Phase I Safety, Pharmacokinetics, and Pharmacogenetics Study of the Anti-Tuberculosis Drug PA-824 with Concomitant Lopinavir/Ritonavir, Efavirenz, or Rifampin

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ABSTRACT

There is an urgent need for new anti-tuberculosis (TB) drugs, including agents that are safe and effective with concomitant antiretrovirals (ARV) and first-line TB drugs. PA-824 is a novel anti-tuberculosis nitroimidazole in late-phase clinical development. Cytochrome P450 (CYP) 3A, which can be induced or inhibited by ARV and anti-tuberculosis drugs, is a minor (~20%) metabolic pathway for PA-824. In a Phase I clinical trial, we characterized interactions between PA-824 and efavirenz (Arm 1), lopinavir/ritonavir (Arm 2), and rifampin (Arm 3) in healthy, HIV-uninfected volunteers without TB disease. Participants in Arms 1 and 2 were randomized to receive drugs via Sequence 1 (PA-824 alone, washout, ARV, ARV plus PA-824) or Sequence 2 (ARV, ARV with PA-824, washout, PA-824 alone). In Arm 3, participants received PA-824, then rifampin, then both. Pharmacokinetic sampling occurred at the end of each dosing period. Fifty-two individuals participated. Compared to PA-824 alone, plasma PA-824 values (based on geometric mean ratios) for maximum concentration ($C_{\text{max}}$), area under the concentration-time curve ($\text{AUC}_{0-24h}$), and trough concentration ($C_{\text{min}}$) were reduced 28%, 35%, and 46% with efavirenz; 13%, 17%, and 21% with lopinavir/ritonavir; and 53%, 66%, and 85% with rifampin, respectively. Medications were well tolerated. In conclusion, lopinavir/ritonavir had minimal effect on PA-824 exposures, supporting PA-824 use with lopinavir/ritonavir without dose adjustment. PA-824 exposures, though, were reduced more than expected when given with efavirenz or rifampin. The clinical implications of these reductions will depend upon data from current clinical trials defining PA-824 concentration-effect relationships.
INTRODUCTION

In 2012 there were 8.6 million cases of tuberculosis and 1.3 million tuberculosis-related deaths (1). “Short course” treatment of drug-sensitive tuberculosis requires six months. Multidrug-resistant (MDR) tuberculosis (i.e. resistant to isoniazid and rifampin) is a growing public health threat, with therapeutic options limited by drug availability, acceptability and efficacy (2).

Current MDR-tuberculosis therapy requires ≥18 months of multidrug therapy with at least 6 months of an injectable agent (3), is poorly tolerated, and is successful in only 48% of patients (2). Almost one-third of tuberculosis-related deaths globally are in patients with HIV-co-infection (1). There is an urgent need for novel anti-tuberculosis regimens to shorten treatment duration for drug-sensitive tuberculosis and improve the efficacy and safety for MDR-tuberculosis. The utility of novel drugs will be significantly enhanced if they are safe and effective among patients who require anti-HIV therapy.

The investigational nitroimidazole PA-824 has potent in vitro activity against M. tuberculosis and no cross-resistance with marketed anti-tuberculosis drugs (4, 5). Its activity against metabolically active and non-replicating M. tuberculosis (5) suggests likely bactericidal and sterilizing activity. The latter is critical for treatment shortening. In mouse models of tuberculosis, PA-824 given with rifampin and pyrazinamide reduced curative treatment duration from 6 months to 4 months (6). In mice, PA-824 with moxifloxacin and pyrazinamide was similarly potent; in humans, this same three-drug combination reduced sputum mycobacterial colony counts more effectively than standard treatment over two weeks’ time when PA-824 was given at a dose of 200 mg once-daily (7, 8). A Phase 2B trial of PA-824 over 8 weeks at doses of 100 mg and 200 mg with moxifloxacin and pyrazinamide finished recently (results pending).
PA-824 has not been tested clinically in a combination with rifampin plus pyrazinamide, the key sterilizing drugs in anti-tuberculosis therapy. It is not known whether PA-824 can reduce treatment duration when given with first-line or second-line anti-tuberculosis drugs.

