

Title: The impact of genetic polymorphisms on the pharmacokinetics of efavirenz in African children.

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Abstract:

Aim: To characterise the efavirenz steady-state pharmacokinetics in African children using model-based approach, quantifying demographic and genotypic effects on the drug's disposition, and conduct simulations allowing prediction of optimised doses of efavirenz in this population.

Methods: We modelled the steady-state population pharmacokinetics of efavirenz in Ugandan and Zambian children using nonlinear mixed-effects modelling. Individual mid-dose efavirenz concentrations were derived and simulations explored genotype-based dose optimisation strategies.

Results: A 2-compartment model with absorption through transit compartments well described 2086 concentration-time points in 169 children. The combined effect of SNPs 516GT and 983TC explained 44.5% and 14.7% of the variability in efavirenz clearance and bioavailability, respectively. The detected frequencies of composite *CYP2B6* genotype were 0.33 for 516GG|983TT, 0.35 for 516GT|983TT, 0.06 for 516GG|983TC, 0.18 for 516TT|983TT, 0.07 516GT|983TC and 0.01 for 516GG|983CC. The corresponding estimated clearance rates were 6.94, 4.90, 3.93, 1.92, 1.36, and 0.74 L/h for a 15.4 kg child and median (95% CI) observed mid-dose concentrations 1.55 (0.51-2.94), 2.20 (0.97-4.40), 2.03 (1.19-4.53), 7.55 (2.40-14.74), 7.79 (3.66-24.59) and 18.22 (11.84-22.76) mg/L, respectively. Simulations showed that wild-type individuals had exposures at the bottom of therapeutic range, while slower metabolisers were over-exposed.

Conclusions: Dosage guidelines for African children should take into consideration the combined effect of SNPs *CYP2B6* 516G>T and 983T>C.

46 **‘What is known about this subject’**

- 47 • High variability in efavirenz pharmacokinetics is largely contributed by SNPs in
48 *CYP2B6*: 516G>T and 983T>C.
- 49 • SNP 983T>C is virtually absent in individuals of European ancestry.
- 50 • No previous studies quantified the effect of 983T>C on efavirenz clearance in children using
51 model-based approach or recommended dose optimisation strategies accounting for this
52 SNP.

53 **‘What this study adds’**

- 54 • We propose a model concomitantly accounting for effect of weight and *CYP2B6*
55 516G>T|983T>C variants in African children.
- 56 • Using the model we simulated and compared exposures in weight-bands between *CYP2B6*
57 metabolic subgroups and suggested a dose optimisation strategy adjusting for effect of both
58 516G>T and 983T>C.

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Text:

INTRODUCTION

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) commonly used within first-line antiretroviral treatment (ART) for HIV-1 infected adults and children over 3 years.^{1,2} Due to its ease of dosing (the long half-life allows once daily administration), proven efficacy, ability to be used with anti-TB drugs, and availability of cheap generic formulations, it is especially widely used in Africa.

Suboptimal efavirenz exposures have been previously related to treatment failure and high concentrations to central nervous system (CNS) side-effects.^{3,4} Numerous studies reported very large between-subject variability (BSV) in efavirenz pharmacokinetics (PK).^{3,5-7} This variability is attributed largely to single nucleotide polymorphisms (SNPs) in the *CYP2B6* gene which encodes the key metabolising enzyme. The loss-of-function polymorphism, 516G>T (rs3745274),^{4,7-9} alters drug metabolism to the extent that dose adjustment based on *CYP2B6* 516G>T genotype is currently under investigation in children.^{10,11} The proportion of slow metabolizers varies among different populations and is relatively high among black Africans.^{4,6,8,9,12} In addition, efavirenz concentrations are affected by the functional polymorphisms *CYP2B6* 983T>C (rs28399499),^{8,9} 785A>G (rs2279343)^{13,14} and 15582C>T (rs4803419),^{8,12} which are reported predominantly in black African and African-American patients; by polymorphisms involving its accessory pathways, including *CYP2A6*, *CYP3A4* and *UGT*;¹⁵⁻¹⁷ and in genes coding nuclear receptors CAR (NR1 | 3) and PXR (NR1 | 2), which regulate enzyme expression.^{18,19} PK variability has also been linked to several physiological and environmental factors, such as sex,^{6,13,20} ethnicity,^{6,21,22} formulation type,^{2,23} concomitant food² or co-medication (e.g. zidovudine²², rifampicin and isoniazid^{7,22,24}), and adherence²⁵. However, reports on these effects have been to some extent contradictory and vary between adults and children.

