

**OPTIMIZING ANTIRETROVIRAL THERAPY  
IN CHILDREN AND ADOLESCENTS  
WITH HIV INFECTION**

Optimaliseren van antiretrovirale therapie  
bij kinderen en adolescenten met HIV-infectie

The studies which comprise this thesis and the professional development of the author were enabled by support from the U.S. Department of Health and Human Services, the National Institutes of Health and the Public Health Service. These included grants from the National Center for Research Resources (K12 RR017613 and M01 RR020359) and the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development (5K23 HD0060452 and 5U10 HD45993).

ISBN 978-90-8559-069-9

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Cover: Maternidad (Maternity) also known as Desemparats. Pablo Picasso, 1903.  
Picasso Museum, Barcelona

Lay-out: Margo Terlouw-Willebrand, Nieuwerkerk aan den IJssel

Printed by: Optima Grafische Communicatie, Rotterdam (optima@ogc.nl)

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**THESIS**

to obtain the degree of Doctor from the Erasmus University Rotterdam  
by command of the rector magnificus Prof.dr. H.G. Schmidt  
and in accordance with the decision of the Doctorate Board

The public defence will be held on  
Tuesday, 21 December 2010  
at 15.30 hours

by

**Natella Yurievna Rakhmanina**

born in  
Moscow, Russia



## **DOCTORAL COMMITTEE**

Promotors: Prof.dr. D. Tibboel  
Prof.dr. R. de Groot

Other members: Prof.dr. C. Boucher  
Prof.dr. T. van Gelder  
Dr. D. Burger

To my mother Natella L.

To John and John

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# 1

# chapter

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**INTRODUCTION**

HIV infection became a newly recognized disease in the mid 1980s. High morbidity and mortality associated with it prompted the urgent development of new therapeutic agents and combination therapies. Throughout the next 20 years the hopes for cure have risen and fallen, and the vaccine research has failed to reach the evasive target of HIV prevention. It is the development and optimization of antiretroviral therapy (ART) that formed the roadmap for the universal control and prevention of existing and new infections, respectively. As with any new therapeutic modality, the data guiding the dosing, efficacy and safety of antiretroviral (ARV) drugs for children have lagged substantially behind as compared to the information available for adults. An advanced PubMed search for "HIV pharmacokinetics", restricted to age groups of infant, child or adolescent, and all categories of original clinical studies (e.g. excluding reviews and other publication types) resulted in 326 citations.<sup>1</sup> Removing any age restriction increased the number of citations to 1404. While one can argue about the absolute numbers and whether classification is appropriate for all publications in the PubMed database, the ratio is nonetheless significant and not surprising, with over four times as many pharmacokinetic (PK) studies conducted in HIV-infected adults than in children.<sup>1</sup>

Since the early 1990s administration of prophylactic ART to pregnant women to assure prevention of the mother-to-child transmission (MTCT) has become the most efficient and cost-effective way to achieve global control of pediatric HIV infection. In the countries with consistent access to ART and universal prevention of MTCT the number of pediatric HIV infections has decreased dramatically: only 4,400 children living with HIV infection and fewer than 500 new infections in children younger than 15 years of age were estimated for the North American region in 2007.<sup>2</sup> In the last decade much progress has been made in the universal ART coverage of women during pregnancy and labor in resource-limited settings. Per World Health Organization (WHO) report, an estimated 45% of pregnant women living with HIV received ARV drugs to prevent transmission of HIV to their children in 2008.<sup>3,4</sup> However, nearly 1,200 new infections in children continue to occur daily (with more than 90% of them in the developing world), leading to a stunning estimate of more than 2 million children younger than 15 years of age living with HIV worldwide.<sup>3,4</sup>

In addition to the younger cohort of perinatally infected children, over 1 million new infections are estimated in adolescents aged 15 - 24 years of age.<sup>4</sup> The seroprevalence of HIV among adolescent females remains high (> 50%), reflecting the burden of the epidemic borne by girls and young women and the potential for the continued MTCT.<sup>4</sup> With an estimation of more than 3 million of children and adolescents living with HIV infection, the delivery of ART to the world's pediatric population is of crucial importance. In recent years significant progress has been made in scaling up the access to the ART among children and adolescents. In 2009 355,000 children worldwide received ART, up from nearly 276,000 in 2008, and up from 127,300 in 2006.<sup>2,5</sup> Given the growing number of HIV-infected children and adolescents with access to treatment, a better understanding of the therapeutic targets of ARV drugs in pediatric HIV infection is urgently needed.

