Utility of Quantitative Analysis in Drug Development and Optimization of Anti-Infective Therapy

by
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Abstract

The cornerstone of anti-infective therapy is attainment of the effective target concentration of the drug at the site of infection; achieving this goal requires integration of the pharmacokinetic and pharmacodynamics properties of the anti-infective drug. In this interplay, systemic and local quantitative analysis of the drug plays an integral part in providing information about the pharmacokinetic and pharmacodynamic properties of the anti-infective; this information is critical for drug development as well as evaluation of adequacy of established therapies. In this thesis, we will demonstrate the role of systemic and local quantitative analysis in the development of preventative strategies for HIV and also, the role of quantitative analysis in assessing adequacy of therapy in pediatric tuberculosis (TB).

The projects in this thesis highlight the various points that are key for successful use of anti-infective drugs. The first two projects, CHARM-01 and CHARM-02, focus on use of anti-infectives for prophylaxis; specifically the development of tenofovir (TFV)-containing gels as locally applied (rectal) microbicides. Since these gels are locally-dosed, there are several factors that have to be considered such as the mucosal safety of the formulations, the ability of the formulations to cover all potentially HIV-exposed mucosa, and the ability to reach the optimal concentration of the active drug, TFV diphosphate (TDF-DP) to prevent HIV infection.

In contrast, the PHATISA project looks at systemic (oral) dosing of anti-TB drugs in children for treatment. Children are a unique population in that optimal therapy has to account for the differences in absorption, distribution, metabolism and excretion of xenobiotic in the growing, ever-changing child. For instance, children have a less acidic gastric environment and their gastric motility is slow, which may affect the absorption of drugs. Children, mainly neonates and infants, have different water body composition as compared to older children and adults, which may affect the volume of distribution of drugs. The ontogeny of metabolic enzymes may affect the degree of metabolism that goes on at a specific age, and immaturity of the kidneys will affect the excretion of drugs. Unfortunately, most drug regimens used in children are extrapolated from adult dosing, which does not consider the abovementioned factors that are unique to children9. In the PHATISA study, we sought to evaluate whether a revised WHO-recommendation for TB drugs is able to achieve the presumed optimal concentrations for treatment of TB in children.

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Table of Contents

Chapter 1. Introduction

The cornerstone of anti-infective therapy is attainment of the effective target concentration of the drug at the site of infection; achieving this goal requires integration of the pharmacokinetic and pharmacodynamics properties of the anti-infective drug[1]. In this interplay, systemic and local quantitative analysis of the drug plays an integral part in providing information about the pharmacokinetic and pharmacodynamic properties of the anti-infective; this information is critical for drug development as well as evaluation of adequacy of established therapies. In this thesis, we will demonstrate the role of systemic and local quantitative analysis in the development of preventative strategies for HIV and also, the role of quantitative analysis in assessing adequacy of therapy in pediatric tuberculosis (TB).

The projects in this thesis highlight the various points that are key for successful use of anti-infective drugs. The first two projects, CHARM-01 and CHARM-02, focus on use of anti-infectives for prophylaxis; specifically the development of tenofovir (TFV)-containing gels as locally applied (rectal) microbicides. Since these gels are locally-dosed, there are several factors that have to be considered such as the mucosal safety of the formulations, the ability of the formulations to cover all potentially HIV-exposed mucosa, and the ability to reach the optimal concentration of the active drug, TFV diphosphate (TDF-DP) to prevent HIV infection.[2-5]

In contrast, the PHATISA project looks at systemic (oral) dosing of anti-TB drugs in children for treatment. Children are a unique population in that optimal therapy has to account for the differences in absorption, distribution, metabolism and excretion of xenobiotic in the growing, ever-changing child. For instance, children have a less acidic gastric environment and their gastric motility is slow, which may affect the absorption of drugs.[6] Children, mainly neonates and infants, have different water body composition as compared to older children and adults, which may affect the volume of distribution of drugs. The ontogeny of metabolic enzymes may affect the degree of metabolism that goes on at a specific age, and immaturity of the kidneys will affect the excretion of drugs[6-8]. Unfortunately, most drug regimens used in children are extrapolated from adult dosing, which does not consider the abovementioned factors that are unique to children[9]. In the PHATISA study, we sought to evaluate whether a revised WHO-recommendation for TB drugs is able to achieve the presumed optimal concentrations for treatment of TB in children.

1.1. Microbicide Development for HIV Prevention

1.1. 1. Rationale for microbicide development

Though there has been a global decline in the incidence of HIV over the past decade, there were still more than 2 million new infections worldwide and close to 48,000 just in the United States in 2013[10,11]. Both globally and regionally, the HIV epidemic disproportionately affects subgroups of the population, such as men having sex with men (MSM) [10-12]Hence, methods augmenting current behavioral and biomedical

approaches are needed to control the HIV epidemic, and pre-exposure prophylaxis (PrEP) is one such key biomedical strategy.

HIV PrEP development has been ongoing in the past two decades. Data from animal models[13-16] and experience of using antiretroviral drugs in prevention of mother-to-child transmission as well as use of anti-retroviral drugs (ARVs) for post-exposure prophylaxis gave initial impetus for the hypothesis that ARVs may be efficacious as PrEP. Subsequently, human clinical trials for PrEP have been carried out, most commonly, using TFV with or without emtricitabine. In 2012, the Food and Drug Administration (FDA) approved the fixed dose combination of emtricitabine and TFV, marketed as Truvada™, for PrEP based on two randomized controlled trials (iPrEX study in MSM[17] and the Partner's in Prevention study in discordant couples of heterosexual men and women[18]); these two studies showed HIV risk reduction by 44% and 75%, respectively[19].

1.1.2. Topical microbicides

For PrEP development, attaining an anti-viral concentration that will prevent establishment of infection at the viral route of entry is critical. For example, for individuals at risk of HIV exposure via intravenous drug use, attainment of target concentration in blood is important. While, for those that are exposed to HIV via the sexual route, adequate anti-viral concentration at the site of the exposure to HIV, mainly the genital mucosa and rectosigmoid mucosa, will be essential.

With this in mind, there have been several clinical trials aimed at developing topical microbicides to be applied vaginally or rectally. Topical microbicides have the advantage of minimizing systemic exposure while maximizing the local mucosal concentration, which makes them ideal for PrEP[20-23]. Early on, topical microbicide research focused on drugs presumed to act within the cervicovaginal lumen to prevent HIV from reaching its target CD4+ cells in cervicovaginal tissue. These products included Nonoxynol-9, other surfactants, and polyanions, which were all ineffective; some even increased the risk of HIV acquisition. Following these early disappointments, the field then shifted to formulations with antiretroviral drugs, which act directly on or within CD4+ cells, as topical microbicides[24].

The success of coitally dependent use of TFV 1% gel in reducing HIV in high risk women by 39% in the CAPRISA004 study provided a proof-of-concept for efficacy of topical microbicdes[25]. Given the disproportionate burden of HIV in men having sex with men for whom receptive anal intercourse is the primary route of HIV infection, rectal microbicide development has been a focus of several studies in recent years[12,26-32].

1.1.3. Key features of effective topical microbicide

There are several key factors that determine the success of PrEP, which include adherence, drug concentration at exposure site, and susceptibility of the exposed mucosa to HIV infection, and the viral inoculum (i.e. viral load of the infectious

cervicovaginal fluid or semen of the infected partner)[33]. The critical role of adherence was delineated in the failure of the Fem-PrEP and the VOICES studies to demonstrate HIV prevention benefit[34,35] , mainly resulting from low adherence in the study population. Even in the PrEP studies that showed efficacy (CAPRISA004, iPrEX, Partners in Prevention, and TDF2), those persons with higher adherence had fewer HIV acquisition events[17,18,25,36]. The size of the HIV inoculum varies roughly with the viral load within the blood of the infected sexual partner and reduction of this viral load to undetectable levels reduces HIV transmission dramatically [37]. Conversely, a high viral load is seen at the time of acute HIV infection and sexual exposure to an infected individual at this time could, presumably, overwhelm the effect of the microbicide[38].

Another key factor for success of PrEP is drug concentration at the exposed site. In development of PrEP, it is not the mere presence or absence of the antiviral drug at the mucosa, but the ability to achieve a sustained effective concentration for a period of time sufficient to "outlast" the viral exposure [20]. One of the many advantages of topical microbicides is the ability to achieve a high local concentration of the drug to maximize efficacy while achieving relatively little systemic exposure, thus, minimizing systemic toxicity. For example, we know that tissue TFV concentration is 100 times higher after administration of a single vaginal gel dose than a single oral dose[21-23].

Integrity of the mucosa exposed to HIV also impacts the success of topical microbicides. We know that concurrent STIs that result in mucosal lesions and inflammation, such as

Herpes infection increase the risk of HIV acquisition. On the other hand, microbicide development also has to take into account that some microbicide formulations may increase the risk of HIV infection by damaging the mucosa. We know from prior lubricant and enema studies that formulations with high osmolality result in mucosal damage [39,40].

One of the first trials of a rectal microbicide was MTN-006, which evaluated the TFV1% vaginal gel formulation (VF) for use as rectal microbicide[41]. The study showed that the high osmolality of the formulation resulted in unacceptably frequent gastrointestinal adverse events, albeit minor ones, which may compromise acceptability and the widespread use of the formulation. Following the results of MTN-006, a reduced glycerin formulation (RGVF) was designed and tested in MTN-007[42]. The result showed that the RGVF had less adverse events when compared to the VF formulation. A third formulation designed specifically for rectal use (RF) was evaluated in CHARM 01 and CHARM 02, the focus of this thesis.

1.1.4. CHARM-01 and CHARM-02 studies

The CHARM-01 and CHARM-02 studies address several key determinants of candidate rectal microbicide success as PrEP: mucosal safety, local and systemic concentration, colonic distribution, and effect of study gels on colonic permeability. The CHARM-02 study compares the safety, systemic exposure, distribution in the colonic lumen and colonic permeability effects of a single dose of each of three candidate rectal

microbicide gels of 1% TFV with varying osmolalities: the rectal formulation (RF), the reduced glycerin formulation (RGVF), and the vaginal formulation (VF). On the other hand, the CHARM 01 compared the abovementioned candidate microbicide gels in multiple compartments (plasma, colonic mucosa, mucosal mononuclear cells, PBMCs, rectal and vaginal fluid) after 7 consecutive daily doses of the RF and RGFVF and a single dose of VF.

The result of both studies demonstrated that all three products were safe as no severe adverse events (AE) were reported; however, the hyperosmolal product, VF, had more minor AE's associated with it, mostly gastro-intestinal complaints. The VF was also associated with increased permeability of the colon to the drug surrogate as measured by concentration of the drug surrogate in the blood and urine. Despite the two-fold difference in osmolality, the RF and RGVF did not drastically differ in their achieved concentration in the various compartments, and both have good distribution in the colonic mucosa.

1.2. Assessment of Adequacy of TB drugs in Children

1.2.1. Burden of Pediatric Tuberculosis

One third of the world's population is estimated to be infected with tuberculosis. In 2013, about 9 million new cases of TB and 1.5 million deaths were estimated, with the majority of the burden concentrated in South-East Asia, the Western Pacific, and the African regions[43]. Given their immature immune system, children bear the greatest

burden of morbidity and mortality. In 2013, it is estimated that about 550,000 new cases and 80,000 deaths occurred in children less than 15 years of age. This estimate is likely a gross underestimation of pediatric TB burden, as it does not include HIV-TB co-infected children. In addition, unlike in adults, diagnosis of tuberculosis in children is quite challenging. Unlike adults, children have non-specific symptoms, and also have pauci-bacillary disease. The most widely used diagnostic tool, sputum smear, is only positive in 5-10%, and the gold standard, sputum culture, is only positive in 40% of children with TB.[44,45]

Another distinguishing characteristic of pediatric tuberculosis is that children are more prone to severe forms of tuberculosis due to their immature immune system. In addition, since children have pauci-bacillary disease and are unlikely to transmit TB to others, most of the public health efforts have concentrated on adult TB, ignoring the pediatric disease.

1.2.2. Tuberculosis therapy in adults

Our current first-line tuberculosis (TB) therapy is composed of four-drug therapy: Isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and addition of ethambutol (EMB) (in areas with high INH resistance and for severe cases) for two months, followed by four months of INH and RIF. These four drugs were instituted into TB therapy several decades ago: INH in 1952, RIF in 1966, PZA in 1952, and EMB in 1961.[46] The fact that these drugs remain a first-line regimen is telling of the stagnation in the realm of TB drug development. In fact, there is only one new class of TB drug approved since the last

TB drug approval in 1971[47], bedaquiline, which was approved in 2012[48] . Fortunately, there are a few new drugs in the pipeline, which may be available in the next few years.

1.2.3. TB therapy in Children

There is a paucity of data in regards to optimal TB treatment in children. Most target concentrations for TB therapy in children are developed by extrapolation from adult data. This method is fraught with inaccuracies, as it does not take into account the differences between adults and pediatrics; as mentioned earlier, in addition to the obvious size differences, there are several differences including enzymatic ontogeny maturation, differences in volume of distribution and percent water in the body and maturation of organ system involved in clearance of these drugs.

In 2009, McIlleron, *et al*., published a study looking at the WHO-recommended dosages for isoniazid in a pediatric population. They showed that 70% of the children that received the adult dose were actually underdosed[49]. Based on this information and meta-analysis of the few existing pediatric studies, the WHO released a rapid advice to change the recommended dosages, in some instances, doubling the previously recommended dose. The report emphasized the paucity of data and critical need for PK/PD studies in children[50].

1.2.4. The PHATISA study

We conducted a prospective observational study in the province of Kwa Zulu Natal, South Africa – a region with one of the highest prevalence's of TB – to look at the implementation of the new drug doses, and whether these recommended dosages did actually achieve the supposed therapeutic target concentrations.

We recruited children under10 years of age that presented for care at a tertiary health care center, and initiated on first-line anti-TB regimen. Our study included children with HIV/TB co-infection.

The study indicates that even with the increased dosage recommendation, many children did not achieve the presumed target concentrations for the four first-line TB drugs; the result was more striking for under-dosing of rifampin. Overall, the study highlights the need for continued research in optimizing anti-TB therapy in children.

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Chapter 2: CHARM 02 Study

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Abstract

Objective: CHARM-02 is a cross-over, double-blind, randomized trial to compare the safety and pharmacokinetics of three rectally applied tenofovir 1% gel candidate rectal microbicides of varying osmolalities: vaginal formulation, VF (3111 mOsmol/kg); the reduced glycerin vaginal formulation, RGVF (836 mOsmol/kg); and an iso-osmolal rectal-specific formulation, RF (479 mOsmol/kg).

Materials and Methods: Participants (n=9) received a single, 4ml, radiolabeled dose of each gel twice, once with and once without simulated unprotected receptive anal intercourse (RAI). Safety, plasma tenofovir pharmacokinetics, colonic small molecule permeability, and SPECT/CT imaging of lower gastrointestinal distribution of drug and virus surrogate were assessed.

Results: There were no Grade 3 or 4 adverse events reported for any of the products. Overall, there were more Grade 2 adverse events in the VF group compared to RF (p=0.006) and RGVF (p=0.048). In the absence of simulated unprotected RAI, VF had up to 3.8-fold greater systemic tenofovir exposure, 26-234-fold higher colonic permeability of the drug surrogate, and 1.5-2-fold greater proximal migration in the colonic lumen, when compared to RF and RGVF. Similar trends were observed with simulated unprotected RAI, but most did not reach statistical significance. SPECT analysis showed 86% (standard deviation 19%) of the drug surrogate co-localized with the virus surrogate in the colonic lumen. There were no significant differences

between RGVF and RF formulation, with the exception of higher plasma tenofovir concentration of RGVF in absence of simulated unprotected RAI.

Conclusion: VF had the most adverse events, highest plasma tenofovir concentrations, greater mucosal permeability of the drug surrogate, and most proximal colonic luminal migration compared to RF and RGVF formulations. There were no major differences between RF and RGVF formulations. Simultaneous assessment of toxicity, systemic and luminal pharmacokinetics, and co-localization of drug and viral surrogates, substantially informs rectal microbicide product development.

Introduction

Even though the incidence of HIV is declining in many regions globally, men who have sex with men (MSM) continue to be affected disproportionately and increasingly. Global MSM incidence estimates are difficult due to poor surveillance in this group; however, the limited available data shows that MSM carry a high burden of HIV in high-income countries as well as in low and middle-income countries.[12,26-32,51,52] In the United States, despite an overall decline in incidence of HIV, the incidence of HIV in men having sex with men (MSM) has been increasing significantly, with data from 2010 showing a 12% rise in incidence of HIV.[53] Hence, prevention of HIV in this vulnerable group, including biomedical interventions like rectal microbicides (RM), is vital.

Key features of successful RM development include safety, efficacy and acceptability of the product by the target population. RM have the advantageous feature of directly targeting the colonic mucosa that is at risk of HIV infection with high antiretroviral (ARV) drug concentrations while simultaneously limiting systemic exposure and potential toxicity.[54] High local concentrations may also enable periodic dosing by achieving local tissue concentrations above protective target concentrations more rapidly than can be achieved by oral dosing. However, locally high concentrations need to be developed carefully to rule out local toxicity.

Encouraged by the success of oral pre-exposure prophylaxis (PrEP) with tenofovir (TFV)-containing regimens [17,18,36,55], TFV, a potent nucleotide reverse transcriptase inhibitor (NRTI) with a long intracellular active drug half-life, is being investigated as a RM. RMP-02/MTN-006 evaluated rectal application of the vaginal formulation (VF) TFV 1% gel, the formulation used in CAPRISA 004 and VOICE studies for vaginal application[25,41,56], and found a rate of minor adverse

events too frequent to recommend further development as a RM. The gastrointestinal related adverse events were attributed, in part, to the very high osmolality (3111 mOsmol/kg) of the formulation. Subsequently, a TFV 1% reduced glycerin formulation (RGVF) with far lower osmolality (836 mOsmol/kg) was studied in MTN-007 showing that RGVF was safe and well tolerated [42]. Based on these favorable tolerability results, a phase II trial of the RGVF gel is now underway (MTN-017). A third TFV 1% gel, formulated specifically for rectal use (rectal formulation, RF) has been developed to achieve even lower, near physiologic, osmolality (479 mOsmol/kg) and pH value closer that of the rectum (pH close to 7)[57]. The RF vehicle was selected from among four candidate RM vehicles based on PK/PD, toxicity and acceptability[58]. The current study, Combination HIV Antiretroviral Rectal Microbicide (CHARM) 02 (CHARM-02), is a double-blinded, randomized, pharmacokinetic and safety study of three rectally applied TFV 1% gel candidate rectal microbicide formulations; the VF, RGVF, and, RF are distinguished primarily by their far different osmolalities. The goals of the study were to evaluate the safety, systemic TFV pharmacokinetics (PK), colonic luminal distribution and clearance of the three gels, and their impact on mucosal permeability. In addition, we assessed the degree of overlap in the colonic luminal distribution for each of the gels with a surrogate for HIV-infected ejaculate. CHARM-02 was designed as a complement to, and performed in parallel with, CHARM-01 whose objectives included multi-compartmental PK, a detailed mucosal safety assessment, and an evaluation of the HIV protective effect using an *ex vivo* colorectal HIV-1 challenge assay[59]. These studies represent the first-in-human studies of TFV 1% RF gel.

