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# **In silico dose prediction for long-acting rilpivirine and cabotegravir administration to children and adolescents**

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## Abstract

**Background and Objectives:** Long-acting injectable (LAI) antiretrovirals (ARVs) represent a pharmacological alternative to oral formulations and an innovative clinical option to address adherence and reduce drug costs. Clinical studies in children and adolescents are characterised by ethical and logistic barriers complicating the identification of dose optimisation. Physiologically-based pharmacokinetic (PBPK) modelling represents a valuable tool to inform dose finding prior to clinical trials. The objective of this study was to simulate potential dosing strategies for existing LAI depot formulations of cabotegravir and rilpivirine in children and adolescents (3-18 years) using PBPK modelling.

**Methods:** Whole-body PBPK models were developed to represent the anatomical, physiological, molecular processes and age related changes in children and adolescents through allometric equations. Models were validated for LAI intramuscular (IM) cabotegravir and rilpivirine in adults. Subsequently, the anatomy and physiology of children and adolescents was validated against available literature. The optimal doses of monthly administration of cabotegravir and rilpivirine were identified in children and adolescents, in order to achieve trough concentrations over the target concentrations derived in a recent efficacy trial of the same formulations.

**Results:** Pharmacokinetic data generated through the PBPK simulations were similar to observed clinical data in adults. Optimal doses of LAI ARVs cabotegravir and rilpivirine were predicted using the release rate observed for existing clinical formulations, for different weight groups of children and adolescents. The IM loading dose and maintenance dose of cabotegravir ranged from 200 – 600 mg and 100 – 250 mg respectively and for rilpivirine it ranged from 250 – 550 mg and 150 – 500 mg respectively across various weight groups of children ranging from 15-70 kg.

**Conclusions:** The reported findings represent a rational platform for the identification of suitable dosing strategies and can inform prospective clinical investigation of LAI formulations in children and adolescents.

## **Key Points**

- Mathematical models defining anatomical, physiological and molecular processes were constructed to simulate drug pharmacokinetics in children and adolescents.
- Two clinically available long-acting formulations of cabotegravir and rilpivirine were utilised to report minimum doses needed in paediatric individuals relative to their weight.
- Evaluation of a mathematical model to identify minimum doses in children and adolescents represents an innovative method to inform dosing strategies in various kinds of population over a range of therapeutic areas.

## 1. Introduction

HIV (Human Immunodeficiency Virus) is one of the leading causes of death that is treated as a global priority. Initiation of HAART (Highly Active AntiRetroviral Therapy) has saved millions of lives in the past decade [1]. However, adherence to antiretroviral therapy continues to be one of the major issues hindering treatment efficacy and suboptimal adherence can extremely vary in patients ranging from 50 to 70 % in the clinical setting [2]. Currently available formulations necessitate lifelong, daily dosing and poor adherence has been attributed to numerous factors including pill fatigue, side effects and a range of socioeconomic considerations associated with different populations [3]. Problems can be particularly exacerbated in specific sub-populations of patients such as paediatric patients, where drug administration is additionally influenced by the caregiver, the family or the social environment [4].

Long acting injectable (LAI) formulations have the potential of solving the adherence issues related to oral formulations, reducing the amount of antiretroviral (ARV) used for the therapy and consequently the cost of therapy. The use of LAI formulations in paediatric patients has already been hypothesised in different disease areas and the use of LAI antipsychotics has been recently described in adolescents [5-7].

Two LAI ARV formulations have recently been developed and several others are currently under investigation [1]. Rilpivirine and cabotegravir, due to long half-life and potency, have been selected for monthly and quarterly LA administration, respectively [1, 8]. Clinical studies investigating the combination of cabotegravir and rilpivirine LAI formulations are currently ongoing to assess its safety and efficacy in adults [9]. Recent clinical trials (LATTE and LATTE-2) conducted in HIV infected adults show that cabotegravir and rilpivirine combination are safe and efficacious which provide similar antiviral activity as efavirenz plus NRTIs – tenofovir and emtricitabine [10]. The combination of rilpivirine and cabotegravir has the potential of being the first long-acting antiretroviral regimen that will not require daily oral dose of any companion drugs, representing a pivotal achievement in the antiretroviral pharmacology. However, the identification of safe and effective dosing strategies for paediatric patients is complicated by multiple factors. Differences in anatomical and physiological characteristics of children and adolescents compared to adults have a relevant effect on ADME processes and are not correctly captured through traditional allometric scaling approaches [11]. Additionally, logistic and ethical challenges in designing dose finding/optimisation studies have limited medical guidance [12].

Physiologically based pharmacokinetic (PBPK) modelling represents a valuable tool to optimise doses prior to clinical trials in paediatric patients thus minimising the time and cost invested in optimising doses. PBPK modelling is the mathematical description of anatomical, physiological and molecular processes defining pharmacokinetics. Compared to techniques usually used to select paediatric doses of adult formulations [13-16], PBPK modelling is a bottom up approach which integrates *in vitro* data such as apparent intestinal permeability, intrinsic clearance, protein binding, etc. in a mathematical description of ADME to predict *in vivo* pharmacokinetics [17].

Previous studies identified trough concentration of 1.2 µg/ml and 17 ng/ml for cabotegravir and rilpivirine respectively need to be achieved to warrant efficacy [18, 19]. No toxicity limited concentrations has been reported previously [1]. Therefore, the aim of this study was to simulate pharmacokinetics and inform optimal doses of LAI intramuscular (IM) formulations of cabotegravir and rilpivirine in at 95 % of children and adolescents aged 3 to 18 years through PBPK modelling for HIV treatment.

## 2. Methods

The PBPK models were constructed using Simbiology<sup>®</sup> v.4.3.1, a product of Matlab<sup>®</sup> v.8.2 (MathWorks, Natick, MA, USA 2013). Instant and uniform distribution of drugs into tissues, no reabsorption of the drug from the large intestine and a blood-flow limited model [20] were assumed. A previously published adult IM PBPK model was used in this study [21]. Cabotegravir and rilpivirine LAI IM pharmacokinetics were simulated and validated in adult PBPK models and later optimised for different weight categories of children (3-12 years) and adolescents (12-18 years). Children and adolescents between the ages 3-18 years were divided into WHO weight groups [22] and 100 virtual individuals were generated in each weight category.

### 2.1 Anatomy

Adult PBPK models were defined by key characteristics such as age and weight of the individual. These defining characteristic values were further used for the computation of organ and tissue volumes, as well as blood flow rates through allometric equations described by Bosgra et al. [23]. The anatomy and physiology of children and adolescents were obtained from various literature sources, validated against available clinical data prior to dose optimisation [23-33]. To improve the confidence of the constructed paediatric PBPK models, validation against intravenous lorazepam and intramuscular ceforanide as reference drugs was also conducted [34]. The various equations used for the construction of paediatric PBPK models and validation across different ages are available in Online Resource 1.

### 2.2 Simulation of ADME processes

Drug diffusion from the IM compartment was assumed to obey first order rate kinetics and the equation was obtained from Tegenge et al.[35]. The release rate of cabotegravir was obtained from the literature [36] and for rilpivirine, was derived using 48-week clinical data from LATTE-2, a recent Phase 2 efficacy trial of these two formulations used in combination [19]. The intrinsic clearance values derived from *in vitro* data were obtained from the literature [37] and extrapolated to systemic clearance [38]. The distribution of drug to different organs and tissues was simulated using previously published equations [21].