The introduction of combination ART into pediatric care has led to a dramatic decrease in HIV mortality (> 80% - 90%) and morbidity among HIV-infected children.<sup>6,7-10</sup> The main strategy of HIV therapy in the pediatric patient focuses on early initiation of ART regimen in order to achieve maximal suppression of viral replication, prevent disease progression, preserve immunologic function, and reduce the emergence of viral resistance, while allowing normal growth and development of the child. Since HIV infection represents an infectious inflammatory disease generated by a retrovirus, the pathogenesis and the general virologic and immunologic principles underlying the use of ART are similar for all HIV-infected people. However, the important etiological, physiological, psychological and social differences between children and adults create unique considerations for HIV-infected pediatric patients, and require a population specific approach to ART.

As mentioned above, the majority of pediatric HIV infection is acquired through perinatal transmission of the virus from an HIV-infected mother. Important differences in the clinical and virologic manifestations of perinatal HIV infection secondary to the occurrence of primary infection in growing, immunologically immature infants create unique treatment initiation criteria that are significantly different from those guiding the start of ART in HIV-infected older children and adults. Secondly, the perinatal exposure to the ART administered to mother *in utero*, intrapartum and/or postpartum may present additional challenges to the emergence of resistance and drug associated toxicities in this vulnerable population. Third, the immunologic parameters (CD4+ cell count) of HIV disease are closely related to the child's growth and create age specific parameters for the evaluation of pharmacodynamic (PD) response to ART in pediatric HIV disease. Most importantly, significant changes in the PK parameters of ARV drugs are caused by the continuing development and maturation of organ systems involved in drug absorption, distribution, metabolism, and elimination. Moreover, the extensive physiologic changes during a child's maturation do not occur with precisely predictable timing or magnitude, and developmental rather than chronological age needs to be considered.<sup>11,12</sup> All of these factors strongly oppose the notion of "one-size fits all" dosing for pediatric HIV-infected patients, and mandate a rational consideration of the optimal dose for every child.<sup>1</sup>

Several pediatric HIV research networks (Pediatric AIDS Clinical Trials Group (PACTG)/International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT), Collaborative HIV Pediatric Study (CHIPS), Pediatric European Network for Treatment of AIDS (PENTA), the HIV Netherlands Australia Thailand Research Collaboration (HIVNAT), and Adolescent Medicine Trials Network (ATN)) have addressed issues of developmental PK/PD of ART in HIV-infected pediatric patients. While many of these studies have contributed to the development of the HIV pediatric treatment guidelines in the United States,<sup>13</sup> Europe<sup>14</sup> and WHO,<sup>3</sup> significant gaps of knowledge of ART still exist.

The overwhelming majority of the ARV PK studies in children have been designed to find the dose which provides ARV exposures (e.g. peak and trough plasma concentrations, and area under the concentration-time curve) similar to those already known to be safe and effective in adults.<sup>1</sup> Very limited information on the potential differences in the PD of ART in children is available. The effect of puberty on ARV drug disposition has not been investigated to date. While considered useful in pediatric populations by the European and US guidelines, the published ARV therapeutic drug monitoring (TDM) experience in children, with active dose management in response to measured drug concentrations, is almost non-existent.<sup>1</sup> Little is known about the relationships between ARV PK and ART associated toxicity, and only a few publications have addressed the long-term consequences of pediatric ART exposure. Equally, a limited number of pediatric studies have addressed the association of pharmacogenetics (PG) of ART with toxicities and outcome, and questions about developmental differences in drug metabolizing and drug transporter gene expression remain to be answered.