To include PA-824 in anti-tuberculosis regimens in HIV-infected patients, the safety and pharmacokinetics (PK) of drug combinations must be assessed. PA-824 is extensively metabolized via a combination of reductive metabolism and oxidative metabolism with no one single metabolic path that can be considered major. In vitro studies suggest that cytochrome P450 (CYP) 3A contributes up to 20% to overall metabolism; PA-824 is not a substrate of CYP2C9, 2C19, or 2D6 metabolizing enzymes (personal communication, TB Alliance).

Rifampin induces many metabolizing enzymes including CYP3A (9). Efavirenz is included in first-line regimens for HIV in many settings, and lopinavir (with ritonavir, lopinavir/r) is the most widely prescribed HIV-1 protease inhibitor globally. Efavirenz induces CYP3A enzymes, while lopinavir and ritonavir can inhibit or induce CYP3A (10). In addition, CYP2B6 genotype is a key determinant of efavirenz concentrations (11-15), while SLCO1B1 polymorphisms impact lopinavir exposures (16-18). In this Phase I trial we investigated the safety and PK interactions of PA-824 with efavirenz, lopinavir/r, and rifampin, taking into account pharmacogenetics.

MATERIALS AND METHODS

Study population

Healthy adults 18 to 65 years were recruited at AIDS Clinical Trials Group (ACTG) sites in the US. Eligible participants had negative HIV and hepatitis C antibody tests, normal ALT, and
creatinine clearance values >50 mL/min. Volunteers were excluded for hemoglobin ≤12.0 g/dL (male) or ≤11.0 g/dL (female), absolute neutrophil count <1250 cells/mm³, platelets <125,000 cells/mm³, electrocardiogram (ECG) with QTc >450 or PR >200 milliseconds, or active tuberculosis. Frequent headaches was another exclusion criterion. The study was approved by institutional review boards of participating sites. All participants provided written informed consent. ACTG A5306 was registered at clinicaltrials.gov (NCT01571414).

Experimental protocol

Study design. Phase I, open-label PK and safety study. PA-824 was dosed 200 mg once-daily, efavirenz 600 mg once-daily, rifampin 600 mg once-daily, and lopinavir/r 400/100 mg every 12 hours. Efavirenz was taken in the evenings. PA-824 and rifampin were taken in the mornings. All medications were taken on an empty stomach. Participants were sequentially assigned to Arm 1 (efavirenz), Arm 2 (lopinavir/r), or Arm 3 (rifampin) (Figure 1). In Arm 1, participants were randomized to Sequence 1 (PA-824 for 7 days, 2-week washout period, efavirenz for 14 days, efavirenz with PA-824 for 7 days) or Sequence 2 (efavirenz, efavirenz with PA-824, washout, then PA-824 alone). Arm 2 participants were randomized to two sequences, as follows: Sequence 1 (PA-824 for 7 days, two-week washout period, lopinavir/r for 14 days, then lopinavir/r with PA-824 for 7 days) or Sequence 2 (lopinavir/r, lopinavir/r with PA-824, washout, PA-824 alone). In Arm 3, participants received PA-824 for 7 days, rifampin for 7 days, then PA-824 with rifampin for 7 days. Adherence was assessed by pill counts and medication diaries. All doses prior to PK sampling were observed by study staff. Serial plasma sampling for PK was performed at the end of each dosing period for PA-824, efavirenz, and lopinavir. For PA-824, plasma was obtained pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours post-dose. For
efavirenz, plasma was obtained pre-dose and 1, 2, 3, 4, 8, 12, and 24 hours post-dose. For lopinavir, plasma was obtained pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours post-dose.

Rifampin concentrations were not measured in this small PK study because the effects of PA-824 on rifampin PK were expected to be small. In addition, since rifampin concentrations are highly variable and that variability is not well-explained by known genetic polymorphisms, even large changes would be unlikely to be detected.

Safety monitoring. Participants underwent weekly safety evaluations. ECG evaluations were performed at baseline and on the final day of PA-824 dosing periods given that some nitroimidazole antibiotics can cause QT prolongation. Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0 (December 2004, August 2009 clarification) (19).