### 2.3 Model validation

The physicochemical properties of cabotegravir and rilpivirine used in the model are presented in Table 1. The validation of the drug properties against clinical data was conducted in 100 virtual adults for a 800 mg quarterly dose of cabotegravir (from weeks 12-28) and for a subsequent monthly dose of 900 mg rilpivirine (after the

initial dose of 1200 mg) [1]. The release rate of rilpivirine was identified from the clinical data using the PBPK model [1]. The release rate was also validated against the LATTE-2 pharmacokinetic curve of cabotegravir and rilpivirine. The cabotegravir release rate was assumed to be  $4.54 \times 10^{-4} \text{ h}^{-1}$  to be same as in LATTE-2 (or prior adult studies); however there was a decrease in the release rate of rilpivirine from  $9 \times 10^{-4}$  to  $5 \times 10^{-4} \text{ h}^{-1}$ , since the rilpivirine formulation included in LATTE-2 was different from previous investigation and [19] with a slower release rate [1]. A schematic of the LATTE-2 dosing regimen implemented in this study is shown in Fig 2.

## 2.4 Dose prediction

After the validation of the physicochemical parameters, the anatomy and physiology were modified to describe children and adolescents using appropriate allometric equations obtained from the literature, as described in the Online Resource 1 [12, 23, 25-27, 39, 40]. Following IM injection, dose optimisation in healthy paediatric individuals was conducted such that at least 95 out of the 100 virtual individuals had a mean trough concentration ( $C_{\text{trough}}$ ) over the target trough concentrations for the required duration. Based on the LATTE-2 study, a target  $C_{\text{trough}}$  of 1.35  $\mu\text{g/ml}$  was used as the minimum target trough concentration for cabotegravir dose predictions following 10 mg oral dose, and 70 ng/ml was used as the average concentration ( $C_{\text{av}}$ ) for rilpivirine following a 25 mg dose [19]. An oral dosing regimen for 4 weeks (steady state) followed by a loading dose and eleven maintenance doses for a 4-weekly IM administration of rilpivirine and cabotegravir were simulated, for a total period of 52 weeks.

## 2.5 Sensitivity analysis

A differential sensitivity analysis was performed to identify the key parameters that impact the pharmacokinetic profiles of LA formulations [41]. Analysis was performed for the loading dose and the first maintenance dose of cabotegravir and rilpivirine LAI IM formulation in adults. Sensitivity was analysed using the provided inbuilt feature of Simbiology at user-defined values without normalisation in the computation. Six parameters – blood-to-plasma ratio, cardiac output, plasma clearance, liver weight, fraction unbound and release rate were analysed against drug plasma concentrations. Each parameter was varied by 20% from its mean value and 100 simulations were conducted while keeping the rest of the parameters constant. The sensitivity coefficient ( $\phi_i$ ) indicates the change of plasma concentration values (Y) with respect to a unit change in a parameter (X) as shown in equation 1 [41].



$$\phi_i = \frac{\% \Delta Y}{\% \Delta X} \quad (1)$$

### 1 3. Results

2 The structure and equation of the current PBPK model are based on a previous publication and modified to  
3 represent antiretroviral distribution in paediatric and adolescent individuals [21]. The anatomy and physiology  
4 of children and adolescents was also validated against literature and the results are presented in Online Re-  
5 sources 1. PBPK models were initially qualified by validation against available clinical data for both cabo-  
6 tegravir and rilpivirine in adults to ensure that the selected drug properties were appropriate. The mean simu-  
7 lated pharmacokinetic parameters for maximum concentration ( $C_{max}$ ),  $C_{trough}$  and area under the concentration-  
8 time curve (AUC) were compared against available clinical data for the LA formulations for both drugs used in  
9 adults (cabotegravir – second IM dose of 800 mg and rilpivirine – 900 mg after the initial dose of 1200 mg)  
10 (shown in Table 2 and Fig. 1). A stringent qualification of accuracy was applied whereby PBPK models were  
11 considered validated only if the mean value was within 0.5-fold from the clinical value, rather than the conven-  
12 tional 2-fold agreement limits [42].

13 The formulation characteristics were maintained equal to the adult formulation for the simulations in chil-  
14 dren and adolescents assuming a similar release rate of the drugs from the formulations, and the use of the same  
15 formulations in adult, children and adolescents. IM doses were optimised to have a pharmacokinetic profile  
16 with concentration exceeding the 10 mg PO  $C_{trough}$  for cabotegravir over the duration of treatment and an aver-  
17 age concentration over the  $C_{trough}$  of 25 mg PO rilpivirine for the first 12 IM doses (Fig. 3). For rilpivirine, it was  
18 also ensured that the concentrations were always above the 90% protein binding adjusted inhibitory concentra-  
19 tion (PAIC<sub>90</sub>) value of 12.1 ng/ml [18] subsequent to the loading dose. Summary of predicted doses for both  
20 cabotegravir and rilpivirine for different weight categories are shown in Table 3.

#### 21 3.1 Cabotegravir

22 The validation for 800 mg IM cabotegravir resulted in mean predicted AUC,  $C_{max}$  and  $C_{trough}$  values that  
23 were +15.6 %, +6.1 % and +9.1 % compared to clinical values, respectively [1]. A target trough concentration  
24 of 1.35 µg/ml (10 mg PO  $C_{trough}$ ) was chosen from the literature [1]. The doses for different weight groups were  
25 informed such that at least 95 out of the 100 virtual individuals had a  $C_{trough}$  value over the target trough concen-  
26 tration for a duration of 48 weeks (Fig 4). The daily oral dose administered for a period of 4 weeks was 10 mg  
27 for weights ranging between 14-50 kg and 20 mg for weights between 50-70 kg. For IM cabotegravir, the load-  
28 ing dose ranged between 200-600 mg and maintenance doses between 100-250 mg for the simulated plasma  
29  $C_{trough}$  to stay over the 10 mg PO  $C_{trough}$  as described in Table 3.

### 30 3.2 Rilpivirine

31 The simulated mean AUC,  $C_{max}$  and  $C_{trough}$  values were +13.2 %, -6.5 % and -8.8 %, compared to the clinical data [1]. After the validation of rilpivirine PBPK model, the first order kinetic release rate was identified to be  $9 \times 10^{-4} \text{ h}^{-1}$  [1]. The validation was then performed to find the optimal release rate for rilpivirine pharmacokinetics from the LATTE-2 study. Due to reformulation of the rilpivirine, the optimal release rate was observed to be  $5 \times 10^{-4} \text{ h}^{-1}$ . The optimal doses were informed for different weight categories such that the average drug  $C_{trough}$  plasma concentrations of 48 weeks remained over 70 ng/ml (25 mg PO  $C_{trough}$ ) [19]. A fixed daily oral dose of 25 mg was administered for 4 weeks prior to IM doses. The loading dose ranged from 250 – 550 mg and the maintenance doses from 200 – 500 mg across weight groups from 15-70 kg individuals. The optimal doses ensured plasma concentrations over the PAIC<sub>90</sub> value and average IM concentrations over 25 mg PO  $C_{trough}$  for at least 95 out of 100 individuals (Fig 5).

### 41 3.3 Sensitivity Analysis

42 Fig. 6 shows the mean differential sensitivity analysis plot of 100 runs for six chosen parameters with respect to time. The analysis was performed for two successive (loading and maintenance) monthly IM doses of cabotegravir and rilpivirine in adults.

45 For cabotegravir, the analysis indicated that the plasma concentration is sensitive to only two of the six factors and higher influence was observed in the first days following administration. Cardiac output and systemic clearance of drug had higher sensitivity towards the variation in plasma concentrations. Protein binding, release rate, liver weight and blood-to-plasma ratio were negligibly sensitive. This indicates that physiological factors and the UGT content in the liver had a higher potential to influence the simulated pharmacokinetics. Sensitivity against cardiac output was negative for most of the duration indicating an increased effect against plasma concentration even when the value changes by  $\pm 20\%$  from the mean. Sensitivity against systemic clearance had a similar trend to cardiac output but with lower intensity. During the initial days after the administration of the maintenance dose, both these factors showed a positive relationship against plasma concentration indicating a lower effect.

