

1 **Efavirenz is predicted to accumulate in brain tissue: an *in silico*, *in vitro* and *in vivo***  
2 **investigation**

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27

28 **Abbreviations:** cisterna magna (CM), extra cellular fluid (ECF), intracellular space (ICS), left  
29 ventricle (LV), nevirapine (NVP), permeability surface area product (log PS), physiologically based  
30 pharmacokinetic (PBPK), rapid equilibrium dialysis (RED), sub arachnoid space (SAS), third and  
31 fourth ventricles (TFV), van der Waals polar surface area (TPSA) and van der Waals surface area of  
32 the basic atoms ( $va_{base}$ ).

33

34

35

36 **Abstract**

37 **Introduction:** Adequate concentrations of efavirenz in the central nervous system (CNS) are  
38 necessary to suppress viral replication but high concentrations may increase the likelihood of CNS  
39 adverse drug reactions. The aim of this investigation was to evaluate efavirenz distribution into the  
40 cerebrospinal fluid (CSF) and brain using a physiologically based pharmacokinetic (PBPK)  
41 simulation for comparison with rodent and human data.

42

43 **Methods:** Efavirenz CNS distribution was calculated using a permeability-limited model in a virtual  
44 cohort of 100 patients receiving efavirenz (600 mg once-daily). Simulations were then compared  
45 with human data from the literature and rodent data. Wistar rats were administered with efavirenz  
46 (10 mg kg<sup>-1</sup>) once daily over 5 weeks. Plasma and brain tissue was collected for analysis via LC-  
47 MS/MS.

48

49 **Results:** Median C<sub>max</sub> was predicted to be 3184 ng mL<sup>-1</sup> (IQR 2219-4851), 49.9 ng mL<sup>-1</sup> (IQR 36.6-  
50 69.7) and 50,343 ng mL<sup>-1</sup> (IQR 38,351-65,799) in plasma, CSF and brain tissue respectively, tissue  
51 to plasma ratio 15.8. Following 5 weeks of oral dosing of efavirenz (10 mg kg<sup>-1</sup>), the median plasma  
52 and brain tissue concentration in rats was 69.7 ng mL<sup>-1</sup> (IQR 44.9 – 130.6) and 702.9 ng mL<sup>-1</sup> (IQR  
53 475.5 – 1018.0) respectively, median tissue to plasma ratio was 9.5 (IQR 7.0 – 10.9).

54

55 **Conclusion:** Although useful, measurement of CSF concentrations may be an underestimation of the  
56 penetration of antiretrovirals into the brain. Limitations associated with obtaining tissue biopsies and  
57 paired plasma and CSF samples from patients make PBPK an attractive tool for probing drug  
58 distribution.

59 **Introduction**

60

61 Despite its widespread use, patients receiving efavirenz-containing therapy frequently report central  
62 nervous system (CNS) disturbances. Symptoms of efavirenz-associated adverse drug reactions  
63 (ADRs) occur with a high frequency and can include depression, anxiety, abnormal dreams and  
64 hallucinations (1). The majority of patients report development of CNS disorders shortly after  
65 commencing efavirenz therapy with symptoms dissipating during the initial months of therapy. A  
66 minority of patients continue to experience symptoms for the duration of efavirenz use (2). More  
67 recently, efavirenz CNS ADRs have been shown to have more long-term effects (3).

68

69 In addition to the negative impact on the quality of the patient's life, CNS ADRs may also lead to a  
70 decrease in patient adherence. Poor patient adherence to antiretroviral medication is a major concern,  
71 in particular drugs displaying a low genetic barrier to resistance such as efavirenz (4). The impact of  
72 CNS side effects on patient adherence is not clearly defined. Some previous studies indicate that  
73 patients demonstrate tolerance to CNS side effects with minimal impact on patient adherence (5, 6).  
74 However, a recent study demonstrated 60% of patients reported CNS side effects as the primary  
75 reason for discontinuation vs. 3% of patients receiving alternative antiretroviral therapies (3).

76

77 There is a paucity of information regarding distribution of efavirenz into brain tissue. Due to  
78 impracticalities in obtaining brain tissue from patients, some groups have used concentrations in  
79 cerebrospinal fluid (CSF) as a surrogate for brain concentrations. The majority of pharmacokinetic  
80 (PK) studies have focused on describing efavirenz plasma concentrations and elucidating genetic  
81 factors that contribute to the variability in efavirenz PK or genetic associations to predict patients at  
82 risk of developing CNS toxicity (1, 7, 8). However there are a few small studies that investigated  
83 efavirenz PK in both plasma and CSF. CSF concentrations have been shown to be much lower  
84 (around 0.5%) than plasma. However, even at 0.5% of the plasma concentration efavirenz  
85 concentrations in the CSF exceed the IC<sub>50</sub> of efavirenz for wild type HIV (9).