Drug concentration analysis

Plasma assay for PA-824. PA-824 and the internal standard, triazolam, were isolated from EDTA-plasma by liquid-liquid extraction. The organic phase was removed, transferred to a clean test tube, and evaporated under nitrogen. The residue was reconstituted in MeOH:water (1:1), transferred to autosampler vials for injection onto a Chromolith SpeedROD-18 high-performance liquid chromatograph (HPLC) column, and eluted with a linear gradient of 10 mM ammonium acetate and methanol (30:70). The ion pairs 359.8/174.7 for PA-824 and 342.8/307.7 for triazolam were selected for tandem mass detection in MRM mode. Quantification of PA-824 was performed with an LC-tandem mass spectrometer system comprising two PerkinElmer series 200 micro LC pumps and a series 200 autosampler coupled with an AB Sciex API2000 tandem mass
spectrometer. For calibration curves, spiked concentrations and peak area ratios of PA-824 and internal standard were fitted by linear least squares regression, weighted by $1/x$. The method was validated over a linear range of 10-10,000 ng/mL with the correlation of $R=0.9984$. For the measurements of PA 824, the inter-assay precision (% CV) ranged from 2.65 to 4.71% and the percent deviation ranged from 0.8 to 5.2% of the nominal values of the control concentrations. The Intra-assay precision (% CV) ranged from 1.58 to 6.29% and the percent deviation ranged from -0.4 to 11.19% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 10 and 10,000 ng/mL, respectively. Plasma aliquots of 50 µL were sufficient for analysis.

Plasma assays for efavirenz and lopinavir. Efavirenz was quantified by reversed phase HPLC following extraction from human plasma by simple protein precipitation. Detection involved a photodiode-array detector, scanning at wavelength of 247 nm, and reserpine as the internal standard. The calibration curve concentration range was 100 ng/mL to 6,000 ng/mL. For calibration curves, spiked concentrations and peak height ratios of efavirenz and internal standard were fitted by linear least squares regression, weighted $1/x$. Efavirenz concentrations were calculated from regression parameters using peak height ratios. The method was validated over a linear range of 100-10,000 ng/mL with the correlation of $R=0.9995$. For the measurements of EFV, the inter-assay precision (% CV) ranged from 2.4 to 4.5% and the percent deviation ranged from -0.4 to 3.3% of the nominal values of the control concentrations. The Intra-assay precision (% CV) ranged from 0.6 to 5.4% and the percent deviation ranged from -1.7 to 6.1% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 100 and 10,000 ng/mL, respectively.
Lopinavir and its deuterated internal standards were extracted from 50µL EDTA human plasma by protein precipitation with acetonitrile followed by centrifugation. The clear supernatant was transferred into autosampler vials for a 10 µL injection onto an Agilent Zorbax XDB-C8 (5µ, 2.1 x 50 mm HPLC column). Mobile phase comprised 10 mM ammonium formate buffer (pH 4.0) and acetonitrile containing 0.1% formic acid. Elution was performed using a gradient flow rate of 400 µL/minute with MS/MS detection on an ABSCIEX API 2000 mass spectrometer (MS) using electrospray in positive ion mode. The ion pairs 629.2/429.0 for Lopinavir and 637.2/429.0 for LPV-D8 were selected for tandem mass detection. For calibration curves, spiked concentrations using peak area ratios of lopinavir were fitted by 1/x linear regression. The method was validated over a linear range of 50-8,000 ng/mL with the correlation of R=0.9988. For the measurements of LPV, the inter-assay precision (% CV) ranged from 4.50 to 5.11% and the percent deviation ranged from -8.15 to 0.037% of the nominal values of the control concentrations. The intra-assay precision (% CV) ranged from 2.35 to 6.39% and the percent deviation ranged from -9.33 to 2.72% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 50 and 8,000 ng/mL, respectively.

Pharmacogenetic testing

Genetic polymorphisms reported to predict plasma PK of efavirenz (11-15), rifampin (20, 21), and lopinavir (17, 18) were genotyped in duplicate. For efavirenz, CYP2B6 516G→T (rs3745274), 983T→C (rs28399499) and 15582C→T (rs4803419) were assayed using MassARRAY® iPLEX Gold (Sequenom Inc., San Diego, California, USA). Composite CYP2B6 genotype was defined as follows (15): extensive metabolizer, 516 G/G, 983 T/T, with either
15582 C/C or C/T; intermediate metabolizer, 516 G/T or 983 T/C but not both, or homozygosity for 15582 T/T; and slow metabolizer, 516 T/T, 983 C/C, or the combination of 516 G/T with 983 T/C. For rifampin, \textit{SLCO1B1} rs4149032C$\rightarrow$T, and for lopinavir/r, \textit{SLCO1B1} 521T$\rightarrow$C (rs4149056) were genotyped by TaqMan™ (Applied Biosystems, Inc., Foster City, CA). The \textit{CYP3A5}*3 variant 6986A$\rightarrow$G (rs776746) (22) was genotyped by MassARRAY® iPLEX Gold.