55 For rilpivirine, the change in plasma concentration was not sensitive when cardiac output, liver weight and release rate varied  $\pm 20\%$  from the mean. Blood-to-plasma ratio had a higher positive effect immediately after dosing implying a lower influence on plasma concentration. Blood-to-plasma ratio and systemic clearance showed a positive relationship over the entire dosing period indicating a decreased effect against plasma concentration.

59 tration. Protein binding fluctuated between positive and negative, however the variation is minimal signifying  
60 minimal or no effect on plasma concentration.

## 61 4. Discussion

62 Optimal treatment adherence is essential for effective inhibition of viral replication and to mitigate devel-  
63 opment of resistance to ARVs. Although oral formulations have been demonstrated to result in therapeutic con-  
64 centrations, sub-optimal adherence in patients who are receiving oral daily dosing for treatment and prevention  
65 have been described [2, 43-46]. Alternative administration strategies could support higher adherence reducing  
66 the frequency of administration and addressing pill fatigue. More specifically, formulations allowing a monthly  
67 or quarterly administration could address the adherence issue, thus decreasing the risk of drug resistance. ARVs  
68 with high potency and favourable pharmacokinetics are essential for the development of the LAI strategy. The  
69 recent development of novel formulations of cabotegravir and rilpivirine constitute a remarkable step towards  
70 the definition of LAI strategies, providing innovative pharmacological tools for adults [1]. Dose optimisation in  
71 special populations of patients such as children and adolescents is complex due to their unique physiological and  
72 anatomical characteristics compared to adults. Traditionally, clinical trials have not been frequently conducted  
73 in these patient populations due to ethical and logistical considerations [47]. However recent regulations pro-  
74 mote clinical studies in paediatric patients to evaluate safety and efficacy prior to therapy [48, 49]. The present  
75 study focuses on the identification of dosing strategies of cabotegravir and rilpivirine in children and adolescents  
76 using computational PK modelling for HIV treatment.

77 Various PBPK models have been developed for adults and recently this modelling technique has also been  
78 used for a variety of special populations including children and adolescents [50, 51]. Drug distribution can be  
79 simulated in special populations of patients through the integration of age-related anatomical and physiological  
80 changes into the mathematical PBPK framework. PBPK modelling has been recently used for the prediction of  
81 midazolam and theophylline in neonates, infants and children [12]. In two other studies, the relationship be-  
82 tween adult and paediatric clearance rates was established using cytochrome P450 ontogeny for six compounds  
83 and then simulations were performed for five different drugs at different age groups [52, 53]. An oseltamivir  
84 PBPK model was used to predict pharmacokinetics in neonates and infants with influenza [54] and a disease-  
85 specific model was also recently developed in children with and without liver cirrhosis [55].

86 Both cabotegravir and rilpivirine are characterised by long-half lives and physicochemical properties that  
87 are compatible with nanoformulations for LAIs, represent attractive options for continuous therapy [1, 56]. Us-  
88 ing physicochemical properties and *in vitro* data, the pharmacokinetics of cabotegravir and rilpivirine in adults  
89 was validated against available clinical data. The model validation was conducted at the second dose of the LAI

90 ARVs to have a mathematical representation of the pharmacokinetics at steady-state. Low accuracy and preci-  
91 sion was observed in the ÉCLAIR study where the simulated  $C_{\text{trough}}$  value of cabotegravir was 1.35  $\mu\text{g/ml}$  com-  
92 pared to the observed value which was less than 0.66  $\mu\text{g/ml}$  ( $4 \times \text{PAIC}_{90}$ )[57]. Hence, stringent guidelines were  
93 applied for the validation process where  $\pm 50\%$  deviation from the mean clinical values was considered accept-  
94 able instead of the conventional 2-fold deviation [42]. The pharmacokinetic parameters – AUC,  $C_{\text{max}}$  and  $C_{\text{trough}}$   
95 simulated through the PBPK approach were in agreement with the clinical data and therefore our PBPK model  
96 was considered robust for predicting the LAI IM doses in children and adolescents. In the simulation of LAI  
97 pharmacokinetics in children, the release rates of the LAI formulations were maintained equal to the validation  
98 in adults, to facilitate bridging to paediatric simulation. Although the physiology of the muscular tissues is dif-  
99 ferent between adults and children, this could potentially support the use of the existing formulations in paediat-  
100 ric clinical studies with no further reformulation [58]. However additional studies are required since there is a  
101 possibility that smaller doses with less injection volume could decrease total surface area and strain in the mus-  
102 cle, thereby altering the pharmacokinetic profile. The doses were optimised such that cabotegravir and ril-  
103 pivirine concentrations were over the target trough concentrations (described in methods section) for the dura-  
104 tion of the dose. Although  $\text{PAIC}_{90}$  values indicate a trough concentration to suppress the virus *in vitro*, this does  
105 not translate in effective therapeutic activity *in vivo* [59]. Therefore, the dose optimisation was conducted con-  
106 sidering LATTE-2 study target trough concentrations.

107 The required dose was proportional to the weight of the individual which indicates increase in volume of  
108 distribution and systemic clearance in adolescents. As the weight of the individual increased from 15 to 70 kg,  
109 the required dose of cabotegravir tripled in an individual weighing 70 kg compared to a 15 kg individual  
110 whereas the dose needed was just over double in the case of rilpivirine. Fluctuation in maximum and trough  
111 plasma concentration of cabotegravir is  $>2 \mu\text{g/ml}$  compared to rilpivirine ( $<100 \text{ ng/ml}$ ). Also cabotegravir is  
112 more sensitive to variations in clearance and cardiac output compared to rilpivirine (as shown in Fig. 6) and due  
113 to these physiological variations across weight groups, higher dose is required in case of cabotegravir for ado-  
114 lescents compared to children. This indicates that doses cannot be linearly extrapolated based on weight and a  
115 deeper understanding of important mechanistic processes influencing the pharmacokinetics in children and ado-  
116 lescents is required. The loading doses are higher compared to the maintenance doses as the extra dose is essen-  
117 tial to maintain drug plasma concentrations over the  $C_{\text{trough}}/\text{PAIC}_{90}$  values. Since the maintenance dose for cabo-  
118 tegravir is low compared to rilpivirine, they could be more suitable for a less frequent (bimonthly or quarterly)  
119 administration.

120 LAI formulations may improve the problems faced with low adherence of therapies in children and adoles-  
121 cents. The identification of optimal doses in healthy paediatric individuals should be given priority as most of  
122 the doses for prescribed drugs are simply scaled from adult doses with varying success. However pain involved  
123 during the administration of IM injections has the potential to refrain children from preferring this route and  
124 opting for oral dosing regimens. Chloramphenicol dose scaling from adults in neonates and infants reached toxic  
125 levels which led to higher mortality rate, an example where the developmental pharmacology of paediatric pa-  
126 tients was ignored [60]. Mortality rate was high in neonates affected with kernicterus who were administered  
127 penicillin/sulfisoxazole than with oxytetracycline in an another case [61]. In both these cases, an immature glu-  
128 curonidation system led to the accumulation of drug, resulting in high plasma concentrations and conclusively  
129 demonstrating that the physiological processes of the child cannot always be accounted for by scaling adult  
130 doses [61, 62].

