Antiretroviral Concentrations and Surrogate Measures of Efficacy in the Brain Tissue and CSF of Preclinical Species – Supplementary Material

Animal models and dose selection

This analysis utilized three preclinical models from two species of humanized mice: stem cell hemopoietic/RAG 2- (hu-HSC-Rag, n=36) and bone marrow-liver-thymus (BLT, n=13) and one species of nonhuman primate (NHP): rhesus macaques (n=18). Humanization of the mice was by protocols that have been previously described (Melkus et al. 2006; Berges et al. 2006). The extent of humanization for the mouse models was assessed by quantifying the human T-cell populations using flow cytometry. All the humanized mice were female, while six (33.3%) NHPs were female. Half of the animals were left uninfected while the other half were infected with 200 μL of 2.1 x 10^6 IU/mL of $HIV_{Bal\ D7}$ intraperitoneally (hu-HSC-RAG) or 200 μL of 90,000 tissue culture infectious units (TCIU) of HIV_{JRcsf} intravenously (BLT) or $10^{4.5}$ median tissue culture infective dose (TCID50) RT-SHIV intravenously (NHPs) (North et al. 2005; North et al. 2010) for four weeks. The hu-HSC-RAG and BLT mice underwent humanization when aged three to six months and were then dosed with various ARV regimens for a period of ten days. Hu-HSC-RAG mice were dosed with EFV 10 mg/kg only (six uninfected and six infected animals) or atazanavir (ATZ) 140 mg/kg only (six uninfected and six infected animals) or a combination of tenofovir (TFV) 208 mg/kg, emtricitabine (FTC) 240 mg/kg, raltegravir (RAL) 56 mg/kg and maraviroc (MVC) 62 mg/kg (six uninfected and six infected animals). BLT mice were dosed with a combination of TFV, FTC, RAL, MVC and ATZ at equivalent doses but not EFV due to toxicity concerns. All drugs were administered by oral gavage. Dosing regimens for each of the animal models are summarized in **Supplementary Table 1**. Dosage selection for all drugs in the animal

models were based on clinically relevant doses for the treatment of HIV infection. The dosing periods for all animals were chosen to achieve pharmacokinetic steady-state conditions in tissues based on known half-lives of the drugs used and previous studies performed with these models.

The macaques were dosed with a backbone of TFV 30 mg/kg daily and FTC 16 mg/kg daily, both administered subcutaneously. These drugs were administered with a combination of either EFV 200 mg/day given orally and RAL 200 mg/day (n=9) given orally or MVC 150 mg/day given orally and ATZ 270 mg/kg (n=9) given orally. One SHIV-infected female macaque dosed with TFV, FTC, EFV, and RAL developed liver failure before necropsy and the measured drug levels in the plasma and tissue matrices in this animal were >100-fold higher than in the other NHPs. As a result, the drug concentration data from this animal were excluded from the final LC-MS/MS analysis. All animal studies were performed in concordance with institutional animal care and use committee (IACUC) protocols from the University of North Carolina at Chapel Hill (protocol 15-168), Colorado State University (protocol 16-6998A) and the University of California at Davis (protocol 18345).

References:

Berges BK, Wheat WH, Palmer BE, Connick E, & Akkina R, 2006. HIV-1 infection and CD4 T cell depletion in the humanized Rag2-/-gamma c-/- (RAG-hu) mouse model. *Retrovirology*, 3:76. Available from: https://doi.org/10.1186/1742-4690-3-76 [Accessed January 11 2018]

Melkus MW, Estes JD, Padgett-Thomas A, Gatlin J, Denton PW, Othieno FA, Wege AK, Haase AT, & Garcia JV, 2006. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nature Medicine*, 12(11), pp.1316–1322.

North TW, Van Rompay KK, Higgins J, Matthews TB, Wadford DA, Pedersen NC, & Schinazi RF, (2005). Suppression of virus load by highly active antiretroviral therapy in rhesus macaques infected with a recombinant simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type 1. *Journal of Virology*, 79(12), 7349–54.

North TW, Higgins J, Deere JD, Hayes TL, Villalobos A, Adamson L, Shacklett BL, Schinazi RF, & Luciw PA, (2010). Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *Journal of Virology*, 84(6), 2913–22.

Supplementary Table 1.

Sample size and dosage regimens for the preclinical models[1]

Dosing Regimen	Humanized Mice				Rhesus macaques			
	Hu-HSC-RAG		BLT		Male		Female	
	HIV-	HIV+	HIV-	HIV+	RT-SHIV-	RT-SHIV+	RT-SHIV-	RT-SHIV+
EFV	N=6	N=6						
ATZ	N=6	N=6						
TFV/FTC/RAL/MVC	N=6	N=6						
TFV/FTC/RAL/MVC/ATZ			N=6	N=7				
TFV/FTC/EFV/RAL					N=3	N=3	N=2	N=1
TFV/FTC/MVC/ATZ					N=3	N=3	N=2	N=1

1. Dosage selection for all drugs in the animal models were based on clinically relevant doses for the treatment of HIV infection. Dosing periods for all animals were chosen to achieve PK steady-state conditions in tissues based on known half-lives of the drugs used and previous studies performed with these models.

Supplementary Table 2.

