47	40.68
100	147.90	45.19	38.79	1.358	24.86	19.01	173.96	40.61

Abbreviations are: GFR Glomerular filtration rate, BSA Body surface area, TBW Total body water, TBF Total body fat, CO Cardiac Output CHSA Albumin molar concentration

Table AII- 4. Reference Adult body composition parameters

Parameter	Caucasian female adult of reference	Caucasian male of reference
Age (Years)	30-50	
GFR (L/h)	5.98	6.78
CO (L/h)	318.19	352.11
CHSA (g/L)	44.86	
BSA (m^2)	1.66	1.89
TBW (L)	30.65	42.31
TBF (kg)	23.57	17.01
Weight (kg)	62.89	73.55
Height (cm)	161.24	175.02
fu (Jauregizar et al., 2009)	0.13	
Abbreviations are: GFR Glomerular filtration rate, CO Cardiac Output, CHSA Albumin molar concentration, BSA Body surface area, TBW Total body water, TBF Total body fat, fu Unbound fraction		

The relevant metrics included in the constructed metadatabase were used to inform the Senescence model by including their numeric value into the generated equations (Table AII-5) applicable to each ADME process used to scale the intravenous parameters from adults into geriatrics (Table AII-6-11). Considering that bilastine is administered orally, all individual parameters were corrected by the known bioavailability (61%) (Sadaba et al., 2013b) and the resulting individual are shown in each table summarizing the different PK parameters.

Table AII- 5. Semi-Physiological Scaled Equations Used in the Extrapolation of Bilastine PK Parameters to Elderly

PK Parameter	Equation to scale absolute PK in elderly
fu	$\mathrm{fu_{ad}} = \dfrac{1}{1 + \dfrac{\mathrm{CHSA_{ger}\ x\ (1 - fu_{ad})}}{\mathrm{CHSA_{ad}\ x\ fu_{ad}}}}$
CLr iv (L/h)	$\mathrm{CLr_{ger}\ iv} = \frac{\mathrm{GFR_{ger}\ x\ fu_{ger}}}{\mathrm{GFR_{ad}\ x\ fu_{ad}}}\ \mathrm{x\ CLr_{ad}\ iv}$ $\mathrm{Clr_{ad}}$ iv 8.17 L/h ratio $\dfrac{\mathrm{CLiv}}{\mathrm{CLr\ iv}}$
Q iv (L/h)	ratio $\dfrac{\mathrm{CO_{ad}\ male}}{\mathrm{Q_{ad}\ iv}} = 348.62$ ratio $\dfrac{\mathrm{CO_{ad}\ female}}{\mathrm{Q_{ad}\ iv}} = 315.04$ Qiv ad 1.01
Vss, Vc and Vp-iv (L)	$\mathrm{Vss} \cong \mathrm{TBW} + \mathrm{TBF}$ $\mathrm{V_c} = 0{,}65\ \mathrm{x}\ Vss\ \mathrm{iv}$ $\mathrm{V_p} = Vss\ \mathrm{iv} - \mathrm{V_c\ iv}$
ka (h^{-1})	The ka was estimated with the Geriatric PopPK model

Abbreviations are: V Volume of distribution, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, ad Adult, ger Geriatric, iv intravenous, fu Unbound fraction, GFR Glomerular filtration rate, CHSA Albumin molar concentration, TBW Total body water, TBF Total body fat, BSA Body surface area, CO Cardiac Output, Q Intercompartmental Clearance, k_a Absorption rate constant

Table AII- 6. Absolute and bioavailability corrected individual Clearance (CL) parameters of Bilastine estimated in the virtual elderly male subjects using the Senescence model. The physiological parameters used to scale the PK parameters are shown

Caucasian elderly male											correction by F (61%)
Age	**GFR (mL/min)**	**GFR (L/h)**	**$CHSA_{ger}$ (g/L)**	**GFR_{ad} (L/h)**	**$CHSA_{ad}$ (g/L)**	**fu_{ad}**	**fu_{ger}**	**CLr_{ad} iv (L/h)**	**CLr_{ger} iv (L/h)**	**CL_{ger} iv (L/h)**	**CL_{ger}/F (L/h)**
65	82.6	4.96	43.0915	6.78	44.86	0.130	0.1346	8.27	6.26	11.71	19.19
66	81.35	4.88	43.0206	6.78	44.86	0.130	0.1348	8.27	6.17	11.55	18.93
67	80.08	4.80	42.9497	6.78	44.86	0.130	0.1350	8.27	6.09	11.38	18.66
68	78.85	4.73	42.8788	6.78	44.86	0.130	0.1352	8.27	6.00	11.22	18.40
69	77.61	4.66	42.8079	6.78	44.86	0.130	0.1354	8.27	5.92	11.06	18.13
70	76.41	4.58	42.737	6.78	44.86	0.130	0.1356	8.27	5.83	10.91	17.88
71	74.72	4.48	42.6661	6.78	44.86	0.130	0.1358	8.27	5.71	10.68	17.51
72	73.00	4.38	42.5952	6.78	44.86	0.130	0.1360	8.27	5.59	10.45	17.13
73	71.37	4.28	42.5243	6.78	44.86	0.130	0.1362	8.27	5.47	10.23	16.77
74	69.73	4.18	42.4534	6.78	44.86	0.130	0.1364	8.27	5.35	10.01	16.41
75	68.15	4.09	42.3825	6.78	44.86	0.130	0.1366	8.27	5.24	9.80	16.06
76	66.62	4.00	42.3116	6.78	44.86	0.130	0.1368	8.27	5.13	9.59	15.72
77	65.08	3.90	42.2407	6.78	44.86	0.130	0.1370	8.27	5.02	9.38	15.38
78	63.59	3.82	42.1698	6.78	44.86	0.130	0.1372	8.27	4.91	9.18	15.05
79	62.12	3.73	42.0989	6.78	44.86	0.130	0.1374	8.27	4.80	8.98	14.73
80	60.71	3.64	42.028	6.78	44.86	0.130	0.1376	8.27	4.70	8.79	14.41
81	59.54	3.57	41.9571	6.78	44.86	0.130	0.1378	8.27	4.62	8.63	14.16
82	58.41	3.50	41.8862	6.78	44.86	0.130	0.1380	8.27	4.54	8.48	13.91
83	57.34	3.44	41.8153	6.78	44.86	0.130	0.1382	8.27	4.46	8.34	13.67
84	56.26	3.38	41.7444	6.78	44.86	0.130	0.1384	8.27	4.38	8.19	13.43
85	55.2	3.31	41.6735	6.78	44.86	0.130	0.1386	8.27	4.31	8.05	13.20
86	54.16	3.25	41.6026	6.78	44.86	0.130	0.1388	8.27	4.23	7.91	12.97
87	53.16	3.19	41.5317	6.78	44.86	0.130	0.1390	8.27	4.16	7.78	12.75
88	52.17	3.13	41.4608	6.78	44.86	0.130	0.1392	8.27	4.09	7.64	12.53
89	51.19	3.07	41.3899	6.78	44.86	0.130	0.1394	8.27	4.02	7.51	12.31
90	50.24	3.01	41.319	6.78	44.86	0.130	0.1396	8.27	3.95	7.38	12.10

91	49.54	2.97	41.2481	6.78	44.86	0.130	0.1398	8.27	3.90	7.29	11.95
92	48.88	2.93	41.1772	6.78	44.86	0.130	0.1400	8.27	3.85	7.20	11.81
93	48.22	2.89	41.1063	6.78	44.86	0.130	0.1402	8.27	3.81	7.12	11.67
94	47.57	2.85	41.0354	6.78	44.86	0.130	0.1404	8.27	3.76	7.03	11.53
95	46.94	2.82	40.9645	6.78	44.86	0.130	0.1406	8.27	3.72	6.95	11.39
96	46.32	2.78	40.8936	6.78	44.86	0.130	0.1408	8.27	3.67	6.87	11.26
97	45.71	2.74	40.8227	6.78	44.86	0.130	0.1410	8.27	3.63	6.79	11.13
98	45.10	2.71	40.7518	6.78	44.86	0.130	0.1413	8.27	3.59	6.71	10.99
99	44.52	2.67	40.6809	6.78	44.86	0.130	0.1415	8.27	3.55	6.63	10.87
100	43.95	2.64	40.610	6.78	44.86	0.130	0.1417	8.27	3.51	6.56	10.75

Abbreviations are: GFR Glomerular filtration rate, ad Adult, ger Geriatric, iv Intravenous, BSA Body surface area, TBW Total body water, TBF Total body fat, CO Cardiac Output, CHSA Albumin molar concentration, fu Unbound fraction, CL Clearance, r Renal, F Bioavailability

Table AII- 7. Absolute and bioavailability corrected individual Clearance (CL) parameters of Bilastine estimated in the virtual elderly female subjects using the semi-mechanistic approach. The physiological parameters used to scale the PK parameter of interest are shown

Caucasian elderly female											correction by F 61%)
Age	**GFR (mL/min)**	**GFR (L/h)**	**$CHSA_{ger}$ (g/L)**	**GFR_{ad} (L/h)**	**$CHSA_{ad}$ (g/L)**	**fu_{ad}**	**fu_{ger}**	**CLr_{ad} iv (L/h)**	**CLr_{ger} iv (L/h)**	**CL_{ger} iv (L/h)**	**CL_{ger}/F (L/h)**
65	63.86	3.83	43.0915	5.98	44.86	0.130	0.1346	8.27	5.49	10.21	16.73
66	63.52	3.81	43.0206	5.98	44.86	0.130	0.1348	8.27	5.47	10.17	16.67
67	63.18	3.79	42.9497	5.98	44.86	0.130	0.1350	8.27	5.44	10.13	16.60
68	62.84	3.77	42.8788	5.98	44.86	0.130	0.1352	8.27	5.42	10.09	16.54
69	62.51	3.75	42.8079	5.98	44.86	0.130	0.1354	8.27	5.40	10.05	16.47
70	62.17	3.73	42.737	5.98	44.86	0.130	0.1356	8.27	5.38	10.01	16.41
71	61.56	3.69	42.6661	5.98	44.86	0.130	0.1358	8.27	5.34	9.92	16.27
72	60.96	3.66	42.5952	5.98	44.86	0.130	0.1360	8.27	5.29	9.84	16.13
73	60.35	3.62	42.5243	5.98	44.86	0.130	0.1362	8.27	5.25	9.76	15.99
74	59.75	3.59	42.4534	5.98	44.86	0.130	0.1364	8.27	5.20	9.67	15.86
75	59.14	3.55	42.3825	5.98	44.86	0.130	0.1366	8.27	5.16	9.59	15.72
76	58.53	3.51	42.3116	5.98	44.86	0.130	0.1368	8.27	5.11	9.50	15.58
77	57.93	3.48	42.2407	5.98	44.86	0.130	0.1370	8.27	5.06	9.42	15.44
78	57.32	3.44	42.1698	5.98	44.86	0.130	0.1372	8.27	5.02	9.33	15.30
79	56.72	3.40	42.0989	5.98	44.86	0.130	0.1374	8.27	4.97	9.25	15.16
80	56.11	3.37	42.028	5.98	44.86	0.130	0.1376	8.27	4.93	9.16	15.02
81	55.87	3.35	41.9571	5.98	44.86	0.130	0.1378	8.27	4.91	9.14	14.98
82	55.63	3.34	41.8862	5.98	44.86	0.130	0.1380	8.27	4.90	9.11	14.94
83	55.39	3.32	41.8153	5.98	44.86	0.130	0.1382	8.27	4.88	9.09	14.89
84	55.15	3.31	41.7444	5.98	44.86	0.130	0.1384	8.27	4.87	9.06	14.85
85	54.91	3.29	41.6735	5.98	44.86	0.130	0.1386	8.27	4.86	9.03	14.81
86	54.67	3.28	41.6026	5.98	44.86	0.130	0.1388	8.27	4.84	9.01	14.77
87	54.43	3.27	41.5317	5.98	44.86	0.130	0.1390	8.27	4.83	8.98	14.72
88	54.19	3.25	41.4608	5.98	44.86	0.130	0.1392	8.27	4.81	8.95	14.68
89	53.95	3.24	41.3899	5.98	44.86	0.130	0.1394	8.27	4.80	8.93	14.64
90	53.71	3.22	41.319	5.98	44.86	0.130	0.1396	8.27	4.79	8.90	14.59

91	52.86	3.17	41.2481	5.98	44.86	0.130	0.1398	8.27	4.72	8.77	14.38
92	52.01	3.12	41.1772	5.98	44.86	0.130	0.1400	8.27	4.65	8.65	14.17
93	51.16	3.07	41.1063	5.98	44.86	0.130	0.1402	8.27	4.58	8.52	13.96
94	50.3	3.02	41.0354	5.98	44.86	0.130	0.1404	8.27	4.51	8.39	13.75
95	49.45	2.97	40.9645	5.98	44.86	0.130	0.1406	8.27	4.44	8.26	13.53
96	48.6	2.92	40.8936	5.98	44.86	0.130	0.1408	8.27	4.37	8.13	13.32
97	47.75	2.87	40.8227	5.98	44.86	0.130	0.1410	8.27	4.30	8.00	13.11
98	46.89	2.81	40.7518	5.98	44.86	0.130	0.1413	8.27	4.23	7.86	12.89
99	46.04	2.76	40.6809	5.98	44.86	0.130	0.1415	8.27	4.16	7.73	12.68
100	45.19	2.71	40.61	5.98	44.86	0.130	0.1417	8.27	4.09	7.60	12.46

Abbreviations are: GFR Glomerular filtration rate, ad Adult, ger Geriatric, iv Intravenous, CHSA Albumin molar concentration, fu Unbound fraction, CL Clearance, r Renal, F Bioavailability

Table AII- 8. Absolute and bioavailability corrected individual Volume of distribution (Vss, Vc, Vp) parameters of Bilastine estimated in the virtual elderly male subjects using the semi-mechanistic approach. The physiological parameters used to scale the PK parameters are shown

Caucasian elderly *male*						correction by F 61%)	
Age	**TBW (L)**	**FAT (kg)**	**Vss iv (L)**	**Vc iv (L)**	**Vp iv (L)**	**Vc/F (L)**	**Vp/F (L)**
65	38.73	18.84	57.57	37.42	20.15	61.35	33.03
66	38.57	19.01	57.58	37.42	20.15	61.35	33.04
67	38.41	19.17	57.58	37.42	20.15	61.35	33.04
68	38.25	19.32	57.57	37.42	20.15	61.34	33.03
69	38.09	19.48	57.57	37.42	20.15	61.34	33.03
70	37.93	19.64	57.57	37.42	20.15	61.34	33.03
71	37.71	19.54	57.26	37.22	20.04	61.01	32.85
72	37.50	19.45	56.95	37.02	19.93	60.68	32.67
73	37.29	19.35	56.64	36.81	19.82	60.35	32.50
74	37.08	19.25	56.33	36.61	19.71	60.02	32.32
75	36.86	19.15	56.01	36.41	19.60	59.68	32.14
76	36.65	19.05	55.70	36.21	19.50	59.35	31.96
77	36.44	18.95	55.39	36.00	19.39	59.02	31.78
78	36.23	18.85	55.08	35.80	19.28	58.69	31.60
79	36.01	18.76	54.77	35.60	19.17	58.36	31.43
80	35.80	18.66	54.46	35.40	19.06	58.03	31.25
81	35.58	18.53	54.11	35.17	18.94	57.66	31.05
82	35.36	18.40	53.76	34.94	18.82	57.28	30.85
83	35.14	18.28	53.42	34.72	18.70	56.92	30.65
84	34.84	18.55	53.40	34.71	18.69	56.90	30.64
85	34.70	18.02	52.72	34.27	18.45	56.18	30.25
86	34.48	17.89	52.37	34.04	18.33	55.80	30.05
87	34.26	17.77	52.03	33.82	18.21	55.44	29.85
88	34.04	17.64	51.68	33.59	18.09	55.07	29.65
89	33.82	17.52	51.33	33.37	17.97	54.70	29.45
90	33.60	17.39	50.98	33.14	17.84	54.33	29.25
91	33.13	17.11	50.24	32.66	17.58	53.53	28.83

92	32.66	16.82	49.49	32.17	17.32	52.73	28.40
93	32.20	16.55	48.74	31.68	17.06	51.94	27.97
94	31.73	16.26	47.99	31.19	16.80	51.13	27.53
95	31.26	15.98	47.24	30.71	16.53	50.34	27.11
96	30.79	15.70	46.50	30.22	16.27	49.54	26.68
97	30.32	15.41	45.73	29.73	16.01	48.73	26.24
98	29.85	15.13	44.99	29.24	15.75	47.94	25.81
99	29.39	14.86	44.24	28.76	15.48	47.14	25.39
100	28.92	14.58	43.50	28.27	15.22	46.35	24.96

Abbreviations are: TBW Total body water, TBF Total body fat, iv Intravenous, V Volume of distribution, ss Steady state, c Central, p Peripheral, F Bioavailability

Table AII- 9. Absolute and bioavailability corrected individual Volume of distribution (Vss, Vc, Vp) parameters of Bilastine estimated in the virtual elderly female subjects using the semi-mechanistic approach. The physiological parameters used to scale the PK parameters are shown