86

87 The appropriateness of CSF concentrations as a surrogate for brain concentrations is currently the  
88 subject of debate (10-12). It has been demonstrated in guinea pigs that brain tissue concentrations of  
89 nevirapine (NVP) not only differ from those in the CSF but also vary between brain regions (10).  
90 NVP uptake was shown to be  $0.32 \text{ mL g}^{-1}$  in the CSF whereas NVP uptake was lower in the choroid  
91 plexus ( $0.25 \text{ mL g}^{-1}$ ) and higher in the pituitary ( $1.61 \text{ mL g}^{-1}$ ) when compared to the CSF (10).  
92 Indeed, concentrations within CSF have been shown to vary depending on where the sample was  
93 taken for other antiretroviral drugs. Lamivudine has been shown to be 5-fold higher in CSF sampled  
94 from the lumbar region compared to ventricular CSF in rhesus monkeys (11). Although there are no  
95 comparable data for efavirenz in the literature, these data exemplify the challenges associated with  
96 predicting brain tissue concentrations in CSF.

97

98 PBPK modelling is a bottom up approach to simulate drug distribution in virtual patients. The  
99 approach mathematically describes physiological and molecular processes defining PK, integrating  
100 drug-specific properties (such as logP, Caco-2 apparent permeability and affinity for transporters and  
101 metabolic enzymes) and patient-specific factors (such as height, weight, sex, organ volumes and  
102 blood flow) (13). The model presented here is based on a full body PBPK model, supplemented with  
103 a 6-compartment model of the CNS and CSF as previously described (14).

104

105 The aim of this investigation was to evaluate efavirenz distribution into the CSF and brain using  
106 PBPK. Simulated efavirenz PK data were then compared to available experimental data from rodents  
107 and clinical data from humans.

108

## 109 **Materials & Methods**

110

### 111 **Animals and treatment**

112 Male Wistar rats (Charles River UK) weighing 180 – 220 g on arrival were used for PK analysis of  
113 efavirenz. Food and water were provided *ad libitum*. Following completion of the dosing all animals  
114 were sacrificed using an appropriate schedule 1 method (via exposure to CO<sub>2</sub> in a rising  
115 concentration). All animal work was conducted in accordance with the Animals (Scientific  
116 Procedures) Act 1986 (ASPA), implemented by the United Kingdom Home Office.

117

### 118 **Drug Treatment**

119 Eight male Wistar rats were dosed with efavirenz (10 mg kg<sup>-1</sup>, 2 mL kg<sup>-1</sup> 0.5% methylcellulose in  
120 dH<sub>2</sub>O) based on individual weight taken prior to dosing. The selected dose was based on scaling  
121 down the dose administered to adult humans (600mg once daily given to an adult weighing 60/70kg).  
122 The dose was also selected as it has been administered to rats previously in a study examining the  
123 angiogenic effects where it was shown to induce anxiety in Wistar rats (15). Dosing was  
124 administered once daily *via* oral gavage over 5 weeks. The animals were terminated (via exposure to  
125 CO<sub>2</sub> in a rising concentration) 2 hours after the final dose and blood was collected *via* cardiac  
126 puncture. Blood samples were centrifuged at 2000g for 10 minutes at 4°C to separate plasma. Plasma  
127 was immediately frozen at -80°C and stored for later analysis. Brain tissue was also collected and  
128 following washing in phosphate buffered saline for 30 seconds 3 times, immediately stored at -30°C  
129 for analysis.

130

### 131 **Rapid Equilibrium Dialysis**

132 The protein binding of efavirenz in brain tissue was performed using rapid equilibrium dialysis  
133 (RED) as described by Liu *et al.* (16). Untreated rat brain tissue was homogenised in 2 volumes  
134 (W:V) of 1% saline solution. Since efavirenz is highly protein bound, a dilution of brain tissue (10%  
135 and 20% brain tissue were prepared with 1% phosphate buffered saline [PBS]) was used. 200 µl of  
136 brain homogenate was spiked with 5000 ng mL<sup>-1</sup> efavirenz and added to the donor chamber. The  
137 receiver chamber contained 350 µl of Sorensens buffer. The RED plate (Thermo, UK) was then  
138 placed in a shaking incubator for 4 hours at 37°C at 100 rpm. 250 µl were removed from the receiver

139 chamber and frozen at -80°C for analysis. The fraction of drug unbound ( $f_u$ ) in brain tissue was then  
140 calculated from the diluted brain tissue using the following formula (17):

141

$$\text{Undiluted } f_u = \frac{\left(\frac{1}{D}\right)}{\left[\frac{1}{f_u(\text{apparent})} - 1\right] + \left(\frac{1}{D}\right)}$$

142

143 Where  $f_u$  = fraction unbound and  $D$  = dilution factor.

144

#### 145 **Sample preparation for bioanalysis**

146 Efavirenz was extracted by protein precipitation. 20 $\mu$ l of internal standard (lopinavir 1000ng mL<sup>-1</sup>)  
147 was added to 100 $\mu$ l of sample, standard or QC which was then treated with 400 $\mu$ l of ACN. Samples  
148 were then centrifuged at 4000g for 10 minutes at 4°C. The supernatant fraction was transferred to a  
149 fresh glass vial and evaporated, samples were placed in a rotary vacuum centrifuge at 30°C and then  
150 reconstituted in 140  $\mu$ l of H<sub>2</sub>O:ACN (60:40). 100 $\mu$ l of the sample was then transferred into 200 $\mu$ l  
151 chromatography vials. 5 $\mu$ l of each sample was injected for analysis by LC-MS/MS.

