

Table 1 Demographic characteristics and model-derived PK parameters in different suppression groups.								
		Treatment-naïve at enrolment					Treatment – experienced at enrolment	
		Suppressed	Single Rebound	Multiple Rebound	Never Suppressed	p*		
Number of children /109		73 67%	19	10	7 6%		14	p**
Baseline	Age [years]	4.3 (3.5-4.7)	3.9 (3.6-4.5)	3.5 (3.3-3.8)	3.5 (3.2-8.5)	0.208	7.3 (5.7-8.5)	<0.001
	Weight [kg]	14.0 (12.4-16.0)	14.5 (13.5-16.0)	13.4 (12.8-15.7)	12.3 (12.0-17.5)	0.513	20.1 (19.3-22.6)	<0.001
	CD4% [%]	18.5 (11.0-24.0)	17.2 (7.3-22.1)	19.5 (15.5-24.5)	19.5 (10.3-19.5)	0.565	35.6 (31.4-37.8)	<0.001
	CD4 [cells/mL]	707 (497-977)	648 (204-904)	684 (477-1047)	618 (201-913)	0.429	915 (807-1249)	<0.001
	Viral Load [copies/mL]	172165 (63250-338685)	93160 (23415-218830)	175390 (130080-606275)	149080 (69065-354885)	0.268	< 100	<0.001
	Sex (M/F)	29/44	10/9	8/2	4/3	0.133	9/5	0.091
Metabolic Subgroup †	FM	24	7	1	2	0.073	5	0.173
	IM	28	6	8	3		6	
	SM	21	6	--	2		3	
	USM	--	--	1	--		--	
NRTI	Stavudine	23	7	5	1	0.738	5	0.908
	Zidovudine	26	6	2	4		4	
	Abacavir	24	6	3	2		5	
PK	AUC ₀₋₂₄	57.1	57.8	46.6	36.8	0.360	77.7	0.142

measure	[mg·h/L]	(37.1-101.4)	(45.9-121.8)	(42.8-78.0)	(13.6-74.0)		(58.5-114.2)	
	C12h [mg/L]	2.25 (1.43-4.11)	2.22 (1.77-4.96)	1.93 (1.65-3.08)	1.40 (0.5-2.83)	0.367	3.12 (2.38-4.43)	0.155
	C24h [mg/L]	1.54 (0.95-3.15)	1.43 (1.19-3.69)	1.24 (1.01-1.77)	0.76 (0.31-1.82)	0.223	2.27 (1.630-3.53)	0.123
	Cmax [mg/L]	4.20 (3.03-6.28)	4.42 (3.22-6.59)	3.81 (3.24-6.99)	3.50 (1.07-5.62)	0.606	5.33 (4.70-6.66)	0.159
	CL [L/h]	5.6 (3.2-7.7)	5.6 (2.4-7.3)	6.2 (5.2-7.6)	9.1 (5.9-9.2)	0.382	6.5 (4.3-8.3)	0.479
	Adherence (MEMScaps)‡	1.00 (0.97-1.00)	0.98 (0.95-1.00)	0.98 (0.91-0.99)	0.87 (0.69-0.95)	0.010	0.97 (0.91-1.00)	<0.001

1 Presented values are number or median (IQR)

2 *Kruskal Wallis or Fisher's Exact test comparing 4 groups of originally treatment-naïve children only.

3 **Kruskal Wallis or Fisher's Exact test comparing 5 groups including children who were treatment-
4 experienced at enrolment.

5 †EM (extensive metabolisers) - 516GG|983TT; IM (intermediate metabolisers) - 516GG|983TC or
6 516GT|983TT, SM (slow metabolisers) - 516TT|983TT or 516GT|983TC; USM (ultra-slow
7 metabolisers) - 516GG|983CC;

8 ‡Data from 104 patients (91 treatment-naïve and 13 treatment-experience at enrolment)

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Table 2 Univariable Cox proportional hazards regression models for C12h, C24h and AUC, with lowest AIC values indicating the models best describing the association with viral non-suppression.				
PK Par		Change in risk per unit increase in absolute exposure	Change in risk per doubling of exposure (per unit increase in log transformed exposure)	Change in risk change at threshold for dichotomized exposure variables (Supplement 1)
C12h [mg/L]	HR (95% CI)	0.87 (0.69-1.10)	0.58 (0.43-0.77)	6.14 (2.64-14.27) (vs C12h>1.12mg/L)
	p-value	0.241	< 0.0001	< 0.0001
	AIC	324.11	305.76	304.55
C24h [mg/L]	HR (95% CI)	0.86 (0.67-1.11)	0.60 (0.46-0.78)	6.57 (2.86-15.10) (vs. C24h>0.65mg/L)
	p-value	0.246	< 0.0001	< 0.0001
	AIC	324.67	304.18	302.82
AUC [mg·h/L]	HR (95% CI)	0.9941 (0.9843-1.0040)	0.57 (0.42-0.76)	5.77 (2.28-14.58) (vs. AUC>28mg·h/L)
	p-value	0.247	< 0.0001	< 0.0001
	AIC	324.04	305.70	307.74

10 HR=Hazard ratio.

11 Note: log transform was the best fitting fractional polynomial for C24h; for C12h and AUC the best

12 fitting transform was inverse square root. However, the difference in AIC compared to log-transform

13 was very small in both cases (+0.47 and +0.97) and so the log transform is presented above for
14 comparability with C24h and ease of interpretation.