**Pharmacokinetic and statistical analyses.**

**Sample size** Thirteen volunteers per arm were estimated to provide 80% power to detect a 20% mean difference in 24-hour area under the concentration-time curve (AUC$_{0-24h}$) for PA-824 when co-administered with companion drug versus given alone, using a two-sided t-test at a significance level of 0.05. We targeted enrollment of 16 participants per arm.

**Pharmacokinetic and statistical evaluation** PK parameters for PA-824, efavirenz, and lopinavir, including AUC, maximum plasma concentration ($C_{\text{max}}$), time of maximum plasma concentration ($T_{\text{max}}$), half-life ($T_{1/2}$), and oral clearance (CL/F) were determined using standard noncompartmental methods performed in SAS (SAS Institute Inc., Cary, NC). Statistical analyses were based on nonparametric tests. The p-values evaluating changes in PK of PA-824 co-administered with efavirenz, lopinavir/r, or rifampin to PA-824 alone using Wilcoxon signed-rank test and comparing changes in PK parameters of these drugs among metabolizer groups using Wilcoxon rank-sum test or Kruskal-Wallis test are reported. Calculated geometric means of ratios (GMR) and 90% confidence interval based on log-transformed PK parameters were also used for PK comparisons. Associations between genotypes and PK parameters were assessed using the Jonckheere-Terpstra trend test and assuming additive genetic models.
RESULTS

Study Subjects. Fifty-two participants enrolled. Median age was 34 years (range 19-63), median weight was 83 kg (range 47-119), median body mass index was 27 kg/m² (range 18-41), and 30 (58%) were male. Thirty-one (60%) were white, 17 (33%) African-American or black, and two each were Asian or not reported. Of 52 participants, 48 completed all PK visits. There were two early discontinuations each in Arms 1 and 2. In Arm 1, one individual had efavirenz-related side effects, and another was a passenger in a motor vehicle accident. In Arm 2, two participants self-administered a lower-than-prescribed lopinavir/r dose so were discontinued. Thus, 48 participants were eligible for PK analyses.

Pharmacokinetics of PA-824, Efavirenz, and Lopinavir. Compared to PA-824 alone, plasma exposures (AUC₀₋₂₄h) of PA-824 (based on GMR) was reduced by 35% with efavirenz, 17% with lopinavir/r, and 66% with rifampin, respectively (Table 1). Plasma concentration-time curves for PA-824 alone versus PA-824 with efavirenz, lopinavir/r, and rifampin are shown in Figures 2 and S1. Plasma efavirenz and lopinavir concentrations were not appreciably affected by PA-824 (Table 2). In a post-hoc nonlinear mixed effects modeling analysis of our Phase 1 trial data plus raw data from Phase 1 and 2 trials of PA-824 supplied by TB Alliance, we found that PA-824 exposures were similar for a 200 mg dose taken together with efavirenz and a 100 mg dose taken without efavirenz. The same was true for PA-824 co-administered with rifampin, except that while overall exposures were similar, trough concentrations remained modestly reduced with rifampin coadministration (data not shown).
Pharmacogenetic associations. Of 16 subjects evaluable for PK in Arm 1 (efavirenz), 6 (38%) were CYP2B6 extensive metabolizers, 10 (63%) were intermediate metabolizers, and none were slow metabolizers. Changes in PK parameter values for PA-824 (based on geometric means of ratios (GMR)) with concomitant efavirenz did not differ significantly between CYP2B6 intermediate and extensive metabolizers (e.g. PA-824 C$_{\text{min}}$ reduced by 44% and 49%, respectively, $p = 0.692$). Of 16 subjects evaluable for PK in Arm 2 (lopinavir/r), 12 (75%) were homozygous for SLCO1B1 521 T/T, and 4 (25%) were heterozygous for SLCO1B1 521 C/T. Regarding CYP3A5, 5 (31%) had rs776746 extensive, 3 (19%) had intermediate, and 8 (50%) had slow metabolizer genotypes. Changes in PK parameter values for PA-824 (based on GMR) with concomitant lopinavir/r did not differ consistently comparing SLCO1B1 521 C/T versus SLCO1B1 521 T/T or by CYP3A5 genotype. Of 16 subjects evaluable for PK in Arm 3 (rifampin), 4 (25%) were homozygous for SLCO1B1 rs4149032 T/T, 5 (31%) were heterozygous for rs4149032 C/T, and 7 (44%) were homozygous for SLCO1B1 rs4149032 C/C. Regarding CYP3A5, 4 (25%) had rs776746 extensive, 6 (38%) had intermediate, and 6 (38%) had slow metabolizer genotypes. Changes in PK parameter values for PA-824 (based on GMR) with concomitant rifampin did not differ significantly by SLCO1B1 rs4149032 or by CYP3A5 genotype. Relationships between polymorphisms and PK parameters of efavirenz and lopinavir as well as relationships between polymorphisms and magnitude of drug-drug interactions when PA-824 was administered with efavirenz, lopinavir/r, or rifampin are described in Supplemental On-line Materials.
To explore genetic associations we considered PA-824 PK data without concomitant efavirenz, lopinavir/r, or rifampin in all 48 subjects. We found no apparent association between *CYP2B6*, *SLCO1B1*, and *CYP3A5* polymorphisms and PA-824 PK parameters. The relationship between these polymorphisms and \( C_{\text{min}} \) is shown in the **Supplemental On-line Materials**.