131 Although the simulated doses for children and adolescents could represent a valuable guideline for drug  
132 safety and efficacy clinical studies, the applied modelling strategies have some limitations. Numerous barriers  
133 can complicate the implementation of dosing recommendations for special populations. Since anatomical and  
134 physiological changes in children follow non-linear trend, pharmacokinetic, pharmacodynamic investigations  
135 need to be conducted to evaluate the safety, efficacy and tolerability profiles in children and the current model-  
136 ling approach can support a rational identification of suitable dosing strategies [63]. Especially in infants and  
137 neonates younger than three years, ontogeny of CYP450 expression in the liver and wide variation in organ  
138 weights and volumes could lead to low accuracy in model prediction and hence this study focuses on children  
139 older than three years. Some anatomical and physiological features and the associated complex biological proc-  
140 esses have not been simulated due to a paucity of relevant data [35]. Absence of information on drug transport-  
141 ers at the injection site could alter the absorption, distribution and metabolic processes which could not be cap-  
142 tured in the current PBPK model. Evidence suggests that cabotegravir undergoes enterohepatic recirculation,  
143 however quantitative evaluation of this physiological process is absent and hence could not be incorporated in  
144 the PBPK model [64]. Recent investigation with paliperidone LAI micro suspension revealed formation of a  
145 granuloma due to macrophage accumulation surrounding the site of injection. This phenomenon further con-  
146 trolled drug release from the depot and evidence also showed drug uptake and release from macrophages [65].  
147 The extent of the occurrence of this phenomenon and the size of the depot could alter the release rates and  
148 thereby drug pharmacokinetics which was not accounted for in this study. Physiological and metabolic variation  
149 of muscle composition in children compared to adults was not accounted during the dose optimisation process

150 [58]. Low clinical  $C_{\max}$  compared to the simulated pharmacokinetic curve (Fig. 1) could be due to the fraction of  
151 drug distributed through the lymphatic circulation. Additionally, the potential adverse effects considering the  
152 differences in the anatomy and physiology of children compared to adults, prolonged exposure and inability to  
153 discontinue therapy once administered are important factors to be assessed before drug administration [3].

154 LAI therapy has attracted a great deal of attention in various therapeutic areas, including chronic HIV infec-  
155 tion. For example, the National Institutes of Health (NIH) recently provided support to set up a worldwide team  
156 involving researchers from academia and the pharmaceutical industry to facilitate development of LAI formula-  
157 tions for HIV. This Long-Acting/Extended Release Antiretroviral Resource Program (LEAP;  
158 [www.longactinghiv.org](http://www.longactinghiv.org)) includes a PBPK modelling service to facilitate the design of long-acting formulations  
159 for HIV and related infectious diseases. This kind of support may improve the efficiency of selection of formu-  
160 lations, doses, and dose intervals for paediatric and other special populations.



161 **5. Conclusion**

162 PBPK models were successfully validated for both cabotegravir and rilpivirine LAI formulations against  
163 available clinical data in adults. A novel PBPK model for the prediction of PK in children and adolescent indi-  
164 viduals was developed to simulate dose selection in this vulnerable group. Dosing strategies for cabotegravir  
165 and rilpivirine were estimated in different weight groups of children and adolescents considering two efficacy  
166 target trough concentrations. From this modelling study, the predicted paediatric dosing of cabotegravir and  
167 rilpivirine differ for each weight category and scaling adult dose could have led to plasma concentration either  
168 below PAIC<sub>90</sub>/MEC value or above safe level. Different dosing fractions compared to adult dosages for  
169 cabotegravir and rilpivirine indicate that drug specific physicochemical parameters and ADME characteristics  
170 play a key role in controlling pharmacokinetics. PBPK predictions from this study could potentially inform  
171 reference doses required to conduct paediatric clinical trials for various weight categories.

172 **Compliance with ethical standards:**

173 **Source of Funding:**

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175 **Conducting of research:**

176 Rajith KR Rajoli has done the PBPK modelling. Rajith KR Rajoli and Marco Siccardi have written the manu-  
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178 manuscript.

179 **Conflict of Interest:**

180 David J Back receives consulting or advisor fees from Abbvie, Boehringer Ingelheim, Gilead, Janssen, Merck  
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182 and ViiV. Steve Rannard received funding from ViiV and AstraZeneca and have many HIV nanomedicine pat-  
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185 Merck, ViiV Healthcare, Janssen, Pfizer and AstraZeneca, consultancy from Merck and Norgine, and is a co-  
186 inventor of patents relating to HIV nanomedicines. Marco Siccardi has received research funding from ViiV and  
187 Janssen. Rajith KR Rajoli and Caren Freel Meyers have no conflicts of interest to declare.

188 **6. Tables**189 **Table 1** Physicochemical properties, *in vitro* and population pharmacokinetic data of ARVs

	<b>Cabotegravir</b>	<b>Rilpivirine</b>
<b>Molecular weight</b>	427	366
<b>log P<sub>o:w</sub></b>	1.04 [66]	4.32 [67]
<b>Protein binding</b>	99.30% [56]	99.70% [67]
<b>pK<sub>a</sub></b>	10.04 [66]	3.26 [67]
<b>R</b>	0.441 [68]	0.67 [67]
<b>Polar surface area</b>	99.2	-
<b>Hydrogen bond donors</b>	2	-
<b>Caco-2 permeability (cm/s)</b>	-	$12 \times 10^{-6}$ [67]
<b>CYP3A4 CL<sub>int</sub></b>	-	2.04 [67]
<b>UGT1A1 CL<sub>int</sub></b>	4.5 [37]	-
<b>UGT1A9 CL<sub>int</sub></b>	2.2 [37]	-
<b>Release rate (h<sup>-1</sup>)</b>	$4.5 \times 10^{-4}$ [36]	$9 \times 10^{-4}$ [1]

190 log P<sub>o:w</sub> – Partition coefficient between octanol and water; pK<sub>a</sub> – logarithmic value of the dissociation constant;  
 191 R – blood-to-plasma drug ratio; CL<sub>int</sub> – intrinsic clearance; CYP – cytochrome P450 (μl/min/pmol); UGT -  
 192 uridine diphosphate glucuronosyltransferase (μl/min/mg)

193 **Table 2** Validation of cabotegravir and rilpivirine after the second IM dose in adults: Clinical [1] vs. simulated  
 194 pharmacokinetic data

Drug	Dose (mg)	AUC		C <sub>max</sub>		C <sub>trough</sub>	
		Clinical	Predicted	Clinical	Predicted	Clinical	Predicted
<b>Cabotegravir</b>	800 mg quarterly	4467 (52)	5166 (23)	3.3 (59)	3.5 (21)	1.1 (140)	1.2 (24)
<b>Rilpivirine</b>	900 mg monthly	74,420 (35)	84,270 (44)	168 (37)	157 (42)	79.1 (44)	72.1 (45)

195 Values are represented as Geometric mean (% CV – coefficient of variation expressed as a percentage), AUC –  
 196 area under the concentration-time curve, C<sub>max</sub> – maximum plasma concentration, C<sub>trough</sub> – trough plasma concen-  
 197 tration. For Cabotegravir C<sub>max</sub> and C<sub>trough</sub> are µg/ml and AUC is µg × h/ml at day 84. For rilpivirine C<sub>max</sub> and  
 198 C<sub>trough</sub> are ng/ml and AUC is ng × h/ml at day 28

199 **Table 3** Prediction of the dose (in mg) for cabotegravir and rilpivirine for different weight categories of children  
 200 and adolescents with initial 4 weeks of oral dose followed by IM loading dose and 11 maintenance doses lasting  
 201 4-weeks each