Materials and Methods

Study design and participants

The Johns Hopkins Medicine Institutional Review Board approved this single-center, randomized, double-blinded, crossover study of three TFV 1% gel formulations. All research participants completed a written informed consent prior to screening. Eligible participants were healthy, male, HIV seronegative adults with history of consensual receptive anal intercourse (RAI) at least once within the six months prior to screening. All participants received each study gel twice, once with and once without simulated unprotected RAI. There was a minimum of 11 days washout period between each gel administration (Supplemental Appendix 1: Protocol). The primary safety endpoint was Grade 2 or higher clinical or laboratory adverse events as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric adverse events, version 1.0, December 2004 as well as addendum 3 (Rectal Grading Table for Use in microbicide Studies)[60]. Primary pharmacokinetic endpoints include plasma TFV concentration, luminal distribution of the drug and viral surrogates and impact on mucosal permeability of the three gel formulations.

Dose preparation and administration

The three rectally applied TFV 1% formulations in this study are a vaginal formulation (VF), a reduced-glycerin vaginal formulation (RGVF) and a rectal-specific formulation (RF). Study investigators administered all doses in the research clinic. Each dose of the study gels was prepared by mixing 100 microCurie (□Ci) ^{111}In-diethylene-triamine-pentaacetic acid (^{111}In-DTPA, Cardinal Health, Halethorpe, MD) with 4mL of the study gel as the radiolabeled study drug surrogate. In addition, for the visits with simulated RAI, 500 □Ci ^{99m}Tc-sulfur colloid (^{99m}Tc-SC)

was mixed with 2.5mL of autologous seminal plasma, and administered 60 minutes after gel product dosing as the HIV surrogate (based on similar 100 nm sulfur colloid particle size in a colloidal suspension). The seminal plasma was collected prior to the study dosing visits in one or several outpatient visits to the research clinic until adequate semen volume was acquired. In order to quantitatively describe the distribution of the formulation following addition of ejaculate and the potential for mixing due to the coital forces, simulated unprotected RAI with autologous semen was carried out. All participants received a bowel preparation using a Normosol-R (Abbott Laboratories) enema to remove bowel contents from the distal colon and to more closely match realistic clinical conditions in which these rectal products will be used. Normosol, a pH and salt-balanced electrolyte solution for licensed intravenous administration and fluid replacement, was chosen in order to reduce confounding toxicity to the colonic mucosa. The research participant then inserts a single-use artificial phallus with catheter in urethral position into rectum and cycles the devices in and out of the rectum to its full extent once each second for 5 minutes. With the phallus remaining in situ, the autologous semen sample, radiolabeled with 99m-Tc-sulfur colloid, is injected by the study team member through catheter within the device. The subject then resumes simulated intercourse with 10 more in/out cycles of the device and then removes the device. This procedure has been used successfully in previous studies.[61]

Safety and Acceptability: Safety of the three products was assessed during the entire study period; participants were asked about any adverse event during each study visit, which were followed by a directed physical examination and safety laboratory examination. They were also instructed to contact the investigators should any adverse event occur while they were at

home. Acceptability of each study product was evaluated through administration of a brief questionnaire after each dose.

Drug concentration analysis

Blood samples (4mL) were drawn in K2EDTA vacutainer tubes (BD, Franklin Lakes, NJ) at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.33, 2.66, 3, 3.5, 4, 8, 12 and 24 h post dose; plasma was separated from the tubes after centrifugation at 1000x g for 10 minutes at 4 ^{o}C. Aliquots were set aside for gamma counting (permeability) and aliquots were stored at -80 ^{0}C for batched TFV analysis. TFV concentrations were determined by a previously validated ultra performance-liquid chromatographic-tandem mass spectrometric (UPLC-MS/MS) method at The Johns Hopkins University Clinical Pharmacology Analytical Laboratory (CPAL)[21,62]. The assay had a lower limit of quantification of 0.31 ng/mL. Peak concentration (C_{max}), times to peak concentration (T_{max}), and area under the concentration-time curve for 24 hours (AUC0-24) were calculated using WinNonlin (Pharsight, 6.3, Cary, NC).

SPECT/CT Imaging distribution

Two hours and 24 hours after each gel administration, participants underwent single photon emission computed tomography with transmission computed tomography (SPECT/CT) to determine the luminal distribution and clearance of each study gel radiolabel (^{111}In-DTPA) and whole semen radiolabel (^{99}Tc-Sulfur colloid). Participants were imaged using a dual-head VG SPECT series system (GE Medical Systems, Waukesha, WI) equipped with a CT unit (Hawkeye) as previously described.[61,63] CT images were reconstructed with a filtered back projection algorithm onto a 256 × 256-matrix size. After SPECT acquisition, images were reconstructed using the OSEM algorithm and fused with CT images, into a 128 x 128 x 128 matrix size with

each voxel representing 3.45 mm^3, using the General Electric eNTEGRA workstation, software version 1.04 (GE Medical Systems, Waukesha, WI) [64].

Curve-fitting and concentration-by-distance calculations were performed using R version 3.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) per previously described algorithms[63,65]. Briefly, a flexible principal curve algorithm was used to construct a three-dimensional curve based on the colon images. After the centerline was constructed, a concentration-by-distance curve was estimated along the centerline using the orthogonal projections. For standardizing distances within and among research participants, the readily identifiable coccygeal plane in the CT (axial view) was used as the origin (z=0 value) of the centerline as previously described[66]. The distance along the centerline between the origin of the radiolabel signal and the coccygeal plane was recorded as D_{min} (minimum distance associated with the closest, most distal, point where radiolabel was detected within the lumen of the colon) with negative values indicating radiolabel origin below the coccyx and positive values indicating centerline origin above the coccyx in the cranio-caudal axis. Previously defined imaging pharmacokinetic-distance parameters – D_{max} (distance associated with the most proximal radiolabel signal within the colon), DC_{max} (distance associated with maximum concentration), and D_{ave} (mean residence distance) – were calculated for further analysis[66].

Mucosal permeability. Blood samples were collected at the same 17-time points as for plasma TFV PK. Urine samples were collected in three intervals: 0-2hrs, 2-4hrs, and 4-8 hours post dose. Gamma emissions in 1 ml aliquots were measured on a gamma counter (Wizard2 automatic gamma counter model 2480, PerkinElmer, Waltham, MA) within a 110–150-keV energy window, and data corrected for decay relative to the time of dosing. Urine gamma

emission results were also volume-corrected. Radioactivity was expressed as a fraction of the dose administered in order to normalize readouts among subjects and products. Plasma ^{111}In-DTPA results were analyzed by calculating the C_{max}, T_{max}, and $AUC_{0\text{-}24}$. For urine, maximum observed urine excretion rate (Max rate), area under urinary excretion curve (AURC) and percent of dose recovered in urine (%recovered) were calculated. Both plasma and urine analysis were carried out using WinNonlin (Pharsight, 6.3, Cary NC).

Dual Isotope ^{111}In and ^{99m}TC Image Analysis

We determined the fraction of the HIV surrogate (^{99m}Tc-SC) co-located with microbicide surrogate (^{111}In-DTPA) to delineate the adequacy of the study product distribution relative to the HIV surrogate distribution. Cross-talk correction was performed using previously described methods[67,68]. Using R (version 3.1.2), all voxels with high ^{99m}Tc were selected and defined as "voxels at risk" (VAR). In order to remove scattered voxels far from the region of interest, only 200 or more contiguous voxels among the VAR, named contiguous VAR (cVAR), were considered.

For this analysis, we used the 99.99% quantile of the intensities of a pure background signal (abdominal location inconsistent with colon distribution) for ^{99m}Tc and ^{111}In, respectively, as a scan-specific threshold. Within the cVAR in each scan, two quantities p_v and p_i were calculated: p_v is the proportion of voxels with both high ^{99m}Tc and high ^{111}In among all the cVAR; p_i is similar to p_v, but indicates the gamma signal intensity based proportion which is the sum of intensities of ^{99m}Tc of voxels with high ^{99m}Tc and high ^{111}In among the total sum of intensities of ^{99m}Tc in cVAR. Both quantities indicate the proportion of ^{99m}Tc covered by ^{111}In among all the

^{99m}Tc within cVAR, the only difference is that p_v is voxel-based while p_i is an intensity (mass)-weighted version of p_v.

*Data analysis and sample size: A s*ample size of 9 research participants was calculated to detect a 0.7 difference in proportion of adverse events and a standardized mean difference of 0.93 in the pharmacokinetic-distance or permeability outcomes between any of the study gel formulations in a paired analysis with 80% power using 2-sided, 5% alpha error. Data were analyzed using the statistical package STATA/IC 13.1 software (StataCorp LP, College Station, TX). Statistical significance was defined as a p-value < 0.05.The number and frequency of Grade 2 or higher AEs were tabulated for each of the 3 study formulations after the final dosing visit. The proportion of events was compared between each pair of formulation using McNemar's test. Friedman test was used to assess differences in frequency of AEs among study products, and based on the result, a Wilcoxon Rank Sum test was utilized for pairwise analysis. For comparison of plasma TFV PK, pharmacokinetic-distance, and mucosal permeability outcomes, Wilcoxon rank sum paired analysis was used. In addition, to delineate linear correlation between plasma TFV concentrations and mucosal permeability, a Pearson's correlation coefficient was calculated, with data transformation as needed.

Results

Subjects

Seventeen men provided written informed consent and were screened (Figure 1). Of these, 9 fulfilled the inclusion and exclusion criteria and were enrolled. Mean age of the research

participants was 41.8 years (standard deviation [SD] 9.3). Three were European American and 6 were African American by their own report. Data from all nine participants were included for adverse event analysis (safety cohort). Data from 8 were included in the other analyses (PK cohort). One research participant was excluded from the PK cohort due to laboratory evidence that he was surreptitiously taking tenofovir/emtricitabine during the study period.

Adverse Events

Overall, there were 54 adverse events (AE) and there were no Grade 3 or 4 AEs. AEs were more common when participants were receiving VF (6/9) as compared to RF (1/9) or RGVF (3/9)(table 1). Pairwise comparison revealed a statistically significant higher number of overall Grade 2 AEs in the VF group as compared to RF (13 vs. 1, p=0.006) and RGVF (13 vs. 5, p=0.048). Twenty-three of the AEs (41.8%) were deemed related to the study gels, and all but one of these events were Grade 1. All of the 23 AEs were gastro-intestinal in nature, including abdominal cramps (34.8%), diarrhea (26%), bloating/flatulence (21.7), urgency (8.7%), proctalgia (4.4%) and rectal bleeding (4.4%). There were numerically higher number of AEs in VF as compared to RGVF and VF, which did not reach statistical significance in pairwise analysis (Figure 2 and Table 1).

Plasma pharmacokinetics of Tenofovir

In the absence of simulated unprotected RAI, the median C_{max} of TFV for the VF formulation was 6.4-fold higher than for the RF (p=0.009)(Table 2). VF also had a 4-fold higher median C_{max} than RGVF, but this did not reach statistical significance (p=0.06). Median C_{max} for RGVF was also 1.6-times higher than RF (p=0.005). With simulated unprotected RAI, the trend of higher

median C_{max} for VF was also observed, but only the difference in C_{max} for VF and RGVF was statistically significant (36.5 ng/mL vs. 6.87ng/mL, respectively, p=0.03)(Figure 3 and Table 2). In addition, there was a statistically significant shorter T_{max} observed for VF when compared to the RF formulation (1.18 hrs vs. 2.85 hrs, p=0.005 without simulated RAI, 1.26 vs. 1.65hrs, p=0.016 with simulated unprotected RAI).

Similar to the trend noted for C_{max}, there was an overall trend of higher AUC_{0-24} for the VF formulation, both in the absence and presence of simulated unprotected RAI; however, only the comparison of VF and RGVF yielded a statistically significant difference, with VF having a 3.8-fold higher AUC_{0-24} than RGVF (p=0.027).

Imaging Distribution

Of the forty-eight 2-hour post dose SPECT/CT scans that were scheduled, all were completed. Three (2 RF, 1VF) did not show any microbicide or HIV surrogate signal due to loss of isotope as a result of a bowel movement prior to imaging. The 24-hours post dose scans were discontinued after the first 5 scans in which there was no signal detected due to a combination of radioactive decay and bowel movements.

For the analysis of the drug surrogate (^{111}In-DTPA) in the absence of simulated RAI, there was a statistically significant difference in D_{max} and D_{ave} for VF when compared to RF and RGVF; D_{max} for VF was 1.5-times and 2-times higher than RF and RGVF, respectively (p=0.04 and 0.002)(Table 3). Similarly, D_{ave} for VF was 2.9- and 2.1-times higher than RF and RGVF, respectively (p=0.015 and 0.02). In contrast, there was no statistically significant difference in DC_{max} among the three products, although VF medians were higher than the other formulations. There was also no difference in D_{min} among the products. In the presence of

simulated RAI, VF had numerically higher medians of D_{max}, DC_{max}, D_{min}, and D_{ave} when compared to RF and RGVF, but none of these reached statistical significance.

When comparing the distribution of the drug and the HIV surrogate, there was no statistically significant difference in D_{max} and DC_{max}. There was a trend of higher D_{ave} for the drug surrogate in RF and RGVF, but it did not reach statistical significance (p=0.06 and 0.07, respectively)(Table 4). The drug surrogate was closer to the anus when compared to the HIV surrogate for the RF and RGVF (p=0.004 and 0.002, respectively). Sample SPECT images and distance-concentration plots are depicted in Figure 4 a-c.

Adjusted for the mass of the HIV surrogate in each voxel, 86% (SD 0.19) of the HIV surrogate was co-located with the drug surrogate; without the mass adjustment (simply comparing coincident radiolabel voxel-by-voxel, regardless of the amount in each voxel), the mean percentage coverage goes down to 36.2% (SD 0.13). There was no statistically significant difference in percent coverage of the HIV surrogate among the three gel formulations using either co-localization method.

Mucosal Permeability

Plasma ^{111}In-DTPA PK. In the absence of simulated RAI, dose-adjusted median C_{max} for VF was 34-fold and 7-fold higher than RF and RGVF, respectively (p=0.006 and 0.02)(Table 5a). A larger difference was noted with AUC, with VF 234-fold, and 26-fold higher when compared to RF and RGVF, respectively (p=0.005 and 0.02). Median C_{max} and AUC, larger for RGVF compared to RF, nearly achieved statistical significance (p=0.06 and 0.08, respectively).

With simulated RAI, a similar pattern was noted with the dose-adjusted median C_{max} for VF being 7-fold and 8-fold higher than RF and RGVF, respectively (p=0.02 and 0.03). The median AUC for VF was 63-times and 32-fold higher than RF and RGVF (p=0.02 for both). There was no difference in AUC between RF and RGVF. There was also no statistically significant difference in regards to permeability T_{max} among the three products, with or without simulated RAI. Comparing the permeability PK parameters in the presence and absence of simulated RAI for each product, there was a pattern of numerically higher median C_{max} and AUC for all three products with coital simulation; however, only median C_{max} for RF, comparing with and without simulated RAI, reached statistical significance, with a 9-fold increase in C_{max} with coital simulation (p=0.03).

We also found a significant linear correlation(r=0.83, p<0.001) between plasma TFV concentration and plasma ^{111}In-DTPA(Figure 5a).

Urine ^{111}In-DTPA PK. In the absence of simulated RAI, maximum observed excretion rate for VF was 6.6-times and 3.2-times higher than RF and RGVF (p=0.016 and 0.046)(Table 5b). The area under the urinary excretion rate curve (AURC) for VF was 5-times and 2.7-times higher than RF and RGVF, respectively (p=0.01 and 0.03). The percent of ^{111}In-DTPA recovered in urine for VF was also significantly higher for VF as compared to the RF and RGVF, 1.75-times and 4.7-times higher, respectively (p=0.046 and 0.009).

With simulated RAI, similar results were seen with 2.8-times and 7.25-times higher maximum observed excretion rate for VF as compared to RF and RGVF, respectively (p=0.036 and 0.021). The AURC for VF was 2.8-times, and 5.8-times higher than RF and RGVF, respectively (p=0.027

and 0.016). Also, the percent of drug surrogate recovered for VF in urine was higher than RF and RGVF by 1.75-fold and 4.7-fold, respectively (p=0.046 and 0.009).

There was no difference noted between the maximum observed excretion rate, area under the urinary excretion rate curve or percent recovery of the In-DTPA from the urine when comparing the RF and RGVF. Among and between products, there was no statistical difference between median maximum excretion rate, AURC and % recovered from urine when comparing values in the presence and absence of simulated RAI. There was a significant correlation between plasma TFV concentration and percent urine recovery of ^{111}In-DTPA ($r=0.92$, $p<0.001$)(Fig 5b)

Discussion

The CHARM-02 study showed that a single rectal dose of the three TFV gel formulations under study, was safe as there was no Grade 3 or 4 toxicity reported. However, minor adverse events were more common with VF as compared to the RGVF and RF. Similar results were observed in the companion study, CHARM-01, with VF accounting for 48% of reported adverse events in the entire study, despite only one VF dose being administered, compared to 7 consecutive doses of each for RF and RGVF[59].

Systemic TFV exposure was greater following VF dosing compared to the other formulations without simulated RAI, but depended on which PK parameter was compared. With the VF formulation, TFV C_{max} was 6-fold higher and twice as rapid when compared to RF in the absence of simulated RAI. TFV AUC was 3.8-fold higher with VF than RGVF. RGVF also achieved higher peak concentrations than RF. This general trend of greater systemic exposure correlating with increased osmolality is seen to an even greater extent with permeability for DTPA (discussed below). With simulated unprotected RAI, these patterns generally persisted, but lost statistical significance. As simulated unprotected RAI generally increased permeability of TFV and DTPA, this may have had a leveling effect on the differences seen without RAI. Also, plasma TFV correlated with the ^{111}In-DTPA permeability estimates, though TFV permeability was of much smaller magnitude compared to DTPA. The difference could be partly attributed to the relatively poor bioavailability of the charged TFV molecule relative to DTPA. The high correlations for DTPA permeability measurements and plasma TFV concentration suggests Indium-DTPA can serve as a reasonable model for permeability measurement for TFV

Imaging of the drug surrogate in the absence of simulated unprotected RAI revealed significantly higher colonic mucosal distribution (D_{max} and D_{ave}) of VF when compared to RF and RGVF. This may best be explained by the far greater osmolality of VF which draws significantly more fluid into the colonic lumen, thus, increasing the spread of the radiolabel after dosing relative to the lower osmolality RGVF and RF formulations. It is noteworthy that RF and RGVF were not different in their luminal distribution in the colon.