**Safety and tolerability.** PA-824 was well tolerated alone and with concomitant efavirenz, lopinavir/r, and rifampin. There were two grade \( \geq 3 \) adverse events. In Arm 2, one participant had an asymptomatic elevation of AST following vigorous exercise (PA-824 alone, Sequence 2). One subject in Arm 3 experienced grade 3 neutropenia on the last day of dosing, likely due to rifampin. Both adverse events resolved quickly after drug discontinuation. There were no Grade \( \geq 2 \) QTc events.

**DISCUSSION**

For the first time in decades, there is a robust drug development pipeline for tuberculosis. The nitroimidazole PA-824 is poised to enter Phase 3 clinical trials. Anticipating the need to treat patients co-infected with HIV-1, we examined the safety, tolerability, and PK of PA-824 given with commonly-used antiretrovirals that induce or inhibit P450 metabolizing enzymes (efavirenz and lopinavir/r), and with the essential first-line tuberculosis drug, rifampin. We showed substantial reduction of plasma PA-824 exposure by efavirenz and rifampin, but modest changes with lopinavir/r. The combinations were safe and well tolerated, and PA-824 did not affect plasma efavirenz or lopinavir exposures.

Concurrent treatment of HIV and tuberculosis reduces mortality and new AIDS-defining illnesses (23-25). Because co-treatment can be complicated by drug-drug interactions,
overlapping drug toxicities, immune reconstitution syndrome, and high pill burden (26), late-
phase clinical trials of anti-tuberculosis drugs typically exclude patients requiring antiretroviral
therapy. In the present study, co-administration of PA-824 with lopinavir/ritonavir did not
increase PA-824 concentrations; rather, exposures were modestly reduced. Ritonavir is a mixed
inducer and inhibitor, and for PA-824 induction apparently dominated. This demonstrates the
importance of empiric data when ritonavir is used with other drugs, as it is difficult to make \textit{a}
\textit{priori} predictions about its likely effects on companion drugs (10, 27). The modest reductions in
PA-824 with lopinavir/r were statistically significant but are likely not clinically relevant. In
contrast, efavirenz substantially decreased PA-824 exposure, likely by upregulating CYP3A or
other metabolizing enzymes. While CYP3A contributes only 20\% to overall metabolism of PA-
824, this percentage may increase when CYP3A is induced. Whether interactions of efavirenz
and rifampicin with PA-824 will be clinically important can only be answered by PK/PD
analysis of trials data in which different doses of PA-824 are tested for longer durations. PK and
outcomes data from an eight-week Phase 2 clinical trial are expected soon, and with these data in
hand, concentration-effect relationships can be explored more fully.