Several investigators have reported a high proportion of sub-therapeutic efavirenz concentrations in children, highlighting the need for optimisation of paediatric dosing guidelines.²⁶⁻²⁸ The aim of this

analysis was therefore to characterise the steady-state PK of efavirenz in the largest cohort of African children reported so far, quantifying demographic and genotypic effects on efavirenz disposition, and thus allowing prediction of optimised doses of efavirenz in this population.

METHODS

PK and other data from two studies in African children from Uganda and Zambia were pooled together: CHAPAS-3²⁹ (Children with HIV in Africa – Pharmacokinetics and Adherence/Acceptability of Simple antiretroviral regimens) and ARROW^{28,30} (Anti-Retroviral Research for Watoto).

CHAPAS-3

Efavirenz was dosed once daily, *mane* or *nocte*, following modified WHO 2010 guidelines (Table 4), using a new paediatric double-scored 600 mg efavirenz tablet (provided by Cipla Pharmaceuticals, India) that can be split into 2 or 3 parts enabling administration of doses of 200, 300, 400, or 600 mg.

All included patients took part in sparse PK-sampling on clinic visits at week 6, 36, and every 24 weeks thereafter until the end of the study. The self-reported time of the last dose was recorded.

Additional intensive PK-sampling was conducted in the first patients enrolled in each WHO weight-band at week 6 and in all patients who acquired tuberculosis during the study (4-10 weeks after tuberculosis treatment initiation and again 4-10 weeks after tuberculosis treatment cessation).

Children in this intensive PK sub-study were advised to take efavirenz in the morning for 6 weeks prior to sampling and the drug intake on the PK day was observed. Plasma was separated and stored at –80°C until transportation on dry ice for drug concentration assay.

Plasma efavirenz concentrations from the intensive PK were assayed using ultra high-performance liquid chromatography at the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. The method produced linear results over the range of 0.0517 to 15.51 mg/L. The lower limit of quantification was 0.05 mg/L. The intraassay and interassay coefficients of variation (CV) were 1.01% to 5.31% and 0.1% to 1.63%, respectively. Relative error of

the method ranged from 97.7% to 104.5%.³¹ Plasma efavirenz concentrations from sparse PK were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the Division of Clinical Pharmacology, University of Cape Town, South Africa. The method was accurate over the range of 0.0195 to 20 mg/L. The lower limit of quantification was 0.0195 mg/L. The interassay coefficient of variation (CV) and residual error (RE) were 3.59%–5.78% and –2.57% to 0.92%, respectively, and the intraassay CV and RE were 1.50% to 9.67% and –6.00% to 4.28%, respectively.³²

Both laboratories participate in international quality assurance and proficiency testing schemes and are expected to have comparable standards, although no cross-validation was performed for the assays. Systematic differences between the labs and assays were tested as covariates in the PK model (see Population Pharmacokinetic Analysis/Covariates).

ARROW

Efavirenz was dosed once-daily, *mane* or *nocte*, according to modified WHO 2006 paediatric recommendations. The tested formulations included 50, 100, and 200 mg capsules; and half or whole 600 mg tablets (provided by the national ART programme in Uganda).

Children in a PK sub-study²⁸ were sampled on 2 occasions: 36 and 40 weeks after starting ART. Eligible children were advised to take efavirenz in the morning for 4 weeks prior to sampling and drug intake on the PK day was observed. Samples were stored and assayed at the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre, Nijmegen using the same methods as described above.