Our weighted dual isotope analysis showed that 86% of the viral surrogate was co-located or "covered" by the drug surrogate and was not different among the formulations. We believe this to be a critically important variable since the goal of rectal microbicide development is to develop a formulation that can outdistance and outlast HIV. This dual isotope analysis reflects a high degree of concordant drug-HIV distribution within the lumen, but it doesn't assess mucosal coverage, *per se*, given the resolution of the radiographic method. Animal studies using fluorescent labeling and histologic imaging enable a more direct assessment of mucosal coverage[69]. These studies indicate optimal mucosal coverage with iso-osmolar and slightly hypotonic products. Finally, none of these methods address diffusion of drug or HIV into the mucosal tissue over time.

The striking difference in mucosal permeability among the study gels was evidenced by the plasma and urine concentration of the drug surrogate (^{111}In-DTPA). Plasma C_{max} and AUC of the drug surrogate for VF were greater than 30-fold and 200-fold, respectively, when compared to the RF, in the absence of simulated RAI. Statistically significant, but smaller magnitude differences, were seen for RGVF compared to VF. RGVF trended toward values greater than RF. Similar patterns were seen with simulated RAI. These DTPA permeability differences are

consistent with the osmolality differences among the study products. Generally, for both TFV and DTPA colonic mucosal permeability, the greater the osmolality, the greater the systemic exposure: VF > RGVF > RF. This suggests that the predominant effect of the hyperosmolar gels was increased colonic mucosal permeability, which more than counterbalanced the competing physiologic effect of increased fluid from colon tissue into the colonic lumen with higher osmolarity products. Besides osmolality, there may be other differences between products (e.g., pH and viscosity) that contributed to the results, although given size of the compartment and the rectum's ability to buffer pH, such contributions are presumed to be minimal.[70] It is notable, that there are not more consistent differences between the RGVF and RF given the nearly 2-fold difference in osmolality. This may be due, in part, to mitigation of some anticipated mucosal integrity-related differences by offsetting hyperosmolarity-related fluid fluxes into the colonic lumen.

Since we did not assess histologic damage or HIV infectivity, we cannot tell if these permeability differences increase HIV infection risk. Our previously published works with hyperosmolar sexual lubricants and hyperosmolar enemas are consistent with our CHARM-02 permeability observations[40,71]. Unlike CHARM-02, both of those earlier studies included colon biopsies and both demonstrated greater loss of the colonic single columnar epithelial layer associated with very high osmolality products - 2,100 mOsmol/kg Fleet enema[40] and 3,429 mOsmol/kg commercial sexual lubricant [71] – when compared to iso-osmolar controls.

Hence, a significant limitation of the current study is that no biopsies were obtained; so, histologic toxicity, tissue PK, and susceptibility to ex-vivo HIV infection were not assessed. Other than inferring potential mucosal alteration based on the TFV and drug surrogate concentrations

in plasma and urine, there was no histological examination performed to evaluate structural changes in the mucosa. The companion study, CHARM-01, included intensive safety analyses which included histology, microbiology, and susceptibility to ex-vivo HIV infection. We chose not to perform intraluminal manipulations to capture biopsies given our primary goal of assessing colonic luminal drug and HIV surrogate distribution, both of which we wanted to assess unperturbed by endoscopic instrumentation.

VF is no longer under development as a rectal microbicide given the adverse effect profile and safety concerns with rectal use, some of which are reinforced in this study. The incorporation of simulated RAI in CHARM-02 proves critical in the comparison of the novel RF formulation being compared to RGVF for the first time in CHARM-01 and CHARM-02. CHARM-02 demonstrated that while RGVF demonstrated greater plasma TFV concentrations and a trend toward greater mucosal permeability compared to RF, these differences disappeared with simulated RAI. Further, RGVF and RF had similar, excellent co-distribution of drug and HIV surrogates. Of note, there were slightly more frequent minor adverse events reported in RGVF group compared to RF, but these differences were not statistically significant. On the basis of these observations and the CHARM-01 findings, we do not find a compelling advantage of RF over RGVF. There are two ongoing clinical studies of the RGVF formulation, PROJECT GEL and MTN-017. The results of these studies, especially MTN-017, which is an international, multi-center phase II trial, will inform the potential benefit and future development of rectal microbicides.

Table 1. Proportion and frequency of overall Grade 2 adverse events and frequency of AEs deemed related to study product

	RF	RGF	VF	p-values RF vs. RGVF	p-values RF vs. VF	p-values RGVF vs. VF
Participants (n,%) with Grade 2 AE(n=9)	1(11.1%)	3(33.3%)	6(66.7%)	0.63*	0.063*	0.38*
Number of Grade 2 AEs(n, %)(n=18)	1(5.3%)	5(26.3)	12(68.4%)	0.5**	0.006**	0.048**
Number of Grade 1 and 2 AEs deemed study-product related, (n, %)(n=23)	4(17.4%)	6(26.1%)	13(56.5%)	0.58**	0.09**	0.19**

*p-values derived from pairwise comparison of formulations using McNemar's test

**p-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products

Table 2. Plasma TFV pharmacokinetic parameters by product; median (25th percentile, 75th percentile)

	RF	RGVF	VF	p-value RGVF vs. RF	p-value VF vs. RF	p-value VF vs. RGVF
Cmax(ng/ml)*						
No SURAI	3.65 (1.35, 4.55)	5.95 (5.07, 7.99)	23.3 (12.93-30.6)	**0.035**	**0.009**	0.06
SURAI	12.4 (3.1, 31.7)	6.87 (3.71, 23.5)	36.45 (22.75-65.8)	0.53	0.093	**0.03**
Tmax(hr)*						
No SURAI	2.85 (1.89, 6.23)	1.03 (0.95, 2.53)	1.18 (0.92, 1.23)	0.07	**0.005**	0.67
SURAI	1.65 (1.54, 5.63)	1.53 (1.5, 1.64)	1.26 (0.8, 1.54)	0.19	**0.016**	0.1
$AUC_{0\text{-}24}$* (ng.hr/ml)						
No SURAI	30.13 (14.9, 55)	39.17 (19.1, 57.4)	81.64 (48.8, 137.1)	0.67	0.09	0.14
SURAI	46.51 (20.8, 71)	23.13 (19, 53.5)	87.83 (73.5, 122.5)	0.46	0.09	**0.027**

SURAI: Simulated unprotected receptive anal intercourse; *Comparison of SURAI vs. no SURAI for each PK-parameter yielded p-value>0.05.

P-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products.

Table 3. Drug surrogate (^{111}In-DTPA) imaging pharmacokinetic-distance parameters in centimeter by product at 2 hours after dosing; median (25th percentile, 75th percentile)

	RF	RGVF	VF	P-value RGVF vs. RF	P-value VF vs. RF	P-value VF vs. RGVF
Dmax						
No SURAI	13.9 (9.86, 18.8)	10.1 (9, 12.5)	21.1 (16.9, 27.6)	0.16	**0.037**	**0.0023**
SURAI	12.3 (10.2,20.3)	13.77 (11.1, 18.5)	18.16 (10.6, 26.2)	0.64	0.28	0.42
DCmax						
No SURAI	1.38* (-1.3, 4.15)	2.08 (-0.84, 5.5)	3.16 (1.82, 5.6)	0.73	0.25	0.67
SURAI	4.34* (2.47, 6.42)	3.79 (1.52, 6.04)	5.5 (1.2, 6.91)	0.64	0.95	0.56
Dmin						
No SURAI	-5 (-6.3, -1.98)	-4.28 (-6.78, -2.43)	-3.72 (-5.22, -0.87)	0.64	0.25	0.46
SURAI	-3.77 (-5, 1.86)	-3.63 (-4.17, -1.56)	-2.66 (-4, -0.13)	0.64	0.28	0.56
Dave						
No SURAI	2.51 (1.2, 2.36)	3.43 (1.23, 4.72)	7.31 (5.48, 9.29)	1	**0.015**	**0.021**
SURAI	5.33 (3.73, 7.9)	5.86 (4.24, 6.36)	6.62 (5.27, 14.8)	0.92	0.42	0.30

Note: The coccyx is the reference point for all the distance variables above.

SURAI: Simulated unprotected receptive anal intercourse; Dmax: furthest point where radiosignal was detected; DCmax: distance at maximum concentration; Dave: mean residence distance; Dmin: distance associated with the most distal signal; CDS: Coital dynamic simulation

*The only comparison between CDS vs. no CDS that yielded p-value<0.05 was DCmax for RF (p=0.035)

P-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products.

Table 4. Comparison of the pharmacokinetic-distance parameters of the virus surrogate (Tc-Sulfur colloid) and drug surrogate (In-DTPA) in centimeter by product at 2 hours after dosing; median (25th percentile, 75th percentile)

	RF	RGVF	VF	P-value RGVF vs. RF	P-value VF vs. RF	P-value VF vs. RGVF
Dmax						
Tc	13.29 (12.1,15.4)	15.84 (12.2,16.4)	15.1 (13.2,26.4)	0.25	0.14	0.42
In	12.29 (10.2,20.3)	13.77 (11.1, 18.5)	18.16 (10.6, 26.2)	0.64	0.28	0.42
DCmax						
Tc	2.97 (1.6, 4.21)	2.91 (1.37, 4.4)	4.01 (3.24, 5.82)	0.91	0.22	0.20
In	4.34 (2.47, 6.42)	3.79 (1.52, 6.04)	5.5 (1.2, 6.91)	0.64	0.95	0.56
Dmin						
Tc	-8.55* (-8.91,-5.61)	-7.782** (-11.2, -6.67)	-5.63 (-11.4,-1.62)	0.64	0.85	0.25
In	-3.77* (-5, 1.86)	-3.632** (-4.17, -1.56)	-2.66 (-4, -0.13)	0.64	0.28	0.56
Dave						
Tc	3.73 (2.39,4.07)	3.51 (2.95, 3.96)	4.77 (4.21, 6.21)	0.91	**0.025**	0.064
In	5.33 (3.73, 7.9)	5.86 (4.24, 6.36)	6.62 (5.27, 14.8)	0.91	0.41	0.3

Note: The coccyx is the reference point for all the distance variables above.

Dmax: furthest point where radiosignal was detected; DCmax: distance at maximum concentration; Dave: mean residence distance; Dmin: distance associated with the most distal signal

*, **: Comparison of Dmin for Tc and In for RF, p=0.004, and RGVF, p=0.002

P-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products

Table 5a. ^{111}In-DTPA permeability parameters by product (plasma). Median (25th percentile, 75th percentile)

		RF	RGVF	VF	P-value RGVF vs. RF	P-value VF vs. RF	P-value VF vs. RGVF
Cmax (□curie/ml)(E-08)							
SURAI	No	2.23* (0, 6.25)	10.2 (3.15, 16.4)	75.4 (32.5, 123)	0.055	**0.0056**	**0.024**
SURAI		20.2* (9.5, 50.3)	18.1 (0, 48.5)	140 (80.1, 213)	0.75	**0.021**	**0.030**
Tmax(hr)							
SURAI	No	1.93 (0,4.23)	2.35 (1.03,2.69)	1.3 (1.18,1.49)	0.83	0.83	0.56
SURAI		1.58 (0.74,2.57)	1.33 (0,1.57)	1.35 (0.92,1.65)	0.31	0.34	0.71
AUC (□curie.hr/ml)(E-06)							
SURAI	No	0.58 (0,5.44)	5.31 (2.15,17.4)	135 (68.6,265.3)	0.088	**0.0056**	**0.024**
SURAI		3.9 (1.1,48.3)	7.65 (0.49.8)	246.1 113,395.2)	0.83	**0.021**	**0.023**

SURAI: Simulated unprotected receptive anal intercourse; *Comparison of Cmax for SURAI vs. no SURAI for RF formulation: p=0.03

P-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products.

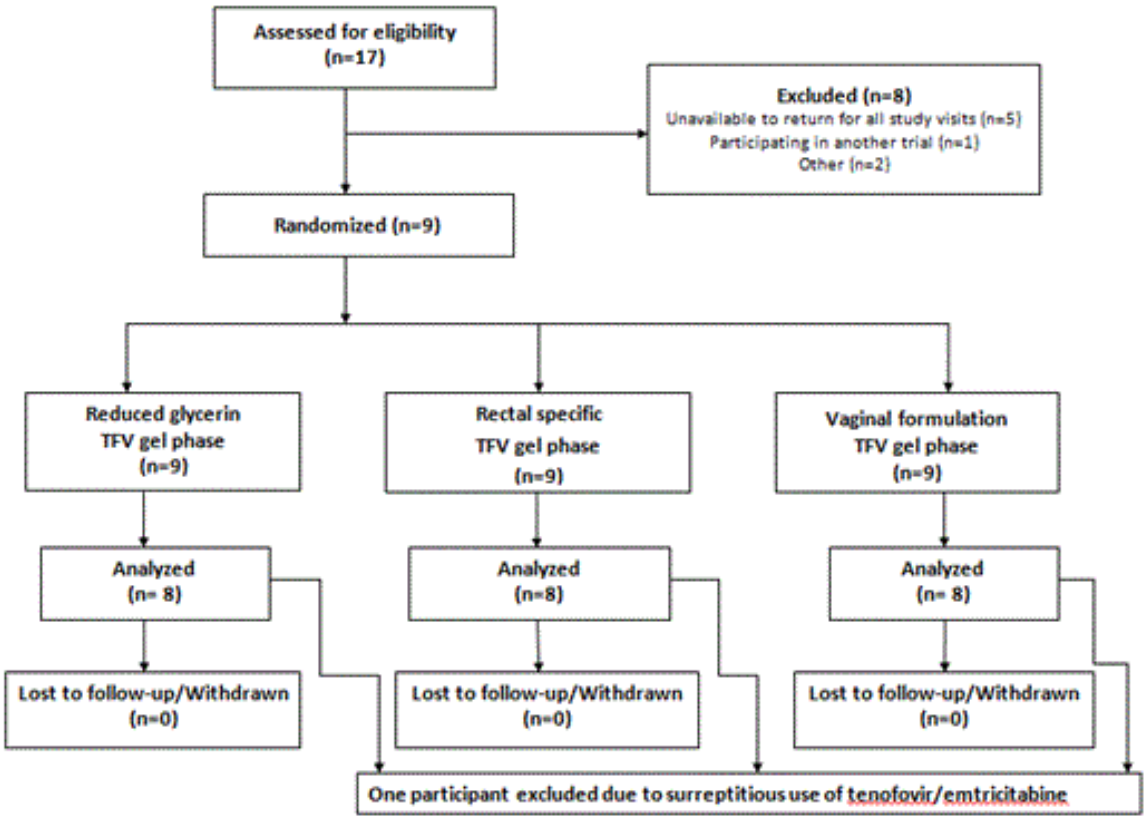
Table 5b. ^{111}In-DTPA permeability parameters by product (urine); median (25th percentile, 75th percentile)

	RF	RGVF	VF	P-value RGVF vs. RF	P-value VF vs. RF	P-value VF vs RGVF
Max rate (□curie/hr)						
No SURAI	0.058 (0.037,0.11)	0.12 (0.068,0.2)	0.38 (0.19,0.48)	0.093	**0.016**	**0.046**
SURAI	0.21 (0.042,0.29)	0.082 (0.066,0.22)	0.58 (0.33,0.98)	0.53	**0.036**	**0.021**
AURC (□curie)						
No SURAI	0.26 (0.16,0.39)	0.5 (0.3,0.85)	1.34 (0.84,1.6)	0.093	**0.012**	**0.036**
SURAI	0.75 (0.16,1.16)	0.36 (0.24,0.84)	2.09 (1.09,3.46)	0.46	**0.027**	**0.016**
% recovered						
No SURAI	0.45 (0.23,0.59)	0.74 (0.4,1.24)	2.2 (1.13,2.71)	0.12	**0.012**	**0.046**
SURAI	1.37 (0.29,1.40)	0.51 (0.32,0.96)	2.4 (1.57,3.58)	0.14	**0.046**	**0.009**

SURAI: Simulated unprotected receptive anal intercourse; Max rate: maximum observed excretion rate; AURC: Area under the urinary excretion rate curve from 0 to last measurable rate; % recovered: Percent of initial dose of 111In-DTPA recovered in the urine

P-values derived from pairwise comparison of formulations using Wilcoxon Rank Sum test; these were performed after a Friedman test showed differences in frequency of AEs among the study products.