Rifampin has unique sterilizing activity against \textit{M. tuberculosis}, making it a mainstay of first-
line tuberculosis treatment. To date there are no drugs clinically proven to have sterilizing
activity equal to rifampin. Rifampin is, however, a potent inducer of metabolizing enzymes and
drug transporters (28). Rifampin induced drug interactions complicate drug development efforts
for drug-sensitive tuberculosis in two ways. First, promising investigational drugs cannot be
added to first-line anti-tuberculosis regimens without evaluating PK effects of rifampin and other
coadministered anti-tuberculosis drugs. For example, rifampin reduces concentrations of the
newest TB drug, bedaquiline, by 50\% (29). Conversely, isoniazid can inhibit metabolizing
enzymes, and unexpected effects may occur when rifampin and isoniazid are co-administered with a third drug (30, 31). Secondly, rifamycin antibiotics like rifampin and rifapentine have dose-dependent treatment-shortening potential, but evaluating high-dose rifamycins is challenging because the magnitude of drug interactions at increased rifamycin doses is unknown. That is, while it is generally believed that rifamycins’ inductive capabilities are maximized at currently-used doses, recent preliminary studies in human hepatocytes suggest that mRNA expression of CYP3A increases with higher rifamycin concentrations, within clinically-relevant ranges (32). Whether or not higher mRNA expression will lead to greater enzyme activity or higher risk for clinical drug interactions is unknown.

Interpretation of drug interaction study results requires understanding of study drug pharmacodynamics (PD, i.e. correlations between PK parameters and efficacy). For tuberculosis, lack of a reliable biomarker of treatment response makes it difficult to define PK-PD relationships. PA-824 is being tested in a two-month Phase 2B treatment trial at doses of 100 mg and 200 mg daily, because doses from 100 mg to 1000 mg had similar activity in the two-week dose-ranging Phase 2A monotherapy studies; only at 50 mg daily was EBA decreased (33, 34). Preclinical studies suggest time-dependent activity of PA-824 against *M. tuberculosis* (35), suggesting that AUC$_{0-24h}$ may be a key pharmacodynamic parameter; however, target AUC values have not been defined. The Phase 2B study may help define dose-effect or concentration-effect relationships that will give our results context.

There are well-replicated associations between *CYP2B6* polymorphisms and efavirenz PK (11-15), and between an *SLCO1B1* polymorphism and lopinavir PK (16-18). An association has been
reported between an *SLCO1B1* polymorphism and rifampin PK (20, 21). It is important to consider whether these polymorphisms affect drug interactions. In addition, because PA-824 is metabolized by CYP3A, we assessed a *CYP3A5* loss-of-function polymorphism. Our study did not show magnitudes of effects of efavirenz, lopinavir, and rifampin on PA-824 PK parameters to differ by the above genetic polymorphisms. In addition, we found no apparent associations between these polymorphisms and PA-824 Cmin, though the sample size was limited. We suspect that the apparent association between *CYP3A5*<sup>*</sup>3 and lopinavir PK is spurious, since this association was not seen elsewhere (16-18).

There were limitations to the present study. Because study drugs were given for relatively brief intervals, the full safety profile could not be assessed. The effect of rifampin on PA-824 was not assessed in the context of full multidrug first-line anti-tuberculosis treatment. It is possible that effects of rifampin alone differ from effects when combined with other first-line anti-tuberculosis drugs. None of the multiple metabolites of PA-824 were measured in this study, and the specific metabolizing enzyme(s) that mediate reductions in PA-824 are not known.

In conclusion, PA-824 was well-tolerated when given with efavirenz, lopinavir/r, and rifampin. Concomitant lopinavir/r only modestly reduced PA-824 plasma exposures, suggesting that they can be coadministered without dose adjustment. Efavirenz reduced PA-824 exposures more substantially, and rifampin reduced PA-824 exposure even more. The clinical implications of these findings should be interpreted in light of results of ongoing Phase 2 dose-ranging trials that will define dose-response relationships and identify target concentrations for maximal PA-824 effect, so that use of PA-824 in first line regimens and in patients requiring HIV-1 therapy can be optimized.
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Table 1: Pharmacokinetic parameters of PA-824 when PA-824 is administered alone (200 mg once daily) or co-administered with steady-state efavirenz (600 mg once daily), lopinavir/ritonavir (400 mg/100 mg twice daily), or rifampin (600 mg once daily).