Genotyping

Genotyping was performed by allelic discrimination real-time PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 15 s and final annealing at 60°C for 1 min. TaqMan® Genotyping Master Mix and assays for *CYP2B6*-516G>T

(rs3745274, C_7817765_60), *CYP2B6*-983T>C (rs28399499, C_60732328_20), *CYP2B6*-c.485-18C>T (rs4803419; C_7817764_10), *CYP2B6*-499C>G (rs3826711, C__27522377_10; ARROW patients only), *CYP3A4**22 (rs35599367, C__59013445_10), *CYP3A5*-6986G>A (rs776746, C__59013445_10), *NR1I3* (rs3003596, C__16194070_10; rs2307424, C__25746794_20), *NR1I2*-63396C>T (rs2472677, C__26079845_10) were obtained from Life Technologies Ltd (Paisley, Renfrewshire, UK). *CYP2B6**4 (785A>G, rs2279343) and *CYP2B6**29 copy number assay were performed on samples from ARROW study only using previously described custom TaqMan assays.^{33,34} Opticon Monitor® version 3.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to obtain allelic discrimination plots and make allele calls. The assays were performed at the Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK.

The distribution of the genotypes was evaluated for compliance with Hardy-Weinberg equilibrium using the exact test conducted using R-package “genetics”.³⁵

Population pharmacokinetic analysis

Model building

The steady-state efavirenz PK was analysed using nonlinear mixed-effects modelling with software NONMEM VII (version 7.3)³⁶ and the first-order conditional estimation method with interaction. PsN 4.4.0, Pirana, and Xpose were used to facilitate modelling and for model diagnostics.³⁷ The model was developed and validated in accordance with standard methods described in the literature.³⁸ For the structural model, 1-, 2-, and 3-compartment models with first-order absorption and elimination were tested, as well as time lag or transit-compartment absorption³⁹ and hepatic first-pass model.⁴⁰ Between-subject and -occasion variability (BSV, BOV) were tested on PK parameters assuming lognormal distribution. Residual unexplained variability (RUV) was tested using a combined proportional and additive error. Data below level of quantification (BLQ) were included in the analysis by imputing half of the LLOQ of the corresponding assay as suggested in Beal (M6

method).⁴¹ Implausible samples and PK-profiles were identified using extreme values of CWRESI and their exclusion was evaluated based on visual checks.

Model development and covariate selection was guided by the NONMEM objective function value (OFV), inspection of goodness-of-fit (GOF) plots and visual predictive checks (VPCs), biological plausibility, and clinical relevance. OFV (proportional to -2 log-likelihood of the data) was assumed to be χ^2 -distributed and a drop of 3.84 or more between two hierarchical models after inclusion of one additional parameter (df=1) was considered a significant improvement (p=0.05). Stability and robustness of the final model, together with precision of its parameter estimates, was evaluated through a nonparametric bootstrap (n=200).

Intensive and sparse data were included in the model development process in a stepwise manner as suggested in Svensson *et al.*,⁴² starting with intensive PK data from CHAPAS-3, followed by the intensive data from ARROW, and finally the sparse PK data from CHAPAS-3.

The model-derived Empirical Bayesian Estimates for the individual parameters were used to predict steady-state mid-dose concentrations (measures 12h after dose) for each sampling occasion and patient.

Covariates

Allometric scaling was added to the model at an early development stage as previously suggested.⁴³ The effect of maturation of metabolic pathways on PK parameters was tested using post-menstrual age (gestation-adjusted age) as a predictor. Both a power function or a sigmoidal model with and without Hill coefficient were tested.⁴³ Besides weight and age, the other covariates tested were: tuberculosis co-treatment, study site, nucleoside reverse transcriptase inhibitor (NRTI) backbone, sex, weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ), drug formulation, the effect of splitting tablets used in CHAPAS-3 (inferred from total daily dose), and genotyping information (SNPs

listed above). The potential difference between assays and lab procedures for the quantification of drug concentrations was tested in the model as proportionality and correction factors on RUV.

