494

Relationship between Efavirenz Trough & *CYP2B6, NAT2,* and *SLCO1B1* Genotypes with Rifampin-based TB Therapy in STRIDE (A5221)

AF Luetkemeyer[1], S Rosenkranz[2], D Lu[2], B Grinsztejn[3], J Sanchez[4], M Ssemmanda,[5] I Sanne[6], H McIlleron[7] ,DV Havlir[1], DW Haas[8], Adult AIDS Clinical Trials Group (ACTG) A5221 and A5243 study teams.

[1]University of California, San Francisco, US [2]Harvard School of Public Health, Boston, US [3] Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz (FIOCRUZ), Rio de Janiero, Brazil,[4]Asociación Civil Impacta Salud y Educacioó, Lima, Peru,[5] Joint Clinical Research Center, Kampala, Uganda , [6] University of the Witwatersrand, Johannesburg, South Africa, [7] University of Cape Town, Cape Town, South Africa, [9]Vanderbilt University, Nashville, US.

Anne Luetkemeyer
995 Potrero Ave, Box 0874
San Francisco, CA 94110
415 476 4082 ext 130 (ph)
415 476 6953 (fax)
aluetkemeyer@php.ucsf.edu

Introduction Rifampin (RIF) induces cytochrome P450 (CYP) isoforms, which can lower plasma EFV exposure. However, in STRIDE, RIF-containing tuberculosis therapy was associated with increased EFV C_{min} (1). As *CYP2B6* polymorphisms predict EFV exposure (2), we conducted a nested study to evaluate EFV C_{min} by *CYP2B* metabolizer genotype in STRIDE. We also evaluated single nucleotide polymorphisms (SNPs) which affect isoniazid (*NAT2*) metabolism (3), and rifampin (*SLCO1B1*) transport into hepatocytes (4), thus potentially impacting rifampin levels (5) and EFV metabolism.

Methods: EFV C_{min} was measured by HPLC (LLQ = 0.1 µg/mL) in samples 20-28 hours post-dose following no missed doses in prior 3 days (self report). Subject-level on- and off-RIF EFV C_{min} values are means of available C_{min} values at weeks 4, 8, 16 and 24 on-RIF and at weeks 4 and 8 off-RIF. *CYP2B6* genotyping was by MassARRAY iPLEX Gold (Sequenom, Inc). *SLCO1B1* and *NAT2* SNPs were genotyped by TaqMan (Applied Biosystems, Inc., Foster City, CA).

CYP2B6: 3 SNPs; 15582C→T, 516G→T, and 983T→C: *extensive* =CC-GG-TT or CT-GG-TT; *intermediate* = TT-GG-TT, CC-GT-TT, CC-GG-TC, CT-GT-TT, or CT-GG-TC; *slow* = CC-TT-TT, CC-GT-TC, or CC-GG-CC.

NAT2: 4 SNPs : *NAT2*14* (rs1801279), *NAT2*5* (rs1801280), *NAT2*6* (rs1799930), *NAT2*7* (rs1799931). *Slow*=homozygous for slow allele at any locus (AA, CC, AA, AA, respectively) or 2 intermediate alleles. *intermediate*=heterozygous at a single locus. *extensive*= GG, TT, GG, GG, respectively at all loci.

SLCO1B1: 1 SNP rs4149032: *extensive*=TT, *intermediate* = CT, and *slow* = CC.

Statistical Methods: Between- and within-subject on- and off-RIF differences in EFV C_{min} values were evaluated by Wilcoxon rank sum (WRS) test and Wilcoxon signed-rank test (WSR) , respectively. Differences between metabolizer groups were evaluated with the WRS and 2 sample t-test.

Baseline Characteristics	**Total (n=42)**
Male	22 (52%)
Median BMI (min, max)	21.5 (14.5, 18.8)
Black	30 (71%)
Hispanic	12 (29%)
South Africa	22 (52%)
Peru	12 (29%)
Uganda	8 (19%)

Results::*CYP2B6* metabolizer genotypes were extensive (11), intermediate (19), and slow (12). On-RIF EFV C_{min} median was 1.96 (min 0.05, max 19.71) and off-RIF 1.85 (0.73, 11.69) (within-subject median difference=0.11; WSR P-value= 0.17). Slow metabolizers were consistently elevated vs. intermediate and extensive metabolizers, both on-RIF and off-RIF (**Fig 1.**) 7/12 (58%) of slow metabolizers had higher on-RIF values than off-RIF.

Figures 1-3 indicate individual EFV C_{min} on-RIF and off -RIF by metabolizer or transporter genotypes. The red line connects between subjects medians. The dotted horizontal line indicates EFV 1.0 ug/ml. 1.0 ug/ml. Within each metabolizer/transporter group, paired (within-subject) on- and off-RIF differences in EFV C_{min} were not significantly different from zero; neither were aggregate on- and off-RIF EFV levels significantly different.

Fig 2: Asterisks identify subjects with 2 *NAT2* intermediate alleles, categorized as slow metabolizers in this analysis

Fig 1.