152

153 Rat brain tissue was homogenised in 3 volumes (W:V) of plasma for 1 minute at maximum power  
154 using a Minilys® homogeniser (Bertin technologies, FR). Extraction was performed using protein  
155 precipitation detailed in the previous section. Recovery was tested at 3 levels (400 ng mL<sup>-1</sup> 100 ng  
156 mL<sup>-1</sup> and 20 ng mL<sup>-1</sup>). Mean recovery was 95% (standard deviation 8.9) and 91% (standard deviation  
157 7.8) for plasma and brain, respectively. Samples generated from the RED experiment were pretreated  
158 with 20% ACN (PBS and Sorensens buffer were spiked with 20% ACN in order to aid efavirenz  
159 solubility in these matrices) and mean recovery was 84% (SD% 11.6).

160

#### 161 **Quantification of Efavirenz**

162 Quantification was achieved via LC-MS/MS (TSQ Endura, Thermo Scientific) operating in negative  
163 mode. The following ions were monitored for quantification in selected reaction monitoring scan:  
164 efavirenz ( $m/z$  315 > 242.1, 244.0 and 250.0) and internal standard, lopinavir ( $m/z$  627 > 121.2,  
165 178.1 and 198.1). A stock solution of 1 mg mL<sup>-1</sup> efavirenz was prepared in methanol and stored at  
166 4°C until use. A standard curve was prepared in plasma by serial dilution from 500 ng mL<sup>-1</sup> to 1.9 ng  
167 mL<sup>-1</sup> and an additional blank solution was also used.

168

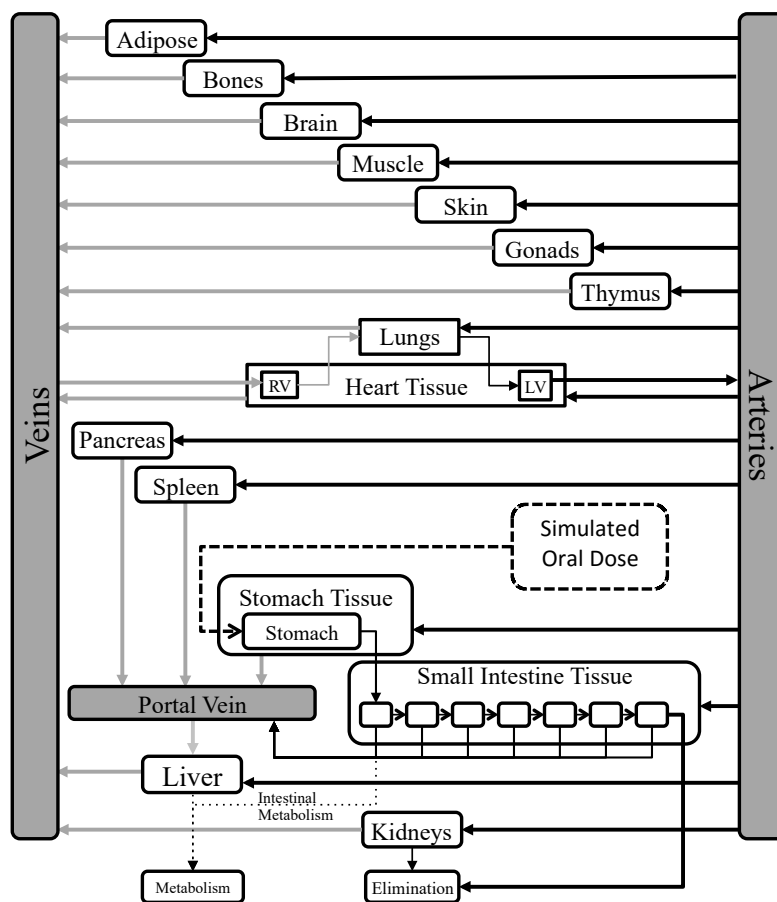
169 Chromatographic separation was achieved using a multi step gradient with a Hypersil gold C-18  
170 column (Thermo scientific) using mobile phases A (100% H<sub>2</sub>O, 5mM NH<sub>4</sub>HCO<sub>2</sub>) and B (100%  
171 ACN, 5mM NH<sub>4</sub>HCO<sub>2</sub>). Chromatography was conducted over 8.55 minutes at a flow rate of 300 µl  
172 min<sup>-1</sup>. At the start of each run, mobile phase A was 90% until 0.1 minutes when mobile phase B was  
173 increased to 86% at 0.5 minutes. Mobile phase B was then gradually increased to 92% over 4.5  
174 minutes. Mobile phase B was then increased to 97% at 5.1 minutes which was held until 6 minutes.  
175 Mobile phase A was then increased to 90% and held till the termination of the run at 8 minutes.  
176 Inter- and intra- assay variance in accuracy and precision were <15%.