Missing genotype values were imputed using mixture modelling with frequencies fixed to those observed in the rest of the cohort as previously suggested by Keizer et al.⁴⁴

Simulations

The final model was used to simulate exposures after administration of efavirenz with the formulation given in CHAPAS-3 and using a dataset of subjects with a uniform distribution of weights ranging from 10 to 40 kg, in 0.1 kg steps (300 individuals simulated 100 times). Several dosing strategies were explored. To avoid generating implausibly extreme values, the maximum variability for each random effect was limited to 3 standard deviations. Data were analysed and plots generated using R.³⁵

RESULTS

Demographic results and samples

This analysis included data from 128 children from CHAPAS-3, and 41 children from ARROW. Relevant subject characteristics including the genotype frequencies for the tested SNPs are presented in Table 1. All tested genotypes were in Hardy-Weinberg equilibrium (HWE). For SNPs rs35599367, rs3826711 and *CYP2B6**29 all patients were homozygous for the common allele and HWE was not calculated. The genotype information was missing for 5 children from ARROW and 2 from CHAPAS-3, who were assigned by the mixture model as follows: 2 as 516GG|983TT, 4 as 516GT|983TT, and one as 516TT|983TT.

From CHAPAS-3, 61 intensively sampled PK-profiles (a total of 474 samples) and 510 sparse PK-profiles (1002 samples, 1-2 per occasion) were available. The PK data were collected from 6 weeks after starting efavirenz up to a maximum of 132 weeks. There were up to 7 PK-sampling visits per child. Of 14 children who acquired tuberculosis, 9 had at least one intensively sampled PK-profile on

efavirenz with tuberculosis treatment. The only BLQ measurement from intensive PK sub-study was a pre-dose measurement and all 8 samples from that PK visit for that patient were excluded from the analysis as it was deemed likely not to be in steady-state due to poor adherence. Within the sparse data, 15 samples were BLQ and were included by imputing half LLOQ, i.e. 0.00975 mg/L. From the ARROW study, 611 intensive PK samples from 82 PK visits (2 visits per patient) were available. Data from one visit were discarded due to an implausible PK profile, possibly caused by mismatch of samples. No samples were BLQ.

Population pharmacokinetics

The data were best described using a 2-compartment model with first-order elimination and transit compartment absorption.³⁹ Final parameter estimates, their precision (obtained through a bootstrap) and statistical significance for the inclusion of the covariate and random effects (based on drop in OFV) are presented in Table 2. The PK parameters were estimated relative to oral bioavailability whose typical value was fixed to 1 due to lack of intravenous data. Adequate fit of the model was confirmed by a GOF plots and VPC (see Supplementary Figure 1 and 2).

The effect of body size on all clearance and volume parameters was accounted for using allometric scaling, which significantly improved model fit (18 points drop in OFV).⁴³ No effect of age on the maturation of clearance could be detected. After adjusting for body size, the main predictor of clearance was the effect of *CYP2B6* genotype, categorised into 6 subgroups based on the combined effect of 516G>T and 983T>C SNP-variants present in our population (Table 2 and 3). *CYP2B6* genotype explained 44.5% and 14.7% of BSV in clearance and oral bioavailability respectively. Exclusion of individuals with missing genotype did not have a significant effect on final results.

The absorption rate constant (k_a) and the absorption mean transit time (MTT) were 1.6-fold larger and 1.4-times longer in ARROW compared with CHAPAS-3. Splitting of the new double-scored efavirenz tablets used in the CHAPAS-3 was not found to affect efavirenz bioavailability. No other

covariate (see Methods) was found to significantly improve the model fit. We did not detect any systematic differences between the assays and labs employed in the analysis.

The model fit was markedly improved by inclusion of a correction parameter to allow for larger residual unexplained variability for all samples obtained after self-recorded efavirenz intake. This includes all pre-dose intensive PK-samples and all the sparse PK-samples from CHAPAS-3. The residual variability for those samples was twice as large as in the rest of the data.

Simulations

Simulations were performed to predict exposures in African children based on their weight and genotype, when dosed according to the regimen used in CHAPAS-3. Median mid-dose concentrations were comparable across weight-bands but noticeable differences were observed between the *CYP2B6* genotype subgroups (Figure 1, Table 3).