Weight (kg)	Rilpivirine			Cabotegravir		
	Oral	Loading dose	Maintenance dose	Oral	Loading dose	Maintenance dose
<b>14 - 19.9</b>	25	250	150	10	200	100
<b>20 - 24.9</b>		250	200		250	100
<b>25 - 29.9</b>		250	200		250	100
<b>30 - 34.9</b>		300	250		350	150
<b>35 - 39.9</b>		350	300		350	150
<b>40 - 44.9</b>		400	300		400	150
<b>45 - 49.9</b>		450	350		450	150
<b>50 - 54.9</b>		450	400	20	450	200
<b>55 - 59.9</b>		500	400		500	200
<b>60 - 64.9</b>		500	450		550	200
<b>65 - 69.9</b>		550	500		600	250
<b>Target concentration in ng/ml (achieved by a PO dose in mg) [Reference]</b>	70 (25 mg PO C <sub>trough</sub> ) [19]				1370 (10 mg PO C <sub>trough</sub> ) [19]	

202 PO – oral route, C<sub>trough</sub> – trough concentration

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385

## 386 **Figure Legends**

387 **Figure 1** Validation of the PBPK model parameters against clinical data for the second IM administration in  
388 adults. a) Cabotegravir (800 mg followed by 800 mg quarterly) b) Rilpivirine (1200 mg followed by 900 mg  
389 monthly) [1]

390 **Figure 2** Validation of adult PBPK model using LATTE-2 dosing regimen. Oral dosing regimen was followed  
391 for 4 weeks, followed by a single 4-weekly intramuscular dose and eleven 4-weekly intramuscular maintenance  
392 doses. CAB – cabotegravir, RPV – rilpivirine, QD – once daily, Q4W – 4-weekly dose

393 **Figure 3** Validation of the release rate against clinical data from 48-week LATTE-2 study in adults. a) Cabo-  
394 tegravir b) Rilpivirine [19]. The target trough concentration is 1.35 µg/ml for cabotegravir and 12 ng/ml for ril-  
395 pivirine

396 **Figure 4** Plasma concentrations of cabotegravir loading and maintenance doses from week 4 to week 52 for  
397 different weight categories of children and adolescents. a) 14 - 19.9 kg, b) 25 - 29.9 kg, c) 35 - 39.9 kg, d) 45 -  
398 49.9 kg, e) 55 - 59.9 kg and f) 65 - 69.9 kg. The mean plasma concentrations are over the target trough concen-  
399 trations of 1.37 µg/ml. Concentration data were derived from optimized dosing strategies calculated for each  
400 weight band, as described in the Results section.

401 **Figure 5** Plasma concentrations of rilpivirine loading and maintenance doses from week 4 to week 52 for differ-  
402 ent weight categories of children and adolescents. a) 14 - 19.9 kg, b) 25 - 29.9 kg, c) 35 - 39.9 kg, d) 45 - 49.9  
403 kg, e) 55 - 59.9 kg and f) 65 - 69.9 kg. The mean plasma concentrations are over the target trough concentrations  
404 of 17 ng/ml. Concentration data were derived from optimized dosing strategies calculated for each weight band,  
405 as described in the Results section.

406 **Figure 6** Differential sensitivity analysis of plasma concentration against key parameters (blood-plasma ratio,  
407 cardiac output, fraction unbound, liver weight, release rate and systemic clearance) in adults for the 4-weekly  
408 intramuscular loading dose and the first maintenance dose. a) Cabotegravir, b) Rilpivirine

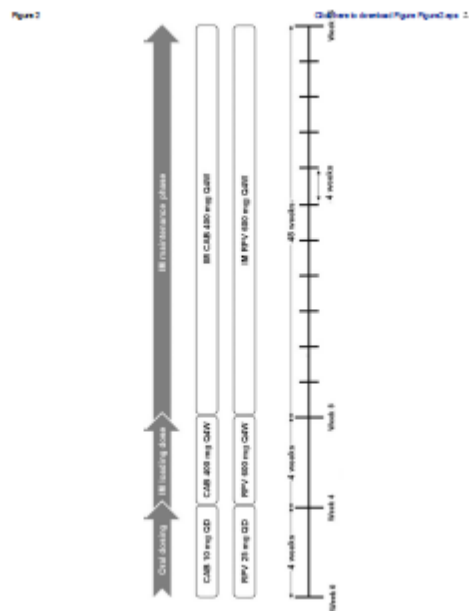
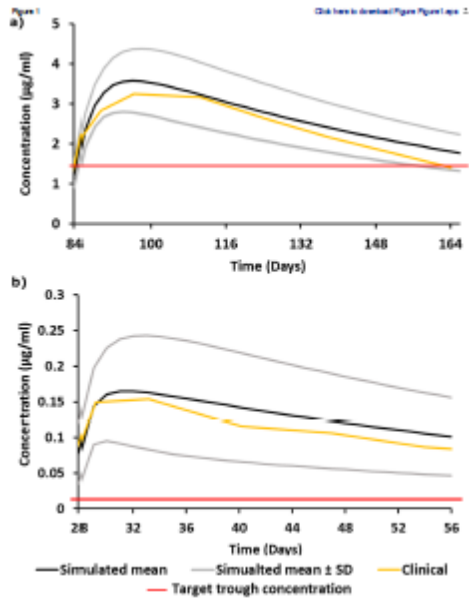


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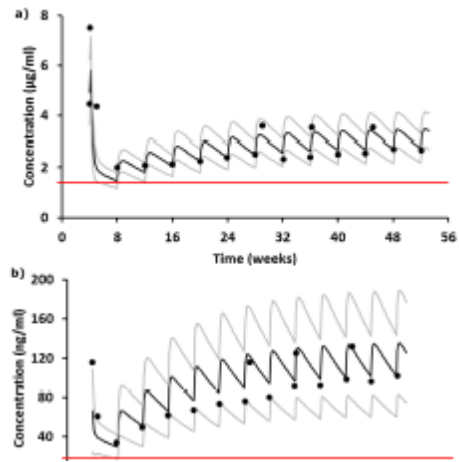


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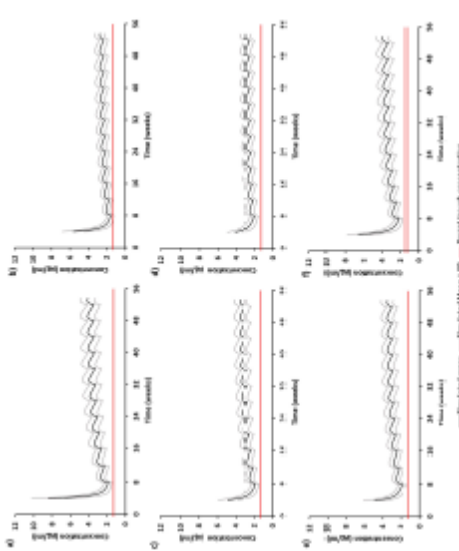


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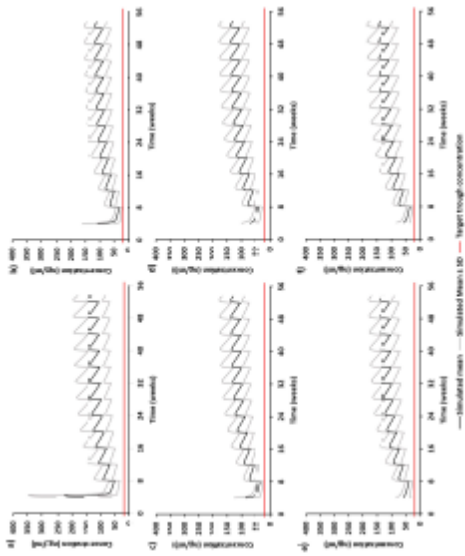
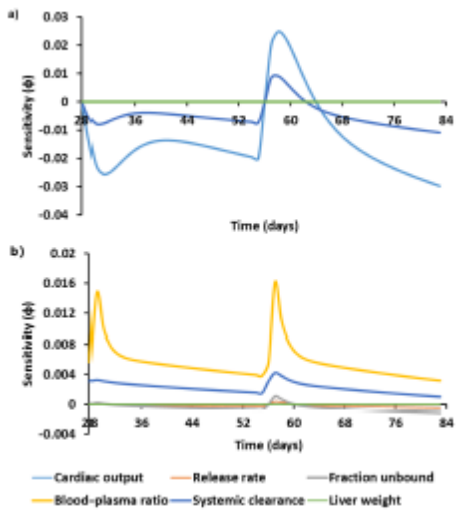


Figure 4

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1 **In silico dose prediction for long-acting rilpivirine and**  
2 **cabotegravir administration to children and adolescents**

3 Journal: Clinical Pharmacokinetics

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17 **Caption: PBPK model development and validation for paediatrics**

18

19 **1. Methods**

20 Anatomy and physiology of children between the ages 2-18 years were validated. The simulated  
21 pharmacokinetics were validated against published clinical data for lorazepam, an anti-anxiety agent and  
22 ceforanide, an anti-bacterial agent as model drugs [1, 2]. The physicochemical and drug specific parameters values  
23 were identified using standardised terminology. For the reported drugs, only a single value for each parameter  
24 could be identified.