With changes in patients' age and maturity, ART evolves from the meticulous, twice-daily dosing of milliliters of liquid preparations to co-formulated tablet preparations. Simultaneously, pediatric adherence barriers evolve during child's growth from spitting and vomiting of liquid preparations in infancy to the rejection of ART and increased peer pressure during puberty. Although some strategies for adherence interventions and simplified ART regimens in pediatric HIV management have been proposed, many of them have not been rigorously evaluated to date. Moreover, developmental and psychosocial factors affecting ART adherence in children and adolescents from different cultural and ethnic regions of the world are not well described and need to be evaluated. Culturally sensitive and acceptable adherence interventions need to be developed.

As the access to ART is increasing among the pediatric population worldwide, the potential role of malnutrition and concomitant infections in altering the PK of ARV drugs in pediatric patients needs to be recognized. The co-morbidity with certain infections such as tuberculosis leading to concomitant therapy with potential for significant drug-drug interaction with ARV drugs pose an additional challenge for the choice of proper ART in these settings.<sup>15</sup> Decreased weight and delayed growth among children with HIV infection represent common health challenges in resource-limited settings.<sup>16-18</sup> The co-morbidity with gastrointestinal illnesses, hepatitis, malaria and tuberculosis may lead to altered absorption of ARV drugs. The loss of fat and lean body mass, and associated metabolic and endocrine abnormalities have the potential to influence the volume of distribution and the total body clearance of ARV drugs, particularly of lipophilic drugs, such as protease inhibitors.<sup>19-20</sup> Currently, the data about regional differences in children's growth are very limited, and accurate growth curves and sexual maturation staging of HIV-infected children and adolescents of various ethnic and racial backgrounds urgently need to be developed.

Finally, multi-drug ARV resistance represents a significant challenge for ART management in the resource-rich settings. Despite the low burden of pediatric HIV infection in developed countries, the most treatment-experienced children and adolescents presently reside in North America and Europe. From 1997 to 2001, among a cohort of more than 2000 U.S. HIV-infected children, the proportion receiving three or more sequential triple-ART regimens increased from 4 to 17%, while the durability of triple-therapy regimens declined from 13 months for the first to 7 months from the first to third regimen.<sup>6</sup> In a European cohort of 654 perinatally infected children from the United Kingdom and Ireland, 52% and 12% of the 166 patients with resistance assays had dual- or triple class resistance mutations, respectively.<sup>21</sup> With the increasing availability of ART to the pediatric population in developing countries, the number of treatment-experienced children will continue to rise globally. The availability of the newer potent classes of ARV drugs in those regions, however, is quite remote due to their high cost and manufacturing restrictions. The optimization of the ARV dose needs to be evaluated to address the need of successful virologic suppression in children with viral resistance.

As new ART studies in children and adolescents become available, the pediatric ARV treatment guidelines continue to evolve throughout the world with the most recent WHO and US pediatric guidelines updates released in the summer of 2010 and European guidelines updated in the fall of 2009. The goal of this thesis is to contribute to the ongoing work by many excellent researchers and practitioners in an effort to achieve universal success in the management of pediatric HIV infection.

## **AIM AND OUTLINE OF THIS THESIS**

**The overall aim of this thesis** is to increase efficacy of ART in HIV-infected children and adolescents, leading to improved outcomes and decreased drug toxicity in pediatric HIV-infected children worldwide.

Given the universal need for the optimization of antiretroviral therapy in the pediatric population and the limited number of pediatric studies available as compared to adults a series of studies were performed to evaluate the developmental PG/PK/PD changes, the safety and toxicity of ART in children and the role of adherence and dose optimization.

**PART I** focuses on the developmental changes from infancy to adolescence and its impact on ARV drug disposition and efficacy. Chapter 2 reviews the history and current progress in pediatric pharmacological research and pediatric drug development. Chapter 3 focuses on the developmental changes during puberty and provides justification for an individualized therapeutic approach in HIV-infected adolescents. The current challenges in the availability, formulations and regimens of pediatric ART are discussed in Chapter 4.