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<th>Arm 1: PA-824 with efavirenz</th>
<th>PA-824 Alone</th>
<th>PA-824 with Efavirenz</th>
<th>GMR</th>
<th>90% CI</th>
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<td>Pharmacokinetic parameter</td>
<td>Medians (IQR)</td>
<td>Medians (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-24}(ng*h/mL)</td>
<td>36,495 (30,853, 53,857)</td>
<td>24,917 (19,094, 34,257)</td>
<td>0.65</td>
<td>(0.56, 0.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>2,035 (1805, 2840)</td>
<td>1510 (1225, 2025)</td>
<td>0.72</td>
<td>(0.62, 0.83)</td>
<td>0.001</td>
</tr>
<tr>
<td>C_{min} (ng/mL)</td>
<td>1110 (892, 1650)</td>
<td>653 (502, 936)</td>
<td>0.54</td>
<td>(0.45, 0.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>4.0 (4.0-5.0)</td>
<td>4.0 (3.5-5.0)</td>
<td>0.88</td>
<td>(0.65, 1.20)</td>
<td>0.383</td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>24.8 (18.9, 27.1)</td>
<td>16.2 (14.9, 21.0)</td>
<td>0.74</td>
<td>(0.68, 0.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>5.48 (3.71, 6.48)</td>
<td>8.03 (5.87, 10.6)</td>
<td>1.53</td>
<td>(1.31, 1.78)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arm 2: PA-824 with lopinavir/r</th>
<th>PA-824 Alone</th>
<th>PA-824 with LPV/r</th>
<th>GMR</th>
<th>90% CI</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic parameter</td>
<td>Medians (IQR)</td>
<td>Medians (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-24}(ng*h/mL)</td>
<td>39,035 (24,295, 42,187)</td>
<td>29,899 (20,691, 37,949)</td>
<td>0.83</td>
<td>(0.71, 0.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>2,130 (1,440, 2,425)</td>
<td>1,770 (1,285, 2,245)</td>
<td>0.87</td>
<td>(0.75, 1.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>4.0 (3.5, 5.0)</td>
<td>4.5 (3.5, 5.0)</td>
<td>1.10</td>
<td>(0.88, 1.38)</td>
<td>0.47</td>
</tr>
<tr>
<td>C_{min} (ng/mL)</td>
<td>1,085 (708, 1320)</td>
<td>838 (509, 1,155)</td>
<td>0.79</td>
<td>(0.66, 0.93)</td>
<td>0.01</td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>21.6 (18.4, 28.7)</td>
<td>16.7 (15.1, 23.7)</td>
<td>0.83</td>
<td>(0.73, 0.94)</td>
<td>0.04</td>
</tr>
<tr>
<td>Arm 3: PA-824 with rifampin</td>
<td>PA-824 Alone</td>
<td>PA-824 with rifampin</td>
<td>GMR</td>
<td>90% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
<td>----------------------</td>
<td>-----</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>AUC_{0-24} (ng*h/mL)</td>
<td>42,495 (29,501, 48,661)</td>
<td>13,659 (9,981, 19,070)</td>
<td>0.34</td>
<td>(0.27, 0.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>2,490 (1925, 2,885)</td>
<td>1,165 (769, 1,520)</td>
<td>0.47</td>
<td>(0.39, 0.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>4.0 (3.0, 6.0)</td>
<td>4.0 (3.5, 4.5)</td>
<td>1.00</td>
<td>(0.81, 1.22)</td>
<td>0.58</td>
</tr>
<tr>
<td>C_{min} (ng/mL)</td>
<td>1,080 (722, 1,300)</td>
<td>173 (93, 320)</td>
<td>0.15</td>
<td>(0.11, 0.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>19.25 (15.66, 20.78)</td>
<td>8.07 (6.28, 9.22)</td>
<td>0.41</td>
<td>(0.36, 0.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>5.13 (4.74, 8.23)</td>
<td>6.69 (5.28, 9.67)</td>
<td>1.20</td>
<td>(1.03, 1.41)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Geometric mean of ratios (GMR) of pharmacokinetics of PA-824 co-administered with a companion drug to PA-824 alone.