25 **1.1 Anatomy**

26 The weight and body mass index of the individuals were defined using CDC growth charts. The charts were  
27 digitalised using plot digitizer tool and polynomial trend line feature in Microsoft excel was used to obtain the  
28 relation between age and other parameters [3]. Using these defined parameters as reference, height and body  
29 surface area [4] were calculated using allometric equations from the literature (Table 1).

30 **1.2 Tissue and organ weights**

31 The tissue and organ weights were computed using allometric equations. All the organ weights were validated  
32 against published data for both male and female populations at different age groups [5]. Summary of the equations  
33 used and their references are shown in Table 2a, 2b.

34 **1.3 Blood flow**

35 The cardiac output for different age groups was obtained from literature sources. The blood flow rates of  
36 various organs and tissues were adjusted as the percentage of cardiac output such that they match the clinical  
37 values [6]. The sum of blood flow passing through the gut, pancreas, spleen and stomach was considered as the  
38 portal vein blood flow rate for PBPK models. The blood flows were adjusted due to the unavailability of data in  
39 the literature (Table 3).

40 **1.4 Validation of anatomy and physiology**

41 The paediatric characteristics, individual tissue and organ weights and blood flows were validated against  
42 available literature data [5, 6]. Simulations were performed for a population (100 individuals) by including  
43 standard deviation from the literature or  $\pm 20\%$  was assumed if not available, in each age group and the mean  
44 value was validated against literature data for both male and female groups.

## 45 **1.5 Pharmacokinetic validation**

46 To improve the confidence of the constructed models, it was validated against lorazepam, an anti-anxiety  
47 drug [1]. This drug was chosen due to the availability of physicochemical and pharmacokinetic data required for  
48 the construction and validation of the PBPK model. Dose of 0.05 mg/kg to a maximum of 2 mg was administered  
49 intravenously and the pharmacokinetics were simulated for 100 male individuals in each age group. The PBPK  
50 model was also validated against a single intramuscular 20 mg/kg dose of an anti-bacterial agent, ceforanide [2].  
51 Maximum concentration ( $C_{\max}$ ), area under the curve (AUC) and volume of distribution ( $V_d$ ) were compared  
52 against clinical data [1, 2].

## 53 **1.6 ADME characteristics**

54 The equations describing the ADME processes defining PK were derived from previously published PBPK  
55 models [7, 8]. The physicochemical and intrinsic clearance values of lorazepam were obtained from Maharaj et  
56 al. [9] and the extrapolation to systemic clearance and the distribution of drug to different organs and tissues was  
57 computed using previously published equations [7, 10].



**Table 1** Equations describing the anatomical characteristic features for paediatrics

	Sex	2-7 years	7-18 years
<b>BMI</b>	<b>Male</b>	$(-2E-06*(Age*12)^3+0.0009*(Age*12)^2-0.096*(Age*12)+18.41) \pm (4E-09*(Age*12)^4-3E-06*(Age*12)^3+0.0006*(Age*12)^2-0.0421*(Age*12)+1.9366)$ [3]	$(0.004*Age^2+0.5348*Age+10.92) \pm 3$ [3]
	<b>Female</b>	$(-2E-06*Age^3 + 0.0011*Age^2 - 0.1058*Age + 18.249) \pm (7E-09*Age^4 - 4E-06*Age^3 + 0.0009*Age^2 - 0.0579*Age + 2.2788)$ [3]	$(-0.0204*Age^2 + 1.1067*Age + 7.7386) \pm 3$ [3]
<b>Weight</b>	<b>Male</b>	$(-1E-07*(Age*12)^4+4E-05*(Age*12)^3-0.0052*(Age*12)^2+0.4118*(Age*12)+4.6681) \pm (3E-10*(Age*12)^5-2E-07*(Age*12)^4+5E-05*(Age*12)^3-0.0051*(Age*12)^2+0.2379*(Age*12)-2.3971)$ [3]	$(-0.0419*Age^3+1.684*Age^2-17.334*Age+78.678) \pm 4.5$ [3]
	<b>Female</b>	$(-9E-09Age^4 - 6E-06Age^3 + 0.003Age^2 - 0.1028Age + 13.926) \pm (4E-10Age^5 - 3E-07Age^4 + 5E-05Age^3 - 0.0045Age^2 + 0.1886Age - 1.4623)$ [3]	$(-0.239*Age^2 + 9.6465*Age - 39.288) \pm 4.5$ [3]
<b>BSA</b>	<b>Both</b>	$0.0235*((Height*100)^{0.42246}*(Weight^{0.51456}))$ [4]	
<b>Height</b>	<b>Both</b>	$\text{sqrt}(Weight/BMI)$	

61 **Table 2 a)** Allometric equations describing organ and tissue weights for male children between 2-18 years

	2-7 years	7-18 years
Adipose	$0.534*Weight-1.59*Age+3.03 \pm 0.041$ [11]	$(1.51*BMI-0.7*Age-3.6*Sex+1.4)*Weight/100 \pm 0.041$ (Sex=0 for female, 1 for male) [12]
Blood	$(-0.0623*(Age^5)+2.4425*(Age^4)-31.37*(Age^3)+149.98*(Age^2)+31.305*Age+393.7)/1000 \pm 0.15$ [13]	$(3.33*BSA-0.81) \pm 0.1$ [12]
Bones	$(-0.0306*(Age^5)+0.5222*(Age^4)+9.7109*(Age^3)-197.97*(Age^2)+1089.7*Age+546.6)/1000 \pm 0.15$ [13]	$\exp(0.0689+2.67*\log(Height)) \pm 0.166$ [11]
Brain	$(0.405*\exp(-Age/629))*(3.68-2.68*\exp(-Age/0.89)) \pm 0.084$ [12]	
Glands	$(0.001*(Age^5)-0.0483*(Age^4)+0.8335*(Age^3)-6.6516*(Age^2)+27.512*Age+13.9 \pm 0.015)/1000$ [13]	
Gonads	$(3.3+53*(1-\text{abs}(\exp((-Age/17.5)^{5.4}))/1000 \pm \exp(0.049))$ [12]	
Heart	$(41.70+0.022*Age*365 \pm 25)/1000$ [14]	
Intestines	$(-4.7817e-2*(Age^4)+1.925*(Age^3)-22.382*(Age^2)+107.09*Age+51.125)/1000 \pm 0.05$ [13]	
Kidneys	$(35.29+0.015*Age*365+34.14+0.015*Age*365)/1000 \pm 2.5$ [14]	
Liver	$(271.58+0.163*Age*365 \pm 25)/1000$ [14]	
Lungs	$(41.31+0.039*Age*365+36.92+0.037*Age*365 \pm 5)/1000$ [14]	
Muscle	$0.93*Weight-(\text{Sum of organ weights})$ [12]	
Remaining	$\exp(-0.072+1.95*\log(Height)) \pm 0.049$ [12]	
Skin	$\exp(1.64*BSA-1.93) \pm 0.049$ [12]	$(-0.0992*(Age^4)+4.2762*(Age^3)-62.165*(Age^2)+437.78*Age+203.2)/1000 \pm 0.2*(-0.0992*(Age^4)+4.2762*(Age^3)-62.165*(Age^2)+437.78*Age+203.2)/1000$ [15]
Spleen	$(18.42+0.018*Age*365 \pm 2.5)/1000$ [14]	
Stomach	$\exp(-3.266+2.45*\log(Height)) \pm 0.0965$ [12]	
Thymus	$(14*((7.1-6.1*\exp(-Age/11.9))*((0.14+0.86*\exp(-Age/10.3))))/1000 \pm 0.049$ [12]	