**PART II** is dedicated to the analysis of PK and PD of several ARV drugs in children and adolescents. Chapter 5 reports the data on a study conducted in collaboration with the University of Nijmegen, the Netherlands, on the PK of nucleoside reverse transcriptase inhibitor lamivudine in HIV-infected children. The development of a population PK model for the most widely used protease inhibitor lopinavir in pediatric practice is presented in Chapter 6. Most importantly, Chapter 6 addresses the potential impact of suboptimal therapeutic concentrations of lopinavir predicted by the model in treatment-experienced HIV-infected children. In continuation of the research on lopinavir, the effects of the drug metabolizing (*CYP3A5*) and drug-transporter (*MDR1* and *SLCO1B1*) polymorphisms on the PK/PD of lopinavir/ritonavir therapy in children is investigated in the research presented in Chapter 7.

**PART III** consists of two studies focusing on the role of TDM in the management of ART of pediatric HIV infection. Chapter 8 investigates the non-invasive evaluation of salivary concentrations of non-nucleoside reverse transcriptase inhibitor nevirapine as an alternative to the plasma sampling in pediatric HIV-infected children. The role of TDM in optimization of pharmacotherapy of HIV infection in adolescent patients is further discussed in Chapter 9.

The individualized approach to the management of the HIV infection in children and adolescents is addressed in **PART IV**. Chapter 10 investigates the role of the pharmacy refill mechanism in the adherence to ART and virologic and immunologic outcomes of HIV infection in adolescents. The development and application of biomarkers for the early identification of HIV-associated nephropathy are presented in Chapter 11. Finally, the general discussion in Chapter 12 focuses on the current gaps in the knowledge of antiretroviral therapy in pediatric patients and outlines the roadmap of future studies to develop an optimized approach to ART in HIV-infected infants, children and adolescents worldwide.

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# I P A R T

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**CURRENT  
CHALLENGES IN  
PEDIATRIC  
HIV THERAPY**



# 2 chapter

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## **PHARMACOLOGICAL RESEARCH IN PEDIATRICS: FROM NEONATES TO ADOLESCENTS**

Rakhmanina NY, van den Anker JN.

*Adv Drug Deliv Rev. 2006;58:4-14.*

**ABSTRACT**

The data guiding the dosing, efficacy and safety of medicines for children have lagged substantially as compared to the information available for adults. As a consequence, pediatricians faced with the prospect of confining their practice to medicines with adequate information have frequently resorted to prescribing medicines for unapproved uses (different dose, frequency, age group, route, indication or formulation). Although a long time in coming, the past decade, have witnessed a new era in drug development for children - an era that is still in its infancy, but which is currently showing signs of maturation. This review will give some of the history and current progress in pharmacological research and pediatric drug development.

## HISTORY

The history of drug therapy is replete with examples of adverse reactions to drugs in neonates, infants, children and adolescents. In 1937, 107 people - primarily children - died after taking elixir of sulphanilamide to treat streptococcal infection. Sulphanilamide was not very water soluble, but a chemist at Massengill Co. found that it dissolved well in diethylene glycol (more commonly known as antifreeze), which is now known to be highly toxic. In 1956, Andersen *et al.* at Columbia reported an excessive mortality rate and an increased incidence of kernicterus among premature babies receiving a sulfonamide antibiotic compared with those receiving chlortetracycline.<sup>1</sup> Then, in 1959, Sutherland described a syndrome of cardiovascular collapse in three newborns receiving high doses of chloramphenicol for presumed infections.<sup>2</sup> More recently, the therapeutic misadventures experienced by low birth weight infants exposed to a parenteral vitamin E formulation<sup>3</sup> and the "gaspig syndrome" by infants who received excessive amount of benzyl alcohol<sup>4</sup> all serve to underscore the generally held perception that newborn infants are more likely to experience adverse reactions to drugs. More recently, all therapeutic issues surrounding the retinoic acid embryopathy and maternal antidepressant drug use have refocused attention on the effects of drugs on the fetus and newborn.<sup>5</sup>

As a result of these experiences, pediatricians have become extremely conservative in their use of drug therapy. Although this conservative approach has permitted the fulfillment of the physician's oath to "do no harm," it also has prevented the adoption of newer therapeutic modalities and their adaptation to neonatal patients.

A more specific approach to pediatric therapeutics that will improve the safe use of medicines in this population requires a thorough understanding of human developmental biology as well as insights regarding the dynamic ontogeny of the processes of drug absorption, drug distribution, drug metabolism, and drug excretion. In addition, there must be a rigorous appreciation of the developmental aspects of drug-receptor interactions, including the ontogenetic changes in receptor number, receptor affinity, receptor-effector coupling, and receptor modulation and regulation.