Caucasian elderly female						***correction by F 61%)***	
Age	**TBW (L)**	**FAT (kg)**	**Vss iv (L)**	**Vc iv (L)**	**Vp iv (L)**	**Vc/F (L)**	**Vp/F (L)**
65	30.42	26.69	57.11	37.12	19.99	60.85	32.77
66	30.30	26.63	56.93	37.00	19.92	60.66	32.66
67	30.19	26.56	56.75	36.89	19.86	60.47	32.56
68	30.07	26.50	56.57	36.77	19.80	60.28	32.46
69	29.96	26.44	56.40	36.66	19.74	60.10	32.36
70	29.84	26.37	56.22	36.54	19.68	59.90	32.26
71	29.65	26.17	55.82	36.28	19.54	59.48	32.03
72	29.46	25.97	55.43	36.03	19.40	59.06	31.80
73	29.27	25.76	55.03	35.77	19.26	58.64	31.58
74	29.09	25.56	54.64	35.52	19.13	58.23	31.35
75	28.90	25.35	54.25	35.26	18.99	57.80	31.13
76	28.71	25.14	53.85	35.00	18.85	57.38	30.90
77	28.52	24.94	53.46	34.75	18.71	56.97	30.67
78	28.33	24.73	53.06	34.49	18.57	56.54	30.45
79	28.14	24.53	52.67	34.24	18.44	56.13	30.22
80	27.95	24.33	52.28	33.98	18.30	55.71	30.00
81	27.87	24.26	52.13	33.89	18.25	55.55	29.91
82	27.80	24.19	51.99	33.79	18.20	55.40	29.83
83	27.72	24.12	51.84	33.70	18.14	55.24	29.75
84	27.64	24.05	51.70	33.60	18.09	55.09	29.66
85	27.57	23.99	51.55	33.51	18.04	54.93	29.58
86	27.49	23.92	51.41	33.41	17.99	54.78	29.49
87	27.41	23.85	51.26	33.32	17.94	54.62	29.41
88	27.33	23.78	51.11	33.22	17.89	54.47	29.33
89	27.26	23.71	50.97	33.13	17.84	54.31	29.24
90	27.18	23.64	50.82	33.03	17.79	54.15	29.16
91	26.95	23.18	50.13	32.58	17.54	53.41	28.76

92	26.72	22.72	49.43	32.13	17.30	52.68	28.36
93	26.48	22.25	48.74	31.68	17.06	51.93	27.96
94	26.25	21.79	48.04	31.22	16.81	51.19	27.56
95	26.02	21.33	47.34	30.77	16.57	50.45	27.16
96	25.79	20.86	46.65	30.32	16.33	49.70	26.76
97	25.55	20.40	45.95	29.87	16.08	48.97	26.37
98	25.32	19.93	45.25	29.41	15.84	48.22	25.96
99	25.09	19.47	44.56	28.96	15.59	47.48	25.56
100	24.86	19.01	43.86	28.51	15.35	46.74	25.17

Abbreviations are: TBW Total body water, TBF Total body fat, iv Intravenous, V Volume of distribution, ss Steady state, c Central, p Peripheral, F Bioavailability

Table AII- 10. Absolute and bioavailability corrected individual Intercompartmental Clearance (Q) of Bilastine estimated in the virtual elderly female subjects using the semi-mechanistic approach. The physiological parameters used to scale the PK parameters are shown

Caucasian elderly *male*						**correction by F (61%)**
Age	**CO_{ger} (L/h)**	**CO_{ad} (L/h)**	**Q_{ad} iv (L/h)**	**CO/Qi_{v}**	**Q_{ger} iv (L/h)**	**Q_{ger} /F (L/h)**
65	302.14	352.11	1.01	348.62	0.87	1.42
66	299.96	352.11	1.01	348.62	0.86	1.41
67	297.79	352.11	1.01	348.62	0.85	1.40
68	295.60	352.11	1.01	348.62	0.85	1.39
69	293.42	352.11	1.01	348.62	0.84	1.38
70	291.25	352.11	1.01	348.62	0.84	1.37
71	288.94	352.11	1.01	348.62	0.83	1.36
72	286.63	352.11	1.01	348.62	0.82	1.35
73	284.32	352.11	1.01	348.62	0.82	1.34
74	282.01	352.11	1.01	348.62	0.81	1.33
75	279.71	352.11	1.01	348.62	0.80	1.32
76	277.40	352.11	1.01	348.62	0.80	1.30
77	275.09	352.11	1.01	348.62	0.79	1.29
78	272.78	352.11	1.01	348.62	0.78	1.28
79	270.46	352.11	1.01	348.62	0.78	1.27
80	268.15	352.11	1.01	348.62	0.77	1.26
81	265.81	352.11	1.01	348.62	0.76	1.25
82	263.46	352.11	1.01	348.62	0.76	1.24
83	261.14	352.11	1.01	348.62	0.75	1.23
84	257.92	352.11	1.01	348.62	0.74	1.21
85	256.45	352.11	1.01	348.62	0.74	1.21
86	254.10	352.11	1.01	348.62	0.73	1.19
87	251.77	352.11	1.01	348.62	0.72	1.18
88	249.42	352.11	1.01	348.62	0.72	1.17
89	247.06	352.11	1.01	348.62	0.71	1.16
90	244.71	352.11	1.01	348.62	0.70	1.15
91	240.77	352.11	1.01	348.62	0.69	1.13

92	236.83	352.11	1.01	348.62	0.68	1.11
93	232.88	352.11	1.01	348.62	0.67	1.10
94	228.91	352.11	1.01	348.62	0.66	1.08
95	224.94	352.11	1.01	348.62	0.65	1.06
96	220.97	352.11	1.01	348.62	0.63	1.04
97	216.99	352.11	1.01	348.62	0.62	1.02
98	213.00	352.11	1.01	348.62	0.61	1.00
99	209.01	352.11	1.01	348.62	0.60	0.98
100	205.02	352.11	1.01	348.62	0.59	0.96

Abbreviations are: CO Cardiac Output, ad Adult, ger Geriatric, iv Intravenous, Q Intercompartmental Clearance, F Bioavailability

Table AII- 11. Absolute and bioavailability corrected individual Intercompartmental Clearance (Q) of Bilastine estimated in the virtual elderly female subjects using the semi-mechanistic approach. The physiological parameters used to scale the PK parameters are shown

Caucasian elderly female						correction by F (61%)
Age	CO_{ger} (L/h)	CO_{ad} (L/h)	Q_{ad} IV (L/h)	CO/Qiv	Q_{ger} IV (L/h)	Q_{ger} /F (L/h)
65	273.64	318.19	1.01	315.04	0.87	1.42
66	271.13	318.19	1.01	315.04	0.86	1.41
67	268.62	318.19	1.01	315.04	0.85	1.40
68	266.10	318.19	1.01	315.04	0.84	1.38
69	263.61	318.19	1.01	315.04	0.84	1.37
70	261.10	318.19	1.01	315.04	0.83	1.36
71	258.03	318.19	1.01	315.04	0.82	1.34
72	254.97	318.19	1.01	315.04	0.81	1.33
73	251.90	318.19	1.01	315.04	0.80	1.31
74	248.83	318.19	1.01	315.04	0.79	1.29
75	245.75	318.19	1.01	315.04	0.78	1.28
76	242.66	318.19	1.01	315.04	0.77	1.26
77	239.59	318.19	1.01	315.04	0.76	1.25
78	236.50	318.19	1.01	315.04	0.75	1.23
79	233.42	318.19	1.01	315.04	0.74	1.21
80	230.32	318.19	1.01	315.04	0.73	1.20
81	228.13	318.19	1.01	315.04	0.72	1.19
82	225.93	318.19	1.01	315.04	0.72	1.18
83	223.74	318.19	1.01	315.04	0.71	1.16
84	221.54	318.19	1.01	315.04	0.70	1.15
85	219.35	318.19	1.01	315.04	0.70	1.14
86	217.15	318.19	1.01	315.04	0.69	1.13
87	214.95	318.19	1.01	315.04	0.68	1.12
88	212.75	318.19	1.01	315.04	0.68	1.11
89	210.56	318.19	1.01	315.04	0.67	1.10
90	208.37	318.19	1.01	315.04	0.66	1.08
91	204.99	318.19	1.01	315.04	0.65	1.07

92	201.59	318.19	1.01	315.04	0.64	1.05
93	198.20	318.19	1.01	315.04	0.63	1.03
94	194.76	318.19	1.01	315.04	0.62	1.01
95	191.33	318.19	1.01	315.04	0.61	1.00
96	187.89	318.19	1.01	315.04	0.60	0.98
97	184.43	318.19	1.01	315.04	0.59	0.96
98	180.94	318.19	1.01	315.04	0.57	0.94
99	177.47	318.19	1.01	315.04	0.56	0.92
100	173.96	318.19	1.01	315.04	0.55	0.91

Abbreviations are: CO Cardiac Output, ad Adult, ger Geriatric, iv Intravenous, Q Intercompartmental Clearance, F Bioavailability

- **Extension of the metadatabase according to race/ethnicity (complementary to section 3)**

Table AII- 12. Summary of equation and/or references used to derive the physiological or PK scaled parameters

Parameter	Equation and/or Reference	Parameter related
Height (cm)	Available from study 2808/RI	**BSA (m^2)** **TBW (L)** **TBF (Kg)**
Weight (Kg)	Available from study 2808/RI	**BSA (m^2)** **TBW (L)** **TBF (Kg)**
TBW (L)	Extracted from Chumlea et al. 2001	**Vss, Vc and Vp-iv (L)**
TBF (Kg)	$TBF\ (kg) = (1.39\ x\ BMI)\ +\ (0.16\ x\ Age) - (10.34\ x\ gender) - 9$ $male = 1, female = 0$ Jackson et al., 2002	**Vss, Vc and Vp-iv (L)**
CO_{ger} (L/h)	$CO = HR \times SV$ HR: Available from study 2808/RI SV: Estimated as 81.8 mL for old subjects aged 65+ independently of the renal impairment Bruss et al., 2019	**Q iv (L/h)**
CO_{ad} (L/h)	Taken from Light et al., 1993	**Q iv (L/h)**
GFR_{ger} (mL/min)	Available from study 2808/RI	**CLr iv (L/h)**
GFR_{ad} (mL/min)	Obtained from values described in software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH - Leverkusen, Germany) of Schlender et al. 2016 according to race/sex. Adult of reference were considered aged 30-50 years	**CLr iv (L/h)**
Abbreviations are: V Volume of distribution, ss Steady state, c Central, p Peripheral, F Bioavailability, CL Clearance, r Renal, ad Adult, GFR Glomerular filtration rate, TBW Total body water, TBF Total body fat, BSA Body surface area, Cp Plasma Concentration, CO Cardiac Output, Q Intercompartmental Clearance		

Table AII- 13. RI Senescence model scaled equations used in the extrapolation of bilastine PK parameters to elderly of study 2808/RI

PK Parameter	Equation to scale absolute PK in elderly
fu	Individual fraction unbound (fu) data were available from an "in vitro" study of plasma protein binding of the drug (study code: FF-0019) with plasma from renal impaired patients of study 2808/RI
CLr iv (L/h)	$\mathrm{CLr_{ger}\ iv} = \frac{\mathrm{GFR_{ger}\ x\ fu_{ger}}}{\mathrm{GFR_{ad}\ x\ fu_{ad}}}\ \mathrm{x\ CLr_{ad}\ iv}$ $\mathrm{Clr_{ad}}$ iv 8.17 L/h $\mathrm{ratio}\ \frac{\mathrm{CLiv}}{\mathrm{CLr\ iv}}$
Q iv (L/h)	$\mathrm{ratio}\ \frac{\mathrm{CO_{ad}\ male/female}}{\mathrm{Q_{ad}\ iv}} = 285.15$ Qiv ad 1.01
Vss iv, Vc iv and Vp iv (L)	$\mathrm{Vss} \cong \mathrm{TBW} + \mathrm{TBF}$
	$\mathrm{V_c} = \ 0{,}65\ \mathrm{x}\ Vss\ \mathrm{iv}$
	$\mathrm{V_p} = Vss\ \mathrm{iv} - \mathrm{V_c\ iv}$
Ka (h^{-1})	The ka was estimated with the Renal Geriatric PopPK model

Abbreviations are: V Volume of distribution, ss Steady state, c Central, p Peripheral, CL Clearance, r Renal, ad Adult, ger Geriatric, iv intravenous, fu Unbound fraction, GFR Glomerular filtration rate, CHSA Albumin molar concentration, TBW Total body water, TBF Total body fat, BSA Body surface area, CO Cardiac Output, Q Intercompartmental Clearance, k_a Absorption rate constant

Table AII- 14. Glomerular filtration rate (GFR)

Reference adult values for GFR and BSA (calculated according to DuBois and DuBois) were extracted from software database PKSIM (component of the Computational Systems Biology Software Suite of Bayer Technology Services GmbH -Leverkusen, Germany) of Schlender et al. 2016 according to the race/ethnicity, sex and age. Considering that the available GFR values of subjects of study 2808/RI were expressed in mL/ min/1.73 m^2 the reference adult values were converted to BSA-indexed GFR as follow: Indexing GFR mL/min/1.73 m^2= (GFR (ml/min) *1.73 m^2)/Patient BSA

	White male			White female		
Age	**GFR (mL/min)**	**BSA (m^2)**	**Indexing GFR for 1.73 m^2**	**GFR (mL/min)**	**BSA (m^2)**	**Indexing GFR for 1.73 m^2**
30	116.31	2	100.61	112.80	1.75	111.51
40	126.51	2.03	107.81	112.35	1.78	109.19
50	124.43	2.02	106.57	112.07	1.82	106.53
Abbreviations are: GFR Glomerular filtration rate, BSA Body surface area						

	Black male			Black female		
Age	**GFR (mL/min)**	**BSA (m^2)**	**Indexing GFR for 1.73 m^2**	**GFR (mL/min)**	**BSA (m^2)**	**Indexing GFR for 1.73 m^2**
30	112.68	2	97.47	105.07	1.83	99.33
40	122.54	2.03	104.43	104.64	1.89	95.78
50	120.56	2.03	102.74	104.33	1.9	95.00
Abbreviations are: GFR Glomerular filtration rate, BSA Body surface area						

Table AII- 15. Reference Adult body composition parameter

Parameter	Black female	Black male	White female	White male
Age (Years)	30-50			
GFR (L/h/1.73m^2)	5.72	6.22	6.54	6.30
CO (L/h)	288			
fu *(Jauregizar et al., 2009)*	0.13			
Abbreviations are: GFR Glomerular filtration rate, CO Cardiac Output, fu Unbound fraction				

Table AII- 16. Total Body Water (TBW)

Age	Black male TBW (L)	Black female TBW (L)	White male TBW (L)	White female TBW (L)
60-69	46.4	34.1	44.8	31.4
70-79	44.2	32.9	44.1	30.9
80-89	na	na	42.5	30.2
Abbreviations are: TBW Total body water, na not applicable				

Table AII- 17. Total Body Fat (TBF)

ID	Age	Sex	Race	Height (cm)	Weight (kg)	BMI (kg/m^2)	TBF (kg)
Group 1							
40	67	male	white	167.6	71.4	25.4	26.69
43	66	female	white	160.0	73.9	28.9	41.73
45	65	male	black	165.1	71.0	26.0	27.20
47	67	male	white	163.0	78.6	29.6	32.52
51	62	male	white	170.0	71.9	24.9	25.19
52	67	male	white	168.0	78.6	27.8	30.02
Group 2							
2	79	female	white	162.6	85.8	32.5	48.82
3	71	male	white	171.0	56.8	19.4	18.99
4	72	male	white	180.0	67.8	20.9	21.23
6	62	male	white	167.6	74.8	26.6	27.55
9	72	male	white	157.5	65.4	26.4	28.88
10	71	male	white	157.5	68.2	27.5	30.25
Group 3							
13	69	male	black	182.9	98.4	29.4	32.57
17	70	male	white	167.6	92.1	32.8	37.45
23	80	female	black	161.0	69.5	26.8	41.05
33	73	male	white	162.6	67.1	25.4	27.65
36	68	male	white	167.6	72.5	25.8	27.40
46	68	male	black	180.3	76.0	23.4	24.07
Group 4							
5	77	male	white	159.0	59.8	23.7	25.92
21	61	male	white	170.2	80.5	27.8	29.06
22	71	male	white	165.0	83.7	30.7	34.69
24	77	female	white	152.0	55.8	24.2	36.96
26	66	female	black	154.9	57.5	24.0	34.92

- **Total body fat (TBF) was estimated using the equations described by Jackson et al. in the 2002 Heritage medical study.**
- **Body Mass Index (BMI) was calculated as weight(kg)/ height (m^2).**
- **Height, weight, sex and age were available from study BILA-2808/RI.**

Table AII- 18. Cardiac Output (CO)

ID	HR (b*min)	SV Mean (mL)	CO (mL/min)	CO (L/h)
Group 1				
40	52.25	81.80	4274.05	256.44
43	74.25	81.80	6073.65	364.42
45	67.50	81.80	5521.50	331.29
47	53.75	81.80	4396.75	263.81
51	50.00	81.80	4090.00	245.40
52	62.60	81.80	5120.68	307.24
Group 2				
2	64.25	81.80	5255.65	315.34
3	56.00	81.80	4580.80	274.85
4	59.75	81.80	4887.55	293.25
6	55.50	81.80	4539.90	272.39
9	72.00	81.80	5889.60	353.38
10	57.00	81.80	4662.60	279.76
Group 3				
13	72.50	81.80	5930.50	355.83
17	64.75	81.80	5296.55	317.79
23	61.25	81.80	5010.25	300.62
33	72.00	81.80	5889.60	353.38
36	59.50	81.80	4867.10	292.03
46	72.75	81.80	5950.95	357.06
Group 4				
5	52.75	81.80	4314.95	258.90
21	50.75	81.80	4151.35	249.08
22	61.75	81.80	5051.15	303.07
24	67.67	81.80	5535.13	332.11
26	71.00	81.80	5807.80	348.47

- **Heart rate (HR) were available from study BILA 2808/RI and calculate as (b*min= beats per minute)**
- **Stroke volume (SV) were estimated as 81.8 mL (Giles N. Cattermole et al., 2017)**
- **CO= HR x SV**

Annex III

Annex I. Methodology of non-linear mixed-effect analysis (complementary to methodology Section I, II and III).

1.1. Population pharmacokinetic analysis

Population modelling represent a tool to identify and describe relationship between a subject's physiologic characteristics and observed drug exposure or response.

Population models are comprised of several components: structural models, stochastic models, and covariate models. Structural models are functions that describe the time course of a measured response, and can be represented as algebraic or differential equations. Stochastic models describe the variability or a random effect in the observed data, and covariate models describe the influence of factors such as demographics or disease on the individual time course of the response.

PK/PD population models will be performed within the Nonlinear Mixed Effects Modelling software package NONMEM that allow to estimate within subject and between subjects variability when fitting a pharmacokinetic and/or pharmacodynamic (PK/PD) model to data (Upton, 2012, 2013).