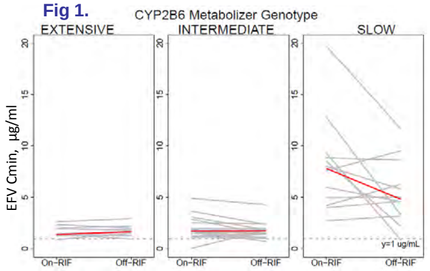


Fig 2.

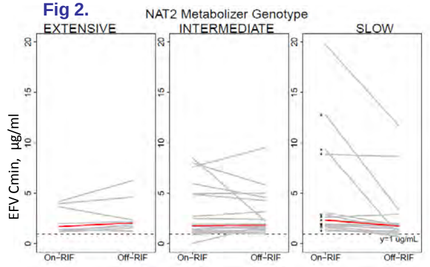


Fig 3.

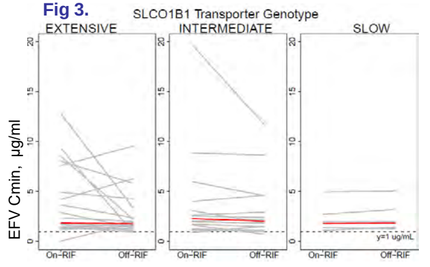


Figure 4. Within subject EFV C_{min} estimates on- and off-RIF, grouped by *CYP2B6* and NAT2 (INT+INT=SLO) genotypes (EXT=extensive; INT=intermediate; SLO = slow). Bolded red line connects between subject medians.

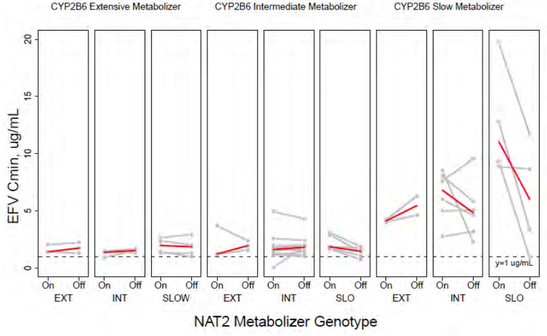
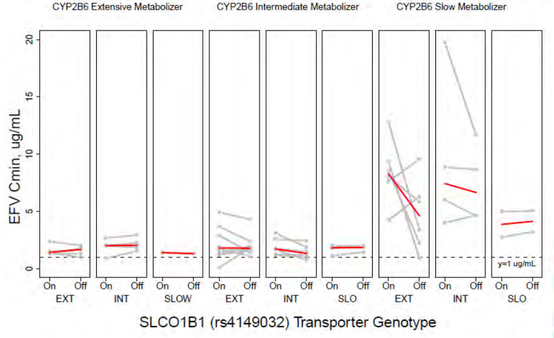


Fig 5. Within-subject EFV C_{min} estimates on- and off-RIF, grouped by *CYP2B6* and *SLCO1B1* genotype (EXT=extensive; INT=intermediate; SLO = slow). Bolded red line connects between subject medians.



Conclusions In a subset of STRIDE subjects genotyped for *CYP2B6*, slow metabolizers had: (a) higher off-RIF EFV C_{min} than intermediate and extensive metabolizers and (b) more than half with even higher on-RIF EFV C_{min} compared to off-RIF. This suggests that increased EFV C_{min} with rifampin is driven in part by slow metabolism genotypes. *NAT2* slow metabolizer genotype may further contribute to elevated EFV C_{min} in *CYP2B6* slow metabolizers while on-RIF. This may be due increased isoniazid concentrations inhibiting CYP2A6, an accessory EFV metabolic pathway. *SLCO1B1* genotype status did not appreciably affect EFV concentrations; however, rifampin concentrations were not measured. The analysis suggests that during TB treatment with rifampin and isoniazid, EFV concentrations are affected by both *CYP2B6* and *NAT2* genotypes.

References

1. Luetkemeyer AF, et al., *Relationship Between Weight, Efavirenz Exposure, and Virologic Suppression in HIV-Infected Patients on Rifampin-Based Tuberculosis Treatment in the AIDS Clinical Trials Group A5221 STRIDE Study. Clin Infect Dis* 2013; **57**: 586-93
2. Holzinger ER, et al. Genome-Wide Association Study of Plasma Efavirenz Pharmacokinetics in AIDS Clinical Trials Group Protocols. *Pharmacogenet Genom* 2012; 22: 858-67.
3. Lee L, et al. *Differential Induction of Efavirenz Metabolism by Rifampin without and with Isoniazid in Healthy Volunteers with CYP2B6 516GG and TT Genotypes (Abstract 516).* in *20th Conference on Retroviruses and Opportunistic Infections* 2013. Atlanta, GA
4. Niemi M, et al., *Effect of SLCO1B1 polymorphism on induction of CYP3A4 by rifampicin. Pharmacogenet Genomi* 2006; **16**: 565-8.
5. Chigutsa E, et al., *The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin concentrations: dosing implications. Antimicrob Ag Chemother* 2011; **55**: 4122-7

ACTG AIDS CLINICAL TRIALS GROUP

University of California San Francisco UCSF