A dose optimisation strategy for African children was devised by categorising subjects into four phenotypic subgroups based on their composite genotype vector 516G>T|986T>C, similarly to Dooley *et al.*³² (presented in Table 3). The proposed dose-adjustment between metabolic subgroups is based on optimal ratios of 1:0.66:0.33:0.1 for EM:IM:SM:USM (extensive, intermediate, slow and ultra-slow metabolisers), respectively, and is outlined in Table 4.

The predicted exposures based on the suggested dose-optimisation algorithm are presented in Figure 2 and Supplementary Table 1. The suggested dosing approach ensured adequate drug exposure in all simulated weight-bands (Figure 2, left panel), and reduced the differences due to metabolic status (Figure 2, right panel).

DISCUSSION

Efavirenz pharmacokinetics in Africans has been previously shown to be affected by the combined effect of SNPs 516G>T and 983T>C.^{8,12,14,17,32} The current investigation confirms those findings and is

253 the first analysis to quantify the effect of the *CYP2B6* 516G>T|983T>C SNP-vector on efavirenz
 254 clearance in African children using nonlinear mixed-effects modelling. The use of modelling provides
 255 a tool to concomitantly account for multiple effects such as genotype and weight, and a platform to
 256 derive a dose adjustment strategy based on these effects.

257 Numerous studies in adults and children have reported a significant effect of *CYP2B6* 516G>T on
 258 efavirenz clearance. Our analysis shows that presence of one variant allele in 516G>T causes
 259 clearance to drop by 34%, while the reduction reaches 72% for homozygous mutants, which is in line
 260 with previously reported reductions of 20-47% and 58%-80%, respectively.^{13,20,24,26,45-47} Our findings
 261 also show that the effect of SNP 516G>T is significantly modified by the 983T>C (i.e. in wild type
 262 516G>T individuals presence of a single variant allele in 983T>C causes a 43% drop in clearance and
 263 89% if no functional allele is present), confirming associations found by *Holzinger et al.*⁸ and in a
 264 number of African studies.^{12,14,32} This polymorphism is virtually absent in individuals of European
 265 ancestry^{8,9} and was not detected in a study of Cambodian patients.²⁴ The combined effect of the
 266 *CYP2B6* 516G>T|983T>C SNP-vector on efavirenz clearance has been previously reported
 267 distinguishing 4 phenotypic subgroups.^{17,32} In our study, we were able to further characterise this
 268 effect using six *CYP2B6* 516G>T|983T>C variant subgroups. Similarly to previous reports we show
 269 that, despite its low prevalence, SNP 983T>C is not only a significant predictor of efavirenz clearance,
 270 but it is responsible for a larger drop in metabolic rate than 516G>T (i.e. 29% drop in clearance in
 271 516G>T heterozygote vs 43% drop in 983T>C heterozygote, when no other polymorphisms were
 272 present).^{8,12} No further significant genetic associations were detected.

273 A genome-wide association study by *Holzinger et al.* identified rs4803419 as another significant
 274 polymorphism in *CYP2B6* affecting efavirenz clearance.⁸ The effect of SNP rs4803419 becomes
 275 significant only for homozygous mutants who are wild type for 516G>T and 983T>C and no such
 276 variants were present in our population. Although this finding was recently replicated in South

277 African patients, the investigators concluded this effect was negligible in comparison to 516G>T and
278 983T>C.¹²

279 Results from the simulations (Figure 1) showed that, even though the dosage guidelines tested in
280 CHAPAS-3 result in average mid-dose concentrations within target range of 1.0-4.0 mg/L,³ the effect
281 of *CYP2B6* genotype leads to large differences within each weight-band. In particular, children with
282 slower *CYP2B6* genotypes (516GG|983CC, 516GT|983TC, and 516TT|983TT) were over-exposed,
283 while the fastest metabolisers (516GG|983TT) achieved exposures at the bottom of the therapeutic
284 range. Over 20% of children in the study with the 516GG|983TT genotype had efavirenz
285 concentrations below the proposed minimum target concentration of 1.0 mg/L (Table 3). Moreover,
286 our model indicates that disregarding the effect of the 983T>C SNP and basing dose optimisation
287 only on 516G>T could lead to exposures significantly higher than the therapeutic range³ in ~14% of
288 African patients with 983TC or 983CC genotypes (Table 3 in grey). This suggests that genotype-based
289 dose optimisation in African children should take into account both 516G>T and 983T>C SNPs.