The PK model for the observed concentration for the *i*th subject at the *j*th time point (Cij) can be expressed as follows.

$$C_{ij} = f(\theta_i, t_{ij}) + \varepsilon_{ij} \qquad \varepsilon = N(0, \sigma^2) \qquad \textbf{Eq. AIII-1}$$

Where f is a function representing the PK model (e.g., one or bi-compartmental model) compartment model), θi represents the model parameters and ε*ij* accounts for the model residual error obtained from the deviations between observed and predicted plasma concentration *vs.* time data, which is assumed to arise from a normal distribution with zero mean and variance σ^2.

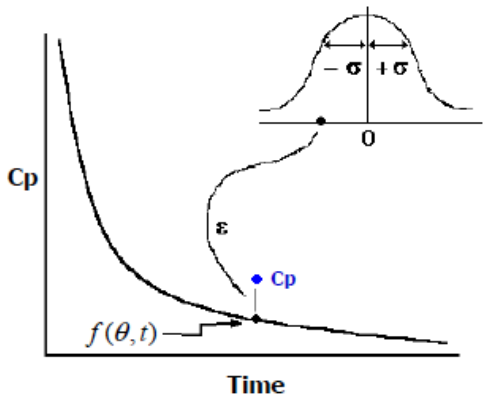


Figure AIII- 1. In modelling residual variability, differences between the measured and predicted concentrations for all the individuals within a population are defined by ε. ε is assumed to arise from a symmetric distribution with a mean of zero and a variance of σ 2) (Adapted from Grasela & Sheiner 1991)

The **intraindividual error** (expressed as ε-σ^2) also called "residual error" or "within-subject" variability includes the error in the assay, errors in drug dose, errors in the time measurement, etc

The **interindividual error** (expressed as η -ω^2) also called "between-subject" variability refers to the natural variability existing between the different subjects within a population, due to pathologies, lifestyle, etc. μ is assumed to arise from a normal distribution with zero mean and variance ω^2. PK parameters exhibit interindividual variability, which can be modelled as follows:

$$\theta = \overline{\theta}_i + \eta_i \qquad \eta = N(0, \omega^2) \qquad \textbf{Eq. AIII-2}$$

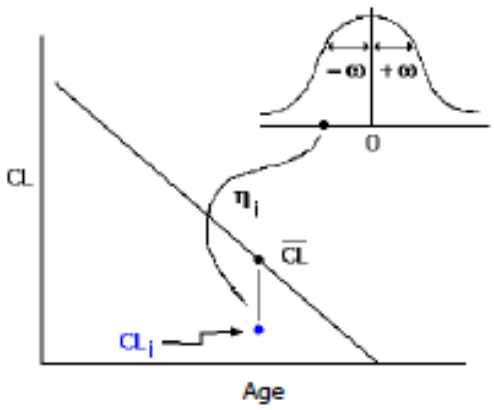


Figure AIII- 2. η is the difference between the individual PK parameter (θi) and the typical parameter value in the population ($\overline{\theta}$). For each individual in the population, an η arises from a symmetric distribution with a mean of zero and variance of ω^2. This η interacts with the typical value to generate the true value and the nature of this interaction must be included in the pharmacostatistical model (Adapted from Grasela et al., 1991)

Furthermore, by means of parametric population methods (i.e., those assuming a normal or log-normal distribution for the PK parameters), such as those implemented in the software package NONMEM®, it is also possible to calculate the standard error of estimate (SEE) associated to each PK parameter, thus giving an idea of the precision and validity of the model.

As a summary, the following three types of parameters are to be defined in the context of a population analysis:

- The structural model as defined by the typical population PK parameters (θ: CL, Vd, etc.), also known as fixed effect parameters.
- The statistical model, which accounts for the random effect parameters, including:
  - Normal distribution for residual error of the model (ε), whose standard deviation is given by σ. Therefore, σ provides information about the typical magnitude of residual

variability consisting of combined intraindividual and measurement error variability (non-controlled error due to analytical technique or experimental design).

- Normal distribution for residual error of each PK or PD parameter (η), whose standard deviation is given by ω, thus providing estimate of the typical magnitude of interindividual variability in the parameter across the members of a population.

- The SEE for all the structural and statistical parameters

1.1.1. Selection of the structural model

First step in the population PK/PD analysis is the selection of the structural model best fitting the observations, since an incorrect choice entails a significant increase of residual error.

Structural model for bilastine

A two-compartmental kinetics with 1st order absorption and elimination (FOCE method) best described the kinetics of bilastine after oral administration. NONMEM_ subroutine ADVAN8 TRANS1was used.

a. Assessing the goodness of fit

- ***Analyzing the residuals***

The concept of a residual, which corresponds to the vertical difference between an observed concentration and the concentration predicted by the model, is straightforward and fundamental to modelling. If the residual is positive, it means that the observed point is above the predicted curve, whereas a negative residual indicates that the point is below the curve. Examining residuals is a key part of all statistical modelling, and carefully looking at them can give a sign whether assumptions are reasonable and the choice of model is appropriate. For a model to be considered as adequate to describe the data, residuals should ideally be as low as possible and be uniformly and randomly distributed about the zero line when scatter plotted against the values of an independent variable (a fixed effect). If they do not, this can suggest that the effect of the variable is not appropriately modelled, and the pattern of the scatter may suggest a more appropriate model (Gabrielsson et al., 2006).

In order to check randomness of residuals, one thing to look for is whether or not there are any runs (i.e., sequences of residuals having the same sign) in the residuals. Although there are statistical methods for testing for significance of the number of runs, these methods generally require large number of observations. For smaller datasets such as data commonly obtained in kinetic and dynamic studies, the visual judgement of the residuals in the program output as well as well as of their graphical

representation versus the independent variable (e.g., time), the observed dependent variable (e.g., observed concentration) or the calculated dependent variable is considered enough (Upton, 2012).

- ***Graphical presentation of relative residuals (rel.res) against predictions***

The relative residual is obtained by dividing the absolute residual by its corresponding predicted concentration.

$$rel.res = \frac{observed - predicted}{predicted} \qquad \textbf{Eq. AIII-3}$$

The advantage of using the relative residual rather than the absolute residual is that one observes more easily where one has the largest contributions to the residuals (deviations) on a relative scale.

For the model to be accepted, these graphs must show deviations of similar magnitude about zero. A relative residual that displays a non-random pattern indicates problems with the structural model (kinetic or dynamic) or with the error model (variance). To improve the adjustment in the latter case, a weighting scheme can be used that allows overcoming the issue that the magnitude of the residual depends on the magnitude of the data, by giving a different emphasis along the concentration-time data (e.g., more emphasis to the smaller concentrations if the model tends to overestimate the larger concentrations while underestimating the smaller concentrations).

b. **Discrimination between models**

- ***Akaike information criterion (AIC) and Schwarch criterion (SC)***

Complex models with more parameters are generally better able to describe a given data set (there are more "degrees of freedom", allowing the model to take different shapes). When comparing several plausible models, it is necessary to compensate for improvements of fit due to increased model complexity. The Akaike information criterion (AIC) and Schwarz criterion (SC, also known as Bayesian information criterion) are useful for comparing structural models.

AIC and CS attempt to quantify the information content of a given set of parameter estimates by relating the residual values to the number of parameters that were required to obtain the fit.

$$AIC = N_{obs} \times Ln(WRSS) + 2 \times N_{obs} \qquad \textbf{Eq. AIII-4}$$

$$SC = N_{obs} \times Ln(WRSS) + N_{par} \times Ln(N_{obs}) \qquad \textbf{Eq. AIII-5}$$

where N_{obs} is the number of experimental data observations, N_{par} is the total number of parameters in the model and WRSS corresponds to the residual values.

The most appropriate model is the one with the smallest value of AIC and SC, even if comparisons of AIC or SC cannot be given a statistical interpretation. Some authors categorized differences in SC

between models of >10 as "very strong" evidence in favour of the model with the lower SC, 6–10 as "strong" evidence, 2–6 as "positive" evidence and 0–2 as "weak" evidence. In practice, a drop in AIC or SC of 2 is often a threshold for considering one model over another (Upton, 2013).

These criteria are useful for selecting from various models based on goodness of fit, but they do not provide information whether the selected model adequately describes the experimental data. While the model associated with the smallest value of AIC or SC may give the best fit of compared models, it still may not give an adequate fit (as determined by residual analysis and other means). The AIC and SC do not require nested models (where one is a subset of another as has different numbers of parameters) but it is necessary to apply equal weighting of data.

- ***F-test***

When comparing hierarchical or nested models (e.g., covariate models to base models), the probability that additional parameters are without effect on the sum of squares (i.e., that they provide the same description of the data) is defined by an *F* distribution. A system of hierarchical or nested models (also called full and reduced models) is used. If the reduced model is identical to the full model when one or more parameters are fixed to a specific value (usually zero), there is an *F* distribution. For instance, the mono-exponential model is the reduced form (B set to 0) of the biexponential model.

$$\boldsymbol{C = A \times e^{-\alpha t} + B \times e^{-\beta t}} \qquad \textbf{Eq. AIII-6}$$

$$\boldsymbol{C = A \times e^{-\alpha t}} \qquad \textbf{Eq. AIII-7}$$

The F statistic is calculated according to Eq. AIII-8 below

$$\boldsymbol{C = \frac{\frac{|WRSS_1 - WRSS_2|}{|df_1 - df_2|}}{\frac{WRSS_2}{df_2}}} \qquad \textbf{Eq. AIII-8}$$

where $WRSS_1$ and $WRSS_2$ are the objective functions of the reduced and full models, respectively, and df_1 and df_2 are their corresponding degrees of freedom. Degrees of freedom are calculated as:

$$\boldsymbol{df = N_{obs} - N_{par}} \qquad \textbf{Eq. AIII-9}$$

where N_{obs} is the number of experimental data and N_{par} is the number of parameters to be estimated by the model. If *F* obtained is larger than the tabulated *F* value (p=0.05) it means that the full model is statistically superior to the reduced model.

1.1.2. Selection of the intraindividual error model in NONMEM®

The predicted value for y_i (blood concentrations) is obtained according to a structural model f (e.g., bicompartmental), which includes the vectors x_i (time, dose, etc.) and θ, accounting for the PK parameters (clearance, volume of distribution, etc.), as well as the addition of a residual value εi.

$$yi = f(\theta, x_i) + \varepsilon_I \qquad \textbf{Eq. AIII-10}$$

There exist different models describing the residual or intraindividual variability:

- **Additive model (homoscedastic error model)**

$$yi = f + \varepsilon_i \qquad \textbf{Eq. AIII-11}$$

This model assumes that the value for the residual error is independent of the corresponding plasma concentration; what it is to say, that variance is constant across the whole observation range (Figure AIII- 3a).

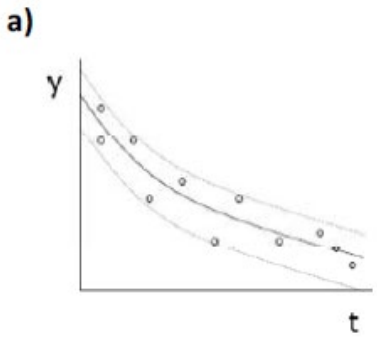


Figure AIII- 3. a-Additive or homoscedastic error

- **Proportional model (Heteroscedastic error model)**

$$yi = f(1+\varepsilon_i) = f + f\varepsilon_I \qquad \textbf{Eq. AIII-12}$$

According to this model, the value for the residual error is dependent on blood concentration. Therefore, variance does not keep constant across the whole observation range, even if the coefficient of variation does (Figure AIII- 3b).

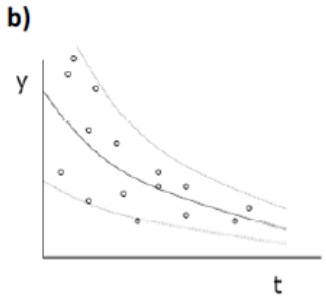


Figure AIII-3. b-Proportional of heteroscedastic error

- **Combined model**

$$yi = f\,(1 + \varepsilon_i) + f\varepsilon_1 \qquad \textbf{Eq. AIII-13}$$

There are some situations in which most observations can be explained through a constant coefficient of variation model, but some of them are close to the lowest limit of quantification of the analytical technique and would thus be better described by means of an additive error model. In these cases, it may be useful to utilize a combined model that includes both additive and proportional error models (Figure AIII- 3c).

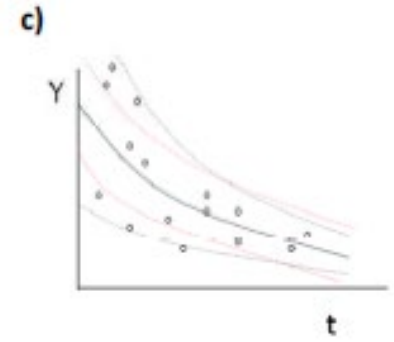


Figure AIII- 3. c-Combined error

- **Exponential model**

$$yi = f\exp(\varepsilon_i) \qquad \textbf{Eq. AIII-14}$$

A different residual error model which may be also used is the exponential model, as displayed (Figure AIII- 3d):

In principle, the selection of the most suitable intraindividual error model will be based on the careful examination of the plots displaying residuals vs. time and residuals vs. predictions. In most PK

studies, residual variability is best described by the proportional error model, as the residual value in this case depends on the magnitude of the measurement.

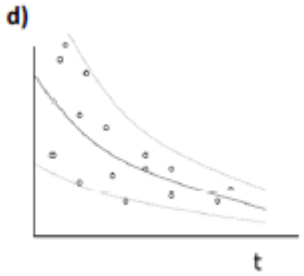


Figure AIII- 3. d-Exponential model

1.1.3. Selection of the interindividual error model in NONMEM®

When considering the variability associated to the observed PK parameters, the typical population value for a given parameter may not match the one observed in a particular individual. This variability between the subjects within a population can be expressed as follows:

$$\theta = \overline{\theta} + \eta_i \qquad \eta = N(0, \omega^2) \qquad \textbf{Eq. AIII-15}$$

Where η is assumed to arise from a normal distribution with zero mean and variance ω^2.

- **Additive model**

According to this model, η is added to the typical population value and its variance remains constant:

$$\theta i = \overline{\theta} + \eta_i \qquad \textbf{Eq. AIII-16}$$

- **Proportional model**

The variance for the individual PK parameter increases with increasing population parameter value.

$$\theta i = \overline{\theta} * (1 + \eta_i) = \theta i + \overline{\theta}\eta_i \qquad \textbf{Eq. AIII-17}$$

- **Exponential model**

Interindividual variability is mostly best described by the exponential error model, which is defined by the following equation:

$$\theta i = \overline{\theta} * \exp^{\eta i} \qquad \textbf{Eq. AIII-18}$$

1.1.4. Nonlinear Regression Minimization Methods

Nonlinear regression is a method of finding a nonlinear model of the relationship between the dependent variable and a set of independent variables. Unlike traditional linear regression, which is restricted to estimating linear models, nonlinear regression can estimate models with arbitrary relationships between independent and dependent variables. and using iterative estimation algorithms. Nonlinear regression programs decrease the value of an objective function by means of an iterative process, until reaching the minimum value. In each iteration, the values of each parameter change, and it is assumed that the estimated values for the last parameter iteration, that is, at the time when the differences between observations and predictions are the minimum possible, are those of the best fit. The differences for each parameter between one iteration and another decrease as the algorithm reaches or reaches the surface minimum sum of squares

a) Ordinary Least Squares (OLS)

Ordinary Least Squares (OLS) or linear least squares, estimates the parameters in regression model by minimizing the sum of the squared residuals. It assumes a condition of homoscedasticity.

$$\boldsymbol{OF_{OLS} = \sum_{j=1}^{n}\left(obs_j - pred_j\right)^2} \qquad \textbf{Eq. AIII-19}$$

b) Weighted Least Squares (WLS)

The Weighted Least Squares (WLS) also known as weighted linear regression, is a generalization of ordinary least squares.

$$OF_{WLS} = \sum_i \left[\frac{\left(obs_j - obs_j\right)^2}{W_1}\right] \qquad \textbf{Eq. AIII-20}$$

c) Extended Least Squares (ELS)

$$OF_{ELS} = \sum_i \left[\frac{\left(\boldsymbol{obs_j - obs_j}\right)^2}{var_1}\right] + \ln var_i \qquad \textbf{Eq. AIII-21}$$

1.1.5. Discrimination between rival models in NONMEM®

a. Estimation methods

NONMEM® is a non-linear regression mixed-effect modelling software where the parameter estimates are provided on a parametric way, according to the maximum likelihood approach within the minimization method known as extended least squares (ELS).

NONMEM® uses various estimation methods for the population parameters, the most common of which are briefly described as follows (Upton, 2013).

- First-order estimation method (FO): all η take the value of zero during the estimation process.
- First-order conditional estimation method (FOCE): η are included in the estimation process
- Laplace conditional estimation method

FO is the least robust method and may occasionally lead to biased population parameter estimates (either structural or statistical). Conditional methods are more robust but, due to their complexity, they take a longer time for convergence of all the parameters. Unlike FO method, conditional methods provide not only the population parameters but also the individual (Bayesian) parameters for every subject within the studied population in a single step, by resolving the following Bayesian equation:

$$BAYES\ OBJ = \sum_i^n \left(\frac{Cobs_i - f_i}{\sigma^2}\right)^2 + \sum_k^m \left(\frac{\bar{\theta}_j - \theta_j}{\omega_j{}^2}\right)^2 \qquad \textbf{Eq. AIII-22}$$

where $Cobs_i$ are the observed concentrations, f_i are the concentrations predicted based on the structural model, $\overline{\theta}$ is the mean population parameter value, θ_j is the parameter value calculated for each subject and σ^2 and ω^2 represent the variance for the intra- and interindividual error, respectively. Once the values for *Cobs*, $\overline{\theta}$, σ and ω are know, it is possible to calculate the individual parameters. Both FOCE and Laplacian methods resolve the Bayesian equation in each iterative step, while individual parameter values are only obtained in the FO method if using the POSTHOC estimation, which calculates them in the last iteration, thus being less reliable (Ette EI, 2007).

Bayesian estimation allows having information about each population component even if individual data points are not available in sufficient amount.

b. Normal distribution of parameters

According to the assumptions made regarding the parameter distribution within the population, methods of analysis may be classified from a statistical perspective into parametric and non-parametric.