177

### 178 **PBPK parameters**

179 The full body PBPK model used here has been previously published using equations from the physB  
180 model (Figure 1) (13, 18). The model generates virtual patients based on a statistical description of  
181 human anatomy. The model simulates flow rates, organ volumes and other tissue volumes based on  
182 anthropometric measures and allometric scaling.

183



184

185 **Figure 1** shows a diagram of the full body PBPK model. Figure adapted with authors permission

186

(18).

187

188 Briefly, the equations required to simulate factors such as volume of distribution were previously

189 published. Physicochemical properties of efavirenz data (including log P, molecular weight, pKa)

190 and *in vitro* data (permeation across Caco-2 cells and protein binding) were gathered from the

191 literature and incorporated into the full body model (19). Volume of distribution was simulated using

192 the Poulin and Theil equation (20). This method describes the tissue to plasma ratio based on the

193 individual organ volumes generated from the physB equations. Elimination clearance was calculated

194 (using equation 1) using allometric scaling of metabolism of efavirenz in microsomes and accounting



195 for activity and abundance of cytochrome P450 (CYP) 2B6, CYP2A6, CYP1A2, CYP3A4 and  
196 CYP3A5, and UGT2B7.

197

$$198 \quad 1. \quad TCL_{int} = Abundance \times Liver\ weight \times MPPGL$$

199

200 Where abundance is the amount of enzyme expressed per microgram of microsomal protein and  
201 MPPGL is the amount of microsomal protein per gram of liver. Apparent clearance was calculated  
202 expressed as the product of the  $TCL_{int}$  of all the enzymes contributing to the metabolism of efavirenz.  
203 Systemic clearance was calculated using equation 2, where  $Q_{hv}$  is the hepatic flow rate and  $fu$  is the  
204 fraction unbound in plasma (18).

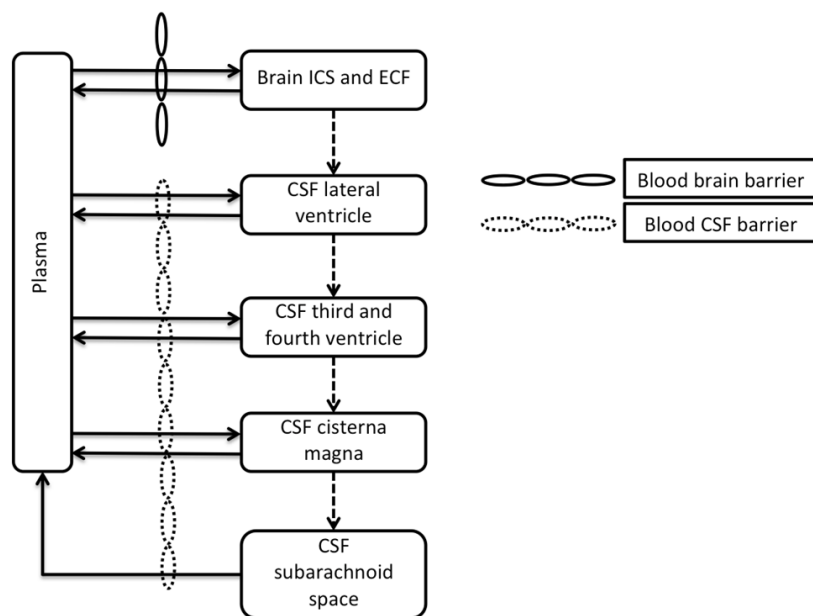
205

$$206 \quad 2. \quad CL = \frac{Q_{hv} \times fu \times CL_{app}}{Q_{hv} + CL_{app} \times fu}$$

207

208 The CNS portion of the model was based on validated parameters describing CNS and CSF  
209 physiology and anatomy (14). A schematic of this model is shown in Figure 2. Physiological and  
210 physicochemical properties used are displayed in Table 1. The equations used in the model presented  
211 here are as follows:

212



213

214 **Figure 2** shows a diagram of the CNS component of the PBPK model to describe efavirenz  
215 movement within the CNS. The brain compartment is comprised of the total volume of extra cellular  
216 fluid (ECF) and intracellular space (ICS).

217

218

219 The equations used in the model presented here are as follow:

220

$$221 \quad 3. \log PS = -2.19 + 0.262 \log D + 0.0583 \text{ vas}_{\text{base}} - 0.00897 \text{ TPSA}$$

222

223 Equation 3 shows a 3-descriptor QSAR model of permeability surface area product (log PS) of the  
224 blood brain barrier (BBB) developed by Liu *et al.* (21). The three descriptors are logD (octanol/water  
225 partition coefficient at pH 7.4),  $\text{vas}_{\text{base}}$  (van der Waals surface area of the basic atoms) and TPSA  
226 (van der Waals polar surface area). Permeability surface area product of the blood CSF barrier was  
227 calculated by dividing the permeability surface area product of the BBB by 1000, to reflect the  
228 smaller surface area of the blood CSF barrier (22).