## OFF-LABEL PRESCRIBING

At intervals since 1968, surveys have documented that only a minority of medicines receive labelling for pediatric use. Even fewer receive labelling for use by neonates and infants.<sup>6</sup> In the period 1973 - 1997, the percentage of approved drugs that contained no labelling information for children remained fairly stable at 71 - 81%.<sup>6</sup> Of the 33 new molecular entities (NMEs) approved in 1997, 27 had potential for pediatric use, but only nine contained any pediatric labelling information.

With so few medicines containing adequate labelling information to guide their use, off-label prescribing became an accepted practice. Off-label prescribing includes the

use of drugs for unapproved indications, or a different age group, dosage, frequency or route of administration. It also includes the administration of extemporaneous formulations with untested bioavailability and stability. Although off-label use for indications beyond the initial labelled indication is seen frequently in adults, there is at least basic information about pharmacokinetics and safety available, and efficacy information is assured for at least one indication. However, for children, off-label prescribing means that there is no labelling information at all. The widespread acceptance of this practice helps perpetuate the lack of information to guide practice, and the disconnection between the label and practice makes the package insert irrelevant for patients and pediatricians as a guide for usage.

### **EXTENT OF OFF-LABEL PRESCRIBING**

Numerous surveys have documented extensive off-label drug use in hospitalized pediatric patients. Conroy *et al.*<sup>7</sup> surveyed off-label use in general pediatric wards in five European countries. Sixty-seven percent of all patients received at least one off-label prescription. 't Jong *et al.*<sup>8</sup> reported an even higher incidence of off-label use in a prospective 19-week survey at a general hospital in The Netherlands, at which 92% of children received at least one off-label prescription. Sicker and younger children, who are more vulnerable, are more likely to receive off-label medicines than less acutely ill children. In specialized children's hospitals in The Netherlands it was found that 66% of all prescriptions were off-label.<sup>9</sup> On general medical wards 59% of prescriptions were off-label compared with 76% in neonatal intensive care units.<sup>9</sup> The high likelihood of neonates receiving an off-label drug was confirmed in an Australian neonatal intensive care unit in which 93% of extremely low birth weight infants received at least one off-label medicine.<sup>10</sup>

In summary, off-label use of medicines has become unfortunately a necessary and accepted part of pediatric medical practice. Children receive ineffective doses of potentially effective medicines and are harmed by medicines that might not be effective for their conditions. The continued off-label use of medicines impedes the ability to organize and learn from experience, and exposes many more children to risk than would be the case in a carefully controlled and monitored clinical trial.

### **NEW DEVELOPMENTS**

During the past two decades, tremendous strides have been made to tailor therapies for the needs of children. As our knowledge of normal growth and development has increased, so has our recognition that developmental changes profoundly affect the responses to medications and produce a need for age-dependent dose requirements.<sup>11</sup>

Prior to the clinical integration of developmental pharmacology into therapeutic decision making, numerous approaches (e.g., Young's Rule, Clark's Rule) for

determining pediatric drug doses were recommended. These approaches vary with some using discrete age points and others using allometric principles that generally assume predictable, linear relationships between mass (e.g., cell mass, body weight) and/or body surface area between infants, children, adolescents and adults. However, as human growth is not a linear process and age-associated changes in body composition and organ function are dynamic and can be discordant during the first decade of life, simplified dosing approaches are not adequate for individualizing drug doses across the span of childhood.<sup>12</sup> As a result, these old "dosing equations" have been abandoned and in most instances, replaced by simple "normalization" of drug dose as a function of either body weight (mg/kg) or body surface area (mg/m<sup>2</sup>). While such guidelines are generally adequate for the initiation of therapy, they may not be sufficient for age-based individualization of continued (e.g., chronic) treatment where dramatic developmental differences in pharmacokinetics and/or pharmacodynamics (i.e., the determinants of appropriate dosing regimen) may occur. Thus, the provision of safe and effective drug therapy for pediatric patients requires a fundamental understanding and integration of the role of development on drug disposition and action.