NONMEM® employs a parametric method and as such is based on the maximum likelihood theory assuming a given distribution, i.e., generally normal or log-normal, for the PK parameters. Therefore, as part of an adequate statistical treatment, it is necessary to verify the validity of the assumptions made by means of standard techniques (histograms, normality tests, etc.). The normal and log-normal distributions are depicted in Figure AIII- 4 below.

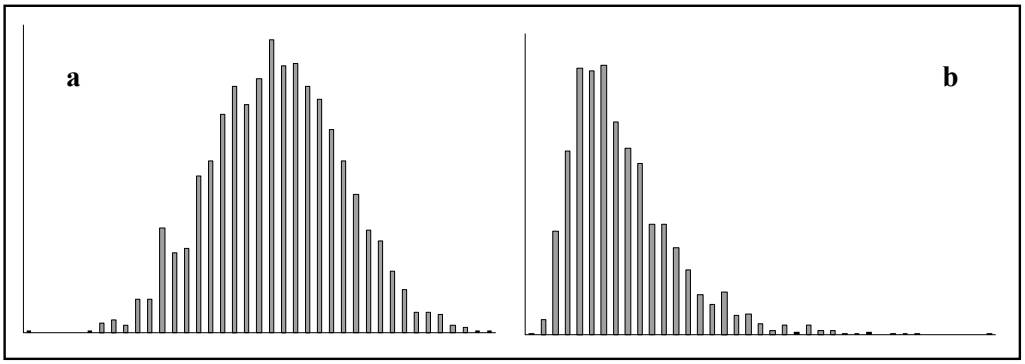


Figure AIII- 4. a) Normal distribution; b) Log-normal distribution

c. Model stability

The model selected for describing the observations should be stable and robust, so that little modifications in the input data and/or initial estimates of structural or statistical parameters do not have a substantial impact in the final estimation.

Sometimes, if the analysis is not sufficiently robust (biased observations, use of first order estimation method FO, etc.) a little change in the population model specification or in the observations (removal or addition of new observational data) would lead to convergence of the minimization process in a local minimum thus leading to erroneous parameter values.

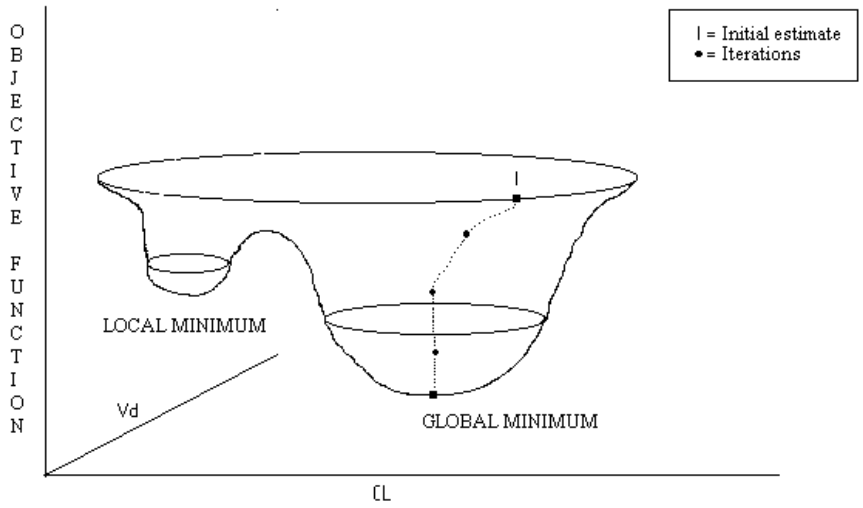


Figure AIII- 5. Multidimensional space of objective function value (OFV) and PK parameters characterizing a monocompartmental model (Vd, CL). The minimization process looks for the minimum OFV. Global minimum is the lowest value that the mentioned objective function can adopt, so that convergence in such global minimum provides the real parameter values defining the observations. May the minimization process end up in a local minimum, the resulting parameters would be misleading

d. Physiological interpretation

The parameters estimated by the software should be reasonable, which means that both the value and its sign should make sense from a physiological perspective and/or be similar to those previously published in scientific literature or obtained in previous experiments concerning the drug. Moreover, between-subject variability, which is expressed either as coefficient of variation (corresponding to the square root of the variance ω^2 multiplied by 100) for proportional and exponential error models or as standard deviation (corresponding to the square root of the variance ω^2) for the additive error model, should not exceed 100%.

e. Objective function value (OFV)

The minimum OFV determined via parameter estimation (OBJ) is important for comparing and ranking models. OBJ is calculated according to equation below.

$$\boldsymbol{OBJ = N_{obs} \times Ln(WRSS)} \qquad \textbf{Eq. AIII-23}$$

where N_{obs} is the number of experimental data observations and WRSS is the sum of squared weighted residuals. When it comes to choose between two models, the one showing a lower OBJ should be generally selected, as long as the remaining discrimination criteria are equivalent. Nonetheless, the OBJ depends on the data set and estimation method used, and consequently the OBJ and its derivations cannot be compared across data sets or estimation methods.

Generally, models with too few compartments describe the data poorly (higher OBJ), showing bias in plots of residuals vs. time, whereas models with too many compartments show trivial improvement in OBJ for the addition of extra compartments (Upton, 2013).

f. Standard error of estimate (SEE) and corresponding estimate precision

Obtaining the standard error for each parameter, either structural or statistical, is of vital relevance as it gives an idea of the statistical significance of the estimates. Any model unable to provide SEE or providing invalid SEE values should be rejected in principle.

Similarly, estimated model parameters have little or no value unless they have a fair degree of precision. This can be assessed by computing each parameter's coefficient of variation (CV%), i.e., the parameter's standard error (SEE) divided by the corresponding parameter estimate.

$$\boldsymbol{CV\% = \frac{SEE}{Par} \times 100} \qquad \textbf{Eq. AIII-24}$$

where Par is the parameter estimate and SEE is the corresponding standard error of estimate.

Precision can also be expressed as confidence intervals (CI), according to equation below.

$$\boldsymbol{CI = Par \pm 1.96 \times SEE} \qquad \textbf{Eq. AIII-25}$$

where generated confidence interval must not include the zero value. CIs that include the null value may imply the estimate is unreliable.

Precise parameter estimates are important (models with poor parameter precision are often overparameterized), but the level of precision that is acceptable depends on the size of the database. For most pharmacokinetic databases, <30% CV for fixed effects and <50% CV for random effects are usually achievable (SEE for random effects are generally higher than for fixed effects) (Upton,

2013). Importantly a large parameter CV does not necessarily imply that the model is incorrect. It may be due to not having collected enough samples or not having collected samples at the appropriate times (Gabrielsson, 2006).

g. Diagnostic plots

Visual examination of key diagnostic plots

- *Graphical evaluation of observed vs. predicted concentrations*

A fundamental plot is a plot of observed, population-predicted and individual- predicted concentrations against time, as shown in Figure AIII- 6 below. These plots should be structured using combinations logscales, faceting, and/or conditioning on explanatory variables to be as informative as possible. Individual-predicted concentrations should provide an acceptable representation of the observed data, whereas the population-predicted concentrations should represent the "typical" patient (reflecting the center of pooled observed data).

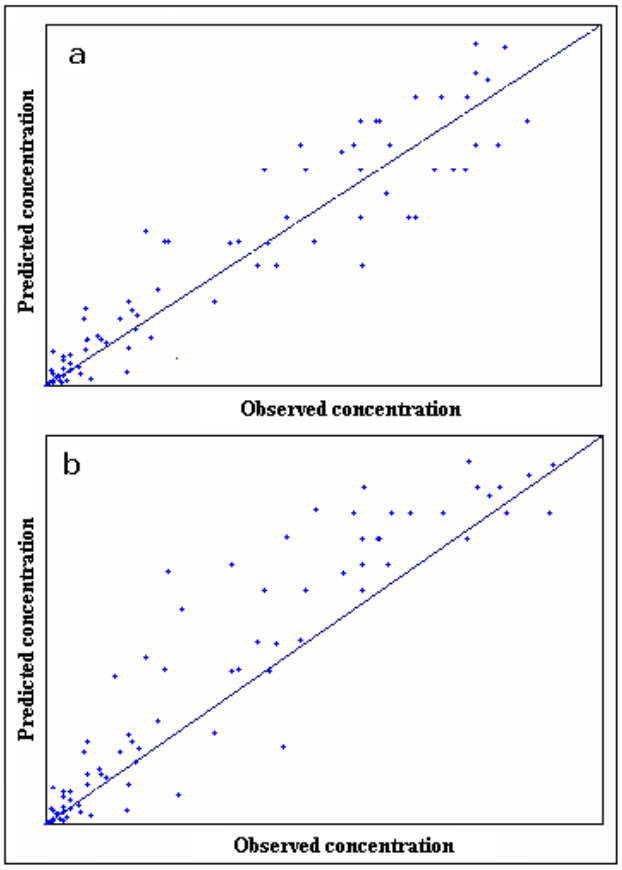


Figure AIII- 6. Relationship between the observed blood concentration and that predicted by the population model. a) Data are evenly distributed about the line of identity, indicating the appropriateness of the structural model selected; b) A large proportion of the observed concentrations are incorrectly distributed around the population model, indicating major bias

- *Weighted residuals (WRES) vs. time*

Plots of WRES against time should be evenly cantered around zero, without systematic bias, and most values within −2 to +2 SDs (marking the ~5th and 95th percentiles of a normal distribution) (Figure AIII- 7). Systematic deviations may imply deficiencies (e.g., over- or underestimation patterns) in the structural model.

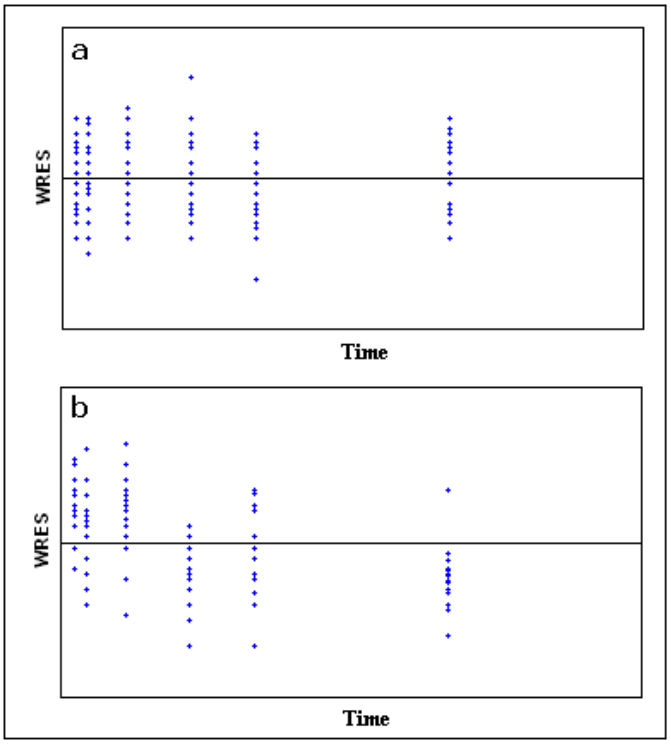


Figure AIII- 7 a) Suitable distribution of the weighted residuals (WRES) against time. Even distribution of data about zero indicates no major bias in the structural model; b) In the latest measurements, WRES are not randomly distributed, which is indicating that most concentrations at the latest timepoints are overpredicted by the selected model

- *Weighted residuals (WRES) vs. predicted concentration*

Plots of WRES against population-predicted concentration should be evenly cantered around zero, without systematic bias, with most values within −2 to +2 SDs. Systematic deviations may imply deficiencies in the residual unexplained variability model. In Figure AIII- 8 below, WRES are randomly distributed across the whole range of predicted concentrations only in part a, whereas the cone-shaped behavior in part b suggests a misspecification in the variance model and the U-shaped disposition in part b underscores an incorrect selection of the structural PK model.

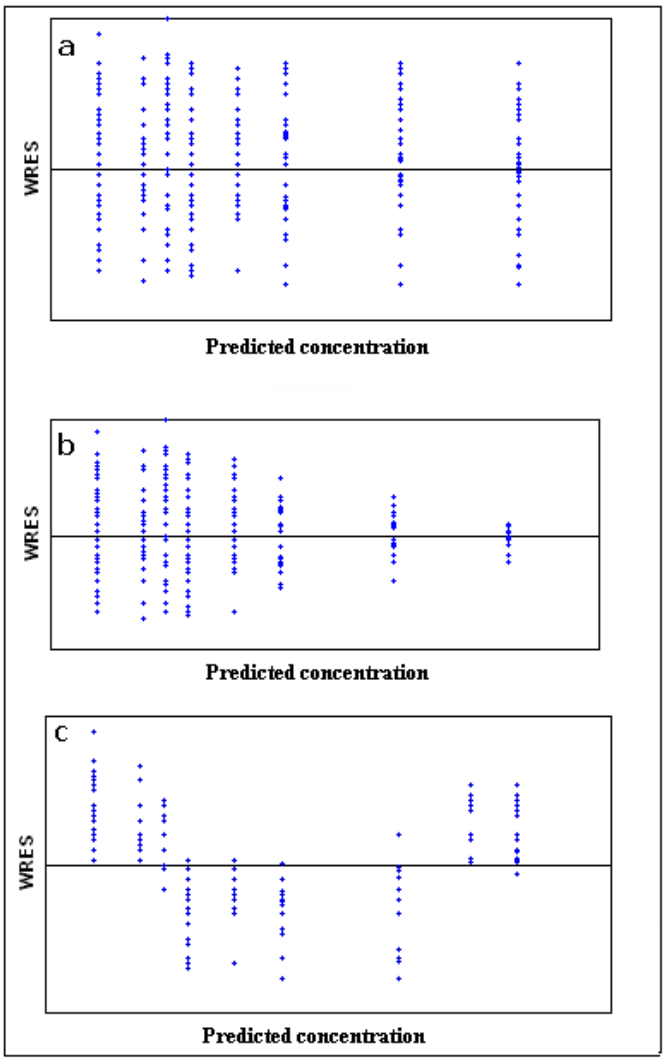


Figure AIII- 8. Weighted residuals (WRES) against the concentrations predicted by the population model. Only in a) data are evenly distributed about zero, indicating no major bias in the residual error model

1.1.6. Development of the covariate model

a. Covariate analysis

Identification of covariates that are predictive of pharmacokinetic variability is important in population pharmacokinetic evaluations. However, analysis of covariates is not an easy task because it does not only comprise identification of those covariates that significantly influence the pharmacokinetic parameters, but also the establishment of the kind of relationship among them. In order to select the adequate covariates, either their clinical relevance or their statistical significance towards the parameter may be considered (PL, 2005).

The general approach for analysis of covariates is outlined below:

1. *Selection of potential covariates:* This is usually based on known properties of the drug, drug class, or physiology. For example, highly metabolized drugs will frequently include covariates such as weight, liver enzymes, and genotype (if available and relevant).

2. *Preliminary evaluation of covariates:* Because run times can sometimes be extensive, it is often necessary to limit the number of covariates evaluated in the model. Covariate screening using regression-based techniques, generalized additive models, or correlation analysis evaluating the importance of selected covariates can reduce the number of evaluations. Graphical evaluations of data are often utilized under the assumption that if a relationship is significant, it should be visibly evident.

3. Build the covariate model: Without covariate screening, covariates are tested separately and all covariates meeting inclusion criteria are included (full model).

With screening, only covariates identified during screening are evaluated separately and all relevant covariates are included. Covariate selection is usually based on OBJ using the F-test for nested models. Thus, statistical significance can be attributed to covariate effects and prespecified significance levels (usually $P < 0.01$ or more) are set prior to model-based evaluations. Covariates are then dropped (backwards deletion) and changes to the model goodness of fit is tested using F-test at stricter OBJ criteria (e.g., $P < 0.001$) than was used for inclusion.

This process continues until all covariates have been tested and the reduced or final model cannot be further simplified.

From a statistical point of view, covariates can be classified into:

- Continuous or quantitative: their values are uninterrupted in sequence, substance, or extent (e.g., weight or age).

- Discrete or categorical: their values constitute individually distinct classes or consist of distinct, unconnected values.

Importantly, evaluating multiple covariates that are moderately or highly correlated (e.g., creatinine CL and weight) may contribute to selection bias, resulting in a loss of power to find the true covariates (Upton, 2014).

b. Development of the final population model

Once the candidate variables to explain the drug PK variability have been identified, they shall then be incorporated into the base model, in one way or another depending on their nature. In this sense, discrete covariates must be handled differently, but it is important for both types of data to ensure that the parameterization of the covariate models returns physiologically reasonable results.

Continuous covariate effects can be introduced into the population model using a variety of functions, including a linear function:

$$\boldsymbol{CL = (\theta_1 + weight \times \theta_2) \times exp(\eta_1)} \qquad \textbf{Eq. AIII-25}$$

This function constitutes a nested model against a base model for CL because θ_2 can be estimated as 0, reducing the covariate model to the base model. However, this parameterization suffers from several shortcomings, the first is that the function assumes a linear relationship between the parameter (e.g., CL) and covariate (e.g., weight) such that when the covariate value is low, the associated parameter is correspondingly low, which rarely exists. Therefore, the use of functions such as a power function or exponential functions is common.

$$\boldsymbol{CL = \left(\theta_1 + weight \times exp(\theta_2)\right) \times exp(\eta_1)} \qquad \textbf{Eq. AIII-26}$$

$$\boldsymbol{CL = \theta_1 + weight^{\theta_2} \times exp(\eta_1)} \qquad \textbf{Eq. AIII-27}$$

Covariates are often cantered or normalized as shown below. Centring should be used cautiously; if an individual covariate value is low, the parameter can become negative, compromising the usefulness of the model for extrapolation and can cause numerical difficulties during estimation. Normalizing covariate values avoids these issues. Covariates can be normalized to the mean value in the database, or more commonly to a reference value (such as 70 kg for weight).

$$\boldsymbol{CL = \theta_1 \times (weight - 70)^{\theta_2} \times exp(\eta_1)} \qquad \textbf{Eq. AIII-25}$$

$$\boldsymbol{CL = \theta_1 \times \left(\frac{weight}{70}\right)^{\theta_2} \times exp(\eta_1)} \qquad \textbf{Eq. AIII-26}$$

For discrete data, there are two broad classes: dichotomous (e.g., taking one of two possible values such as sex) and polychotomous (e.g., taking one of several possible values such as race or metabolizer status). For dichotomous data, the values of the covariate are usually set to 0 for the reference classification and 1 for the other classification. Common functions used to describe dichotomous covariate effects are shown below:

$$\boldsymbol{CL = \theta_1 \times (\theta_2)^{covariate} \times exp(\eta_1)} \qquad \textbf{Eq. AIII-27}$$

$$\boldsymbol{CL = \theta_1 \times (1 + \theta_2 \times covariate) \times exp(\eta_1)} \qquad \textbf{Eq. AIII-28}$$

The introduction of polychotomous covariates into the model requires the use of conditions. For example, in the case of genetic polymorphisms, where poor metabolizers=1, rapid metabolizers=2 and ultra-rapid metabolizers=3, the covariate would be coded as follows in NONMEM®:

- *IF (PHENOTYPE.EQ.1) THEN **TVCL**= THETA (1)*
- *IF PHENOTYPE.EQ.2) **TVCL**= THETA (2)*
- *IF (PHENOTYPE.EQ.3) **TVCL**= THETA (3)*

A usual way to incorporate the candidate covariates into the model is to do it one by one, checking the decrease or increase in the OBJ as well as the following criteria for model discrimination after inclusion of each of them.