229

$$230 \quad 4. \quad \frac{\Delta EFV_{Br}}{\Delta t} = psb * \left( \frac{EFV_{Ar} * fu}{R} - EFV_{Br} * fu_{Br} \right) - Q_{ecf} * EFV_{Br} * fu_{Br}$$

231

232 Equation 4 describes the movement of efavirenz from arterial plasma to the brain where  
233 concentration of arterial efavirenz ( $EFV_{Ar}$ ), fraction unbound in plasma ( $fu$ ), blood to plasma ratio  
234 ( $R$ ), concentration of efavirenz in the brain ( $EFV_{Br}$ ), flow of brain extracellular fluid ( $Q_{ecf}$ ), and  
235 fraction unbound in brain ( $fu_{Br}$ ).

236

$$237 \quad 5. \quad \frac{\Delta EFV_{CSF LV}}{\Delta t} = pse * \left( \frac{EFV_{Ve} * fu}{R} \right) - pse * EFV_{LV} * fu_{CSF} + Q_{ecf} * EFV_{Br} * fu_{Br} - Q_{csf} *$$

$$238 \quad EFV_{LV}$$

239

$$240 \quad 6. \quad \frac{\Delta EFV_{CSF TFV}}{\Delta t} = pse * \left( \frac{EFV_{Ve} * fu}{R} \right) - pse * EFV_{TFV} * fu_{CSF} + Q_{csf} * EFV_{LV} - Q_{csf} * EFV_{TFV}$$

241

$$242 \quad 7. \quad \frac{\Delta EFV_{CSF CM}}{\Delta t} = pse * \left( \frac{EFV_{Ve} * fu}{R} \right) - pse * EFV_{CM} * fu_{CSF} + Q_{CSF} * EFV_{TFV} - Q_{csf} * EFV_{CM}$$

243

$$244 \quad 8. \quad \frac{\Delta EFV_{CSF SAS}}{\Delta t} = Q_{csf} * EFV_{CM} - Q_{csf} * EFV_{SAS}$$

245

246 Equations 5 to 8 describe the movement of efavirenz from the brain to CSF, including movement  
247 across the blood CSF barrier. The CSF is subdivided into 4 compartments left ventricle (LV), third  
248 and fourth ventricle (TFV), cisterna magna (CM) and the subarachnoid space (SAS) where  
249 concentration of efavirenz in veins ( $EFV_{Ve}$ ), fraction unbound in plasma ( $fu$ ), blood to plasma ratio  
250 ( $R$ ), concentration of efavirenz in the brain ( $EFV_{Br}$ ), concentration of efavirenz in the CSF  
251 compartments ( $EFV_{CSF}$ ), flow of brain extracellular fluid ( $Q_{ecf}$ ), flow of CSF ( $Q_{csf}$ ), fraction unbound  
252 in CSF ( $fu_{CSF}$ ) and fraction unbound in brain ( $fu_{Br}$ ).

253

254 **Simulation Design**

255 A virtual cohort of 100 patients was generated and a once-daily dose of efavirenz (600 mg) was  
256 simulated over 5 weeks. Patient age (minimum 18 maximum 60), weight (minimum 40kg, maximum  
257 100kg), height (minimum 1.5 meters maximum 2.1 meters) and body mass index (minimum 18,  
258 maximum 30) were generated from random normally distributed values. The PK in plasma, CSF and  
259 brain tissue were recorded during the final 24 hours at steady state. Plasma and CSF PK simulations  
260 were compared with previous data generated from clinical trials. Brain tissue to plasma ratios were  
261 also calculated and compared to data generated in rodents.

262

263 **Materials**

264 Male Wistar rats were purchased from Charles River (Oxford, UK). Efavirenz powder (>98% pure)  
265 was purchased from LGM Pharma Inc (Boca Raton, USA). All other consumables were purchased  
266 from Sigma Aldrich (Dorset, UK).

267

268 **Results**

269 The protein binding of efavirenz in brain tissue was determined using rapid equilibrium dialysis. The  
270 mean ( $\pm$  standard deviation) concentration of efavirenz detected in the receiver chamber was  $209.7 \pm$   
271  $33.4 \text{ ng mL}^{-1}$ , and  $165 \pm 22.0 \text{ ng mL}^{-1}$  10% and 20% brain homogenate respectively. The fraction  
272 unbound in brain tissue ( $f_{u_{Br}}$ ) was calculated to be 0.00181 and 0.00212 in 10% and 20% brain  
273 homogenate, respectively. The average  $f_{u_{Br}}$  was 0.00197.

274

275 Following 5 weeks of oral dosing of efavirenz ( $10 \text{ mg kg}^{-1}$ ), the median plasma concentration of  
276 efavirenz in rats was  $69.7 \text{ ng mL}^{-1}$  (IQR 44.9 – 130.6). Median efavirenz concentrations in brain  
277 tissue were  $702.9 \text{ ng mL}^{-1}$  (IQR 475.5 – 1018.0). The median tissue to plasma ratio was 9.5 (IQR 7.0  
278 – 10.9).