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## b) Allometric equations describing organ and tissue weights for female children between 2-18 years

	<b>2-7 years</b>	<b>7-18 years</b>
Adipose	$0.642*Weight-0.12*Height-0.606*Age+8.98 \pm 0.041$ [11]	$(1.51*BMI-0.7*Age+1.4)*Weight/100 \pm 0.041$ [12]
Blood	$(0.0018*(Age^5)+0.0959*(Age^4)-4.4055*(Age^3)+44.442*(Age^2)+82.808*Age+292.26)/1000 \pm 0.15$ [13]	$(2.66*BSA-0.46) \pm 0.1$ [12]
Bones	$(-2.831e-3*(Age^5)-0.18184*(Age^4)+10.685*(Age^3)-142.88*(Age^2)+782.05*Age+609.64)/1000 \pm 0.15$ [13]	$\exp(0.0689+2.67*\log(Height)) \pm 0.166$ [11]
Brain	$(0.373*\exp(-Age/629)*(3.68-2.68*\exp(-Age/0.89)) \pm 0.084$ [11]	
Glands	$(0.001*(Age^5)-0.0483*(Age^4)+0.8335*(Age^3)-6.6516*(Age^2)+27.512*Age+13.9 \pm 0.015)/1000$ [13]	
Gonads	$(3.3+90*(1-\exp(-Age/16.8)^6.7))/1000 \pm \exp(0.049)$ [12]	
Heart	$(41.70+0.022*Age*365 \pm 25)/1000$ [14]	
Intestines	$(-0.0513*(Age^4)+2.0352*(Age^3)-23.478*(Age^2)+110.61*Age+49.229)/1000 \pm 0.05$ [13]	
Kidneys	$(35.29+0.015*Age*365+34.14+0.015*Age*365)/1000 \pm 2.5$ [14]	
Liver	$(271.58+0.163*Age*365 \pm 25)/1000$ [14]	
Lungs	$(41.31+0.039*Age*365+36.92+0.037*Age*365 \pm 5)/1000$ [14]	
Muscle	$0.93*Weight-(Sum\ of\ organ\ weights)$ [12]	
Remaining	$\exp(-0.072+1.95*\log(Height)) \pm 0.049$ [12]	
Skin	$\exp(1.64*BSA-1.93) \pm 0.049$ [12]	$(0.00476622*(Age^5)-0.27924*(Age^4)+6.3444*(Age^3)-70.113*(Age^2)+429.85*Age+252.06)/1000 \pm 0.20*(0.00476622*(Age^5)-0.27924*(Age^4)+6.3444*(Age^3)-70.113*(Age^2)+429.85*Age+252.06)/1000$ [15]
Spleen	$(18.42+0.018*Age*365 \pm 2.5)/1000$ [14]	
Stomach	$\exp(-3.266+2.45*\log(Height)) \pm 0.0965$ [12]	
Thymus	$(14*((7.1-6.1*\exp(-Age/11.9))*((0.14+0.86*\exp(-Age/10.3))))/1000 \pm 0.049$ [12]	

**Table 3** Equations for cardiac output and percentages of blood flow rate to each organ from the cardiac output adjusted according to literature data [6]

	<b>2-7 years</b>		<b>7-18 years</b>	
Cardiac output	$60 \cdot (10^{(0.8914 \cdot \log_{10}(\text{Weight}) - 0.654)})$ [16]		$(3.107 + (0.012 \cdot \text{Weight}^{1.369})) \cdot 60$ [17]	
Gender → Organ/Tissue ↓	Male	Female	Male	Female
Adipose	0.04	0.05	0.04	0.06
Bone	0.02	0.02	0.04	0.04
Brain	0.38	0.38	0.24	0.24
Gut ( $Q_{gu}$ )	0.12	0.13	0.14	0.15
Hepatic artery	0.08	0.08	0.06	0.07
Kidneys	0.13	0.11	0.17	0.13
Lungs	0.02	0.02	0.02	0.02
Muscle	0.05	0.06	0.12	0.11
Portal vein ( $Q_{pv}$ )	$Q_{gu} + Q_{st} + Q_{sp}$			
Remaining	0.07	0.06	0.08	0.08
Skin	0.03	0.03	0.04	0.04
Spleen + Pancreas ( $Q_{sp}$ )	0.05	0.05	0.04	0.04
Stomach ( $Q_{st}$ )	0.01	0.01	0.01	0.01

**Table 4 Physicochemical properties, *in vitro* and population pharmacokinetic data of lorazepam and ceforanide**

	<b>Lorazepam</b>	<b>Ceforanide</b>
<b>Molecular weight</b>	321	519
<b>log P<sub>o:w</sub></b>	2.39 [9]	-3.7 [18]
<b>Protein binding (%)</b>	0.93 [19]	80.6 [18]
<b>pK<sub>a</sub></b>	1.3 (base), 11.5 (acid) [9]	2.55 (acid), 9.14 (base) [18]
<b>R</b>	0.642 [9]	†0.1173
<b>UGT2B7 CL<sub>int</sub></b>	0.439 [9]	-
<b>Renal clearance</b>	0.01 [9]	-
<b>Plasma clearance</b>	-	72 ± 21 [2]

log P<sub>o:w</sub> – Partition coefficient between octanol and water; pK<sub>a</sub> – logarithmic value of the dissociation constant; R – blood-to-plasma drug ratio; CL<sub>int</sub> – intrinsic clearance; UGT - uridine diphosphate glucuronosyltransferase (ml/min/g of liver), renal clearance is in ml/min/kg, plasma clearance is expressed in ml/min · 1.73 m<sup>2</sup>; † the value was computed from the correlation provided by Paixão et al. [20]

## 2. Results

The mean simulated values of the anatomy and blood flow rates of children and adolescents were compared against literature values [5, 6]. The validation was performed for ages 2, 5, 10 and 15 years [6]. The simulated paediatric characteristic values for BSA, height and weight are in agreement with literature data as shown in Table 5. Allometric equations from various literature sources describing the organ and tissues weights and blood flow rates of children and adolescents are in agreement with the published data, shown in Table 6 and Table 7. A separate 'remaining' compartment was created to accommodate the unaccounted weight (data not shown) and blood flow rate in order to improve the model prediction. Allometric equations for 2 years were assumed to predict anatomy and physiology between the ages 2 and 5 with relative accuracy and precision. Observed growth pattern was slightly different from 7 years onwards due to which different equations were used for allometric scaling [3]. Due to large variation in anatomical and physiological characteristics among children and adolescents, broader validation range i.e.  $\pm 100\%$  was assumed. The mean values from the chosen allometric equations were between the assumed ranges from the reported literature values except for the lung weight of a 10-year-old child (101.2 %). The mean simulated blood flow rates were  $\pm 50\%$  from the literature value for all the age groups (Table 7).