- Coherence in the obtained parameter estimates.
- Obtaining SEE for all the parameters.
- Absence of significant covariance between parameters.
- Reduction of the interindividual variability in PK parameters.
- Statistically significant decrease in the OBJ (ΔOBJ).

The difference in OBJ between two models (ΔOBJ) is proportional to the -2 times the log of the likelihood (-2LL) and follows an asymptotical chi square distribution ($\chi 2$) with as many degrees of freedom as the number of added parameters. The significance corresponding to different ΔOBJ is shown in Table AIII- 1 below.

Table AIII- 1. Reduction of the -2LL value and corresponding statistical significance

Increase in the number of parameters	Reduction in the -2LL value	P value
1	3.84	<0.05
1	7.88	<0.005
1	10.83	<0.001
2	5.99	<0.05
2	10.60	<0.005
2	13.82	<0.001

Once the final model has been completed, it is possible to estimate the individual (Bayesian) and population PK parameters, as well as to obtain the model predicted concentration profiles and the inter-and intraindividual error values.

1.1.7. Validation of the final model

There are many aspects to the evaluation of a population pharmacokinetic model, which is essential to ensure the model is appropriate for intended use. The OBJ is generally used to discriminate between models during early stages of model development, allowing elimination of unsatisfactory models. In later stages when a few candidate models are being considered for the final model, simulation-based methods such as the visual predictive check (VPC) may be more useful, in addition to the battery of statistical methods previously described.

a. Visual predictive check (VPC)

The visual predictive check (VPC) is a valuable and supportive instrument for evaluating model performance, as it graphically assesses whether an identified model is able to reproduce the variability in the observed data from which it originates. VPCs generally involve simulation of data from the original or new database and offer benefits over standard diagnostic plots, in that they can ensure that simulated data are consistent with observed data.

The final model is used to simulate several replications (i.e., new data sets) based on the structure of the original database (i.e. dosing, timing and number of samples), and prediction intervals (usually 95%) are constructed from simulated concentration time profiles and compared with actual observed data. The most common display of the VPC corresponds to the comparison of the 5th and 95th percentiles of the simulated distribution to the observations. The model is considered to adequately predict the plasma concentration time profiles if most observations lay within the prediction interval. Nonetheless, the VPC largely depends on a subjective comparison of the distribution of the simulated data with the observed data, and does not assess whether the expected random distribution of the observations around the predicted median trend is realized in relation to the number of observations (Post et al., 2008).

Also, VPC plots stratified for relevant covariates (such as age or weight groups), doses, or routes of administration are commonly constructed to demonstrate model performance in these subsets.

A related evaluation is the numerical predictive check which compares summary metrics from the database (e.g., a peak or trough concentration) with the same metric from simulated output (Upton, 2013).

Abstract ModelBio 2018

Title

Population analysis aimed to evaluate the influence of aging on the pharmacokinetics (PK) and pharmacodynamics (PD) of bilastine in healthy volunteers.

Authors and affiliation

Lo Re V[1], Lukas JC[2], Encinas E[2], Campo C[3], Labeaga L[3], Rodríguez M[2].

[1] *Department of Pharmacology, UPV/EHU. 48940 Leioa, Vizcaya, Spain.*
[2] *Dynakin, SL. PTB, Ed. 801-B, 48160 Derio, Vizcaya, Spain.*
[3] *FAES FARMA, SA. 48940 Leioa, Vizcaya, Spain.*

Objective

The aim of this work was to evaluate, using a population modeling based approach, the impact of aging on the PK/PD of bilastine (a H1-antihistamine indicated for the treatment of allergic rhinoconjunctivitis and urticaria) in order to assess the potential need for dosing adjustments in the elderly.

Methods

A population PK/PD model was developed for oral bilastine in NONMEM (FOCE method) using observations (drug plasma levels and inhibition effect on cutaneous reaction) from two phase I studies comprising a combined dataset of 53 healthy subjects aged 18 to 80 years receiving either a single 20 mg dose or multiple ascending doses of 2.5-50 mg.

Upon selection of the base structural model according to statistical and diagnostic criteria, graphical exploration of trends of Bayesian PK and PD parameters with age was undertaken, and the influence of age as a potential covariate was tested within population runs.

Results and discussion

A 2-compartment PK model together with an indirect inhibitory effect PD model including interindividual variability (modelled as exponential) in all parameters best described the observations from both studies, regardless of dose.

Graphical exploration revealed a trend between central volume of distribution (Vc) and age while no clear trend was observed between age and any of the PD parameters (Kon, Koff, IC_{50}). Consistently, inclusion of age as a covariate into Vc led to a significant decrease in the objective function OBJ ($p<0.001$), suggesting a ~30% increase in Vc (~30% expected reduction in C_{max}) from subjects of median age 30 vs. 70 years, which was deemed clinically irrelevant as supported by a dedicated safety study in elderly patients. Age did not show any statistically significant effect on bilastine PD.

Conclusions

Population PK/PD analysis revealed that aging has no clinically relevant impact on the PK or the PD of the antihistamine bilastine, thus supporting the lack of need for dose adjustment in the elderly.

Abstract ModelBio 2019

Title

Population Pharmacokinetic Analysis of Bilastine in subjects with various degrees of renal insufficiency: prediction in elderly populations

Authors and affiliation

Lo Re V[1,2], Rodríguez M[2], Lukas JC[2], Encinas E[2], Campo C[3], Garcia A[3] ,Suarez E[1]

[1] *Department of Pharmacology, UPV/EHU. 48940 Leioa, Vizcaya, Spain.*

[2] *Dynakin, SL. PTB, Ed. 801-B, 48160 Derio, Vizcaya, Spain.*

[3] *FAES FARMA, SA. 48940 Leioa, Vizcaya, Spain.*

Objective

The aim of this work was to evaluate, using a population modeling approach, the relationship between renal function and the pharmacokinetics (PK) of Bilastine also assessing whether posology adjustment is warranted in elderly patients with impaired renal function and the consequent dosing recommendations.

Materials and Methods

A population PK model was developed for oral Bilastine in NONMEM (FOCE method) using observations (drug plasma levels and urinary excretion data) from an open-label, single-dose, parallel-group study comprising a total of four groups (n=6) of subjects with a mean age across the groups between 65 and 72 years either healthy or with various degrees of renal insufficiency (RI) according to their glomerular filtration rate (GFR) values. The analysis included covariate modelling of GFR as a continuous indicator of renal insufficiency within the population runs.

Results and discussion

A two-compartment PK model including interindividual variability (modelled as exponential) in all parameters (except TLag) best described the observations from the study. A base model for the full patient (all GFR levels) population was first developed. Then, the four RI patient subgroups were modelled as a categorical covariate. Consistently, the inclusion of GFR as a covariate into CL/F, V2/F led to a significant decrease in the objective function OBJ. Graphical exploration revealed a trend of increasing AUC and C_{max} across the 4 RI groups. Data from literature suggest that all physiological renal changes age-related (decreased kidney size, decreased renal blood flow, decreased number of functional nephrons) lead to a decrease glomerular filtration rate and thus, to a reduced renal clearance, directly impacting the total clearance for a drug with exclusive renal clearance such as Bilastine.

In fact, a reduction of both plasma and renal clearance linked to the decrease in GFR across the 4 sub-groups lead to an increase in exposure to the drug. However, this increase was deemed clinically irrelevant in terms of efficacy and safety of the drug even in elderly patients.

Conclusions

Population PK analysis revealed that although the effect of aging in addition to RI condition cause increased exposure to Bilastine, the drug can be safely administered, at the therapeutic dose.

Abstract ModelBio 2020

APPLICATION OF A DUAL PBPK-POPPK MODEL BASED APPROACH ACROSS THE AGE-POPULATION OF ADULTS USING BILASTINE AS A PROBE DRUG

Valentina Lo Re[1,2], Chaejin Kim[3], Lukas JC[2], Campo C[4], Garcia A[4], Stephan Schmidt[3], Valvanera Vozmediano[3], Elena Suarez[1], Monica Rodriguez[2]

(1) Department of Pharmacology, UPV/EHU. 48940 Leioa, Vizcaya, Spain.

(2) Drug Modeling & Consulting (DMC), Dynakin, SL, Bilbao, Spain

(3) Center for Pharmacometrics and Systems Pharmacology, Department of Pharmaceutics, University of Florida

(4) Medical Department, FAES FARMA, S.A, 48940, Spain

KEYWORDS: elderly; modelling; pharmacokinetics; semi-physiological model; PBPK model

ABSTRACT

Introduction

Despite the increasing size of the geriatric population and specific guidance on the elderly regarding medicinal products, this patient population is clinically understudied. Gathering an in-depth understanding of physiological changes in a special population, such as geriatric patients would allow a better understanding of age PK and PD relating factors in order to optimize drug therapy in the elderly

Objective

Use of a dual physiologically-based pharmacokinetic model- population pharmacokinetic (PBPK-popPK) model-based approach to integrate bilastine physicochemical, *in vitro* and *in vivo* data in young adults to: 1) enhance the mechanistic understanding of intestinal transporters on drug PK, and 2) predict the PK in elderlies of different biological age (*i.e.*, young old, middle old and oldest old).

Methods

1) PBPK model: Using GastroPlus 9.6® a PBPK model for young adults was developed considering apical efflux and apical and basolateral influx transporters in the enterocyte, using PK data from young adults after IV (10mg SD) and PO (20mg SOD). The model was qualified using an external dataset containing data from 12 Phase-I studies with 13 different SOD and MOD. The model was then used to extrapolate the PK to young olds, which also served to verify the predictive capacity of the model. 2*) PopPK model:* A semi-mechanistic predictive popPK model for elderlies was develop in NONMEM version 7.2 using a previously developed young adult popPK model incorporating declining functions on different physiological systems (glomerular filtration, unbound fraction) and differences in body composition. Model predictive capacity was evaluated using observations from young olds. Both models were qualified by comparing the predicted *vs* observed PK parameters in elderlies, and by comparing the predicted concentration-time profiles to the clinical data.

Results

Final PBPK model predictions showed AUC_{pred}/AUC_{obs} ratios within 0.5 and 2 for all the doses (5mg - 220mg). The final PBPK adequately predicted plasma concentrations in geriatrics. Similar results were also obtained for the semi-mechanistic popPK model where more than 90% of observations were within the 5~95% of simulated confidence intervals.

Conclusions

This study suggests that the developed models can be successfully used to scale the pharmacokinetics of Bilastine from adults to geriatrics.
Moreover, this complementary approach allowed to enhance the accuracy of the predictions and to fine tune them by applying additional physiological based factors and pharmacokinetic based knowledge. The application of both models indicates that 20 mg QD dose is appropriate for geriatrics of any age. Current work is ongoing to establish the influence of pathophysiological conditions that are common in this patient population.

ACoP11

Application of a dual PBPK-popPK model based approach across the age-population of adults using bilastine as a probe drug

Chaejin Kim[1], Valentina Lo Re[2], Monica Rodriguez[2], Lukas JC[2], Campo C[3], Garcia A[3], Stephan Schmidt[1], Valvanera Vozmediano[*1]

(1) Center for Pharmacometrics and Systems Pharmacology, Department of Pharmaceutics, University of Florida

(2) Drug Modeling & Consulting (DMC), Dynakin, SL, Bilbao, Spain

(3) Medical Department, FAES FARMA, S.A, 48940, Spain

* Correspondence: valva@cop.ufl.edu

Objective: Use of a dual physiologically-based pharmacokinetic model- population pharmacokinetic (PBPK-popPK) model-based approach to integrate bilastine physicochemical, *in vitro* and *in vivo* data in young adults to: 1) enhance the mechanistic understanding of intestinal transporters on drug PK, and 2) predict the PK in elderlies of different biological age (*i.e.*, young old (65~74 yrs), middle old (75~84 yrs) and oldest old (> 85 yrs)).

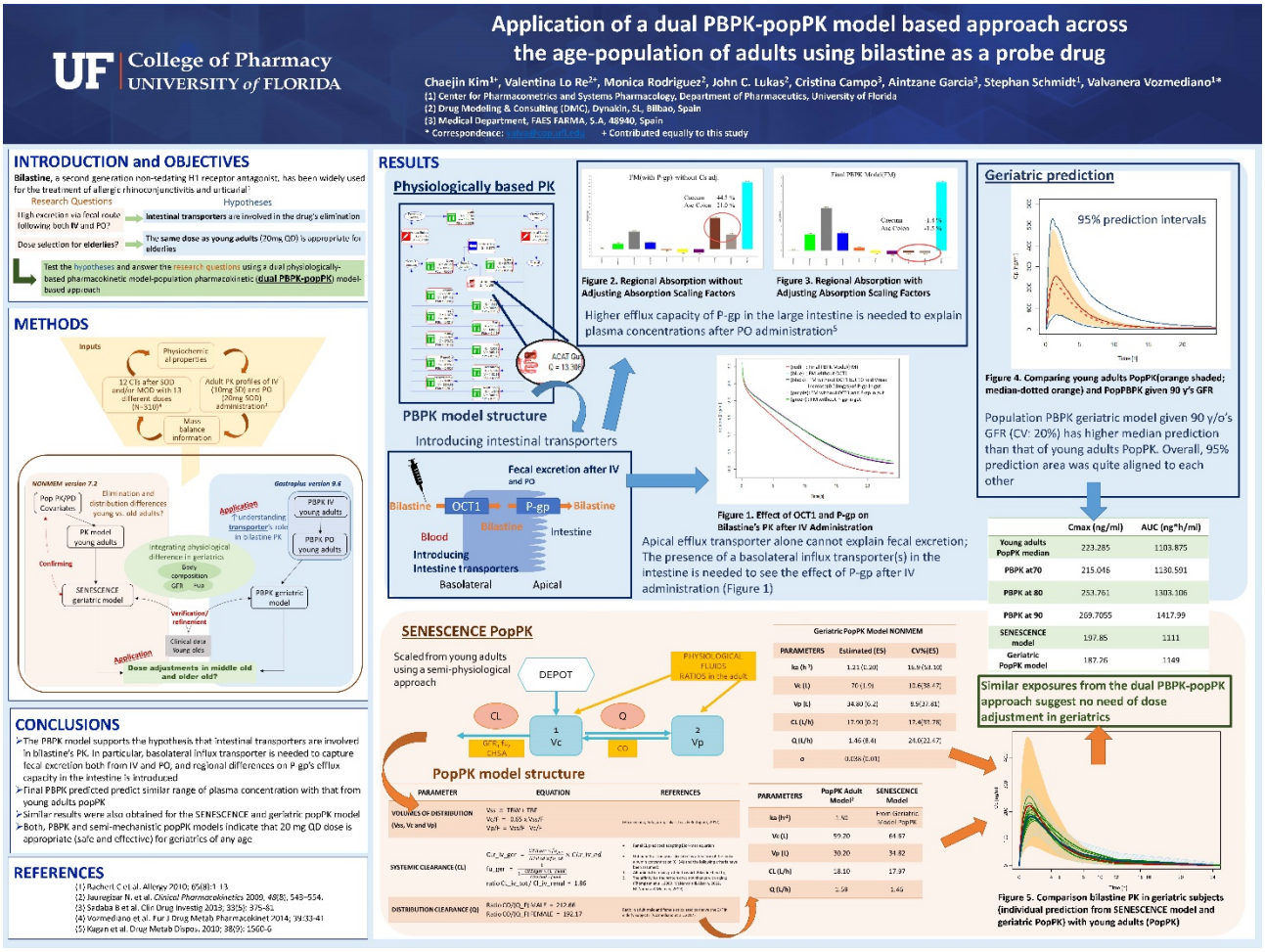
Methods: *1) PBPK model*: Using GastroPlus 9.6® a PBPK model for young adults was developed considering apical efflux and apical and basolateral influx transporters in the enterocyte, using PK data from young adults after IV (10mg SD) and PO (20mg SOD)[1]. Transporters' kinetics and colonic absorption parameters were optimized during the model development process (due to insufficient experimental data available to inform the model). The model was qualified using an external dataset containing data from 12 Phase-I studies with 13 different SOD and MOD[2]. The model was then used to extrapolate the PK to young olds, which also served to verify the predictive capacity of the model. 2*) PopPK model:* A semi-mechanistic predictive popPK model for elderlies was develop in NONMEM version 7.2 using a previously developed popPK model[2] incorporating declining functions on different physiological systems (glomerular filtration, unbound fraction) and differences in body composition. Model predictive capacity was evaluated using observations from young olds. Both models were qualified by comparing the predicted *vs* observed PK parameters in elderlies, and by comparing the predicted concentration-time profiles to the clinical data. Both models were then applied to assess the suitability of the therapeutic dose in middle and oldest olds.

Results: Final PBPK model predictions showed AUC_{pred}/AUC_{obs} ratios within 0.5 and 2 for all the doses (5mg - 220mg). The final PBPK adequately predicted plasma concentrations in geriatrics (n=8 male; mean age= 69.75 yrs; n=8 female: mean age = 67.625 yrs). Similar results were also obtained for the semi-mechanistic popPK model where more than 90% of observations were within the 5~95% of simulated confidence intervals. The application of both models to middle and oldest old led to the conclusion than the 20 mg dose produces plasma concentrations of bilastine within the known therapeutic margin[3].