290 The only available guidelines on genotype-adjusted paediatric dosage were recently formulated for
291 patients under 3 years by the Panel on Antiretroviral Guidelines for Adults and Adolescents and are
292 currently being tested in the IMPAACT study P1070.^{10,11} The dose adjusted strategy was developed
293 based on results of an analysis by Salem *et al.*²³ and preliminary results of IMPAACT P1070⁴⁸ and
294 proposed different dosing for individuals with 516GG or 516GT genotype versus 516TT. In contrast
295 to previous paediatric studies,^{46,49} Salem *et al.* did not detect significant differences in clearance rate
296 between patients with 516GG and 516GT genotypes, and the effect of 983T>C was not evaluated.
297 Their findings might differ from the current investigation for several reasons: the study by Salem *et*
298 *al.* included a smaller number of patients, the lowest age of participants was only 2 months, the
299 tested population comprised patients of various races and the formulations included capsules and
300 liquid. According to simulations from the current model, direct application of that strategy in African
301 children could result in result in exposures above the therapeutic range³ in a large proportion of

patients who are either heterozygous for 516G>T or wild type with 983TC or 983CC genotypes (Figure 3).

Our recommendations were simplified to previously described 4 metabolic subgroups determined by the composite 516G>T|983T>C vector outlined in Table 3.³² The clearance between EM:IM:SM:USM drops as follows: 1:0.6:0.24:0.1, which is remarkably similar to the ratios detected by *Dooley et al.* in African adults (1:0.6:0.26:0.08, respectively).³² Our dosage algorithm presented in Table 4 (1:0.66:0.33:0.1) was adjusted to maximise the use of currently available solid formulations. A similar dose adjustment pattern (1:0.66:0.33) was previously successfully implemented based of phenotypic differences in an adult study by Mello *et al.*⁵⁰

The results of the few dose reduction studies guided by *CYP2B6* genotype conducted in developed countries highlighted improved treatment tolerability and cost-effectiveness in adults.^{50–53} These results were confirmed by recent cost-effectiveness analysis of this practice in American adults.⁵⁴ It could be speculated that in a resource-limited settings, cost and logistical challenges would make implementation of such practice difficult. Nonetheless, decreasing price and broader availability of genotyping technology and economic development open future scenarios in which genotype-based dosing approaches may be economically viable and beneficial even in developing countries. The IMPAACT P1070 study, which is currently being conducted in HIV-infected infants and children, should hopefully give more insight into practical implications of genotyping in low- and middle-income countries.

Similarly to previous paediatric studies, the average clearance value (before inclusion of genotype effect) was higher than findings in adults - 14.34 L/h versus 7.5 to 11.7 L/h (both after scaling with allometry up to 70 kg).^{5,21,24,45,46} This is consistent with reports that efavirenz clearance in children 1-4 years exceeds adult values.^{27,46,55} In keeping with previously published paediatric models, the effect of size on clearance and volume was explained through allometric scaling.^{23,43,46} Unlike Salem *et al.* we did not observe age-related maturation of clearance, but the previous analysis showed that 90%

327 of maturation was reached by the age of 9 months²³ and the majority of patients in the current
328 study were >3 years.

329 We detected significant differences in absorption parameters between CHAPAS-3 and ARROW,
330 possibly related to the use of different formulations (tablets in former and mostly capsules in latter).
331 The formulations were assumed to be bioequivalent and indeed no formulation effect on
332 bioavailability was detected.

333 Due to the availability of data from multiple sampling occasions within the same patient, it was
334 possible to characterise both BSV and BOV in the PK parameters. Large BOV was identified for
335 absorption parameters and bioavailability. Drug absorption is widely known to be a variable
336 phenomenon, depending on occasion-specific factors, such as food intake, gastric emptying times,
337 and GI-tract pH. In the current study, other factors may have contributed to inflating BOV (in
338 particular on bioavailability), including differences between actual and self-reported intake times for
339 the sparse data, lack of accurate intake history before the last dose, lack of information on
340 accompanying food consumption. The fact that the information about intensively sampled occasions
341 was more accurate was accounted for with the introduction of a scaling factor on residual
342 unexplained variability (2-fold larger for sparse data).