Comparison of antiretroviral concentrations in the plasma, CSF (NHP only) and brain tissue and antiretroviral brain tissue:plasma and CSF:plasma penetration ratios (NHP only) across the three preclinical species

ARV/ Animal Model	ARV concentration (ng/mL [plasma and CSF] or ng/g [brain tissue])							ARV penetration ratio			
	Plasma			Brain Tissue			CSF	Brain Tissue:Plasma ratio			CSF:Plasma ratio
	Hu-HSC-RAG	BLT	NHP	Hu-HSC-RAG	BLT	NHP	NHP	Hu-HSC-RAG	BLT	NHP	NHP
TFV	150 (77.1, 368)	125 (89.5, 241)	60.3 (47.8, 84.4)	4.49 (0.62, 18.8)	14.3 (11.9, 47.9)	51.3 (34.9, 57.5)	2.04 (1.40, 2.82)	0.02 (0.01, 0.11)	0.11 (0.07, 0.14)	0.75 (0.59, 0.92)	0.035 (0.016, 0.055)
FTC	24.0 (19.8, 68.4)	46.6 (27.8, 79.2)	13.5 (8.46, 20.1)	1.78 (0.20, 2.46)	8.33 (4.29, 14.2)	26.3 (15.9, 31.9)	3.97 (2.50, 6.48)	0.05 (0.01, 0.12)	0.16 (0.10, 0.18)	1.55 (1.20, 2.43)	0.33 (0.17, 0.42)
EFV[1]	2.5 (0.50, 10.7)	-	187 (71.6, 339)	0.58 (0.27, 19.8)	-	775 (318, 1453)	0.94 (0.50, 1.89)	1.14 (0.25, 1.90)	-	4.26 (4.07, 4.54)	0.007 (0.005, 0.008)
RAL	1.81 (78.6, 297)	21.9 (10.5, 32.2)	157 (78.6, 297)	0.22 (0.19, 0.26)	2.29 (1.53, 3.17)	21.8 (14.2, 67.1)	0.50 (0.50, 1.05)	0.13 (0.05, 0.41)	0.13 (0.07, 0.17)	0.12 (0.05, 0.21)	0.003 (0.001, 0.007)

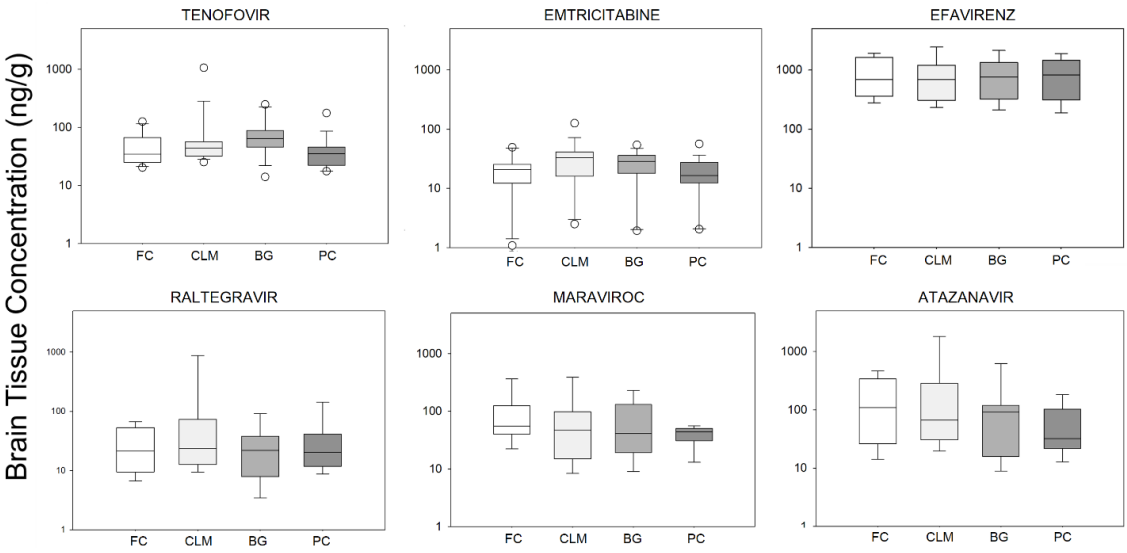
ARV/ Animal Model	ARV concentration (ng/mL [plasma and CSF] or ng/g [brain tissue])							ARV penetration ratio			
	Plasma			Brain Tissue			CSF	Brain Tissue:Plasma ratio			CSF:Plasma ratio
	Hu-HSC-RAG	BLT	NHP	Hu-HSC-RAG	BLT	NHP	NHP	Hu-HSC-RAG	BLT	NHP	NHP
MVC	1.26 (0.50, 5.73)	5.67 (0.94, 23.4)	31.8 (18.0, 80.6)	0.22 (0.20, 1.16)	12.3 (4.44, 19.7)	57.5 (37.6, 108)	0.50 (0.50, 4.96)	0.39 (0.25, 0.44)	1.86 (0.64, 4.84)	1.81 (0.76, 2.14)	0.030 (0.014, 0.082)
ATZ	9.91 (2.50, 18.7)	9.80 (8.64, 14.4)	2.40 (0.50, 106)	0.98 (0.49, 1.54)	2.10 (0.71, 10.1)	84.1 (47.2, 269)	0.50 (0.50, 4.96)	0.13 (0.06, 0.76)	0.12 (0.04, 0.28)	97.4 (0.41, 166)	0.21 (0.039, 0.98)

Data are shown as median (inter-quartile range).

1. EFV was not dosed in the BLT mice due to prior toxicity concerns (personal communication, J. Victor Garcia, 2014).

Supplementary Figure 1.

Antiretroviral concentrations in different parts of the brain in rhesus macaques



ARV concentration was not significantly different across the various parts of the brain ($p>0.08$). FC – frontal cortex, CLM – cerebellum, BG – basal ganglia, PC – Parietal cortex.