Figure 1. CHARM-02 Study design



*PK analysis included 8 participants, and safety analysis included all 9 participants

Figure 2. Number of overall Grade 2 AEs and subset of AEs(Grade 1 and 2) deemed realted to study gels by product

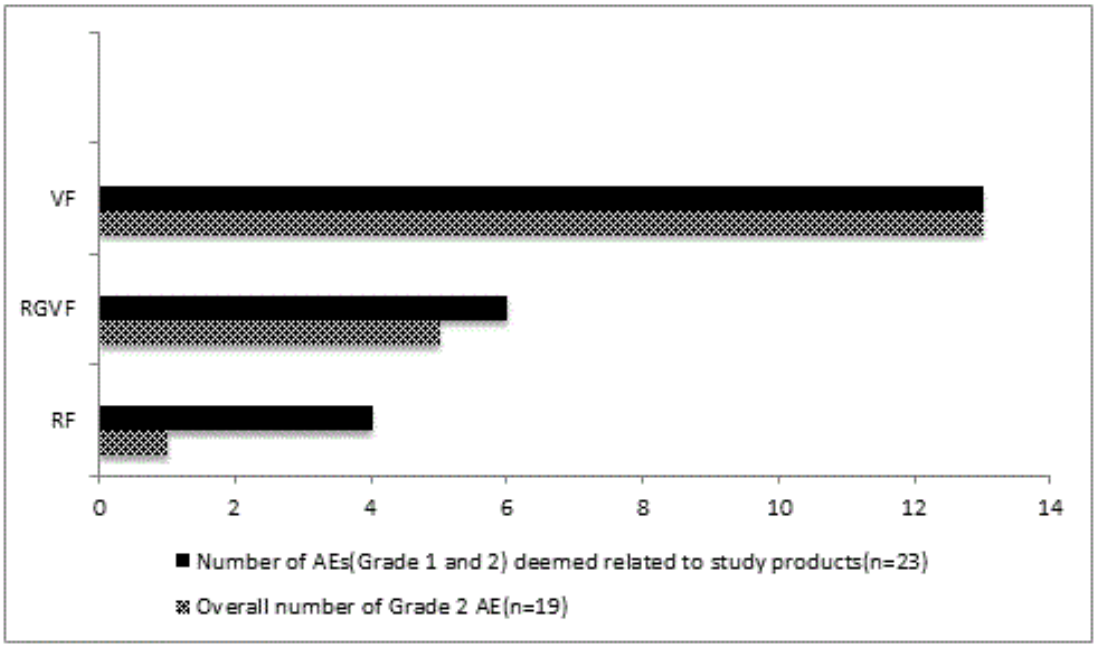


Figure 3a. Median plasma TFV concentration (log-transformed) for each time point by product without simulated unprotected receptive anal intercourse

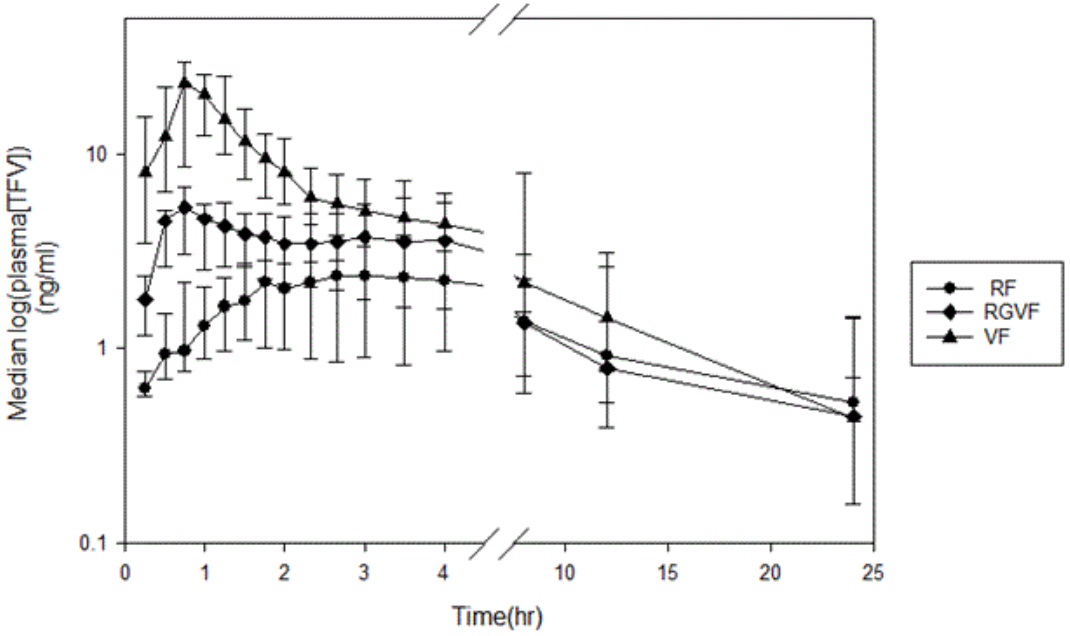


Figure 3b. Median plasma TFV concentration(log-transformed) for each time point by product with simulated unprotected receptive anal intercourse

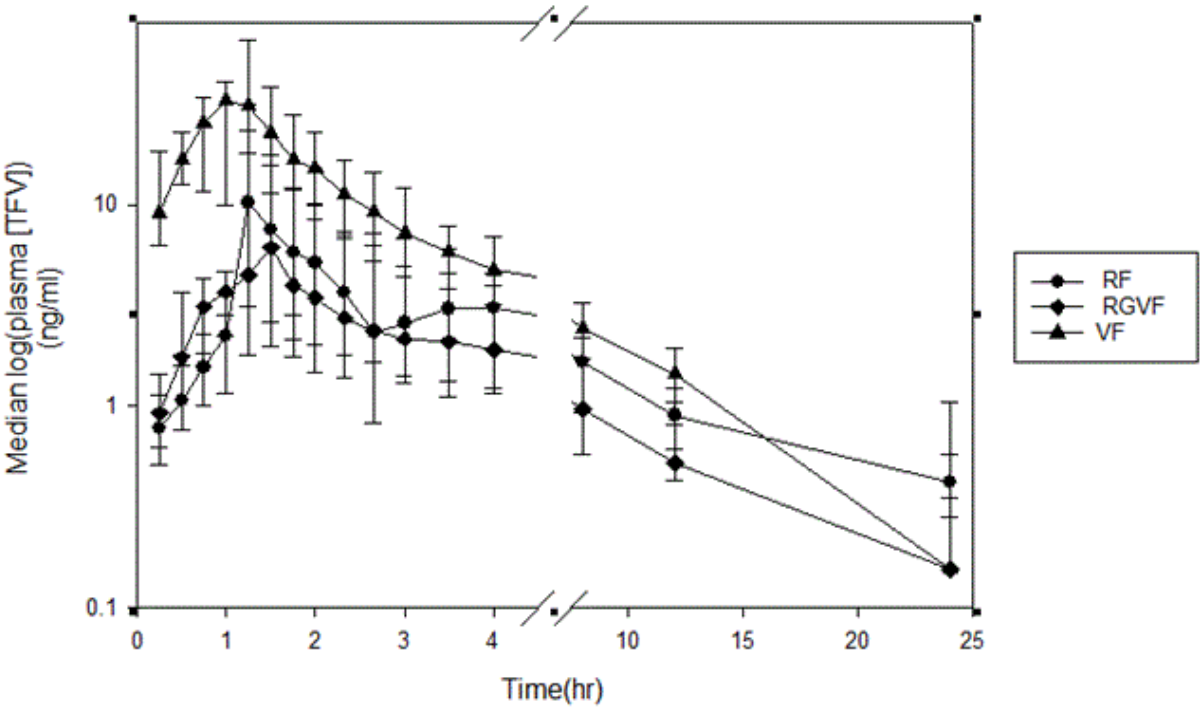


Figure 4a. Sample SPECT Images of the drug("microbicide") surrogate

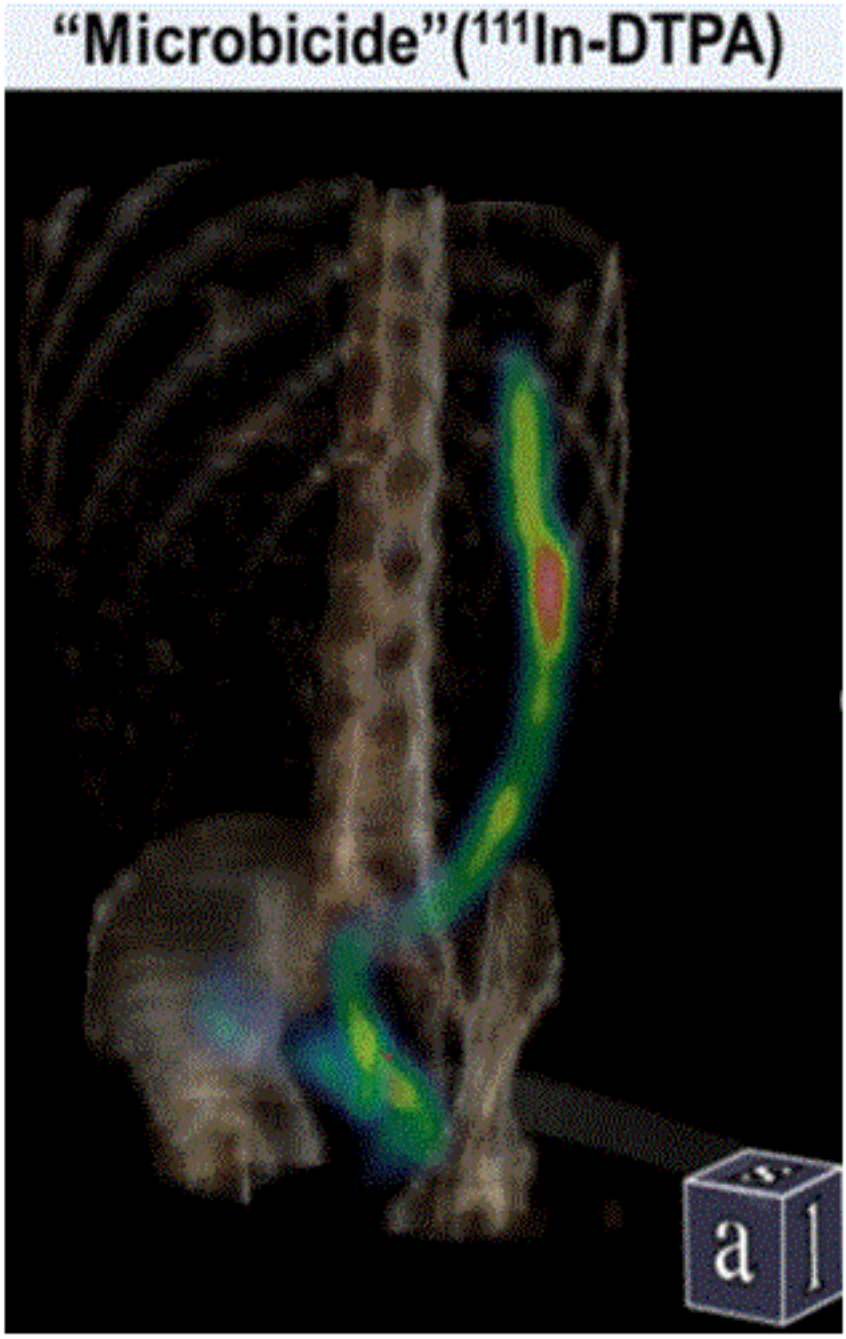


Figure 4b. Sample SPECT Images of the virus("HIV") surrogate

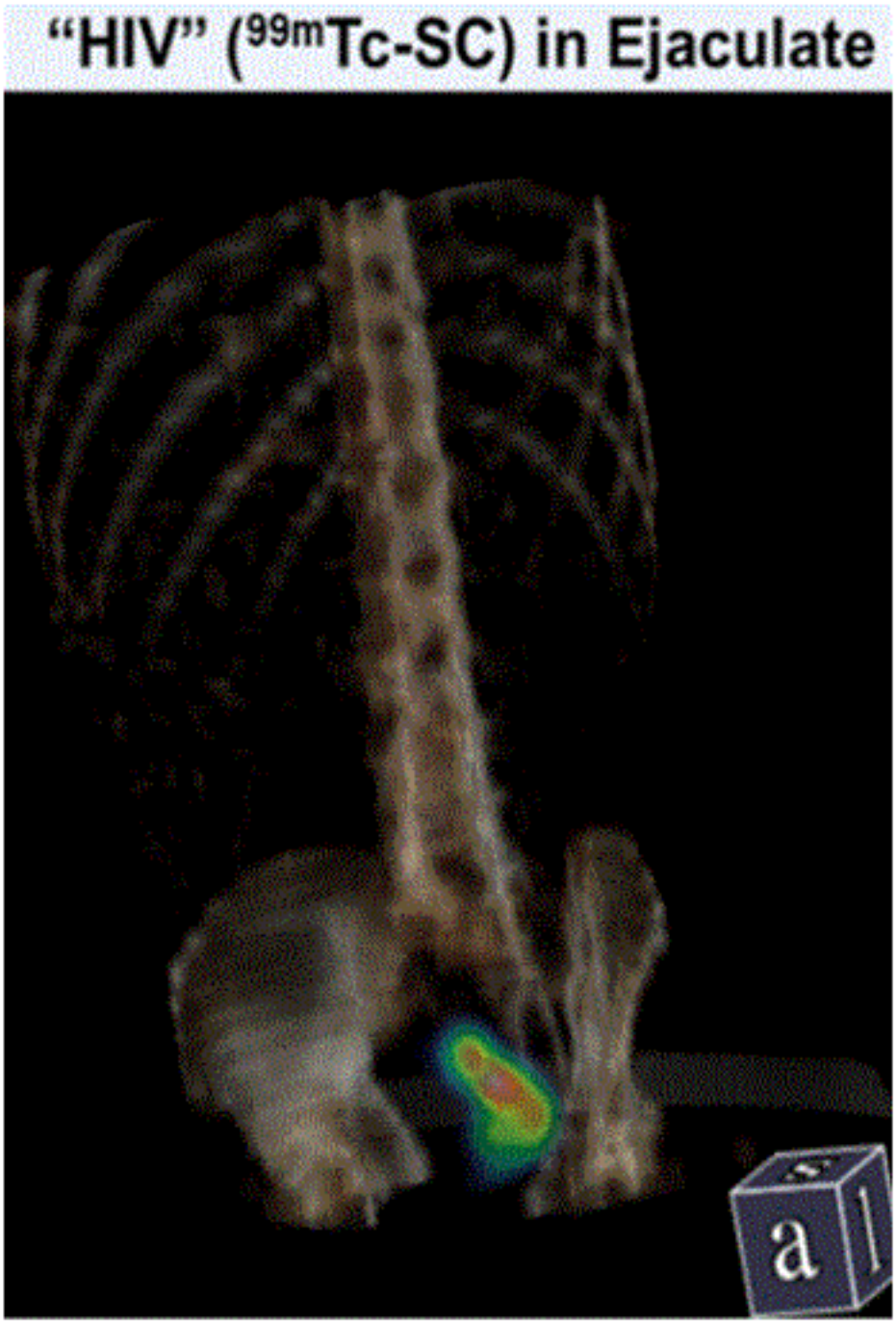
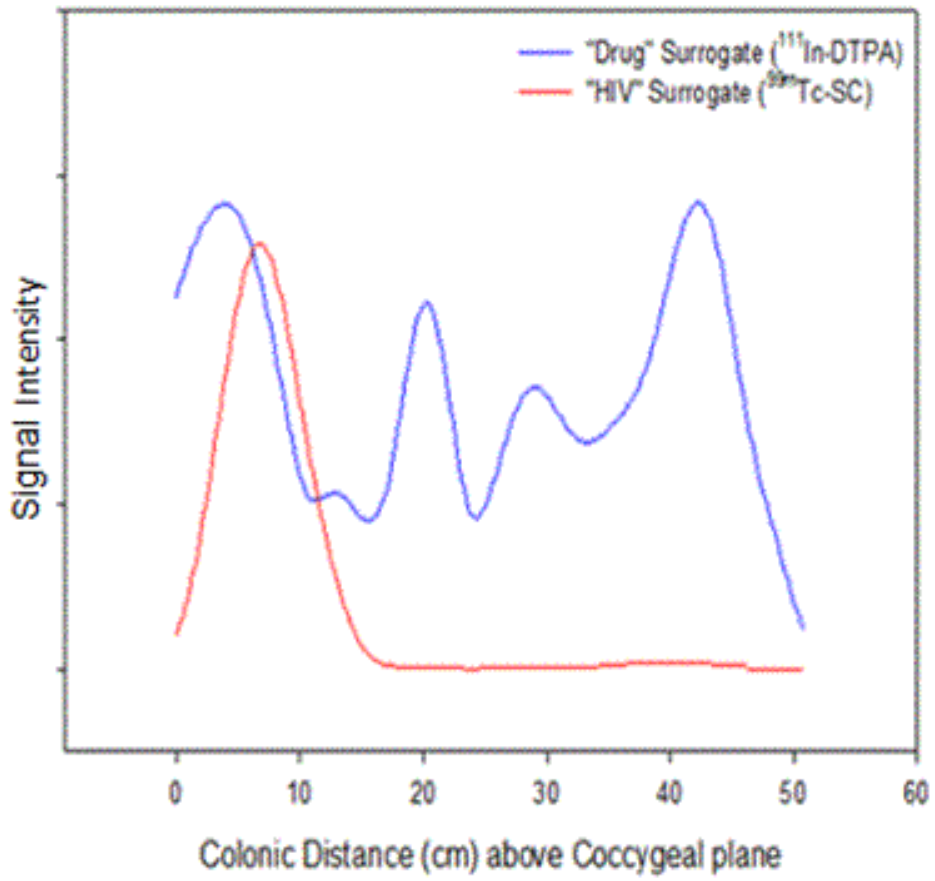


Figure 4c. Sample Concentration-Distance plotcorresponding to SPECT images depicted in Figure 4a and b



Signal intensity for HIV surrogate scaled to fit the image

Figure 5a. Correlation between plasma ^{111}In-DTPA concentration and plasma TFV

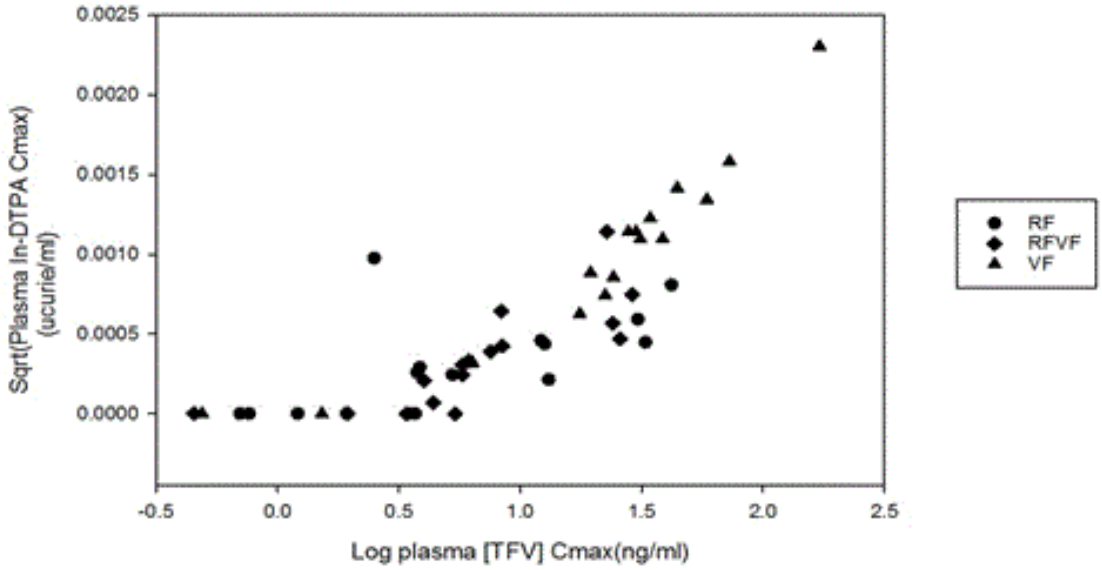
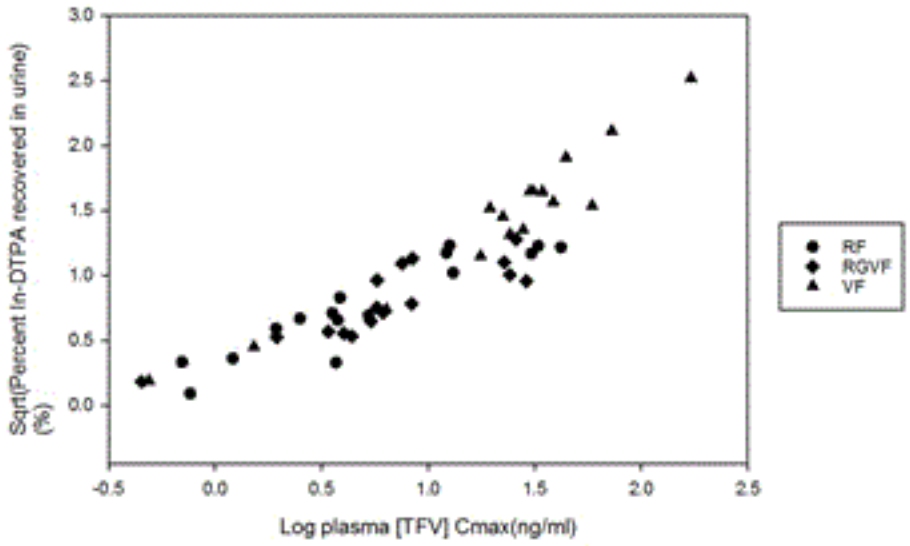


Figure 5b. Correlation between percent In-DTPA recovered from urine and plasma TFV



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Chapter 3: CHAMR 01 Study

This chapter is a part of the published work “Mcgowan I, Cranston RD, Duffill K, Siegel A, Engstrom JC, Nikiforov A, Jacobson C, Rehman KK, Elliott J, Khanukhova E, Abebe K, Mauck C, Spiegel HM, Dezzutti CS, Rohan LC, Marzinke MA, **Hiruy H**, Hendrix CW, Richardson-Harman N, Anton PA. **A Phase 1 Randomized, Open Label, Rectal Safety, Acceptability, Pharmacokinetic, and Pharmacodynamic Study of Three Formulations of Tenofovir 1% Gel (the CHARM-01 Study)**. *PLoS One*. 2015 May 5;10(5):e0125363. PubMed PMID: 25942472; PubMed Central PMCID: PMC4420274”. Permission to use part of the work, as it pertains to H. Hiruy’s contribution, in this thesis provided by PlosOne creative commons legal code.