343 The current study had several limitations. As mentioned, the dosage timing and food co-
344 administration was not recorded beyond the last intake. Previous studies showed that efavirenz PK
345 was affected by adherence and food effects.^{2,21,25} Polymorphisms in accessory pathways including
346 CYP2A6 and CYP3A4 or UGT were not assessed and the effect of *CYP2B6* 785A>G was only evaluated
347 in patients from the ARROW study. Despite several reports suggesting that efavirenz metabolism is
348 affected by polymorphisms in those pathways, the genome-wide association study by Holzinger *et*
349 *al.* showed that their effect was significantly less dramatic than for 516G>T and 983T>C.⁸

350 Furthermore, no PK/PD relationship was explored in this analysis for either efficacy or toxicity, but
351 currently accepted therapeutic ranges were used as cut-offs guiding dose optimisation. These

targets were generated in an adult cohort and have recently been brought into question suggesting that lower efavirenz concentrations might be sufficient to provide viral suppression,¹ however no alternative has been suggested to date.

Lastly our study was underpowered to determine the effect of tuberculosis treatment on efavirenz concentrations, but recent findings suggest that the inducing effect of rifampicin on clearance is counterbalanced by a concentration-dependent inhibitory effect of isoniazid that could explain contradictory conclusions from previous studies.^{7,24,32}

CONCLUSIONS

Our study suggests that genotype-adjusted efavirenz dosage in African children should be based on the composite 516G>T|983T>C SNP-vector, due to significant modification of clearance rates caused by SNP 983T>C genotype, whose prevalence in Africans is much higher than other populations. Using nonlinear mixed-effects modelling we quantified this effect and suggest that a dose optimisation algorithm 1:0.66:0.33:0.1 (EM:IM:SM:USM, respectively) would provide more balanced drug exposures between individuals with a different metabolic status while maximising the potential of using the new double-scored efavirenz tablets tested in the CHAPAS-3 study. The findings warrant further studies evaluating the genotype-based dosing approach and the feasibility of genotyping in resource-limited settings.

FIGURE LEGENDS:

Main article:

Figure 1 Individual mid-dose concentrations estimated by the population pharmacokinetic model (black dots) plotted on top of the mid-dose concentrations (percentile plots) simulated across weight-bands (left) and by different *CYP2B6* 516GT|983TC subgroups (right). Red horizontal lines

correspond to efavirenz concentrations of 1mg/L and 4mg/L.³ Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data.

Figure 2 Simulated mid-dose concentrations across weight-bands (left) and by different 516GT|983TC genotypes (right) based on suggested most optimal dosing. Red horizontal lines correspond to efavirenz concentrations of 1mg/L and 4mg/L.³ Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data

Figure 3 Simulated mid-dose concentrations across weight-bands (left) and by different 516GT|983TC genotypes (right) based on dose recommendations tested in IMPACT study P1070 applied to our population. Red horizontal lines correspond to efavirenz concentrations of 1mg/L and 4mg/L.³ Breaks in the percentile plot correspond to 25th, median and 75th percentile and whiskers correspond to 5th and 95th percentile of the simulated data

SUPPLEMENTARY FIGURES:

Figure 1 Goodness of fit plots. Top left – observations vs population predictions; top right – observations vs individual predictions; bottom left – conditional weighted residuals vs time after dose; bottom right – absolute values of individual weighted residuals vs individual predictions

Figure 2 Visual Predictive Check of the final model by 516GT|983TC SNP vector in semi-log scale. Hollow points – observations, red solid line – median of observed data, red line with breaks – 5th and

399 95th percentile of observed data, orange fill area – 95% confidence interval of simulated median,
400 blue fill area - 95% confidence interval of simulated 5th and 95th percentile

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CONFLICT OF INTEREST:

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