. The pharmacokinetics were predicted across all age groups from 3-17 years and the mean value was compared with clinical data. Simulated lorazepam and ceforanide pharmacokinetics were compared against clinical data as shown in Table 8. For lorazepam, the  $C_{max}$ , AUC and  $V_d$  were +37.5 %, +22.2 % and -14.6 % from clinical values [1]. Validation of intramuscular ceforanide against clinical data had a deviation of +7.4 %, +16.6 % and -8.1 % for  $C_{max}$ , AUC and  $V_d$  respectively [2]. The simulated  $C_{max}$  and AUC values are slightly high which can be explained by the low volume of distribution observed. Due to unavailability of data, the blood-to-plasma ratio, fraction unbound, intrinsic and renal clearance were not altered across age groups.

**Table 5** Validation of characteristic features against literature data for different ages (data represented as Male/Female) [5, 6]

Years →	2		5		10		15	
Characteristic ↓	Simulated	Reference	Simulated	Reference	Simulated	Reference	Simulated	Reference
<b>BSA</b>	0.76/0.74	NA	0.91/0.91	0.78/0.78	1.20/1.14	1.12/1.12	1.67/1.49	1.62/1.55
<b>Height</b>	0.87/0.87	NA	1.09/1.09	1.09/1.09	1.39/1.37	1.38/1.38	1.80/1.53	1.67/1.61
<b>Weight</b>	12.7/12.1	12.6/11.9	18.0/17.2	18.7/17.7	31.0/34.0	31.4/32.6	56.0/52.0	56.7/53.7

**Table 6** Validation of organ weights (kg) against literature data for different ages (Data represented as Male/Female) [5, 6]

Years →	2		5		10		15	
Organs ↓	Simulated	Reference	Simulated	Reference	Simulated	Reference	Simulated	Reference
<b>Adipose</b>	3.17/2.91	3.76/3.72	4.68/4.26	5.50/5.50	7.45/9.28	8.60/8.60	11.6/14.2	12.0/18.7
<b>Bones</b>	0.74/0.74	0.85/0.82	2.49/2.49	2.43/2.43	4.6/4.47	4.50/4.50	8.32/8.32	7.18/7.18
<b>Brain</b>	1.50/1.50	1.12/1.03	1.48/1.36	1.31/1.31	1.47/1.48	1.40/1.40	1.46/1.34	1.30/1.30
<b>Glands</b>	0.02/0.02	0/0	0.06/0.06	0.04/0.04	0.07/0.07	0.06/0.06	0.06/0.06	0.10/0.10
<b>Heart</b>	0.07/0.06	0.07/0.06	0.08/0.08	0.09/0.09	0.12/0.12	0.14/0.14	0.16/0.16	0.22/0.22
<b>Intestines</b>	0.20/0.19	0.19/0.19	0.24/0.24	0.34/0.34	0.33/0.33	0.58/0.58	0.70/0.69	0.82/0.82
<b>Kidneys</b>	0.10/0.10	0.09/0.08	0.12/0.12	0.11/0.11	0.18/0.18	0.18/0.18	0.23/0.23	0.24/0.24
<b>Liver</b>	0.46/0.45	0.48/0.46	0.57/0.57	0.57/0.57	0.87/0.87	0.83/0.83	1.16/1.16	1.30/1.30
<b>Lungs</b>	0.18/0.18	0.24/0.24	0.22/0.22	0.13/0.13	0.38/0.38	0.21/0.21	0.52/0.52	0.29/0.29
<b>Muscle</b>	3.37/2.92	2.83/2.83	5.23/4.67	5.60/5.60	7.90/10.42	11.0/1.01	17.5/14.6	17.0/17.0
<b>Skin</b>	0.52/0.49	0.41/0.39	0.64/0.62	0.57/0.57	1.65/1.65	0.82/0.82	2.19/2.19	1.70/1.70
<b>Spleen</b>	0.04/0.04	0.07/0.07	0.05/0.05	0.05/0.05	0.08/0.08	0.08/0.08	0.12/0.12	0.13/0.13
<b>Stomach</b>	0.03/0.03	0.03/0.03	0.05/0.05	0.05/0.05	0.09/0.09	0.09/0.09	0.16/0.11	0.12/0.12
<b>Thymus</b>	0.02/0.02	NA	0.03/0.03	0.03/0.03	0.03/0.03	0.04/0.04	0.03/0.03	0.03/0.03

**Table 7** Validation of organ blood flows (L/h) for different ages against literature values (Data represented as Male/Female) [6]

Years →	2		5		10		15	
Organs ↓	Simulated	Reference	Simulated	Reference	Simulated	Reference	Simulated	Reference
<b>Cardiac Output</b>	129/122	124/114	176.5/132.7	172.8/157.8	269.5/272.5	234/224.4	363.9/347.2	346.2/309
<b>Adipose</b>	5.1/4.8	5.4/5.8	7.1/8	7.2/7.8	10.8/16.4	10.8/14	14.5/20.8	13.7/25
<b>Brain</b>	41.0/38.7	54.4/50.1	63.2/32	62.7/57.8	65.6/65.8	55.5/51	46.6/42.6	45/39.9
<b>Gut</b>	11.3/10.7	13.1/12.4	24.7/19.9	23.5/22.2	37.7/40.9	35.5/35.5	50.9/52	49.7/47.7
<b>Hepatic Artery</b>	32.1/31.0	43.2/40.8	10.6/9.3	11.3/10.6	16.2/19.1	14.6/15	21.8/24.3	22.7/21.5
<b>Kidney</b>	11.9/11.3	18.5/13.3	30/17.2	22.6/16.7	45.8/35.5	42.6/30	61.8/45.1	63.2/46.8
<b>Lungs</b>	8.4/8.0	9.2/8.28	3.5/2.7	3.3/3.1	5.4/5.5	4.1/4.8	7.3/6.9	8.6/7.2
<b>Muscle</b>	1.6/1.5	2.3/2.3	13.5/14.5	13.1/13.1	31.4/29.8	25.7/25.7	57/45.8	56.2/39.8
<b>Remaining</b>	5.1/4.8	4.9/4.9	14.1/10.6	11/7.3	21.6/21.8	17.7/18.4	29.1/27.7	37/35
<b>Skin</b>	3.4/3.2	3.9/3.7	7.1/5.3	5.3/5.1	10.8/10.9	7.7/7.8	14.5/13.9	14.5/12
<b>Spleen</b>	2.6/2.4	5.0/4.6	7.1/5.3	6.4/6	10.8/10.9	8.9/9.5	14.5/13.9	15.4/13.8
<b>Stomach</b>	1.2/1.1	1.2/1.1	1.8/1.3	2/1.8	2.7/2.7	3.2/3	3.6/3.5	5/4.5



**Table 8** Validation of lorazepam [1] and ceforanide [2] paediatric model against clinical data

<b>Lorazepam</b>			
	<b>C<sub>max</sub></b> (ng/ml)	<b>AUC</b> (ng.h/ml)	<b>V<sub>d</sub></b> (L/kg)
<b>Simulated</b>	77.14 ± 15.82	1005.62 ± 268.48	1.64 ± 0.13
<b>Clinical</b>	56.1 ± 44.9	822.5 ± 706.1	1.92 ± 0.84

<b>Ceforanide</b>			
	<b>C<sub>max</sub></b> (µg/ml)	<b>AUC</b> (µg.h/ml)	<b>V<sub>d</sub></b> (L/kg)
<b>Simulated</b>	60.4 ± 14.4	250 ± 74.0	0.24 ± 0.16
<b>Clinical</b>	56.3 ± 14.0	215 ± 61.0	0.26 ± 0.67

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