Abstract

Objectives

CHARM-01 characterized the safety, acceptability, pharmacokinetics (PK), and pharmacodynamics (PD) of three tenofovir (TFV) gels for rectal application: the vaginal formulation (VF), the reduced glycerin vaginal formulation (RGVF) and the rectal specific formulation (RF) gel. The focus of this thesis is the comparison of the compartmental PK obtained from the six matrices: plasma, peripheral blood mononuclear cells (PMBC), colonic tissue, colonic mucosal mononuclear cells (MMC), rectal and vaginal fluid. CHARM-01 extends the objectives of CHARM-02 to multiple dosing and includes tissue drug concentrations, but does not include the colonic luminal imaging used in CHARM-02.

Methods

Participants received 4 mL of the three TFV gels in a blinded, crossover design: seven daily doses of RGVF, seven daily doses of RF, and six daily doses of placebo followed by one dose of VF, in a randomized sequence. Colonic tissue, blood samples, rectal and vaginal fluids were obtained before any rectal dosing (baseline) and thirty minutes after the 7^{th} dose. In addition, blood samples, vaginal and rectal fluid samples were obtained at 2,4 and 24 hours after the final dose.

Results

TFV moieties were detected in all matrices except for PBMC; all concentrations of TFV-DP in PBMC were below the lower limit of quantification. There were no differences between RF and

RGVF in terms of TFV PK profile in plasma, rectal tissue homogenate, vaginal and rectal fluid. Median tissue mucosal mononuclear cell (MMC) TFV-DP trended higher for RF when compared to RGVF, 1136(IQR: 473-2200) and 320(IQR: 170-1150) fmol per 10^6 cells, respectively; however, this difference did not reach statistical significance (p=0.067).

Conclusion

There were no statistically significant differences between the PK features of TFV in RF and RGVF in plasma, rectal homogenate, colonic MMC, rectal and vaginal fluid.

There was a trend of higher colonic MMC in RF as compared to RGVF, which did not reach statistical significance. Because participants received only a single VF dose, PK after VF dosing couldn't be compared to PK after RGVF and RF dosing.

Introduction

Men having sex with men have a disproportionate burden of HIV globally. Part of the reason for such high prevalence is that the risk of contracting HIV is significantly higher in those that practice unprotected receptive anal intercourse (RAI)[72-74]. Hence, in addition to current biomedical and behavioral prevention strategies, rectal microbicides will provide an additional prophylactic method.

One essential feature for rectal microbicide development is the ability of the candidate RM formulation to be present at a concentration that will inhibit infection at the site at risk of HIV infection. As stated earlier, local dosing has the benefits of attaining higher drug concentration at the mucosa compared to systemic dosing[21,56]. In CHARM-01, we compare the concentration of TFV and its moieties in six matrices, namely, plasma, PBMC, colonic tissue, colonic MMC, rectal and vaginal fluid.

Ethics Statement

The study was designed by the investigators with collaborative input from CONRAD and the NIAID/DAIDS/Prevention Sciences IPCP for HIV Topical Microbicides, as stipulated in the award notice and reviewed by the U.S. Food and Drug Administration (FDA). The study was approved by the University of Pittsburgh Institutional Review Board (IRB) as well as the University of California at Los Angeles IRB. All subjects provided written informed consent. The trial is registered at ClinicalTrials.gov, number # NCT01575405 and is in compliance with the CONSORT 2010 recommendations for reporting of trial results (www.consort-statement.org).

Materials and Methods

The CHARM-01 study was a Phase 1, double blind, randomized crossover trial in which participants received the three TFV gel formulations (VF, RGVF, and RF) in a randomized sequence. Each phase of product administration lasted 7 days with a 21 (± 7) day washout period (Figure 1). The first and seventh doses of study product were administered in the clinic and the remaining five doses were administered by the participant at home, with daily, protocol-defined reminders to encourage product use.

During the RGVF and RF phase of dosing, participants received seven identical doses of either the RGVF or RF TFV gel. However, during the VF phase of dosing, participants received six doses of a hydroxyethyl cellulose (HEC) placebo gel, with only a final dose of VF TFV gel. As the majority of participants in the RMP-02/MTN-006 rectal safety trial who received VF TFV gel experienced gastrointestinal side effects (bloating, abdominal discomfort, and diarrhea), it was considered unethical to ask participants to use more than one dose of VF TFV gel[56].

The study was conducted at two clinical sites (The University of Pittsburgh, Pittsburgh, Pennsylvania. and the David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California). Enrollment began in March 2013 and the last participant completed the study in October 2013. The target sample size was 18 (nine participants at each site) and enrolled participants were assigned at random to one of the three study formulation sequences. Randomization was done in blocks of three at each site to ensure balance between

formulation groups and the sequence of administration between sites. The randomization scheme was stratified by site and generated by the University of Pittsburgh, Center for Research on Health Care Data Center, using computer-generated random numbers. The role of HH was the pharmacokinetic and pharmacodynamics data analysis and interpretation.

The randomization assignments for up to 12 participants (24 total per site) were delivered to the Director of Pharmacy Affairs at the Magee-Womens Research Institute (MWRI) who held primary responsibility for maintaining the blinding and generated the product labels.

Study population

The study population consisted of healthy, RAI-abstinent, HIV-uninfected, adults (male and female) aged 18 years or older at time of screening who had been successfully vaccinated for hepatitis B virus (HBV) or who had naturally acquired immunity to HBV, as evidenced by HBV antibody titers. An inclusion criterion for female participants was the active use of an acceptable form of contraception (e.g., barrier method, intrauterine device, hormonal contraception, surgical sterilization, or vasectomization of the male partner). Individuals with abnormalities of the colorectal mucosa, significant gastrointestinal symptoms (such as a history of rectal bleeding), evidence of anorectal *Chlamydia trachomatis* (CT) or *Neisseria gonorrhea* (GC) infection, chronic HBV infection, or a requirement to use drugs that were likely to increase the risk of bleeding following mucosal biopsy were excluded from the study.

Study products

The VF TFV gel, the RGVF TFV gel, and the Universal HEC placebo gel were manufactured, under direction from CONRAD (Arlington, VA), by DPT Laboratories (San Antonio, TX). DPT Laboratories generated pre-filled RGVF applicators and packaged the RGVF device with a plunger. DPT Laboratories also manufactured the RF TFV gel under direction of Dr. Lisa Rohan's Group at MWRI. The HTI applicators (HTI Plastics, Lincoln, NE) were used in the CHARM-01 study. These applicators had been initially designed for vaginal use and have been used in all of the previous vaginal microbicide trials with TFV gel. They have also been used rectally in the RMP-02/MTN-006 and MTN-007 studies[42,56]. Each opaque pre-filled applicator was packaged with a plunger and labeled with a code to preserve the identity of the formulation. Each pre-filled applicator contained a dose of approximately 4 mL of TFV gel of the HEC placebo. The pre-filled applicators were shipped directly to study site pharmacies and were stored by and dispensed from the site pharmacy.

Each participant was assigned applicators based on the randomization number. At Visits 3, 6, and 9, the participant's first dose of study product was administered by the clinical staff. During the period of daily administration, study participants were instructed to insert one dose of gel into the rectum once daily throughout the seven-day period.

Study procedures

There were a total of eleven study visits and one follow-up phone call. After obtaining informed consent all participants were screened with a thorough medical history, a targeted physical

examination, a digital rectal examination, and rectal swabs for CT/GC nucleic acid amplification testing (NAAT). Urine was also collected for CT/GC NAAT and for pregnancy testing in the female participants (pregnancy testing was repeated at all subsequent clinical visits). Blood was collected for safety labs (complete blood count, urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase) and serology (syphilis, HIV-1, hepatitis B, and herpes simplex 1 and 2). Participants who met the aforementioned inclusion criteria during the Screening Visit were enrolled into the study. The Enrollment Visit occurred within 28 days of screening. At the Enrollment Visit, participants were randomized, and a rectal examination and focused physical examination were performed. Rectal swabs were collected for CT/GC. Rectal sponges for PK were also collected. Participants then received a normal saline pH 7.4 enema. A flexible sigmoidoscope was inserted into the rectum and biopsies were collected at approximately 15 cm from the anal verge. At Visits 3, 6, and 9 (Treatment Initiation Visits), all participants had a single applicator of study gel inserted into the rectum. Within 30 minutes, samples were collected for CT/GC. At Visits 4, 7, and 10 (Last Dose Treatment Visits), a normal saline enema was then administered followed by a single dose of study product. Approximately 30 minutes later (± 15 minutes) blood, and in females, self-collected vaginal sponges were collected for PK studies. A sigmoidoscope was then inserted and the same rectal tissue biopsy samples were collected as described during the Enrollment Visit (with the exception of samples for GC/CT and cytokines). Additional blood and rectal/vaginal sponges were collected at 2 hours (± 30 minutes) and 4 hours (± 30 minutes) after product insertion. At Visits 5, 8, and 11 (conducted 18-30 hours after Visits 4, 7, or 10) blood and rectal/vaginal sponges were collected for PK.

Pharmacokinetic procedures

Blood plasma, peripheral blood mononuclear cells (PBMCs), vaginal and rectal fluid, and rectal tissue were obtained before rectal dosing (Visit 2) and 30 minutes after the seventh dose of the gels (Visits 4, 7, and 10). Additional samples of blood plasma, PBMCs, and rectal/vaginal fluid samples were obtained at 2, 4, and 24h after the final dose (Visits 5, 8, and 11).

Sample Processing: TFV and TFV-DP concentrations were determined via validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods at The Johns Hopkins University Clinical Pharmacology Analytical Laboratory as described previously [23]. All assays were validated following the recommendations of the FDA, Guidance for Industry: Bioanalytical Method Validation guidance document [10]. TFV concentrations were determined in plasma, rectal fluid, and vaginal fluid. TFV-DP concentrations were determined for PBMCs, rectal tissue homogenates, and rectal MMCs. The measured value from each PK assay was used unless the PK value was determined to be between the lower limit of quantification (LLOQ) and the lower limit of detection (LLOD), in which case, a number equal to half that assay's LLOQ was imputed for that PK value.

Analysis of outcomes

Pharmacokinetics: TFV-based gel formulations' PK were evaluated in six compartments (plasma, PBMCs, rectal fluid, rectal tissue, rectal MMCs, and cervicovaginal fluid) after rectal administration of the study product. For matrices other than tissue which were sampled multiple times after the last dose, the 24 hour post-dose concentration vs. time profile was examined for the final rectal dose of each TFV-containing study product (after 7 doses for RF and RGVF, after 1 dose for VF); TFV (or TFV-DP in PBMCs) maximum concentration (C_{max}), time to maximum concentration (T_{max}), and area under the TFV concentration-time curve from 0 to 24h (AUC_{0-24} [log-linear trapezoidal method]) was estimated using non-compartmental methods (WinNonlin v. 6.3 software, Pharsight, St. Louis, MO). Rectal biopsies, which were sampled only once with each product, were taken 30 minutes after each final study product dose to determine TFV and TFV-DP concentrations in tissue homogenates and TFV-DP in MMCs. We performed paired comparisons between RF and RGVF using the Wilcoxon rank sum test with exact two-sided significance test ($p \leq 0.05$). VF was not compared due to non-steady state conditions as only one drug-containing dose was given.

Results

Enrollment and retention

A total of 14 participants (11 men and 3 women) were enrolled and randomized in the study (Figure 2), 12 of whom completed the study. The majority of participants were white (57%) with

a mean age of 37.7 (± 14.3) years (Table 1). There was no statistical difference between sites in gender composition or the proportion of white participants, although there was a marginal difference with respect to age (41.7 versus 23.0; p=0.0414) with UCLA having a slightly older cohort. One female participant was enrolled but developed pyelonephritis prior to product exposure and was removed from the study. A second participant was randomized to receive the RGVF gel as the first study product. The participant completed Visit 5 but was subsequently withdrawn due to gastrointestinal symptoms including bloating and abdominal discomfort suggestive of irritable bowel syndrome. All other participants completed the study. Averaged across all study visits, the proportion of completed administrative procedures, clinical procedures, clinical laboratory sample collection, and research laboratory sample collection was 89%, 87%, 96%, and 86% respectively.

Pharmacokinetics

TFV moieties were detected in all compartments sampled, except for PBMC TFV-DP which was below the LLOQ for all products (Table 3). The plasma TFV concentration-time profile (figure 3), C_{max}, T_{max}, and AUC_{0-24} were not significantly different for the RF and RGVF products (Table xx). There were no differences between RF and RGVF in TFV or TFV-DP in rectal tissue homogenate, though tissue MMC TFV-DP trended toward greater values with RF when compared to RGVF with median (IQR) RF/RGVF ratio of 1.8 (0.4, 3.9) (p=0.07). As mentioned previously, only a single exposure (Day 7) of the VF TFV 1% gel was given to those during their randomization to the VF arm; consequently, the VF product findings for PK are not summarized here.

Discussion

Rectal exposure to study products was associated with the detection of TFV in plasma, rectal fluid, and rectal tissue and TFV-DP in rectal tissue and tissue MMC but not in PBMCs. As previously reported, rectal exposure to TFV gels was also associated with detection of TFV in vaginal fluids.

The compartmental PK data from CHARM-01 are similar to PK data generated in the RMP-02/MTN-006 study (Yang PLOS ONE 2014): rectal exposure to TFV gels is associated with minimal systemic exposure, lack of drug detection in PBMCs, high concentrations in rectal tissue/fluid, and detection in vaginal fluid. MMC TFV-DP trended toward ~2-fold greater concentrations following RF when compared to RGVF. Otherwise, there were no PK differences between these two products.

Single dose VF PK values cannot be fairly compared to the drug accumulation in steady-state RF and RGVF PK values after 7 doses. For example, based on our single dose VF PK data and the long TFV and TFV-DP half-life within most of the matrices tested [30], accumulation of TFV and TFV-DP after 7 daily VF doses would match or exceed the concentrations seen with the RF and RGVF products in this study.

The CHARM-01 PK data do suggest that the RF formulation may deliver higher local concentrations of TFV-DP to the rectal mucosa than the RGVF formulation, although this did not

reach significance. This is the only discriminating parameter between the RF and RGVF TFV gels in the CHARM-01 study and may be insufficient to displace the RGVF TFV gel that is currently being evaluated in an International Phase 2 expanded safety study (MTN-017; ClinicalTrials.gov Identifier: NCT01687218) being conducted in the United States, Peru, Thailand, and South Africa. The results of the MTN-017 study (expected in early 2016), with approximately 192 participants, eight week periods of exposure to daily or pericoital RGVF TFV gel, as well as a PK/PD substudy of 36 participants, will have a critical role in defining the future for the RGVF TFV gel as a candidate rectal microbicide for Phase 3 safety and effectiveness trials. Certainly, with increasing rates of HIV infection in MSM and transgender women there is an urgent need to develop new approaches for the prevention of HIV infection in these highly vulnerable populations.