279

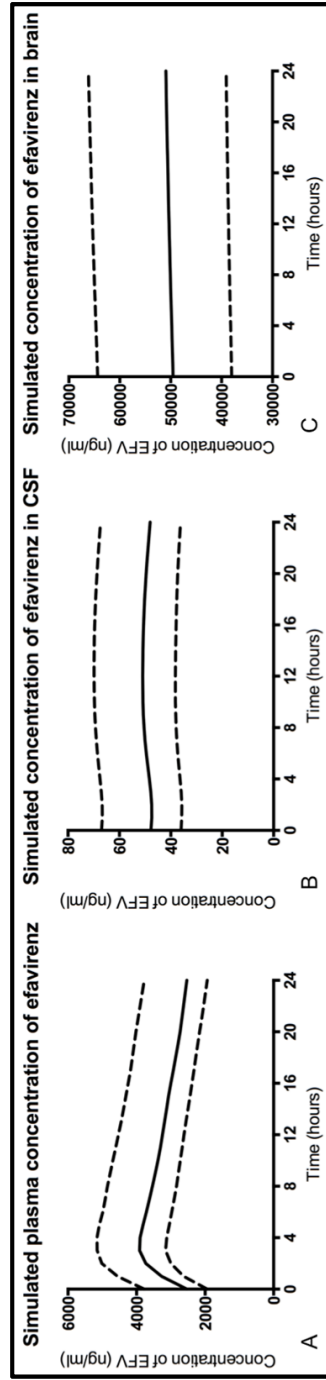
280

281 **Simulation**

282 A standard dosing schedule of efavirenz (600 mg once daily) was simulated in 100 patients for the  
283 duration of 5 weeks. The results for efavirenz concentrations in plasma (Figure 3A), CSF (Figure  
284 3B) and brain tissue (Figure 3C) were all taken from the final 24 hours of the simulation.

285

286



**Figure 3** shows the median (solid line) simulated plasma (a), CSF (b) and brain tissue (c) concentrations of efavirenz during the final 24 hours following 5 weeks of once daily efavirenz (600mg). Also shown is the interquartile range (dotted line).

287

288 The maximum concentration ( $C_{\max}$ ), minimum concentration ( $C_{\min}$ ) and area under the curve  
289 ( $AUC_{24}$ ) of efavirenz in plasma were 3916 ng mL<sup>-1</sup> (IQR 3155-5153), 2537 ng mL<sup>-1</sup> (IQR 1942-  
290 3779) and 76,991 ng.h mL<sup>-1</sup> (IQR 62,170-107,560). The CSF was predicted to have lower  
291 concentrations of efavirenz  $C_{\max}$  50.96 ng mL<sup>-1</sup> (IQR 38.23-69.09),  $C_{\min}$  47.8 ng mL<sup>-1</sup> (IQR 36.1-  
292 66.7) and  $AUC_{24}$  1193 ng.h mL<sup>-1</sup> (IQR 898-1649). At 24 hours efavirenz in the CSF was 1.6% of  
293 plasma concentrations. The simulation predicted efavirenz concentrations in the brain to exceed CSF  
294 and plasma,  $C_{\max}$  50,973 ng mL<sup>-1</sup> (IQR 39,122-66,177),  $C_{\min}$  49,566 ng mL<sup>-1</sup> (IQR 38,044-64,374)  
295 and  $AUC_{24}$  1,207,542 ng.h mL<sup>-1</sup> (IQR 926,900-1,567,974). The brain tissue to plasma partition ratio  
296 at 24 hours was 15.8.

297

298 The absorption constant ( $K_a$ ) was predicted to be 0.19 h<sup>-1</sup> (IQR, 0.18-0.21). Volume of distribution  
299 ( $V_{SS}$ ) and elimination clearance (Cl) were predicted to be 2.15 l kg<sup>-1</sup> (IQR 2.06-2.31) 4.56 l h<sup>-1</sup> (IQR  
300 3.52-5.33) respectively. The fraction absorbed ( $f_a$ ) of efavirenz was predicted to be median 0.46  
301 (IQR, 0.44-0.49) and was used to calculate apparent  $V_{SS}$  and apparent Cl, 323.31 l<sup>-1</sup> (IQR 308.31-  
302 346.28) and 9.79 l h<sup>-1</sup> (7.54-11.41) respectively.

303

#### 304 **Comparison with clinical data**

305 The simulated PK parameters in plasma produced by the model were in agreement with data  
306 published from human trials and population PK studies (popPK). Table 2 shows the results from the  
307 simulation and a number of clinical studies and popPK studies. The mean/median observed plasma  
308 concentrations of EFV ranged from 1973 ng mL<sup>-1</sup> to 3180 ng mL<sup>-1</sup> (9, 23-26). Simulated Cl,  $V_{SS}$  and  
309  $K_a$  were 1.04 fold, 1.28 fold and 0.6 fold different compared to observed data (26). The average  
310 simulated CSF concentrations were 49.9 ng mL<sup>-1</sup> (IQR 36.6-69.7) compared to a range of 11.1 ng  
311 mL<sup>-1</sup> to 16.3 ng mL<sup>-1</sup> observed in previously published clinical studies (9, 23).