Conclusions: The PBPK model supports the hypothesis that basolateral influx transporters are involved in bilastine PK (basolateral influx and apical efflux intestinal transporter were needed to adequately describe the PK) and of regional differences on P-gp's efflux capacity in the intestine. Both, PBPK and semi-mechanistic popPK models indicate that 20 mg QD dose is appropriate (safe and effective) for geriatrics of any age.

References:

[1] Sádaba et al. *Clin Drug Investig*. 2013;33:375-381. doi:10.1007/s40261-013-0076-y
[2] Jauregizar et al. *Clin Pharmacokinet*. 2009;48(8):543-554. doi:10.2165/11317180-000000000-00000
[3] Vozmediano et al. *Eur J Pharm Sci*. 2019;128(November 2018):180-192. doi:10.1016/j.ejps.2018.11.016



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ARTICLE

Application of a dual mechanistic approach to support bilastine dose selection for older adults

Chaejin Kim[1]* | Valentina Lo Re[2,3]* | Monica Rodriguez[2] | John C. Lukas[2] | Nerea Leal[2] | Cristina Campo[4] | Aintzane García-Bea[4] | Elena Suarez[3] | Stephan Schmidt[1] | Valvanera Vozmediano[1]

[1]Department of Pharmaceutics, Center for Pharmacometrics and Systems Pharmacology, University of Florida, Gainesville, Florida, USA

[2]Drug Modeling & Consulting (DMC), Dynakin, SL, Bilbao, Spain

[3]Department of Pharmacology, Faculty of Medicine and Nursing, University of Basque Country UPV/EHU/ Biocruces Health Research Institute, Bizkaia, Spain

[4]Medical Department, FAES FARMA, S.A, Leioa, Spain

Correspondence
Valvanera Vozmediano, Center for Pharmacometrics and Systems Pharmacology, Department of Pharmaceutics, University of Florida, Gainesville, FL 32612, USA.
Email: valva@cop.ufl.edu

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Abstract

The objective of this study was to evaluate bilastine dosing recommendations in older adults and overcome the limitation of insufficient data from phase I studies in this underrepresented population. This was achieved by integrating bilastine physicochemical, in vitro and in vivo data in young adults and the effect of aging in the pharmacology by means of two alternative approaches: a physiologically-based pharmacokinetic (PBPK) model and a semi-mechanistic population pharmacokinetic (Senescence) model. Intestinal apical efflux and basolateral influx transporters were needed in the PBPK model to capture the observations from young adults after single i.v. (10 mg) and p.o. (20 mg) doses, supporting the hypothesis of involvement of gut transporters on secretion. The model was then used to extrapolate the pharmacokinetics (PKs) to elderly subjects considering their specific physiology. Additionally, the Senescence model was develop starting from a published population PK) model, previously applied for pediatrics, and incorporating declining functions on different physiological systems and changes in body composition with aging. Both models were qualified using observed data in a small group of young elderlies ($N = 16$, mean age = 68.69 years). The PBPK model was further used to evaluate the dose in older subjects (mean age = 80 years) via simulation. The PBPK model supported the hypothesis that basolateral influx and apical efflux transporters are involved in bilastine PK. Both, PBPK and Senescence models indicated that a 20 mg q.d. dose is safe and effective for geriatrics of any age. This approach provides an alternative to generate supplementary data to inform dosing recommendations in under-represented groups in clinical trials.

*These authors equally contributed to this research.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Older adults are usually underrepresented in clinical trials limiting the information on pharmacological changes and special dosing needs in this population. New precision medicine tools can help to support dosing recommendations in geriatrics.

WHAT QUESTION DID THIS STUDY ADDRESS?

How to integrate aging-mediated physiological changes in quantitative pharmacology approaches to overcome the lack of sufficient older adults in clinical trials and provide support for precise dosing recommendations.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Aging-mediated changes in physiological functions and body composition can be successfully integrated into quantitative strategies to scale the pharmacological knowledge to geriatric patients. This approach provides the mean to rationally improve posological adequacy in this under-represented population.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The present work pioneers the application of quantitative physiological modeling to address the limitations of including older adults in clinical trials and support dosing needs, using bilastine as an example. The application of similar approaches will help to generate pharmacological knowledge and design more efficient dedicated drug development programs for geriatric patients.

INTRODUCTION

Bilastine is a second-generation, H_1 selective antihistamine approved worldwide for the treatment of allergic rhinoconjunctivitis and urticaria in adults and children.[1,2] It has a well-defined therapeutic window and favorable pharmacokinetic (PK) properties, including (1) no significant hepatic metabolism and (2) no significant brain penetration resulting in nonsedative properties and lack of cognitive impairment.[2–4] Regarding the pharmacodynamic (PD) aspects, drug's potency is high, which is compatible with its three and six times higher affinity for the H_1 receptor when compared to cetirizine and fexofenadine, respectively.[5] In addition, bilastine has a very rapid onset (within an hour) and a long duration of action (26 h).[6,7] Aforementioned pharmacological properties are particularly attractive for geriatrics, a population at a higher risk of suffering adverse reactions and drug-drug interactions (DDIs).[8]

According to the Summary of Product Characteristics of bilastine, no dose adjustment is needed for older adults.[9] However, the dosing recommendation in geriatrics was initially evaluated using data from phase I studies with young adults and a small number ($N = 16$) of older adults (mean age 68.69 years) were included in a conventional population PK (PopPK) analysis.[9,10] This approach may thus not represent the overall geriatric population, which is physiologically diverse due to the heterogeneity of individual aging rate and extent, and the high prevalence of comorbidities/comedications. This situation is frequently encountered in clinical trials despite the efforts from regulatory authorities to include geriatric patients in randomized studies.[11–13] The availability of in silico approaches that integrate aging-mediated changes in physiology with associated effects on drug PKs and PDs, and enhance the understanding of underlying drug and disease mechanisms provide an opportunity to overcome these limitations. Moreover, these approaches may allow the evaluation of comorbidities and DDIs facing the unmet need of appropriate dosing recommendations for geriatrics in the absence of head-to-head clinical trials. In this research, we applied two alternative approaches that integrate physiological characteristics of older adults and the drug properties to characterize the PK and provide dose recommendations. Here, we used bilastine in healthy geriatrics as a case example also motivated by its peculiar secretion involving transporters on the gut wall after i.v. administration. First, we developed a PopPK based semi-mechanistic model, hereafter referred to as Senescence model, by using scaling equations that account for changes on the systemic PK parameters with aging as well as individual subject's demographics. This model is the continuation of a previous model applied to inform bilastine pediatric drug development.[14] Second, we developed a full physiologically-based pharmacokinetic (PBPK) model to evaluate the impact of intestinal transporters on bilastine PKs in adults, which was not possible with the Senescence model, and account for aging-related physiological changes on PK parameters. One remarkable PK feature of bilastine is the high fecal excretion

as an unchanged form after p.o. administration (~ 67% of total dose) and also very likely after i.v. administration (projected for ~ 30% of total dose).[2] Considering that the expected biliary excretion in human is less than 5% and the existing evidences of the drug-intestinal transporter interactions from clinical and in vitro studies, we hypothesize that intestinal transporters play a significant role in both, absorption and secretion.[15] Increasing the knowledge on the involvement of transporters on a drug PKs is crucial to have a deeper understanding of the mechanisms but also to predict the potential for DDIs which is especially important in the polymedicated geriatric population. We first developed the PBPK model in young adults and then, we extrapolated the model to healthy young geriatrics (adults of 65 to 74 years). Finally, both models, Senescence and PBPK, were verified with available data in a limited group of young geriatric volunteers as well as by comparing the predictions with that of a geriatric PopPK model developed using the young geriatrics data. Once this approach was qualified in young geriatric subjects, it was used to evaluate the therapeutic dose in older subjects (>75 years).

METHODS

The overall research strategy is summarized in Figure 1.

Dataset

All the clinical data used in the present study were part of bilastine clinical development and were approved by the corresponding institutional review board (IRB), and conducted in accordance with the principles of the Declaration of Helsinki of 1975 (as revised in 1983).

Young adults bilastine 10 mg i.v. data (BILA-2909/BA)

BILA-2909/BA was designed to investigate bilastine oral bioavailability in humans.[2] It was a randomized, open label, single dose, single center, two-arm crossover-controlled trial under fasting condition. Six male and six female subjects received 20 mg single dose of the p.o. tablet (Bilaxten FAES FARMA) and 10 mg of bilastine i.v. single dose over 5 min. The washout period between the two treatments was of at least 14 days, and the sequence of the treatments was determined by randomization in balanced manner. Subjects aged between 18 ando 24 years (mean 20.8 years), weighed between 50 ando 80.6 kg (mean 65.9 kg), and had body mass index (BMI) between 19.41 ando 25.40 kg/m^2 (mean 22.47 kg/m^2). Detail information can be found in the paper from Sadaba et al. (2013).[2]

Geriatric Bilastine 20 mg p.o. data (BILA/459-05)

BILA/459-05 was an open-label, single-dose, parallel-group study comprising a total of 32 young and elderly subjects. In the present research, data from 16 healthy subjects aged 65 or older (men $n = 8$; women $n = 8$) were used to represent the geriatric population. The elderly subjects were aged between

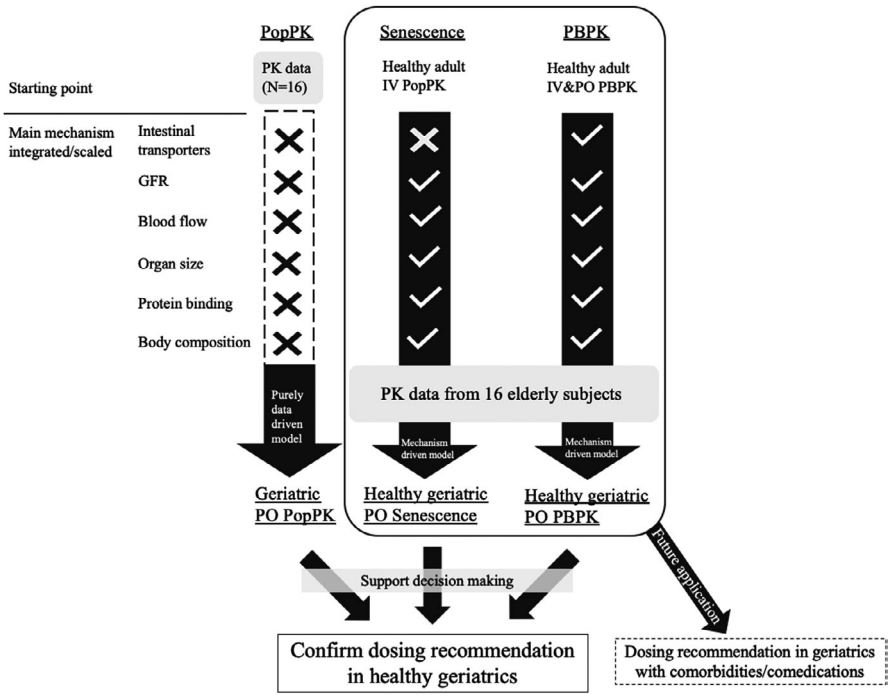

FIGURE 1 Overview of the dual physiologically-based pharmacokinetic model population pharmacokinetic (PBPK-PopPK) model-based approach used to evaluate bilastine dosing recommendation in geriatric subjects. GFR, glomerular filtration rate

65 ando 83 years (mean 68.69 years), weighed between 48.4 ando 85.1 kg (mean 73.06 kg), and had BMI between 21.51 ando 30.15 kg/m^2 (mean 26.33 kg/m^2).

Software used in the analysis

For the Senescence model and the PopPK model, modeling and simulation were performed with NONMEM (version VII; Icon Plc, Dublin, Ireland). Perl-speaks-NONMEM (version 4.6), Pirana (version 2.9.4), and Xpose4 (version 4.7.1) were used for model development and evaluation. For the PBPK model, GastroPlus 9.6 (SimulationsPlus, Inc.) was used for model development and simulation. Data exploration and management and graphics were performed using S-PLUS (version 8.2; TIBCO Software Inc.) and R (version 4.0; R core Team 2019).

Senescence model development

The starting point to build the Senescence model was a published two compartment PopPK model parameterized in terms of absorption rate constant (Ka), volume of the central compartment (Vc), volume of the peripheral compartment (Vp), and intercompartmental clearance (CL/Q).[10] The model was

TABLE 1 Main assumption and conclusion from the Senescence and PBPK models

Main assumptions	Justification	Approach to assess the impact	Conclusion
Senescence			
Changes in the PK as a consequence of aging related changes in albumin, GFR, CO, TBW, and TBF	Known processes involved in bilastine's PK that was also successfully used previously for pediatrics	Comparison of individual parameters predicted with the senescence model compared to EBE from a PopPK model	Individual predictions within the two-fold and less than 30% prediction error in the case of mean parameters (Senescence vs. geriatric popPK). The equation used to predict bilastine CL successfully tested CLr in patients with renal dysfunction.[32] Miss-predictions on CL/F attributable to (1) use of mean F from young adults, and (2) possible changes in F with aging not considered in the model.
F mean in young subjects similar to that in older adults			
PBPK			
Apical and basolateral transporters involved in bilastine secretion and absorption	Only 66% of the drug recovered in urine after i.v. but CLr is the main elimination pathway.[19] Amount recovered in urine after oral: ~42%[19] DDI and in vitro studies evidenced the influence of transporters at an intestinal level	Compare predictions and observations before and after the inclusion of transporters for iv and oral. Comparison with the mass balance results.	Apical and basolateral transporters needed to predict bilastine PK profile after i.v. and p.o. administration. After i.v. administration, only 74% of the drug predicted to be systemically available (in line with observed 66% recovery in urine) and the rest secreted to the GI track by active transporters. After p.o., only about 42.3% predicted to be systemically available; and 40% recovered in urine. This is in line with drug's renal CL and amount recovered in urine in the BA study (42%).[19] These results are also in line with the radio-labeled mass balance study.[20] Bilastine is eliminated by renal filtration in the kidneys. Decrease in renal CL in subjects with renal impairment was proportional to the decrease in the GFR.
Renal CL main route of elimination of bilastine	Mass balance study[19,20]	Comparison of urine recovery in the mass balance studies with the PBPK mass balance	Bilastine plasma concentrations were well predicted in geriatric subjects without the inclusion of aging related changes on drug transporters
No impact of aging on drug transporters	Not enough evidence to inform possible changes	Application of the model to predict the PK in older subjects and comparison with observations	

Abbreviations: BA, bioavailability; CL, clearance; CL/F, total apparent clearance; CLr, renal clearance; CO, cardiac output; DDI, drug-drug interaction; EBE, empirical Bayes estimate; F, bioavailability; GFR, glomerular filtration rate; GI, gastrointestinal; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic; TBF, total body fat; TBW, total body water.

developed and qualified using p.o. data from 310 healthy adult volunteers in NONMEM (first order conditional estimation [FOCE] method) using nonlinear mixed effects (NLME) and standard procedures for population analysis.[10,16,17] Following a previous application of this approach to predict bilastine's PKs in pediatrics, a comprehensive literature search was carried out to design and/or extract appropriate mathematical equations for scaling Vc, Vp, clearance (CL), and Q with respect to age, sex, weight, height, and change of physiological factors with aging. The final equations integrated in the Senescence model are depicted in Table 1. Due to the difficulty of inferring a mathematical function to account for the aging effects on Ka, individual values of this parameter were directly borrowed from the geriatric PopPK model described below (see section "Geriatric population pharmacokinetics model development"). We also considered that the bioavailability (F) of the 20 mg bilastine p.o. dose was 61% (i.e., invariant regardless of aging and demographics).[2]

The Senescence model was then used along with subject specific demographics of elderly subjects ($N = 16$; BILA/459-05) to simulate the individual PK after 20 mg of p.o. bilastine. Simulations were then compared with those from the geriatric PopPK model (section "Geriatric population pharmacokinetics model development") developed using data from the same subjects. Specifically, predicted individual PK parameters (Vci, Vpi, CLi, and Qi) using the Senescence model and divided by a mean F of 61% were compared with the individual parameters estimates from the geriatric PopPK model. The Senescence model was considered appropriate when the ratio between the parameters estimated for each individual from both models was less than two fold.

Geriatric population pharmacokinetics model development

The geriatric PopPK model was developed with data from 16 elderly subjects from study BILA/459-05 using NLME and standard procedures for population analysis.[10,16,17] The purpose of developing the geriatric PopPK model was to estimate a Ka to inform the Senescence model but also to use it as a reference for comparison. Further information on the model development and qualification can be found in section 2 of the supplementary material.

Physiologically-based pharmacokinetic model development

The PBPK model was developed using i.v. and p.o. data from healthy young adults participating in study BILA-2909/BA, which contains plasma concentration and mass balance (cumulative urine excretion) data using GastroPlus 9.6. A key characteristic of the PBPK model is that it considers intestinal transporters. The evaluation of the influence of transporters on secretion and absorption was performed in a step-wise fashion: first, the influence of transporters on bilastine's secretion was evaluated using the i.v. data; second, the need of inclusion of additional transporters involved in the absorption was evaluated using the p.o. data. Based on the evidence from in vitro and clinical data, we introduced an apical efflux transporter, which represents the P-glycoprotein (P-gp). However, due to its location in the apical membrane it was not able to account for the secretion after the i.v. administration, suggesting the need of an additional transporter on the basolateral membrane. The values for maximum value (V_{max}) and kinetic metabolite (Km) were not available from in vitro studies and were thus incorporated in the model using a sensitivity analysis to fit the PK data (Figure S7). After the inclusion of transporters, the PBPK model was further qualified using external data from 12 clinical trials after single and/or multiple p.o. doses in the range between 5 mg and 220 mg per day (Table S4).[10,18] Moreover, the mean area under the curve (AUC) and maximum plasma concentration (C_{max}) values from observations and predictions were also compared (Figure S4 and S5). The model was considered appropriate when the ratio of the PK parameters from mean of observations and mean of predictions was less than twofold. Furthermore, multiple dose of 20 mg p.o. q.d. scenario was simulated, and it compared with observations for additional verification of the PBPK model (Figure S6 and Table S5).[10,18] Available mass-balance data were also used for verification (Figures S8 and S9).[19,20] The PBPK model was then extrapolated to healthy geriatrics. Virtual geriatric subjects were generated using GastroPlus 9.6 built-in Population Estimates for Age-Related (PEAR) Physiology program[21] at mean age of 70 (age range of 65–75 years with 50% men) and at mean age of 80 (age range of 75–85 years with 50% men). Based on age-related information, whole-body tissue estimates are calculated for weight, volume, and perfusion for each tissue and recalculate drug distribution to each specific organ in the model as well as elimination. The age-related population data in GastroPlus comes from the National Health and Nutrition Examination Survey (NHANES) from 11,039 Americans (50% male and female subjects; 1–85 years old). The model predictions ($N = 1500$) in young elderly subjects were visually verified with the observations before proceeding with the extrapolation to the older group.