†p-value of Wilcoxon signed-rank test comparing pharmacokinetics of PA-824 co-administered with the companion drug to PA-824 alone.
Table 2. Pharmacokinetic parameters of efavirenz (when efavirenz is given at a dose of 600 mg daily) or lopinavir (when lopinavir/ritonavir is given at a dose of 400 mg/100 mg twice daily), when administered alone or co-administered with PA-824.

<table>
<thead>
<tr>
<th>Arm 1: Efavirenz with PA-824</th>
<th>Pharmacokinetic parameter</th>
<th>Efavirenz Alone Median (IQR)</th>
<th>Efavirenz with PA-824 Median (IQR)</th>
<th>GMR</th>
<th>90% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCo-24(ng*h/mL)</td>
<td>62,112 (48,568, 72,301)</td>
<td>55,835 (44,347, 73,023)</td>
<td>0.96</td>
<td>(0.91, 1.02)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>4380 (3,712, 4,773)</td>
<td>3945 (2,616, 4,897)</td>
<td>0.86</td>
<td>(0.72, 1.02)</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Cmin (ng/mL)</td>
<td>1868 (1,359, 2,275)</td>
<td>1768 (1,193, 2,270)</td>
<td>0.96</td>
<td>(0.90, 1.04)</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>20.2 (15.6, 24.3)</td>
<td>18.5 (16.5, 33.3)</td>
<td>1.14</td>
<td>(0.93, 1.40)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>3.23 (2.77, 4.12)</td>
<td>3.61 (2.74, 4.51)</td>
<td>1.04</td>
<td>(0.98, 1.11)</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arm 2: Lopinavir/r with PA-824</th>
<th>Pharmacokinetic parameter</th>
<th>LPV when LPV/r is given alone</th>
<th>LPV when LPV/r is given with PA-824</th>
<th>GMR</th>
<th>90% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCo-24(ng*h/mL)</td>
<td>95,689 (71,321, 117,600)</td>
<td>87,092 (58,858, 98,231)</td>
<td>0.86</td>
<td>(0.77, 0.96)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>11,450 (8,955, 13,400)</td>
<td>10,150 (7,400, 10,850)</td>
<td>0.83</td>
<td>(0.76, 0.92)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Cmin (ng/mL)</td>
<td>4,095 (2,245, 6,255)</td>
<td>2,925 (2,200, 5,110)</td>
<td>1.03</td>
<td>(0.60, 1.80)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>6.71 (4.67, 8.76)</td>
<td>6.54 (4.95, 8.22)</td>
<td>0.96</td>
<td>(0.84, 1.09)</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>4.18 (3.41, 5.64)</td>
<td>4.59 (4.07, 6.80)</td>
<td>1.17</td>
<td>(1.05, 1.30)</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE LEGENDS.

Figure 1. Schematic of the dosing regimen and pharmacokinetic sample collection for (A) Arm 1 (PA-824 with efavirenz (EFV)); (B) Arm 2 (PA-824 with lopinavir/ritonavir (LPV/r)); and (C) Arm 3 (PA-824 with rifampin (RIF)).

Figure 2. Mean loge PA-824 plasma concentration versus time curve of PA-824 200 mg once daily alone (solid lines) or together with steady-state (A) efavirenz (EFV) (dotted line); (B) lopinavir/ritonavir (LPV/r) (dotted line); or (C) rifampin (RIF) (dotted line). Values shown represent means with standard error.
Figure 1.

A.

SEQUENCE 1

Period 1: PA-824 Days 1-7

No treatment

Washout Period Days 8-21

Period 2: EFV Days 22-35

Period 3: EFV plus PA-824 Days 36-42

Intensive 24 hr PA-824 PK (day 7)

SEQUENCE 2

Period 1: EFV Days 1-14

Period 2: EFV plus PA-824 Days 15-21

No treatment

Washout Period Days 22-35

Period 3: PA-824 Days 36-42

Intensive 24 hr EFV PK (day 13)

Intensive 24 hr EFV (day 20) and PA-824 PK (day 21)

Intensive 24 hr EFV (day 34) and PA-824 PK (day 41)
C.

**Period 1**
- PA-824
- Days 1-7

**Period 2**
- RIF
- Days 8-14

**Period 3**
- RIF plus PA-824
- Days 15-21

**Intensive 24 hr PA-824 PK**

- (day 7)

- (day 42)