312

313

314 **Discussion**

315 The presented data show that the PBPK model predicts efavirenz to accumulate in the brain in  
316 concentrations that far exceed those in the CSF. Human CSF concentrations were gathered from  
317 relatively small cohorts (Best N=80, Yilmaz N=1 and Tashima N=10) and may not fully represent  
318 CSF concentrations larger populations. Indeed, concentrations of efavirenz in the brain were  
319 predicted to exceed even plasma concentrations, with a brain to plasma ratio of 15.8. The rodent data  
320 presented here supports the model prediction of a higher concentration of efavirenz in brain tissue,  
321 with a median tissue to plasma ratio of 9.5. Recently, efavirenz has been demonstrated to accumulate  
322 in the brain tissue of a macaque. Following 8 days of orally administered efavirenz (60 mg kg<sup>-1</sup>) the  
323 concentrations in plasma and CSF were 541 and 3.30 ng mL<sup>-1</sup> respectively. Concentrations of  
324 efavirenz in the cerebellum and basal ganglia were 6.86 µg g<sup>-1</sup> (tissue to plasma ratio 12.7) and 2.01  
325 µg g<sup>-1</sup> (tissue to plasma ratio 3.7) respectively (27).

326

327 Currently only one study has examined efavirenz concentrations in human brain tissue (28). This  
328 study showed similar brain concentrations to historical CSF values and are in disagreement with the  
329 data presented here. While participants in this analysis had efavirenz detectable in intracardiac serum  
330 using a qualitative assay, reliable dosing information was not routinely available since the final care  
331 setting varied between individuals (home, hospice, or hospital). Given this uncertainty regarding the  
332 final dosing interval, no precise information was available on the time of last dose, which  
333 complicates interpretation of the reported brain concentrations. If the last efavirenz dose was  
334 administered, for example, 3 days prior to death, then the brain tissue concentrations may not  
335 accurately reflect those that occur in living, adherent patients. However, efavirenz has been shown to  
336 display long plasma half-life (40 to 52 hours) (29). This would indicate patients would have had  
337 ceased receiving efavirenz for many days or having poor adherence in order to explain the very  
338 low concentrations observed. Despite this the data predicted by the model is supported by robust  
339 data generated from the brain tissue concentrations from rats and monkeys (27).

340



341 Accumulation of efavirenz in brain tissue may be driven by physicochemical properties of efavirenz,  
342 in particular lipophilicity. Since efavirenz is highly lipophilic (logP 4.6) and has high accumulation  
343 in multiple cell types, it shows high cellular permeation (19). The brain has a high fat content, with  
344 approximately 60% of the brain consisting of fat (30). An additional factor that favours distribution  
345 is the high degree of protein binding of efavirenz. In plasma, efavirenz is highly protein bound ( $f_u$   
346 0.01) (31). Protein binding in the CSF is much lower leading to more free efavirenz,  $f_u$  0.238 (29).  
347 The data presented here from rapid equilibrium dialysis shows efavirenz  $f_u$  in rodent brain tissue to  
348 be 0.00197. Taken collectively, the combination of low  $f_u$  and affinity for the lipophilic environment  
349 of the brain favour accumulation of efavirenz in the CNS. Lipophilicity has been shown to be a  
350 significant factor in uptake of drugs into the brain (32). Lipophilicity, but not plasma protein binding,  
351 was shown to correlate with uptake of benzodiazepines, for example, into the brain. However, this  
352 study did not consider  $f_u$  in the brain and plasma  $f_u$  may not be a good indicator of brain  $f_u$ . Kalvass  
353 *et al* examined the  $f_u$  in plasma and brain tissue of 34 drugs covering multiple drug classes. The data  
354 presented showed that plasma  $f_u$  both under and overestimated brain  $f_u$  depending on the drug (33).  
355  
356 Although this is the first study to employ PBPK modelling to investigate efavirenz distribution into  
357 the CNS, PBPK has been used previously to investigate efavirenz dose optimisation, drug-drug  
358 interactions and PK in special populations (19, 34).  
359  
360 Limitations of this work include that the presented model does not take into account genetic  
361 variability (i.e. *CYP2B6* variants), the brain  $f_u$  values were generated in rodent brain rather than  
362 human brain, the current model is not able to estimate local concentrations in individual brain  
363 regions, and permeability of efavirenz was calculated using a QSAR model of passive permeability  
364 which often rely on extrapolated data from animals with important differences to humans (21, 35).  
365 The CSF concentrations predicted by the model were approximately 3 fold greater than observed in  
366 human patients. This indicates that the interactions with efavirenz and the blood CSF barrier may not  
367 have been accurately represented. The permeability of efavirenz at the blood CSF barrier was

368 adjusted for the decreased surface area of the blood CSF barrier, 1000 times less than the BBB (22).  
369 The assumption that the permeability of the two barriers is equal may be incorrect. However, these  
370 aspects could be expanded in future modelling strategies as the necessary input data emerges.  
371 The BBB is highly effective at excluding xenobiotics from the CNS. Tight cellular junctions prevent  
372 paracellular transport of drugs and the metabolising enzymes and transport proteins remove drugs  
373 from the CNS. As such, another potential limitation of the model that warrants further elaboration is  
374 that distribution of efavirenz across the BBB may not be governed purely by passive permeability.  
375 The potential influence of influx and efflux transporters was not considered because efavirenz is not  
376 classified as substrate of any transporters and effects of transporters on efavirenz PK have not been  
377 described. The model presented here potentially may be improved upon in the future if efavirenz is  
378 demonstrated to be a substrate for such transporters.