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Table 3 Univariate and multivariate predictors of virological suppression.				
Factor	Univariate		Final Multivariate Model	
	HR (95% CI)	p	HR (95% CI)	p
C12h (per doubling)	0.58 (0.43-0.77)	<0.001	0.61 (0.50-0.76)	<0.001
Sex: male vs female	2.77 (1.01-7.64)	0.048	(see interaction below)	
Current age (ref. <8 years)	5.45 (1.85-16.06)	0.002	(see interaction below)	
Sex and age (ref girl <8y)	Boy <8y: 6.14 (2.01-18.77)	0.001	Boy <8y: 5.31 (1.58-17.82)	0.007
	Girl >8y: 16.63 (4.05-68.37)	<0.001	Girl >8y: 15.82 (2.97-84.27)	0.001
	Boy >8y: 25.50 (3.37-193.13)	0.002	Boy >8y: 12.47 (1.31-119.08)	0.028
Site (ref. S1)	S2: 0.22 (0.06-0.77)	0.018	S2: 0.73 (0.18-2.88)	0.653
	S3: 0.39 (0.11-1.38)	0.146	S3: 1.04 (0.23-4.82)	0.956
	S4: 2.48 (0.69-8.99)	0.166	S4: 4.96 (1.38-17.79)	0.014
WAZ (per unit higher)	0.66 (0.49-0.88)	0.005	-	

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Note: as the final multivariable model identified a significant interaction between age and sex, this interaction is also presented unadjusted for other factors in the univariable column.

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Final model selected using backwards elimination, see methods. Interaction between

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continuous age and sex (p=0.01) dichotomised at the optimal age threshold for

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presentation. HR – hazard ratio; CI – confidence interval;

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Table 4 Comparison of efavirenz exposure targets and predictors of virological outcome in paediatric studies

Reference	Derived PK Targets	Predictors of virologic failure		n	Method	VL Target [copies/mL]
		PK	Covariates			
<i>Starr et al.</i> ²²⁺	AUC = 60 – 120 mg·h/mL	Not analysed	Uni: (A)* log ₂ bCD4%, WAZ, bVL / (B)* WAZ, bVL Multi: (A)* WAZ, bVL / (B)* bVL	57	Cox	400 (A)* 50 (B)*
<i>Brundage et al.</i> ¹⁷	AUC > 59 mg·h/mL	AUC	Uni: IPAM, bVL, bCD4%, WAZ Multi: IPAM, bVL, AUC	50	Cox, TSSA	400
<i>Hirt et al.</i> ¹⁰	C _{min} > 1.1 mg/L AUC > 51 mg·h/L	C _{min} , AUC	Not analysed	48	Fisher's exact test	300
<i>Fletcher et al.</i> ⁹	AUC > 49 mg·h/mL	AUC	Not analysed	50	logistic regression	400
<i>Janssens et al.</i> ²¹	Not analysed		Uni: Orphan status, male gender Multi: Orphan status	212	logistic regression	400
<i>Kamaya et al.</i> ^{19‡}	Not analysed		Uni: male gender, bCD4%<5% Multi: male gender, bCD4%<5%	250	logistic regression	400
<i>Jittamala et al.</i> ^{20‡}	Not analysed		Uni: male gender, age, adherence	202	Cox	50

			Multi: none			
Bienczak <i>et al.</i> (this analysis)	C _{12h} > 1.12 mg/L C _{min} > 0.65 mg/L AUC > 28 mg·h/L	C _{12h} , C _{min} , AUC	Uni: male gender, age < 8 years, site, WAZ Multi: male gender, age < 8 years	118	Cox	100

25 † target derived based on adult data, ‡ patients treated with nevirapine or efavirenz – presented results relate to efavirenz only, *two efficacy cut-offs used:

26 (A) 400 copies/mL, (B) 50 copies/mL; bCD4% - baseline (pre-ART) CD4 percentage, bVL – baseline (pre-ART) viral load, Cox – Cox proportional hazards

27 regression, IPAM – integrated pharmacokinetic adherence measure, Multi – multivariate analysis, TSSA - tree-structured survival analysis, Uni – univariate

28 analysis, VL – viral load, WAZ – weight-for-age z-score

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Table 5 Comparison of previously published treatment targets for efavirenz concentrations and AUC and most predictive thresholds derived in this analysis.														
	C12h [mg/L]				C24h [mg/L]				AUC [mg-h/L]					
Threshold	1.0 ³		1.12		1.0 ³		0.65		49 ⁹		60 ²²		28	
HR	6.36		6.14		3.96		6.57		3.16		3.84		5.77	
95% CI	2.53-15.96		2.64-14.27		1.73-9.03		2.86-15.10		1.39-7.16		1.56-9.44		2.28-14.58	
AIC	305.35		304.55		315.07		302.82		319.86		318.12		307.74	
	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T	< T	> T
Samples							19/	19/						
not-sup/sup	17/	21/	19/	19/	21/	17/	32	276	24/	14/	30/	8/	18/	20/
(n)	27	281	34	274	66	242	37%	6.5%	101	207	153	155	32	276
Sensitivity	44.7%		50.0%		55.3%		50.0%		63.2%		78.9%		44.74%	
Specificity	91.2%		88.9%		78.5%		89.6%		67.1%		50.2%		90.23%	
Accuracy	86.1%		84.6%		75.9%		85.2%		66.7%		53.3%		85.22%	
Positive Predictive Value	38.6%		35.8%		24.1%		37.3%		19.2%		16.4%		36.17%	
Negative Predictive Value	93.0%		93.5%		93.4%		93.5%		93.6%		95.1%		92.95%	

31 HR –hazard ratio, 95% CI – 95% confidence interval, AIC – Akaike information criterion, PK –

32 pharmacokinetic, T – cut-off target. In grey – cut-offs proposed by this analysis, in white – previously

33 published cut-offs.

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