Evaluation of the suitability of bilastine 20 mg q.d. oral dose in geriatrics

To test whether bilastine 20 mg q.d. p.o. dose is also appropriate for the overall group of geriatrics, simulated PK profiles from the Senescence ($N = 16$), geriatric PopPK ($N = 1500$), and

TABLE 2 Senescence model scaling equations used in the extrapolation of bilastine PK parameters to elderly

Parameter	Equation and/or reference	PK related parameters	Equation to scale intravenous PK in elderly
CSHA (g/L)	$\text{CHSA} = -0.0709 \times \text{Age(yr)} + 47.7$ Eq. 1[27]	fu	$\text{fu ger} = \frac{1}{1 + \frac{\text{CHSAger} \times (1 - \text{fuad})}{\text{CHSAad} \times \text{fuad}}}$ Eq. 2[33]
GFR (L/h)	$\text{GFR(ml/min/100 g kidney)} = 26.6 \times \left(1 - \frac{0.9 \times (\text{Age(yr)} - 30)^{1.5}}{\text{TA}_{50}{}^{1.5} + (\text{Age(yr)} - 30)^{1.5}}\right)$ Where TA_{50}:54 (male), 59 (female) Eq. 3[25] The GFR equation and relevant age and sex dependent kidney weight is obtained from Schlender et al. 2016	CLr (L/h)	$\text{CLr}_{\text{ger}} = \frac{\text{GFRger} \times \text{fu}_{\text{ger}}}{\text{GFRad} \times \text{fu}_{\text{ad}}} \times \text{CLr}_{\text{ad}}$ Eq. 4 $\text{ratio}\frac{\text{CL}}{\text{CLr}}$ Eq. 5[34,35]
CO (L/h)	$\text{CO} = 159 \times \text{BSA}\left(\text{m}^2\right) - 1.56 \times \text{Age(yr)} + 114$ Eq. 6[27]		$\text{ratio}\frac{\text{CO}}{\text{Q}_{iv}}\text{male} = 348.62$ Eq. 8[14]
BSA (m^2)	$\text{BSA} = 0.007184 \times \text{weight (kg)}^{0.425} \times \text{height (cm)}^{0.725}$ Eq. 7[36]	Q (L/h)	$\text{ratio}\frac{\text{CO}}{\text{Q}_{iv}}\text{female} = 315.04$ Eq. 9[14]
TBW (L) TBF (kg)	$\text{TBW}_{\text{male}} = 1.203 + 0.176 \times \text{weight (kg)} + 0.449 \times S^2/\text{Res}$ Where $\frac{S^2}{\text{Res}}$ (age groups 60 − 69.9) = 67.0, (age groups 70 − 79.9) = 64.3 Eq. 10[37] $\text{TBW}_{\text{female}} = 3.747 + 0.113 \times \text{weight (kg)} + 0.45 \times S^2/\text{Res}$ Where S^2/Res (age groups 60 − 69.9) = 46.2, (age groups 70 − 79.9) = 45.2 Eq. 11[37] $\text{TBF (kg)} = 0.68 \times \text{weight (kg)} - 0.56 \times \text{height (cm)} + 6.1 \times \text{Sex} + 65$Where male = 0, female = 1 Eq. 12[27]	Vss, Vc and Vp (L)	$\text{Vss iv} \cong \text{TBW} + \text{TBF}$ Eq. 13 $\text{Vc iv} = 0.65 \times \text{Vss iv}$ Eq. 14 $\text{Vp iv} = \text{Vss iv} - \text{Vc iv}$ Eq. 15
		k_a	The constant of absorption was taken from the Geriatric PopPK model

Note: Reference Adult body composition parameter: (adult of reference were considered aged 30–50 years).

CO adult man = 352.11, CO adult woman = 318.19[27]; GFR adult man = 113.03, GFR adult woman = 99.66[25] ; fu adult = 0.13[10]; CHSA adult = 44.86.[27]

Abbreviations: V, Volume of distribution; ss, steady state; c, Central; p, Peripheral; F, bioavailability; CL, clearance; r, renal; ad, adult; fu, unbound fraction; GFR, glomerular filtration rate; CHSA, albumin molar concentration; Cp, plasma concentration; CO, cardiac output; Q, intercompartmental clearance; TBW, total body water; TBF, total body fat; k_a, absorption rate constant.

TABLE 3 Summary of bilastine pharmacokinetic parameters in elderly subjects

Senescence model (20 mg p.o.) $F = 61\%$		Geriatric PopPK model (20 mg p.o.)	
Parameter	**Mean of individual subjects' predicted parameters**	**Parameter**	**Mean of individual subjects' predicted parameters**
Vc/F (L)	66.88	Vc/F (L)	77.44
Vp/F (L)	36.01	Vp/F (L)	37.62
CL/F (L/h)	12.78	CL/F (L/h)	18.04
Q/F (L/h)	1.40	Q/F (L/h)	1.57
Ka (1/h)	1.28	Ka (1/h)	1.28
CV (%) Ka	24.67	CV (%) Ka	24.67
CV (%) CL	8.91	CV (%) CL	26.26
CV (%) Vc	10.33	CV (%) Vc	30.50
CV (%) Q	8.51	CV (%) Q	29.22
CV (%) Vp	10.33	CV (%) Vp	38.17

Note: (Left) Mean and CV of individual predicted parameters with the Senescence model in subjects ($N = 16$) from study BILA/459-05; (right) Mean and CV of individual Bayesian estimates with the geriatric popPK model using data from BILA/459-05 ($N = 16$).

Abbreviations: CL, clearance; CL/F, total apparent clearance; CV, coefficient of variation; Ka, absorption rate constant; PopPK, population pharmacokinetic; Q/F, intercompartmental clearance; Vc, central compartment; Vp, peripheral compartment.

PBPK ($N = 1500$; mean age of 70) models were compared with that of young adults PopPK model ($N = 5000$).[10] We compared predicted C_{max} and AUC values as well as visually check the plasma concentration profiles focusing on whether the overall predictions from the three geriatric models were within 95% confidence interval (CI) of young adults PopPK model. PBPK simulated results at mean age of 80 were further compared with that of young adults PopPK model to evaluate whether bilastine 20 mg q.d. is also appropriate in the older geriatric age.

RESULTS

Figure 1 summarizes the different approaches combined in this analysis with a highlight of the main mechanisms on bilastine PK investigated and/or supported by each of these analyses. Moreover, Table 1 summarizes the main assumptions and conclusions taken with each model.

Senescence and geriatric PopPK model

Table 2 shows the scaling equations integrated in the Senescence model. Individual subject's demographics used as input for the extrapolations in the Senescence model are presented in Table S1. Table 3 summarizes the PK parameters of the Senescence and geriatric PopPK model. Additional information on the evaluation of the predictive capacity of the Senescence model is presented in Figure S1. Both models led to very similar PK parameters (all the predicted individual PK parameters predicted with the Senescence model were within the 2-fold range) and model predictions in the population of elderly subjects supporting the validity of the equations and assumptions used to train the Senescence model. A more detailed description of the development and qualification of the geriatric PopPK model is provided in the section 2 of supplementary material.

PBPK model

The final PBPK model parameters and verification, and simulation settings are summarized in section 3 of supplementary material. Two different transporters were included in the final model (Figure 2a), an efflux transporter in the apical membrane which represents the P-gp and an influx transporter in the basolateral site needed to explain the passage from the blood to the enterocyte after i.v. administration. Figure 2b shows the final i.v. PBPK model predictions (black solid line) with subjects' observations superimposed (grey dots). Moreover, different predictions performed during the model development process are also depicted in the same panel demonstrating that both an influx and efflux transporter are needed to fit bilastine PK profile. This result was also supported by the urine excretion data (Figure 2c) where the observed urine data were only well-predicted when both transporters were included in the model. Urine data were overpredicted otherwise. A similar representation is shown in Figure 2d for the p.o. administration where the black solid line represents the final model predictions with subjects' observations superimposed (grey dots). In this specific case, in addition to inclusion of the P-gp, the C3 and C4 parameters of the absorption scale factor (ASF) were manually optimized to

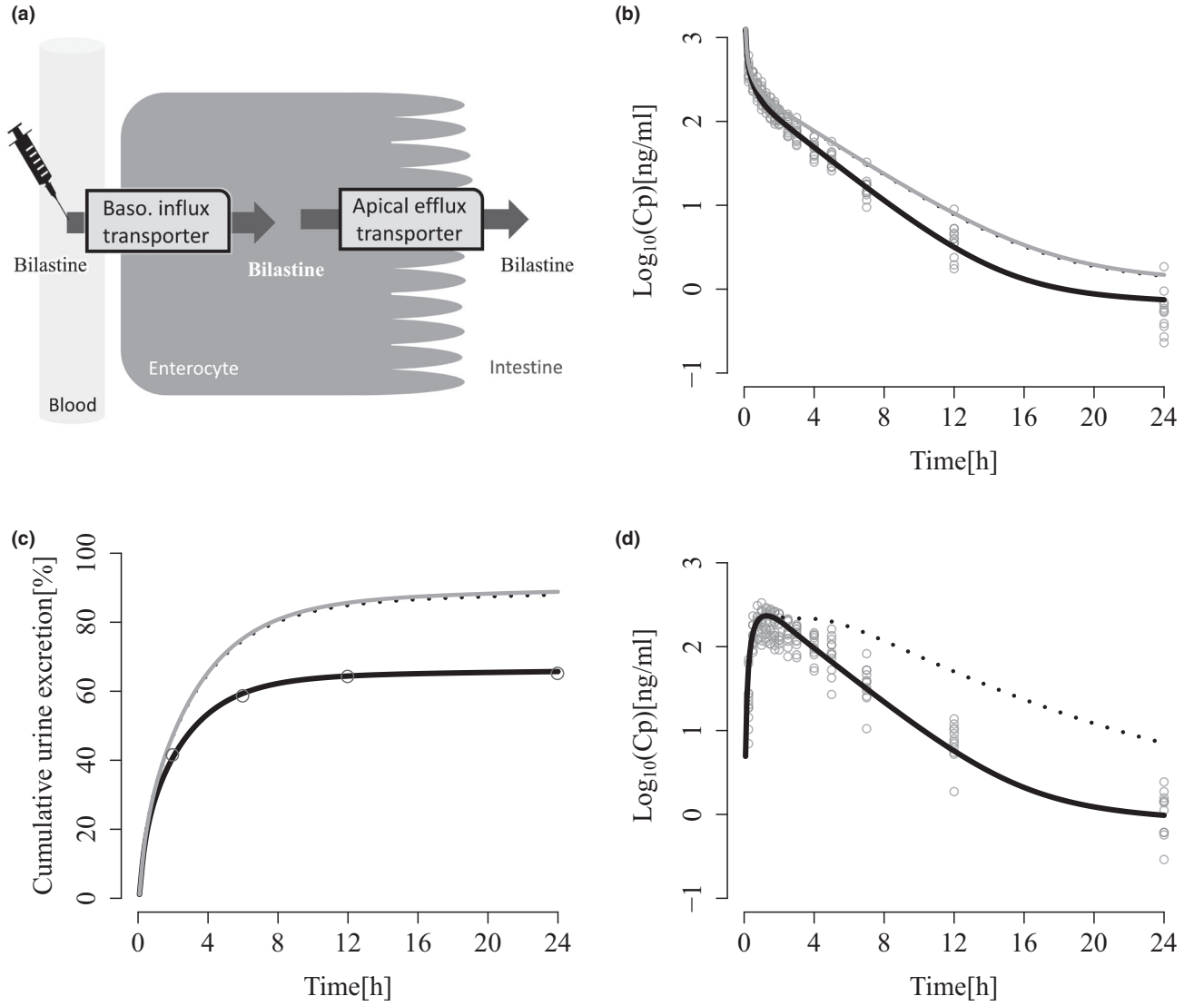

FIGURE 2 Bilastine PBPK model in young adults. (a) Schematic diagram of proposed intestinal transporters involved in bilastine disposition. (b) Predicted versus observed plasma concentrations after 10 mg single i.v. dose (solid black line: final model; dotted line: model without basolateral influx transporter; grey solid line: model without both basolateral influx and apical efflux transporters; open circles: observations). (c) Predicted versus observed cumulative urine excretion after 10 mg single i.v. dose (solid line: final model; dotted line: model without basolateral influx transporter; grey solid line: model without both basolateral influx and apical efflux transporters; open circle: mean observations. (d) Predicted versus observed plasma concentration after single 20 mg p.o. dose (solid line: final model; dotted line: model without Cs adjustment; open circles- observations).

account for lower colonic absorption and avoid overpredictions of bilastine's absorption (black dashed line).

Evaluation of suitability of bilastine 20 mg q.d. oral dose in geriatrics

Figure 3 shows the overlay of predicted median (dashed line) and 95% CIs (light grey shaded area) from young adults with the predicted median (solid black line) and 95% CI (dark grey area) from the geriatric PopPK model (Figure 3a), the Senescence model (Figure 3b), and PBPK mean age of 70 years (Figure 3c), and mean age 80 years (Figure 3d). Moreover, Table 4 summarizes the PK metrics simulated with all the different models, including the extrapolation to older subjects (mean of 80 years). The predictions performed with all the three models felt within the simulations performed with the PopPK model in young adults[10] supporting the adequacy of the 20 mg q.d. oral dose in the geriatric population.

DISCUSSION

Establishing dosing recommendation in underrepresented groups in clinical trials is challenging not only due to lack of data but also due to insufficient understanding on the effect

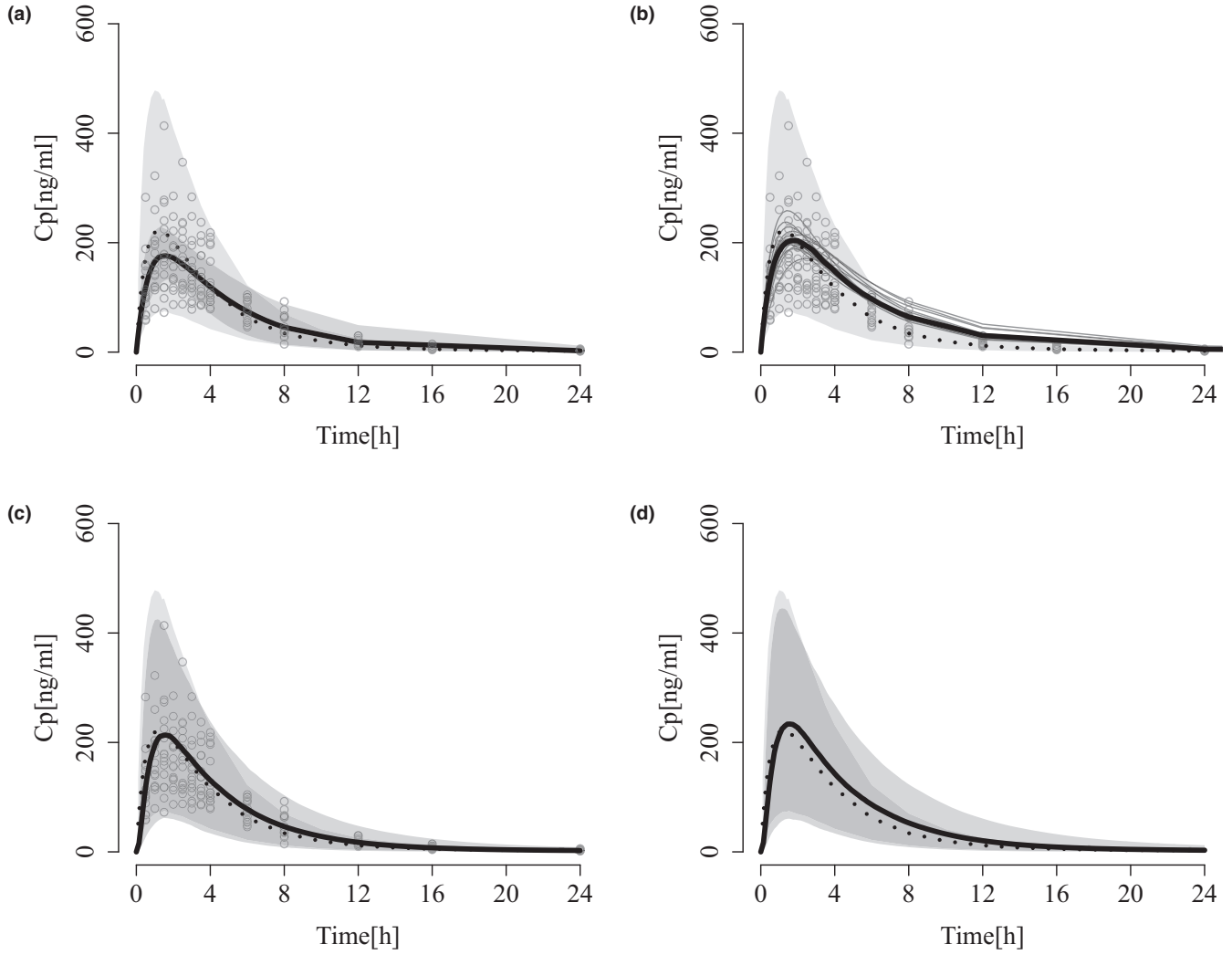

FIGURE 3 Evaluation of the appropriateness of the 20 mg dose in geriatrics with the different models: (a) geriatric PopPK, (b) Senescence, (c) PBPK at mean age of 70 years, and (d) PBPK at mean age of 80 years (dotted line: median PopPK young adults; light grey shaded area: 95% PopPK young adults; solid black lines and dark grey shaded area: median and 95% CI from models (a) Geriatric PopPK, (b) Senescence, and (c, d) PBPK in 70 and 80 year old subjects; light gray lines in (b): individual predictions Senescence model; grey dots: observations). CI, confidence interval; PBPK, physiologically-based pharmacokinetic; PopPK, population pharmacokinetic

TABLE 4 Model prediction of median and 95% CI C_{max} and median AUC

	C_{max} (ng/ml)	AUC (ng·h/ml)
Young adults PopPK ($N = 5000$)	223.29 [74.88–478.12]	1103.88 [370.03–2311.15]
Geriatric PopPK ($N = 1500$)	176.02 [133.04–227.98]	1129.40 [588.65–1891.86]
Senescence at 70 ($N = 16$)	204.06 [170.91–258.51]*	1478.14 [1222.83–2010.15]*
PBPK at 70 ($N = 1500$)	213.39 [62.10–425.26]	1176.82 [280.17–2464.71]
PBPK at 80 ($N = 1500$)	233.63 [60.62–445.22]	1307.59 [278.66–2813.93]

Note: Young adults AUC from the observation: mean = 1160 ng h/ml; range: 481–2528 ng·h/ml.