379

380 Numerous studies have linked efavirenz plasma concentrations to clinical evidence of CNS toxicity.  
381 Other studies have shown that efavirenz readily passes the BBB and is present in CSF. The  
382 simulations presented here indicate plasma and CSF may underestimate efavirenz exposure within  
383 the brain. Limitations associated with obtaining tissue biopsies and paired plasma and CSF samples  
384 from patients make PBPK modelling an attractive tool for estimating such drug distribution.

385

#### 386 **Author Contributions**

387 P.C., R.K.R.R., D.M.M., N.J.L., S.L., A.O. and M.S. wrote the manuscript.

388 P.C., and M.S. designed research.

389 P.C., R.K.R.R., D.M.M. and N.J.L. performed research.

390 P.C., R.K.R.R. and M.S. analysed data.

391

#### 392 **Conflict of Interest/Disclosure**

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398 AstraZeneca, consultancy from Merck and Norgine, and is a co-inventor of patents relating to HIV  
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400

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Model Parameter	Value	Reference
Molecular Weight	315.7	(19)
LogP	4.6	(19)
pKa	10.2	(19)
Caco-2 permeability (10 <sup>-6</sup> cm/s)	2.5	(19)
Fraction unbound		
Plasma	0.01	(31)
CSF	0.238	(29)
Brain tissue	0.00197	
PSB	2.47	
PSE	0.00247	
Qcsf (mL/min)	0.175	(14)
Qecf (mL/min)	0.4	(14)
Brain ICS (mL)	960	(14)
Brain ECF (mL)	240	(14)
CSF LV (mL)	22.5	(14)
CSF TFV (mL)	22.5	(14)
CSF CM (mL)	7.5	(14)
CSF SAS (mL)	90	(14)

530

531 **Table 1** shows the physiological and physicochemical variables used to generate the PBPK model.

532 Intracellular space (ICS), extra cellular fluid (ECF), left ventricle (LV), third and fourth ventricles

533 (TFV), cisterna magna (CM) and sub arachnoid space (SAS).

	Simulated data		Yilmaz <i>et al</i> 2012* (22)	Best <i>et al</i> 2011 (9)	Tashima <i>et al</i> 1999 (23)	Sánchez <i>et al</i> 2011 (24)	Csajka <i>et al</i> 2003 (25)
	Mean	Median	Median	Median	Mean	Mean	Mean
Plasma concentration (ng mL <sup>-1</sup> )	3183 (SD ±447)	3184 (IQR 2219-4851)	3718 (range 2439-4952)	2145 (IQR 1384-4423)	1973.8 (range 792.2-2950.9)	3180 (SD ±1610)	
Plasma AUC (ng.h mL <sup>-1</sup> )	91924 (SD ±51619)	76991 (IQR 62170-107560)	86,280				
Apparent Cl (L h <sup>-1</sup> )	9.29 (SE ±0.26)	9.79 (IQR 7.54-11.44)				9.61 (SE ±0.38)	9.4 (SE ±0.36)
Apparent V <sub>SS</sub> (L kg <sup>-1</sup> )	329.43 (SE ±2.38)	323.31 (IQR 308.31-346.28)				291 (SE ±44.81)	252 (SE ±35.28)
K <sub>a</sub> (h <sup>-1</sup> )	0.20 (SD ±0.02)	0.19 (IQR 0.18-0.21)					0.3 (SE ±0.09)
CSF concentration (ng mL <sup>-1</sup> )	49.9 (SD ±1.2)	49.9 (IQR 36.6-69.7)	16.3 (range 7.3-22.3)	13.9 (IQR 4.1-21.2)	11.1 (SD 2.1-18.6)		
CSF AUC (ng.h mL <sup>-1</sup> )	1401 (SD ±809)	1193 (IQR 898-1649)	380				
Brain tissue concentration (ng mL <sup>-1</sup> )	50312.5 (SD ±438)	50343 (IQR 38351-65799)					
Brain tissue AUC (ng.h mL <sup>-1</sup> )	1397820 (SD ±815657)	1207542 (IQR 926900-1567974)					

534

535 **Table 2** shows the results from the simulation and a number of human trials and POP PK studies. Results are presented as either mean (± standard  
536 deviation [SD] or standard error [SE]) or median (± interquartile range [IQR]). Mean and median are presented to allow comparison of simulated and  
537 clinical. \* all samples in this study were obtained from a single patient over 24 hours.

23