Table 1 Baseline demographic data by site

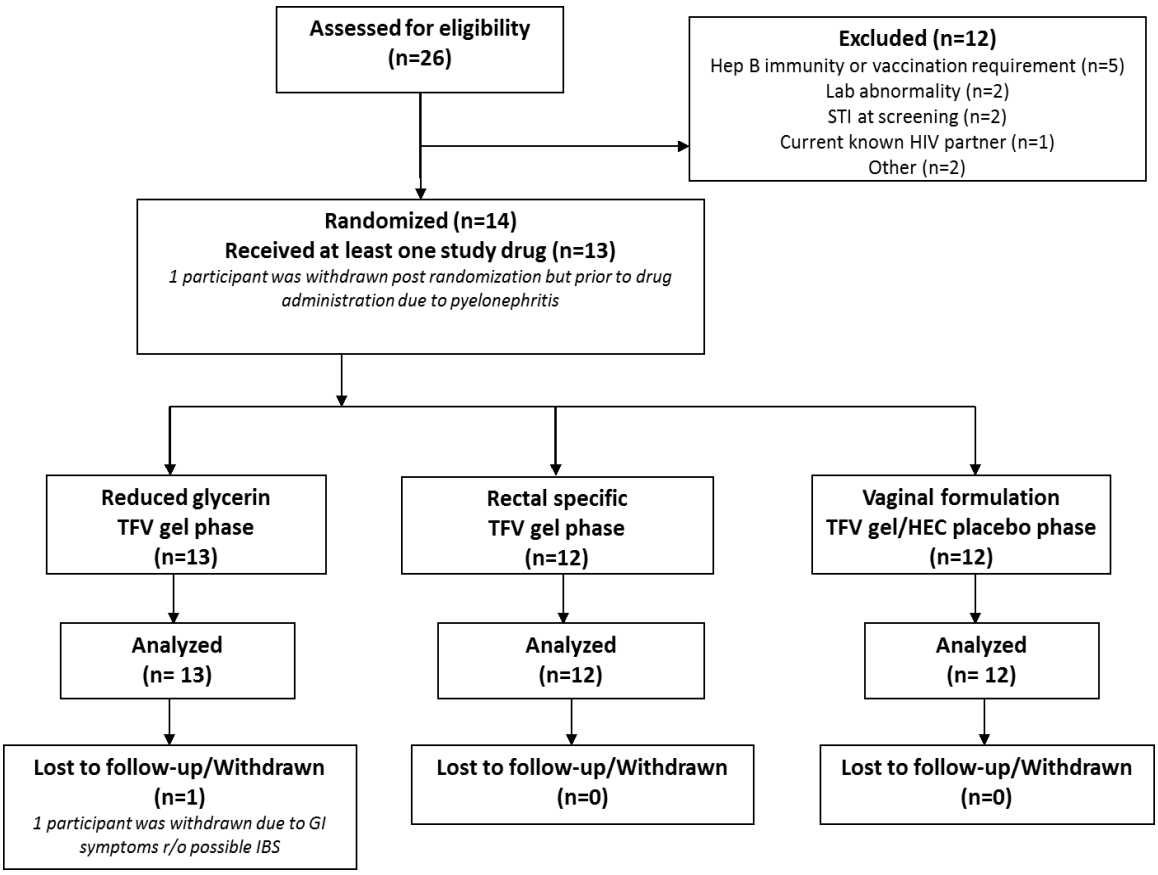
Variables	UCLA (n = 11)	PITT (n = 3)	Overall (n = 14)
Age	41.7 ± 13.6	23.0 ± 1.7	37.7 ± 14.3
Male	9(81.82%)	2(66.67%)	11 (78.57%)
Race			
White	6(54.55%)	2(66.67%)	8(57.14%)
Black or African American	4(36.36%)	1(33.33%)	5(35.71%)
American Indian or Alaska Native	1(9.09%)	0(0.00%)	1(7.14%)
Hispanic			
No, not of Hispanic, Latino/a, or Spanish origin	9(81.82%)	3(100.00%)	12(85.71%)
Yes, Mexican, Mexican American, Chicano/a	1(9.09%)	0(0.00%)	1(7.14%)
Yes, Another Hispanic, Latino/a or Spanish origin	1(9.09%)	0(0.00%)	1(7.14%)

Table 2. Pharmacokinetic data are summarized as median (interquartile range)*

Matrix	Moiety	PK	Units	RF TFV	RGVF TFV	VF TFV
Plasma	TFV	C_{max}	ng/mL	7.1 (3.5-11.9)	6.0 (4.3-7.1)	5.1 (3.3-6.2)
		AUC	ng*hr/ mL	78 (33-135)	64 (28-97)	36 (23-57)
PBMC	TFV-DP		fmol/M	All BLQ	All BLQ	All BLQ
Colon tissue	TFV	30'	ng/mg	2.9 (0.5-5.8)	1.4 (0.7-3.7)	1.0 (0.1-9.2)
	TFV-DP	30'	ng/mg	10.3 (BLQ-36.8)	5.2 (BLQ-12.8)	BLQ (BLQ-6.4)
Colon tissue MMC	TFV-DP	30'	fmol/M	1136 (473-2200)	320 (170-1151)	91 (19-367)
Rectal Fluid	TFV	C_{max}	ng/mL	$8.1x10^5$ ($(1.8 -16) x10^5$)	$9.4 x10^5$ ($(4.3-14)x10^5$)	$3.6x10^5$ $(0.8-8.2)x10^5$)
		AUC	ng*hr/ mL	$1.4 x10^6$ ($(0.45-2.9)x10^6$)	$1.4 x10^6$ ($(0.66-2.5)x10^6$)	$7.9x10^5$ ($(5-14)x10^5$)
Vaginal Fluid[#]	TFV	C_{max}	ng/ sponge	31, 220	133, 172	6, 12
		AUC	ng*hr/ sponge	486, 3,499	922, 1,377	88, 29

*No RF v. RGVF comparisons are statistically significant (all p>0.05, Wilcoxon rank sum test). VF was not compared to other products. [#]Only 2 subjects, both shown.

Figure 1. Flow diagram of participant progress through the CHARM-01 study



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Chapter 4: PHATISA Study

This chapter is published as "**Hiruy H**, Rogers Z, Mbowane C, Adamson J, Ngotho L, Karim F, Gumbo T, Bishai W, Jeena P. **Subtherapeutic concentrations of first-line anti-TB drugs in South African children treated according to current guidelines: the PHATISA study**. *J Antimicrob Chemother.* 2015 Apr;70(4):1115-23. PubMed PMID: 25505005; PubMed Central PMCID: PMC4356201". Permission to reprint in this thesis was provided by Oxford University Press (license ID 3640910510432).

ABSTRACT:

Background: There is a paucity of evidence regarding optimal dosing of anti-tuberculosis drugs in children. The aim of this study was to identify the pharmacokinetic parameters of first-line anti-tuberculosis drugs, and concentrations achieved, after the implementation of the 2010 World Health Organization (WHO)-recommended pediatric dosages.

Methods: We conducted a prospective, observational pharmacokinetic study in children 10-years old, or younger, who were on isoniazid, rifampin, pyrazinamide, and ethambutol therapy in Durban, KwaZulu-Natal, South Africa. Blood was collected at six time points over a 24-hour period, chosen using optimal sampling theory. Drug concentrations were simultaneously modeled to identify the compartmental pharmacokinetics of each drug in each child, using the ADAPT program.

Results: The best six sampling time points in children were identified as 0 (pre-dose), 0.42, 1.76, 3.37, 10.31 and 24 hours post dose. Thirty-one children were recruited and blood drawn at these time points. Rifampin, ethambutol and pyrazinamide were best described using a 1-compartment model, while isoniazid was best described with a 2-compartment model. Only 9.6%, 83%, 64.5% and 30.7% of children attained the WHO 2-hour target therapeutic concentrations of rifampin, isoniazid, pyrazinamide, and ethambutol, respectively. Moreover, only 77%, 19%, and 26% achieved the area under concentration-time curves associated with

optimal clinical response of rifampin, pyrazinamide, and isoniazid, respectively. No single risk factor was significantly associated with sub-therapeutic drug levels.

Conclusion: Drug concentrations of all first line anti-tuberculosis drugs were markedly below the target therapeutic concentrations in most South African children who received the revised WHO-recommended pediatric weight based dosages.

Tuberculosis (TB) continues to be a major global public health threat in which children bear a significant portion of disease mortality and morbidity. In 2012, there were an estimated 8.6 million new cases worldwide, with most cases in several high-burden countries including South Africa, China, India and Russia. In South Africa, childhood TB accounts for 15-20% of the burden. To compound this, the additional problem of multidrug resistant TB (MDR-TB)-, extensively- and totally-drug resistant strains has emerged.[1, 2] Despite these ominous threats, the first-line treatment regimen for TB, comprised of isoniazid, rifampin, pyrazinamide, and often ethambutol, has remained stagnant for several decades. With the failure to ensure adequate control of the childhood TB burden, an evaluation of drug concentrations associated with standard dosing of the existing drugs is paramount, since inadequate drug levels may contribute to treatment failure and the problem of MDR-tuberculosis.[2]

The design of pediatric pharmacokinetic (PK) studies needs to be optimized. Often, pediatric PK studies have relied on a convenience sampling strategy, and a desire to incorporate the 2hr time point. However, this "random" and arbitrary sampling strategy leads to imprecision in PK estimation, and is a common source of error.[3-5] First, there is need to define the full concentration-time profile over a dosing interval so that AUC_{0-24}, T_{max}, and C_{max} can be identified, which *always* vary from child to child. Specifically between-individual PK variability is a fact that must be taken into account in study design. Second, the duration of sampling is most accurate when the sampling time encompasses at least three elimination half-life values for all drugs.[4] An approach that takes these concerns into account is application of optimal sampling

theory, based on Fisher information matrix.[3, 5] Blood draws are performed at particular "information rich" time points, allowing for identification of unbiased PK parameter estimates. The number of sampling times is also minimized, without loss of information since sampling occurs at points that maximize useful information. Here, we applied optimal sampling theory to the sampling strategy design so that more accurate PK parameter estimates could be identified in children.

The importance of accurately identifying PK parameter estimates in children is to ensure that optimal dosing strategies can be designed. An optimal dose is that which achieves a target concentration that is known to be associated with optimal microbial and clinical outcomes. The most commonly utilized reference concentrations by the WHO have been 2hr drug concentrations, with references of rifampin 8 mg/L, isoniazid 3 mg/L, pyrazinamide 20 mg/L, and ethambutol 2 mg/L.[6] In order to achieve these target 2hr drug concentrations, the WHO recently recommended new treatment doses for children.[7] These 2hr concentrations are often confused with "peak" (C_{max}) concentrations, however McIlleron et have shown that they differ.[8] Moreover, 2hr concentrations have been found not to be predictive of clinical outcomes in several studies in adults.[9-11] Furthermore, these 2hr concentrations were not designed to address the question of acquired drug resistance (ADR); ADR is unquestionably driven by low drug concentrations, which initiate a series of molecular events termed "the antibiotic resistance arrow of time".[11-22] On the other hand, studies in the hollow fiber model (HFM) and in murine TB, and our re-analysis of older guinea pig studies, have identified that instead it is AUC_{0-24}/MIC and C_{max}/MIC of these first line drugs that drive efficacy and suppress ADR.[15-18, 22-24]

The HFM studies and computer-aided clinical trial simulations predicted that PK variability was the main driver of therapy failure and ADR in South Africa.[11] This was confirmed in three studies, first a meta-analysis of prospective studies that involved 2,382 patients, and later in 2 prospective clinical studies.[12, 13, 25] In one clinical study of 142 adult South Africans, >90% of therapy failure (death, relapse and microbial failure) and 100% of ADR was explained by having a pyrazinamide $AUC_{0-24} \leq 363$ mg·h/L, a rifampin $AUC_{0-24} \leq 13$ mg·h/L and an isoniazid $AUC_{0-24} \leq 52$ mg·h/L, as well as low C_{max}.[12] These findings have since been validated in a separate prospective clinical study.[25] Moreover, these concentration thresholds predictive of outcome in adult TB were virtually the same as identified in HFM and in mice.[15-18, 22] Here, we investigated how often South African children treated with the new WHO recommended doses achieve the older reference concentrations used by the WHO as well as how often they achieved the AUC_{0-24} thresholds that predicted clinical outcomes in adults.

MATERIALS and METHODS

Regulatory compliance

The Institutional Review Boards (IRB) of the University of KwaZulu-Natal and Johns Hopkins University approved this study.

Study Population and Setting

Pharmacokinetics of Anti-Tuberculosis Medications in South African Children (PHATISA) is a prospective, single-center, observational PK study that was conducted at the King Edward VIII hospital in Durban, South Africa, from May 2012 to March 2013. Children 10 years of age or

younger with the diagnosis of TB were enrolled. The diagnosis of TB was based on clinical symptoms, radiological findings, tuberculin skin testing, history of household contact, and microbiologic confirmation. Children were excluded from the study if they had a hemoglobin level <6 grams per deciliter, alanine aminotransferase (ALT) more than 3 times the normal value for age, evidence of coagulopathy based on an abnormal PT/PTT, probable diagnosis of abdominal TB based on clinical findings, or any history of intolerance or allergy to the first-line anti-TB drugs. Children who were enrolled in another study were also excluded.

Baseline clinical data was obtained for each participant, including age, nutritional status (weight, height, mid upper arm circumference), alkaline aminotransferase, creatinine, blood urea nitrogen (BUN), and chest radiography. HIV testing was performed by antibody testing for children older than 18 months of age, and by HIV DNA PCR for those younger than 18 months of age. Dietary information and concomitant medications were recorded for the 24hrs of the blood sampling.

Definitions

The diagnosis of definite TB was made if there was microbiological evidence (by sputum or tissue culture *Mycobacterium tuberculosis* positivity) or probably TB (by acid-bacillus smear positive and/or a classic radiological finding); those that did not meet these criteria but showed symptoms of TB (possible TB) and were started on treatment were also included in the study.[26] Children were started on standard first-line anti-TB agents, rifampin, isoniazid, and pyrazinamide with addition of ethambutol for severe forms of TB in accordance with the new

WHO guidelines. These guidelines recommend that children receive 10-15 mg/kg of isoniazid, 10-15 mg/kg of rifampin, 30-40 mg/kg of pyrazinamide, and 15-25 mg/kg of ethambutol.[7] Informed consent was obtained from parents or guardians prior to enrollment.

Drug treatments

Drugs were provided by the hospital pharmacy and were obtained from Aspen Pharmacare and Sanofi-Aventis South Africa (Pty) Ltd. Drugs were ground and given as food emulsions for children too young to swallow tablets. The drugs were given as fixed dose combinations. The doses were rounded to the nearest value using the available tablet sizes: combined rifampicin, isoniazid and pyrazinamide 60,30 and 150 mg tablet, combined rifampin and isoniazid 60 and 30 mg or 60 and 60 mg tablets, pyrazinamide 150 and 500 mg tablets, and ethambutol 100 and 400 mg tablets according to weight based charts.

Study and sampling procedures

Blood samples were obtained from each participant between the fourth and twelfth day after initiation of anti-TB therapy. In order to avoid biased PK parameter estimation, optimal sampling theory was utilized to identify information-rich time points for each of the four drugs with the use of ADAPTII software.[3-5, 27] Six time points were identified. Peripheral intravenous catheters were used for sample collections. At each time-point, 2-ml of blood was collected in EDTA-coated tubes. Each specimen was immediately placed on ice until processing. Blood samples were centrifuged at 2,000 x g for 10 minutes. The plasma layer was separated within 30 minutes after sampling and stored in a cryovial at -80ºC until time of analysis.

Drug concentration measurement assays

Measurement of drug concentrations was carried out by a previously published multiplexed three-drug assay using liquid chromatography coupled to tandem mass spectrometry (AB-Sciex Qtrap® 4500 LC/MS/MS system).[28] The internal standard was 6-amino nicotinic acid. Calibration and quality control standards, along with a blank plasma aliquot and an internal standard aliquot, were used for all runs. Dilutions of standard drug solutions were used to cover the range of concentrations expected for each drug. Three quality control solutions were used to span the range of serum drug concentrations: lowest (QL), intermediate (QM), and highest (QH). Inter-day and intra-day coefficients of variation were below 10%. Selected multiple reaction monitoring (MRM) transitions were run in positive ion mode of $[M+H]^+$. Precursor ions to product ions were isoniazid (mass-to-charge ratio [m/z] 138.1→ 51.9), rifampin (m/z, 823.1→791.2), pyrazinamide (m/z, 124.1→ 52.1), and 6-amino nicotinic acid (m/z, 138.7→ 58.9). Analyst® 1.5 software version 1.5.1 was used.

Compartmental PK analyses

All concentrations of rifampin, isoniazid, pyrazinamide, and ethambutol were modeled using the ADAPT 5 software program.[29] First, we utilized the standard two stage estimation method to generate initial PK parameter estimates for each drug for a one-, two-, or three-compartment model, with first-order input and elimination. The compartmental parameter estimates were then used in subroutine POPINIT of ADAPT. Next, each drug was modeled to identify pharmacokinetic parameter estimates for each child using the maximum-likelihood

solution via the expectation-maximization algorithm (MLEM). Choice of best compartmental model was then made based on lowest Akaike information criterion (AIC) score and Bayesian Information Criteria (BIC). While ideally they should agree, the BIC, which penalizes for complexity, was used for the final decision. However, we also applied the rule of parsimony, based on Occam's razor, which was that if AIC and BIC chose the more complex model, but that model did not significantly improve the parameter estimates compared to the less complex model, then the simpler model offered the best explanation.

Statistical analysis

STATA version 12 was used for statistical analysis. The baseline characteristics and secondary PK parameters such as C_{max}, T_{max}, and AUC_{0-24} were summarized as means ± standard deviation. The 2hr concentration values were considered to be binary data: above the reference target concentration or below. The 2 hour reference concentrations for rifampin were 8 mg/L, for isoniazid 3 mg/L, for pyrazinamide 20 mg/L, and for ethambutol 2 mg/L, based on previous studies that utilized these concentrations to design new doses.[30] These were used simply to identify if the intended target concentrations for the new WHO doses had been attained. Next, we wanted to identify the proportion of children in these WHO recommended doses who achieved or exceeded, the AUC_{0-24}s shown to be associated with clinical outcome in pulmonary TB adult patients.[12] These were a pyrazinamide $AUC_{0-24} \leq 363$ mg·h/L, a rifampin $AUC_{0-24} \leq 13$ mg·h/L and an isoniazid $AUC_{0-24} \leq 52$ mg·h/L. For this latter analysis the concentrations were not dose- or weight-normalized.

For each of the four drugs, the C_{max} and AUC were dose-normalized, depicted as C_{max}/D and AUC_{0-24}/D, respectively. Graphical exploratory data analysis showed that the C_{max} and AUC_{0-24} were not normally distributed; hence, non-parametric tests were used to investigate differences in C_{max} and AUC among the covariates. The Wilcoxin rank-sum test was used to delineate differences in C_{max}/D and AUC_{0-24}/D between younger and older children (<2 years vs. >2 years), sex (male vs. female), HIV serostatus (positive vs. negative), and nutritional status (malnourished vs. non-malnourished).

RESULTS

Application of optimal sampling theory revealed that the best six sampling time points in children were 0 (pre-dose), and 0.42, 1.76, 3.37, 10.31 and 24 hours post dose. These sampling times were then used to time blood draws. A total of 36 children, treated between May 2012 and March 2013, were eligible for the study. Three parents declined informed consent. One patient improved with antibiotic therapy for bacterial pneumonia and the clinical diagnosis of probable TB was removed with discontinuation of anti-TB therapy. Another patient was diagnosed with probable gastrointestinal TB after imaging, and was disqualified from the study. The baseline anthropometric and clinical data in the 31 remaining children is summarized in Table 1. 80.6% children presented with pulmonary tuberculosis; 19.4% had disseminated disease.

All children were inpatients and doses were received under supervision of nurses; however 11 children received their doses as outpatients. Dosages received by participants for each of the

four drugs were ascertained by checking the bottles of the drugs as well as the prescribed dosage in the participants' charts (Figures 1-4, Panel A). Overall, 90.3% (28/31), 83% (26/31), 41.9% (13/31), and 84.6% (11/13) of participants received dosages within the revised WHO recommendations for rifampin, isoniazid, pyrazinamide, and ethambutol, respectively (Figures 1-4, Panel A).

Spearman's rank correlation coefficient was used to determine if correlations existed between underdosing of one drug and another. This evaluation revealed a strong statistically significant correlation between underdosing of isoniazid and rifampin (ρ=0.746, p-value 0.001), isoniazid and pyrazinamide (ρ=0.373, p-value 0.039), but not between rifampin and pyrazinamide (ρ=0.278, p-value 0.13). This correlation reflects the fact that fixed dose combinations were used, an effect compounded by weight banding.