Young adults C_{max} from the observation: mean = 260 ng·h/ml; range: 63–924 ng/ml.

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, maximum plasma concentration; PBPK, physiologically-based pharmacokinetic; PopPK, population pharmacokinetic.

*Due to sparse *N* size, minimum value and maximum value are presented.

of their physiological and clinical characteristics on drug PK and/or PD. Conventional PopPK analysis can be conducted if clinical trial data exist. However, there is usually a limitation in the number of participants from special populations in the development of drugs restraining the performance of covariate analysis and reducing the confidence

of dosing recommendations for these populations. To overcome these limitations, physiologically based approaches have been proposed.[17,22,23] Allometric scaling of PK parameters by using predictive biomarkers such as body weight or body surface area (BSA) in children would be one of the well-recognized strategies frequently used for establishing dosing recommendations in pediatrics. However, when a case to be studied is even more complicated, such as geriatrics, establishing dosing recommendations still remains to be solved.[24] Why the need is yet unmet is mainly because of the substantial heterogeneity of the geriatric group derived from various aging rate/extent and high number of comorbidities and comedications.[25] This impedes using common scaling factors of dose adjustment, such as age, weight, or BMI.[24,26] Eventually, this issue requires conducting highly personalized approaches for guiding dosing in older adults based on a thorough understanding of physiological/pathological characteristics in a specific subject. Conducting head-to-head clinical trials examining every possible scenario is neither time nor cost efficient. Hence, testing different dosing scenarios in virtual subjects using modeling and simulation provides a great alternative to support precise dosing recommendations. The present study proposed two mechanistic-based approaches to support dose recommendations in healthy geriatrics using bilastine as a case example. The application is being expanded to patients with comorbidities and comedications.

The strength of the Senescence model includes that it utilizes the existing PopPK model structure in young adults, thereby saving time and efforts. In addition, the model was used to confirmed that key age-dependent variables impacting bilastine disposition already identified in pediatrics, such as glomerular filtration rate (GFR) that were essential to obtain accurate predictions of plasma concentrations in elderly subjects. Interestingly, a previous research showed that the volume of distribution relates with the physiological total body water (TBW) in rats and dogs.[18] This relationship was assumed to be maintained in humans and successfully used to predict the volume in young adults and then also in children.[14,17,18] However, this consideration did not work for elderly subjects where both TBW and body fat were needed for accurate predictions of this parameter. This aging effect was expected considering bilastine lipophilic nature and the 10–15% decrease in TBW and 20–40% increase in the total body fat in older adults with respect to younger subjects.[27] The renal clearance in the Senescence model was scaled by means of a function that incorporates age-related changes in the GFR and in unbound fraction (fu). A ratio CLtotal/CLrenal greater than 1 supported the hypothesis of secretion mechanism in young adults. This ratio was used to empirically correct for additional mechanisms affecting bilastine CL in the Senescence model. Considering that bilastine is mostly eliminated renally by glomerular filtration without significant metabolism or biliary excretion as well as the evidence of clinical involvement of transporters in the PKs, this additional mechanism is expected to be the P-gp, contributing to the fecal secretion of the drug (as was further investigated and confirmed with the PBPK model). The estimated total apparent clearance (CL/F) from the Senescence (12.78 L/h) and geriatric PopPK (18.04 L/h) model were slightly smaller than that of the young adult PopPK model from Jauregizar et al. 2009 (18.10 L/h). The aligned CL/F values (key PK parameter for the exposure of repeated doses) from these three models strongly suggest that there may be no need for dose adjustment in geriatric subjects. The Senescence model could be further applied to predict the PK in older adults by using published demographics from this population, and could be an alternative when the development of a full PBPK model is not doable due to, for example, insufficient data.

The second approach utilized in this project was the PBPK model. Generally, this type of model needs more time and resources to be developed. Nevertheless, once developed, they provide the framework to investigate hypothesis on the mechanisms involved in drugs PK. As an example, the PBPK model developed in the present study aided to investigate the influence of transporters on drug PK and support the dose selection in healthy geriatrics. Additionally, due to the comprehensive and integrative consideration of the physiology in the model, it may also facilitate, with some modifications and adjustments, the prediction of bilastine PK in geriatrics with comorbidities and/or comedications. Based on strong evidence of the interaction between P-gp and bilastine, an apical efflux transporter representing the P-gp was initially introduced.[9,15] However, the sole inclusion of the apical efflux transporter was not able to fit on the i.v. data. Particularly, the cumulative urine excretion data indicated that 66% of the 10 mg i.v. dose was excreted via urine in humans but the model overpredicted the cumulative urine excretion when the basolateral transporter was not considered, or when transporters were considered in isolation.[2] Bilastine permeation into the enterocyte without introducing a basolateral influx intestinal transporter was highly restricted. The consequence was almost no exposure of the drug to the P-gp after i.v. administration. These results suggested that along with the P-gp other basolateral influx transporter in the enterocyte may be involved in bilastine secretion in humans, probably the OCT1, although only moderate evidence was found in vitro with the higher dose tested.[15] Additional research is needed to further support this finding and to investigate the effect of aging on drug transporters in the gut wall. However, the results of the study do not suggest a significant impact of age-related changes at this level.

The initial PBPK p.o. model predicted a substantial amount of bilastine absorbed in the large intestine. Consequently, the predicted plasma profile looked similar to that of a sustained release formulation and was far from the observations. Ungell et al., in 1998, reported that a lipophilic drug, which has relatively high *logD* shows a tendency to have higher colonic permeability than their jejunal permeability. This becomes the basis for developing the ASF model in GastroPlus 9.6.[21,28] The default values of the ASF model parameters were estimated by regression from multiple set of compound data, thus do not guarantee to work for every case. Especially, like in the case of bilastine where intestinal transporters are involved, the prediction of ASF with respect to *logD* alone is no longer valid, and the default fitted constants for ASF need to be drug-specifically adjusted.[21] Even after introducing transporters in the model, the PBPK p.o. model still predicted high absorption in large intestine (caecum: 44.5%, ascending colon: 21.0% of total dose). To solve this issue, we optimized the values for the fitted constants of the colon part, C3 and C4, being the final values 0.05 and 0, respectively. The model could then properly fit the observations with a reasonable regional gastrointestinal (GI) absorption (caecum: −1.4%, ascending colon: −1.5% of total dose). The need of optimizing C3 and C4 to values close or equal to 0 while keeping default values for C1 and C2 supports the hypothesis that P-gp's efflux capacity and/or its distribution within the GI track may not be constant, whereas the impact of the P-gp on the large intestine may be higher than in other GI regions.[29,30]

The predictions of the Senescence, PBPK, and geriatric PopPK models showed that drug exposures were similar to that from the young adults. Median predictions with all the three geriatric models were within the 95% CI of young adults, and the median values were not much deviated from young adult median. As the Senescence model and geriatric PopPK model were based on BILA/459-05 demographics and its PK data, respectively, variabilities of the two model were very narrow (small number of subjects and homogenous demographic distribution). Additionally, although the PBPK model predicted AUC and C_{max} values tend to increase with aging and its 95% prediction interval of the age of 80 years were slightly wider, the prediction interval was very similar to the 95% CI of young adults, supporting that the current dose recommendation of 20 mg q.d. p.o. is also suitable not only for young geriatrics (i.e., elderly subjects in trial BILA/459-05; mean age of 68.69 years) but also for the older subjects. The safety of the 20 mg dose in geriatric has been further evaluated in a safety trial involving 150 elderly subjects as part of bilastine risk management that demonstrated the favorable safety profile with a low incidence of treatment-emergent adverse events.[31]

In conclusion, this study demonstrated the utility of mechanistic modeling in proposing dose recommendations in geriatric subjects. Specifically, we evaluated the posology of bilastine in geriatrics using two different mechanistic-based models, the Senescence (PopPK-based semi-mechanistic model) and the PBPK model. Considering the lack of guidance documents for model-informed dosing recommendation in geriatrics, and the insufficient understanding of aging processes, convergence of the conclusion from the different approaches reinforces and supports each model's output. This research showed that a dual PopPK-PBPK approach can be applied to support clinical decision making for underrepresented groups in traditional clinical trials.

CONFLICT OF INTEREST

C.C. and A.G.-B. are employees of FAES FARMA, S.A. All other authors declared no competing interests for this work.

AUTHORS' CONTRIBUTION

C.K., V.L.R., M.R., J.C.L., N.L., C.C., A.G., E.S., S.S., and V.V. wrote the manuscript. M.R. and V.V. designed the research. C.K., V.L.R., J.C.L., M.R., and V.V. performed the research. C.K., V.L.R., J.C.L., and V.V. analyzed the data.

REFERENCES

1. Rodríguez M, Vozmediano V, García-Bea A, et al. Pharmacokinetics and safety of bilastine in children aged 6 to 11 years with allergic rhinoconjunctivitis or chronic urticaria. *Eur J Pediatr*. 2020;179(5):801-805.
2. Sadaba B, Azanza JR, Gomez-Guiu A, Rodil R. Critical appraisal of bilastine for the treatment of allergic rhinoconjunctivitis and urticaria. *Ther Clin Risk Manag*. 2013;9:197-205.
3. Kawauchi H, Yanai K, Wang D-Y, Itahashi K, Okubo K. Antihistamines for allergic rhinitis treatment from the viewpoint of nonsedative properties. *IJMS*. 2019;20(1):213.
4. Wang DY, Wang XY, Lim-Jurado M, Prepageran N, Tantilipikorn P. Treatment of allergic rhinitis and urticaria: a review of the newest antihistamine drug bilastine. *Therapeut Clin Risk Manage*. 2016;12:585-597.
5. Corcostegui R, Labeaga L, Innerarity A, Berisa A, Orjales A. In vivo pharmacological characterisation of bilastine, a potent and selective histamine H1 receptor antagonist. *Drugs R & D*. 2006;7(4):219-231.
6. Horak F, Zieglmayer P, Zieglmayer R, Lemell P. The effects of bilastine compared with cetirizine, fexofenadine, and placebo on allergen-induced nasal and ocular symptoms in patients exposed to aeroallergen in the Vienna Challenge Chamber. *Inflamm Res*. 2010;59(5):391-398.
7. Simons FER, Simons KJ. H1 antihistamine: Current status and future directions. H 1 Antihistamines: Current Status and Future Directions. World Allergy Organization Journal | Full Text (biomedcentral.com). Published online 2008:11.
8. Schlender J-F, Vozmediano V, Golden AG, et al. Current strategies to streamline pharmacotherapy for older adults. *Eur J Pharm Sci*. 2018;111:432-442.
9. SmPC. Ilaxten 20 mg tablets (SmPC). Published online August 22, 2019. https://www.medicines.org.uk/emc/product/4551/smpc.
10. Jauregizar N, de la Fuente L, Lucero ML, Sologuren A, Leal N, Rodríguez M. Pharmacokinetic-pharmacodynamic modelling of the antihistaminic (H 1) effect of bilastine. *Clin Pharmacokinet*. 2009;48(8):543-554.

11. Herrera AP, Snipes SA, King DW, Torres-Vigil I, Goldberg DS, Weinberg AD. Disparate inclusion of older adults in clinical trials: priorities and opportunities for policy and practice change. *Am J Public Health*. 2010;100(S1):S105-S112.
12. Center for Drug Evaluation and Research. Center for Biologics Evaluation and Research. ICH. Studies in support of special populations: Geriatrics E7. Published online June 24, 1993. E7 Studies in Support of Special Populations: Geriatrics | FDA.
13. Center for Drug Evaluation and Research. Center for Biologics Evaluation and Research. ICH. E7 Studies in Support of Special Populations: Geriatrics Question & Answers. Published online July 6, 2010. E7 Studies in Support of Special Populations; Geriatrics; Questions and Answers | FDA.
14. Vozmediano V, Sologuren A, Lukas JC, Leal N, Rodriguez M. Model informed pediatric development applied to bilastine: ontogenic PK model development, dose selection for first time in children and PK study design. *Pharm Res*. 2017;34(12):2720-2734.
15. Lucero ML, Gonzalo A, Ganza A, et al. Interactions of bilastine, a new oral H1 antihistamine, with human transporter systems. *Drug Chem Toxicol*. 2012;35 (Suppl 1):8-17.
16. Byon W, Smith M, Chan P, et al. Establishing best practices and guidance in population modeling: an experience with an internal population pharmacokinetic analysis guidance. *CPT Pharmacomet Syst Pharmacol*. 2013;2(7):51.
17. Vozmediano V, Lukas JC, Encinas E, et al. Model-informed pediatric development applied to bilastine: Analysis of the clinical PK data and confirmation of the dose selected for the target population. *Eur J Pharm Sci*. 2018;2019(128):180-192.
18. Vozmediano V, Ortega I, Lukas JC, Gonzalo A, Rodriguez M, Lucero ML. Integration of preclinical and clinical knowledge to predict intravenous PK in human: Bilastine case study. *Eur J Drug Metab Pharmacokinet*. 2014;39(1):33-41.
19. Sádaba B, Gómez-Guiu A, Azanza JR, Ortega I, Valiente R. Oral availability of bilastine. *Clin Drug Investig*. 2013;33(5):375-381.
20. Sologuren A, Lucero M, Valiente R, Charles H, Mair S. Human mass balance with [14C]-bilastine following oral administration to healthy volunteers. *Basic Clin Pharmacol Toxicol*. 2009;105(Suppl. 1):106-107.
21. SimulationsPlus, Inc. GastroPlus Manual Version 9.6. Published online May 2018.
22. Emoto C, Johnson TN, McPhail BT, Vinks AA, Fukuda T. Using a vancomycin PBPK model in special populations to elucidate case-based clinical PK observations. *CPT Pharmacometrics Syst Pharmacol*. 2018;7(4):237-250.
23. Liu T, Ghafoori P, Gobburu VSG. Allometry is a reasonable choice in pediatric drug development. *J Clin Pharmacol*. 2017;57(4):469–475.
24. Lau SWJ, Schlender J-F, Slattum PW, Heald DL, O'Connor-Semmes R. Geriatrics 2030: developing drugs to care for older persons—a neglected and growing population. *Clin Pharmacol Ther*. 2020;107(1):53-56.
25. Schlender J-F, Meyer M, Thelen K, et al. Development of a whole-body physiologically based pharmacokinetic approach to assess the pharmacokinetics of drugs in elderly individuals. *Clin Pharmacokinet*. 2016;55(12):1573-1589.
26. Chetty M, Johnson TN, Polak S, Salem F, Doki K, Rostami-Hodjegan A. Physiologically based pharmacokinetic modelling to guide drug delivery in older people. *Adv Drug Deliv Rev*. 2018;135:85-96.
27. Stader F, Siccardi M, Battegay M, Kinvig H, Penny MA, Marzolini C. Repository describing an aging population to inform physiologically based pharmacokinetic models considering anatomical, physiological, and biological age-dependent changes. *Clin Pharmacokinet*. 2019;58(4):483–501.
28. Ungell AL, Nylander S, Bergstrand S, Sjöberg A, Lennernas H. Membrane transport of drugs in different regions of the intestinal tract of the rat. *J Pharm Sci*. 1998;87(3):360-366.
29. Gramatté T, Oertel R, Terhaag B, Kirch W. Direct demonstration of small intestinal secretion and site-dependent absorption of the β-blocker talinolol in humans*. *Clin Pharmacol Ther*. 1996;59(5):541-549.
30. Kagan L, Dreifinger T, Mager DE, Hoffman A. Role of P-glycoprotein in region-specific gastrointestinal absorption of Talinolol in rats. *Drug Metab Dispos*. 2010;38(9):1560-1566.
31. Sologuren A, Viñas R, Cordón E, et al. Open-label safety assessment of bilastine in elderly patients with allergic rhinoconjunctivitis and/or urticaria. *Allergy Asthma Proceed*. 2018;39(4):299-304.
32. Lasseter KC, Sologuren A, La Noce A, Dilzer SC. Evaluation of the single-dose pharmacokinetics of bilastine in subjects with various degrees of renal insufficiency. *Clin Drug Investig*. 2013;33(9):665-673.
33. McNamara PJ, Alcorn J. Protein binding predictions in infants. *AAPS PharmSci*. 2002;4(1):19-26.
34. Edginton AN, Schmitt W, Voith B, Willmann S. A mechanistic approach for the scaling of clearance in children. *Clin Pharmacokinet*. 2006;45(7):683-704.
35. Edginton AN, Schmitt W, Willmann S. Development and evaluation of a generic physiologically based pharmacokinetic model for children. *Clin Pharmacokinet*. 2006;45(10):1013-1034.
36. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition*. 1989;5(5):303-311; discussion 312-313.
37. Chumlea WC, Guo SS, Kuczmarski RJ, et al. Body composition estimates from NHANES III bioelectrical impedance data. *Int J Obesity*. 2002;26(12):1596-1609.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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