Rifampin, pyrazinamide, and ethambutol were best described using a one-compartment model, while isoniazid was best described using a two-compartment model. The mean population PK parameter estimates for all four drugs are shown in Table 2. Secondary PK estimates such as $C_{max,}$ 2hr concentrations, and $AUC_{0\text{-}24}$, are shown in Figures 1-4, which highlight the wide inter-patient variability for each of the drugs. Table 3 shows how poorly either the 2hr concentration and C_{max} were as predictors of $AUC_{0\text{-}24}$. The r^2 were mediocre, except for pyrazinamide which had a moderate r^2. The duration of therapy until blood draws for drug concentration measurement was a median of 7.0 (range:4-12) days. The relationship between duration of therapy and drug concentration is shown in Table 4, which shows none of the slopes significantly differed from zero. Thus, the duration of therapy until blood draws for the PK study

was not associated with low or high serum drug concentration, even for rifampin which undergoes autoinduction.

Table 5 shows a summary of the proportion of children who achieved the reference 2hr drug concentrations that has been used for dose design in children. For all four drugs, a substantial portion of children achieved 2hr concentrations below the reference targets, especially rifampin and pyrazinamide. This means that for children in KwaZulu, the new WHO recommended doses still fail to achieve their intended target concentration. Table 4 also shows that median value and range of 2hr concentration differed from those for C_{max} for all drugs, except the pyrazinamide median (but not range).

Table 5 also shows that for isoniazid and pyrazinamide, most children did not achieve the AUC_{0-24} that have been shown to be associated with optimal long term responses such as cure in adults. In the case of rifampin, a recent clinical study in adults identified a rifampin AUC of 35 mg*h/L as predictive of speed of sterilizing effect and 2-month sputum conversion rates.[25] Twenty-two (71.0%) of the 31 children had a rifampin AUC≤35 mg*h/L, which suggests they would have slow sterilizing effect rates and delayed cure. Thus, overall, rifampin, isoniazid and pyrazinamide exposures were below optimal AUC_{0-24} in a majority of the children.

Next, we performed a univariate analysis for failure to achieve target 2hr drug levels for each of the four study drugs against clinical and demographic factors, only HIV positivity was significantly associated with low 2hr/D for isoniazid ($p = 0.04$, Wilcoxon rank sum comparison).

There was a trend towards significance for low AUC_{0-24}/D for isoniazid and pyrazinamide (p = 0.07 for both drugs).

DISCUSSION

There are several findings in our study. First, we evaluated the plasma concentration of the four first-line anti-tuberculosis drugs that are achieved in children dosed according to the 2010 revised WHO dosing recommendation. The most important finding in our study was the surprisingly high proportion of children with sub-therapeutic plasma concentrations of all four drugs, even in those receiving recommended revised WHO dosages. Our PK parameter estimates and drug concentrations are likely accurate and have minimal bias, given that we employed optimal sampling theory to identify the most information rich sampling times, as opposed to a design based on convenience. Our findings suggest a need to increase the doses of these drugs in children above what is currently recommended by the WHO. On the other hand however, the target concentrations used to make this recommendation assume that the 2hr and AUC concentrations needed for optimal effect in adults are the same as in children. This is currently unknown and should be investigated, given possible differences in bacterial burden between adults and children with tuberculosis.[31]

Secondly, the PK parameter estimates in the children we studied differed from those from other parts of sub-Saharan Africa and from India.[32, 33] A study from Cape Town, South Africa, reported different AUCs and C_{max} concentrations in children who received the revised WHO recommended doses. As a result, the proportion of children who achieved sub-therapeutic drug

concentrations was lower than in our current study. Both studies are correct, and the differences simply illustrate the large between-patient PK variability in children between different regions of the same country, perhaps due to genetic, demographic, and nutritional factors as well as co-morbities. Our current study was conducted among children primarily of Zulu ancestry, who also had a wider age range (3 months to 10 years, with 48% being > 2 years of age) compared to the Cape Town study.[32, 33] Indeed, in a study of ofloxacin in adults from the same two places, PK parameter estimates differed between them in MDR-TB patients.[34] Similarly, PK parameters and their variability are expected to differ in children in different countries. This variability strongly emphasizes the crucial need to establish population PK parameter estimates in children in each different locale where there is a large pediatric tuberculosis burden: children in Mumbai differ from those in KwaZulu-Natal who differ from those in Cape Town. Our findings should be used to allow more targeted local adjustment of doses by clinicians.

In summary, despite implementation of the 2010 WHO dosing guidelines, a considerable proportion of children still achieve sub-therapeutic anti-TB drug concentrations. Since metabolism of each of the drugs is from different xenobiotic metabolism enzymes encoded by unlinked alleles, the subtherapeutic concentrations observed across all four drugs suggest that dosing practices (in this case in pursuance of WHO guidelines) is one of the major reasons for the low drug concentrations, and not pharmacogenetic reasons. The current practice of prescribing first-line TB drugs is weight-based; hence, a 2-month old infant will receive the same 10 mg/kg dose of INH as a 10-year old child. This approach ignores the significant

physiologic differences that exist between infants and children, in addition to the weight difference. As an example, principles of allometric scaling, such as the ¾ power laws mean that children of different weights should be dosed differently based on different mg/kg doses.[35-38] In other words, a 10 mg/kg dose in a 5 kg infant (50 mg) will achieve different concentrations in a 30 kg child who is ten years old (300 mg) because the effect of weight on clearance and volume is non-linear. Thus, further well-powered studies are needed to elucidate optimal age- and weight-based dose schedules in the pediatric population. In addition, studies that also compare the clinical responses in children with and without sub-therapeutic levels are needed to further inform and strengthen the underlying concern that failure to achieve the therapeutic targets in the anti-TB pharmacotherapy for children may be a driver of poor outcomes and ADR.

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Table1. Baseline demographics and clinical characteristics

Clinical factor	Median (range)
Demographics	
Age, years	2.29 (0.25-10.5)
≤ 2 years, n	16
> 2 years, n	15
Weight, kg	11.5 (6.1-19)
Height, cm	84 (66-114)
Malnourished, n (%)	20 (64.5)
HIV+, n (%)	7 (22.6)
Female, n (%)	13 (41.9)
Laboratory Data	
Hemoglobin, g/dL	9.2 (7-11.7)
Alanine aminotransferase	16 (9-71)
Creatinine, □mol/L	28.5 (20-62)
Microbiologic Data, n	
Smear positive	5/23
Culture positive	7/20
Hain positive	7/8
GeneXpert	1/13
Diagnostic Data	
Positive TST, n**	13/17
Chest X ray findings, n	22/31
Cavitary lesion	4/22
Parenchymal consolidation	19/22
Perihilar adenopathy	14/22

**TST=Tuberculin skin test

Table 2. Pharmacokinetic parameter estimates of first line-anti-tuberculosis drugs South African children.

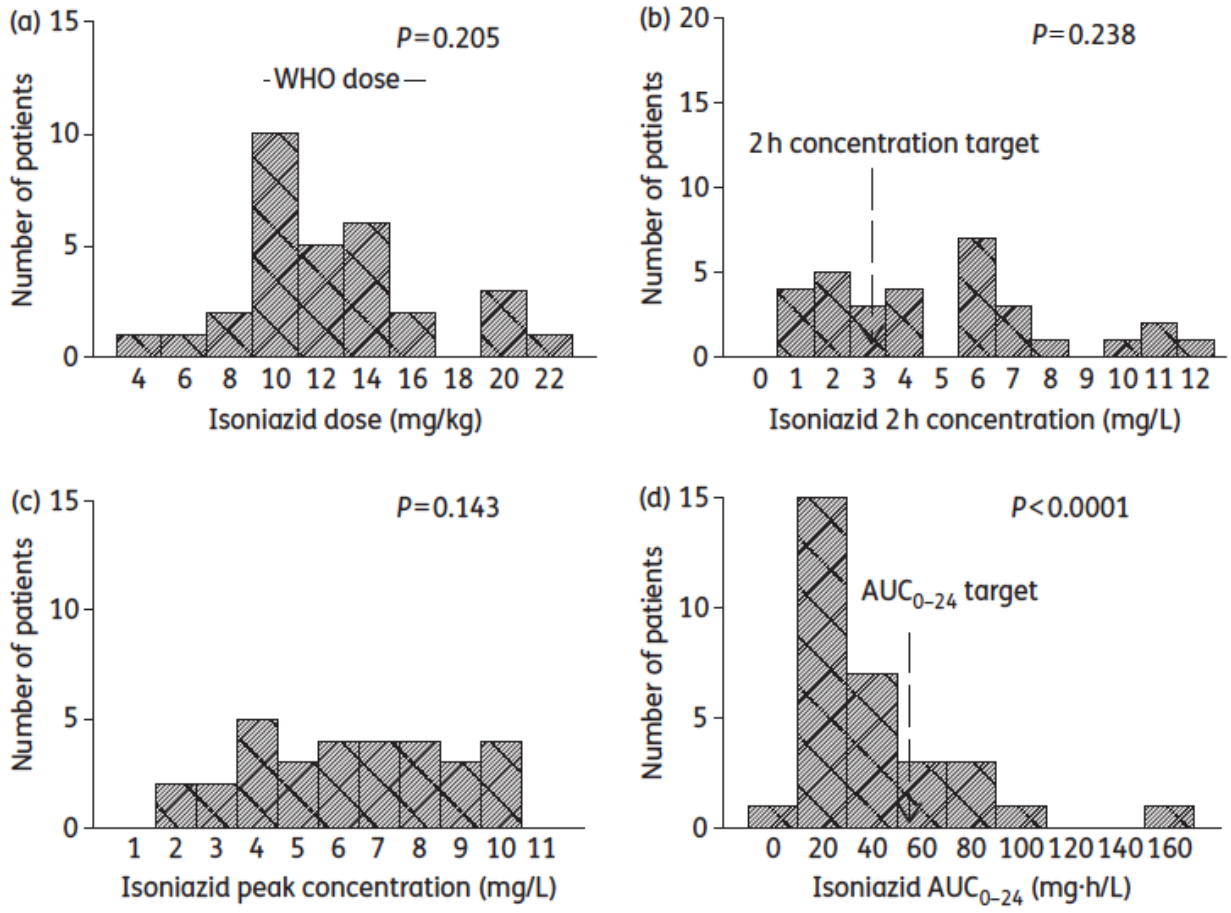
	Isoniazid mean (±SD)	Rifampin mean (±SD)	Pyrazinamide mean (±SD)	Ethambutol mean (±SD)
Total clearance (L*hr^{-1})	12.2 (7.58)	12.7 (9.9)	2.7 (0.9)	20.6 (6.1)
Volume of central compartment (L)	56.4 (7.5)	85.1 (42.7)	24.0 (2.3)	135 (21.2)
Absorption constant (hr^{-1})	10.0 (4.4)	14.8 (7.4)	0.9 (0.3)	1.7 (2.1)
Inter-compartmental clearance (L*hr-1)	10.2 (2.8)	NA	NA	
Volume of peripheral compartment (L)	1.0 (0.3)	NA	NA	

SD=standard deviation; NA= not applicable for a one compartment model

Table 3. Concentrations achieved in South African children treated with World Health Organization recommended dosing

	Pyrazinamide (n=31)	Rifampin (n=31)	Isoniazid (n=31)	Ethambutol (n=13)
Observed 2-hour concentration				
Median (range); mg/L	22.55 (2.35-66.35)	2.87 (0.05-14.18)	4.50 (0.82-11.80)	1.10 (0.02-3.07)
Children with concentration below reference (%)	14 (45%)	29 (94%)	11 (35%)	11 (85%)
Pharmacokinetic model derived peak				
Median (range); mg/L	22.51 (11.18-47.17)	3.47 (0.56-10.20)	6.05 (1.83-10.28)	1.44 (0.62-6.28)
Pharmacokinetic model derived AUC_{0-24}				
Median(range); mg*h/L	233.9 (110.10-525.7)	21.2 (1.8-67.3)	28.7 (6.8-153.0)	10.8 (4.7-22.7)
Children with AUC_{0-24} below optimal (%)	25 (81%)	7 (23%)	23 (74%)	-

Figure 1. Isoniazid doses and concentrations achieved in 31 South African children.



The p-values are for the D'Agostino and Pearson omnibus normality test, whereby a p≥0.05 is significant for a normal distribution, while lower p-vales indicate a non-normal distribution.

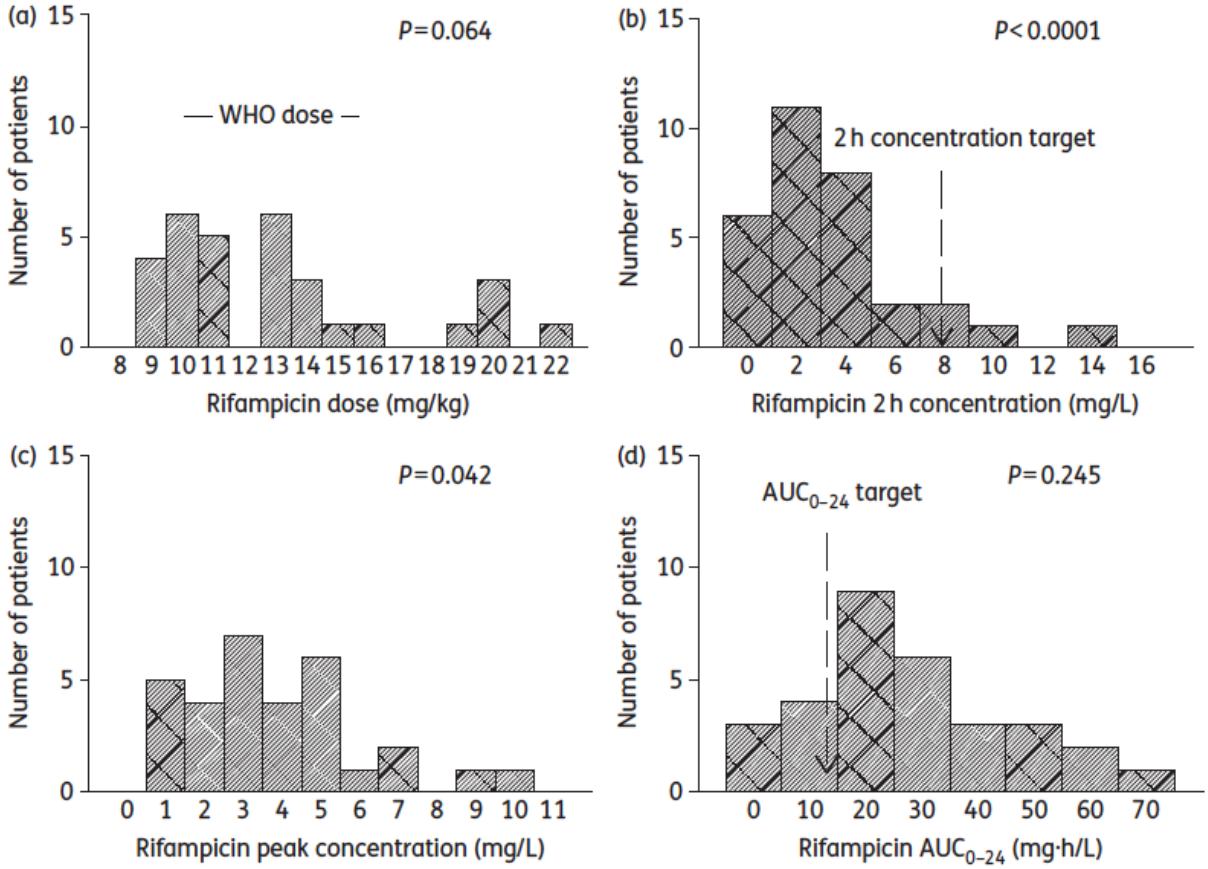
a. Isoniazid doses administered are compared to the WHO recommended doses. The majority of patients were dosed according to the WHO recommended doses. The ratio between the highest and lowest dose administered was 4.7.

b. Isoniazid 2hr concentration had a lowest-to-highest ratio of 14.7, several fold higher than that imposed by dose. The true time to peak concentration (T_{max}) had a median and range of 1.75 (0.33-3.67) hrs, indicating a wide variability in T_{max}, and that the 2hr concentration rarely coincided with isoniazid peak concentration in the children.

c. Isoniazid peak concentration differed from the 2-hour concentration in distribution. The ratio between the lowest and highest peak was 5.6, closely tracking the doses administered.

d. The isoniazid AUC_{0-24} achieved varied 22.5-fold between the lowest and highest, much higher than the ratio for doses.

Figure 2. Rifampin doses and concentrations achieved in 31 South African children.



A p≥0.05 is significant for a normal distribution.

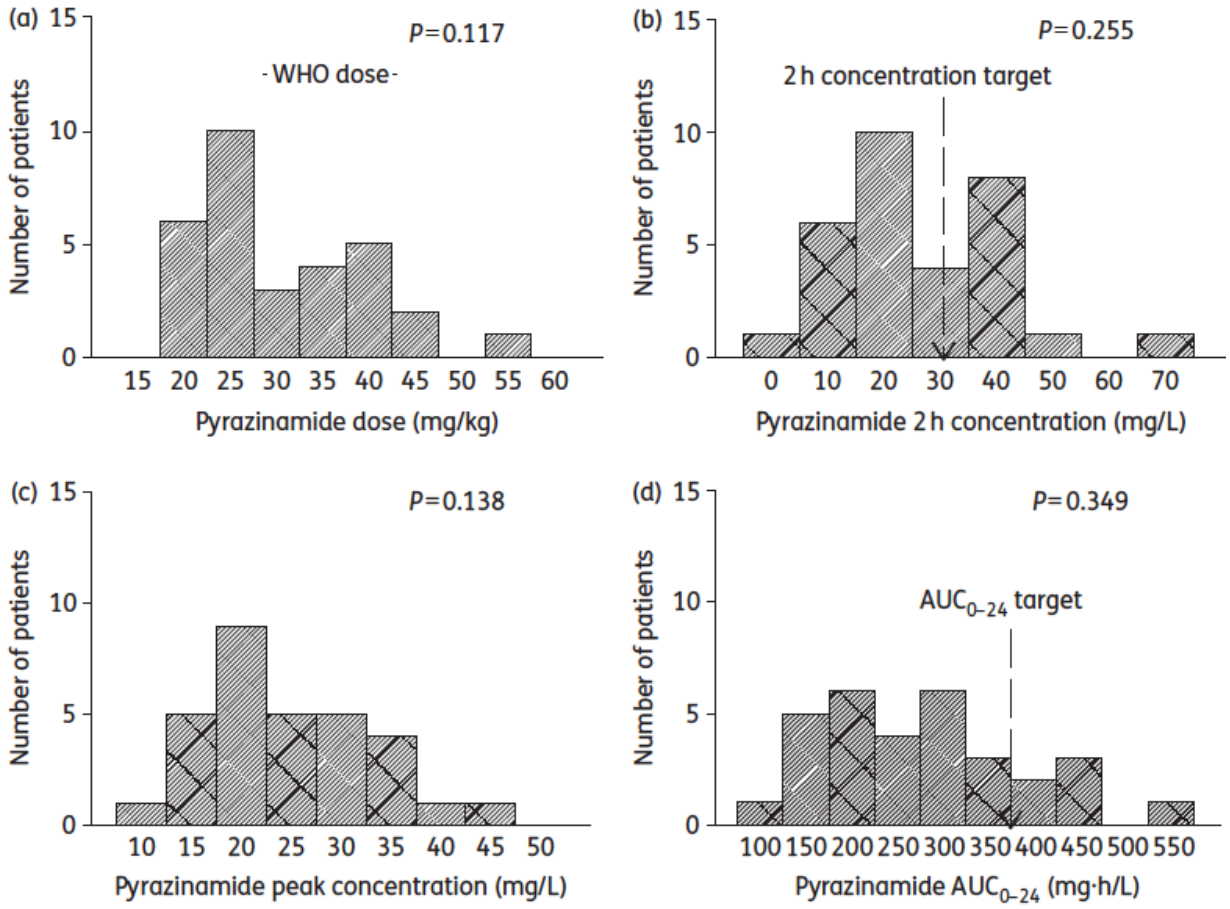
a. The ratio of the lowest-to-highest rifampin dose was 2.5-fold, and mostly was either in the WHO recommended doses or even higher.

b. Rifampin 2hr concentration had a lowest-to-highest ratio of 308.3, more than 100-fold higher than ratio for the dose.

c. Rifampin peak concentration had a lowest-to-highest ratio of 18.2, and thus lower than the variability of the rifampin 2-hr concentration.

d. Rifampin AUC_{0-24} achieved varied 37.8-fold between the lowest and highest. More than10-fold due to dose.

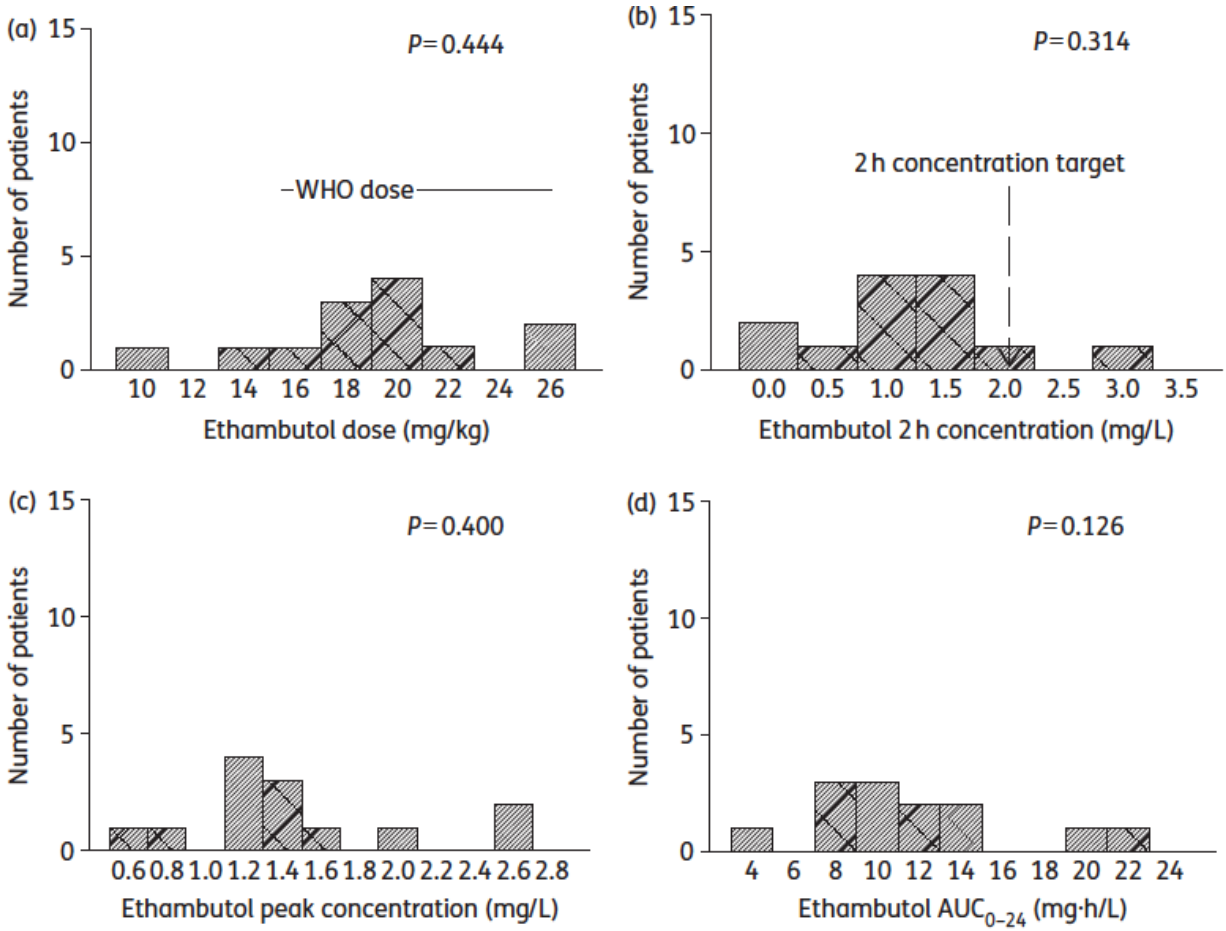
Figure 3. Pyrazinamide doses and concentrations achieved in 31 South African children.



A p≥0.05 is significant for a normal distribution.

a. The ratio of the lowest-to-highest pyrazinamide dose was 2.8-fold, with a large proportion below the WHO recommended doses.
b. Pyrazinamide 2hr concentration had a lowest-to-highest ratio of 28.3, several fold higher than ratio for the dose.
c. Pyrazinamide peak concentration had a lowest-to-highest ratio of 4.2, and thus lower than the variability of the pyrazinamide 2-hr concentration.
d. Pyrazinamide AUC_{0-24} achieved varied 4.8-fold between the lowest and highest concentrations.

Figure 4. Ethambutol doses and concentrations achieved in 13 South African children.



A p≥0.05 is significant for a normal distribution.
a. The ratio of the lowest-to-highest ethambutol dose was 2.5-fold, with a large proportion below the WHO recommended doses.
b. Ethambutol 2hr concentration had a lowest-to-highest ratio of 139.1, >100-fold higher than ratio for the dose.
c. Ethambutol peak concentration had a lowest-to-highest ratio of 4.3, and thus lower than the variability of the ethambutol 2-hr concentration.

d. Ethambutol AUC_{0-24} achieved varied 4.9-fold between the lowest and highest concentrations.

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Chapter 5: Conclusion

Pharmacokinetic analysis is a vital part of drug development and optimization of therapeutic regimens. In this thesis, the role of systemic and local quantitation of tenofovir after local (rectal dosing) was presented. In addition, utility of quantitative pharmacokinetic analysis to evaluate adequacy of therapeutic regimens currently recommended for clinical practice was evaluated in the setting of first-line anti-TB medications in a pediatric population where data-driven dosing regimen recommendations are lacking.

1. Rectal Microbicide Development for Pre-exposure Prophylaxis

1.1. Our Findings

In CHARM-02, we found that all three candidate TFV 1% gel products were safe for one-time dosing. There were more minor gastro-intestinal AEs during VF administration period. CHARM-02 also mainly focused on PK and evaluation of colonic distribution of three candidate TFV 1% gel products: We found that the hyperosmolal product, VF, has the greatest distribution (highest Dmax), and also, as inferred from the permeability and plasma TFV concentration data, is associated with the greatest permeability to small molecules. There was high (86%) co-localization of the drug and virus surrogate and no

difference in co-localization of the drug and virus surrogate between these three study gels. We also noted that all the differences seen in the colonic distribution between VF and the other two study gels becomes insignificant when dosing was followed by simulated coitus compared to no differences seen when dosing is not followed by coitus. We believe PK with and without coitus are both relevant as some doses will not be followed by sex and some will, hence a combination of the sex/no sex differences may combine over time to generate persistent differences between products in a given individual.

CHARM-01 looked at safety and multi-compartment PK after multiple (7-doses) doses of the RF and RGVF TFV 1 % gel, and single dose of VF gel. Daily dosing of the RF and RGVF gels for one week was found to be safe without any histologic evidence of tissue damage (data not included in the thesis, but presented in the published manuscript)[59]. The VF product was associated with increased minor AEs, significant, since this was with only a single dose compared to 7 doses for the other two products. Looking at the PK of TFV in plasma, rectal tissue, rectal and vaginal fluid and its active moiety, TFV-DP, in colonic MMC, PBMC, and rectal tissue, there were no statistically significant differences. Of note, the median concentration of TFV-DP in colonic MMC was numerically higher (but not quite statistically significant at the 5% level) for RF as compared to RGVF with median mucosal mononuclear cell (MMC) TFV-DP RF/RGVF ratio of 1.8 (interquartile range 0.4-3.9) (p=0.07)]. Hence, despite the almost two-fold difference in osmolality

between RF and RGVF, there were no major safety or PK differences between these two study formulations.

Based on these two studies, we conclude that both RF and RGVF are safe to be advanced to phase II trials. Currently, MTN-017, a phase II clinical trial with RGVF is underway.

1.2. Challenges and Future Directions

One major challenge for HIV microbicdes is identifying the target TFV concentration that is protective from HIV infection in the pertinent compartment. This would require bridging studies that can relate concentrations from easily obtainable compartments, such as plasma, to concentrations that are less accessible, but more pertinent to assessing HIV infection, such as colonic/vaginal mucosa. In the CAPRISA 004 study, where women received coitally-depended TFV 1% gel, TFV concentration of at or above 1000ng/ml in cervico-vaginal fluid was associated with increased protection from HIV. In the iPrEx study, where MSM were provided with oral emtricitabine/tenofovir as PrEP, a TFV-DP concentration of 16 fmol/million cells in PBMC was associated with increased protection[17,75]. In order to make sense of these concentrations, we need bridging studies that simultaneously measure concentrations of TFV and its active moiety in several compartments simultaneously, such as the CHARM-01 study, which looked at multi-compartment PK after rectal dosing of TFV 1% gel. Once we have several bridging

studies, we then will be able to integrate the information with pharmacodynamic data obtained from clinical trials to identify the target concentration for prevention of HIV infection. Such a "connect-the-dots" exercise using data across studies is, however, subject to potential drift in PK across studies due to methodologic differences (analytical methods or population differences) that require attention to subtle differences and caution in application. It is most preferable to collect PK samples at all anatomic sites in at least, some subjects in seroconversion outcome studies to minimize known and unknown variables.

Another challenge is our quantification of the distribution of the study gels. In CHARM-02, we used SPECT/CT along with radiolabeled drug and virus surrogate to look at the distribution of the three study products. The main limitation of this method is that it only assesses the distribution of the study gels in the colonic lumen, but does not evaluate mucosal distribution, *per se*. One potential method that may improve our ability to deliver more active drug to the mucosa is use of nanoparticles. With the advances in nanotechnology, novel mucus-penetrating nanoparticles are being investigated for mucosal drug delivery. For instance, Ensign, *et al*., demonstrated vaginal delivery of a mucus-penetrating acyclovir formulation in mice which rapidly penetrates cervicobaginal mucus nearly as quickly as water[76]. In addition, nanoformulations coupled with imaging modalities such as fluorescence particle tracking technology will optimize our ability to quantify drug delivery via mucosal surfaces more effectively by providing far higher resolution of drug on the mucosal

surface (100-1,000 micron resolution) than provided by SPECT/CT (3.54 millimeter resolution scale). Using these microscopic imaging methods, these mucus penetrating nanoparticles are seen to provide a highly uniform distribution across the mucosal surface, in contrast to conventional particles which move more slowly through mucus and rest on the mucosal surface in very heterogenous patterns. Especially in a setting of coitally dependent microbicide use, both speed of mucosal contact and uniform mucosal surface distribution are preferred. Drug diffusion across the mucosal surface and into the tissue may well establish homogeneity of distribution in time, but coitally dependent dosing may not allow this time delay. To this end, anti-retroviral nanoforumulations for mucosal use, such as TFV, are being actively investigated[77,78].

Last, the success of any PrEP product will depend on acceptability and adherence to PrEP regimen. While there were multiple variables affecting adherence in the VOICE and Fem-PrEP studies, product acceptability was one of the relevant factors in its infrequent use and contributed to poor adherence, which hindered the studies' ability to evaluate efficacy of the TFV as PrEP[34,35]. This also highlights the need for objective assessment of drug adherence such as using drug concentration as a reflection of drug use. Though this data was captured in the VOICE trial, TFV concentration in plasma samples was only collected quarterly and was not assayed until the study was complete. Having a more frequent drug level assessment available in real time may aid in providing objective measure of adherence to enable targeted adherence interventions. Additional novel ways to assess adherence are critically needed.

2. TB Therapy in Children

2.1. Our Findings

The results of the PHATISA study shows that even with the revised higher doses of first-line anti-TB drugs, many children are achieving below target concentration for all of the drugs; this was particularly striking for rifampin, one of the backbones of TB therapy, with only 3 out of 31 children achieving the target concentration. Given RIF's ability to develop resistance with just one single mutation and the fact that children will be treated with only INH and RIF for the continuation phase, it would mean that children are receiving INH monotherapy during the continuation phase. For populations such as Kwa Zulu Natal, an epicenter for TB and HIV, and areas with high INH resistance, this may result in major treatment failures and possibly contribute to the already growing problem of mulit-, extensive, and totally-drug resistant TB.

2.2. Challenges and Future Directions

The paucity of data regarding optimal TB therapy in this population results in suboptimal therapeutic regimens in this vulnerable population. Given the major differences in immunology, bacterial burden and distribution of TB disease in adults and children, we need well-designed, well-powered pediatric studies that relate the PK of these first line drugs to clinical outcome (pharmacodynamics).

Since the revised WHO pediatric dosing guideline came to effect in 2011, there have only been two studies, other than the PHATISA study, that evaluated the new dosing regimen[79,80]. Hence, well-designed, well-powered studies that relate PK of these first line drugs to clinical outcome (PD) are urgently needed.

More importantly, as novel therapies for TB are being planned, we need children to be included in the studies so that more accurate dosing regimens, which take into account factors specific to the pediatric population, can be evaluated. Regulations such as the Food and Drug Administration Safety and Innovation Act (FDASIA) of 2012, which require pharmaceutical companies to include pediatric studies prior to submission for new drug application (NDA), may facilitate more inclusion of children in drug development.

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Education and Training

PhD	**Bloomberg School of Public Health, Johns Hopkins University**
Sept '12 – present	Graduate Training Program in Clinical Investigation
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July '10 – July '13	Pediatric Infectious Disease
Residency	**Seattle Children's Hospital and Regional Medical Center**
July '05 – July '08	University of Washington
MD	**Johns Hopkins University School of Medicine, Baltimore, MD**
Sept. '01 – May '05	
BS Biochemistry	**University of Maryland at College Park, MD**
Aug '98 – May '01	
Pre-medicine	**Montgomery College, Takoma Park, MD**
Aug '96 – May '98	

Clinical Experience (International)

Aug '08 – July '09	Pediatric AIDS corps physician, Kilimanjaro Christian Medical Center, Moshi, Tanzania Provided care in HIV clinic, supervised as a consultant in the pediatric Ward, mentored healthcare providers in outreach posts. Funded by Baylor International Pediatric AIDS Initiative,

Baylor
College of Medicine

July ‘09 – May ‘10 Pediatric AIDS corps physician, Gondar University Hospital, Ethiopia

Provided care in pediatric outpatient urgent care and TB clinic; taught didactic and clinical skills to medical students.

Funded by Baylor International Pediatric AIDS Initiative, Baylor College of Medicine

Research Experience

July ’13 – present Co-investigator, Johns Hopkins University School of Medicine, Baltimore, MD.
Part of the Microbicide Trial Network (MTN) Combination HIV Antiretroviral Rectal Microbicide studies: A phase I clinical study evaluating different formulations of tenofovir 1% gel as rectal microbicide

May ’12 – Sept.’13 Co-investigator, Johns Hopkins University School of Medicine, Baltimore, MD
Carried out a prospective cohort pharmacokinetic study to determine the adequacy of the revised WHO dosage recommendations for 1st line anti-TB medications in South African children.

July ’02 – Sept ’02 Research Assistant, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD
Participated in NIH-funded clinical trial of Nevirapine for prevention of mother to child transmission of HIV in Addis Ababa, Ethiopia

June ’00 – July ’01 Research Assistant, Walter Reed Army Institute of Research, Silver Spring, MD
Worked with a team focused on developing a vaccine against *N. meningitides* serogroup B using the NspA membrane protein

Awards

July ’12 - Present Baurenschmidt Fellowship Research Award

Eudowood Board, Johns Hopkins University

Publications

Research paper

Hiruy H, Fuchs E, Marzinke M, Bakshi R et al. A Phase 1 Randomized, Blinded Comparison of the Pharmacokinetics and Colonic Distribution of Three Candidate Rectal Microbicide Formulations of Tenofovir 1% Gel with Simulated Unprotected sex (CHARM-02). *AIDS Res Hum Retroviruses.* 2015;31(11): 1098-108

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Book chapters

Jain SK, **Hiruy H**. 2011. Extra-pulmonary tuberculosis. In: *Clinical Decision Support in Infectious Diseases*, Karchmer A, Southwick F, Wenzel R and Frank I (eds). Decision Support in Medicine, LLC.

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Presentations at National and International Conferences

2012 **Hiruy H**, Mbowane C, Adamson J. et al. Anti-Tuberculosis Agents in Children. *NIAID/IDSA ID Research Careers Meeting*, 2012, Bethesda, Maryland

2013 **Hiruy H**, Mbowane C, Adamson J. et al. Pharmacokinetics of Anti-Tuberculosis Drugs in South African Children.*7th International AIDS Society Conference on HIV Pathogenesis and Treatment and Prevention*, Kuala Lampur, Malaysia

Reviewer

Journal of Acquired Immunodeficiency Syndrome-2012
Pediatric Infectious Disease Journal-2011

Leadership Experience

July '10 – July '15	Pediatric Diversity Council Board Member Johns Hopkins University School of Medicine, Baltimore, MD
August '01 – May '05	Student National Medical Association Johns Hopkins University School of Medicine, Baltimore, MD
August '98 – May '01	Ethiopian Student Association International University of Maryland at College Park, MD

Certification and Licensure

American Board of Pediatric Infectious Disease	2013-present
American Board of Pediatrics	2010-present
Basic Life Support Certificate	2013-present
Pediatric Advanced Life Support Certificate	2013-present
Maryland State Medical license	2013-present
Washington State Medical license	2010-present

Academic Society Memberships

American Academy of Pediatrics
Infectious Disease Society of America
Pediatric Infectious Disease Society
National Institute of Health Women of Color Research Network
Alpha Lambda Delta National Honor Society
Golden Key National Honor Society

Language Skills

Proficient in English and Amharic (national language of Ethiopia)
Basic skill in Swahili