## **ABSORPTION**

For therapeutic agents administered by extravascular routes, the process of absorption is reflected by the ability of a drug to overcome chemical, physical, mechanical and biological barriers. Developmental differences in the physiologic composition and function of these barriers can alter the rate and/or extent of drug absorption. While factors influencing drug absorption are multifactorial in nature (e.g., physical, chemical and biological), developmental changes in the absorptive surfaces (e.g., gastrointestinal tract, skin, pulmonary tree, etc.) can be determinants of bioavailability.

The oral route is the principal means for drug administration to pediatric patients. Changes in intra-luminal pH can directly impact both drug stability and degree of ionization, thus influencing the relative amount of drug available for absorption. Additionally, the ability to solubilize and subsequently absorb lipophilic drugs can be influenced by age-dependent changes in biliary function. Immature conjugation and/or transport of bile salts into the intestinal lumen results in low intraduodenal levels despite blood levels that exceed those of adults.<sup>13,14</sup>

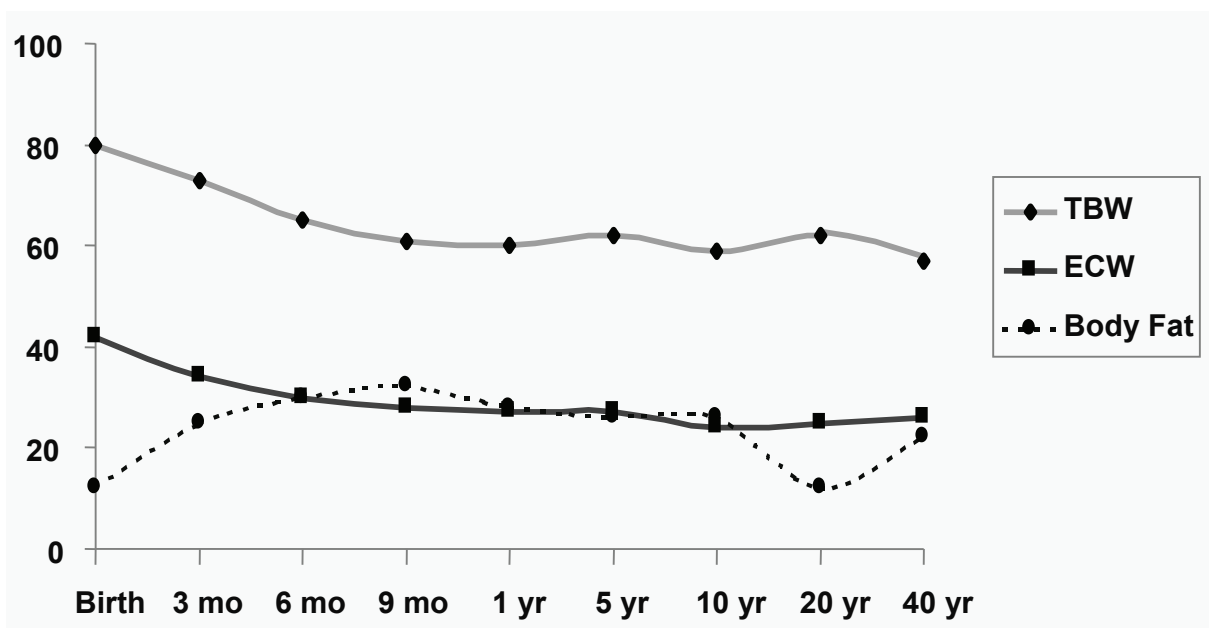
Gastric emptying and intestinal motility are primary determinants of the rate at which drugs are presented to and dispersed along the mucosal surface of the small intestine. Unfortunately, few studies have systematically evaluated the effect of developmental changes in gastric emptying and intestinal motility on drug absorption in infants and children. Generally, the rate at which most drugs are absorbed is generally slower and thus, the time to achieve maximum plasma concentrations is prolonged in neonates and young infants relative to older infants and children.

Despite their incomplete characterization,<sup>15</sup> developmental differences in the activity of intestinal drug metabolizing enzymes and efflux transporters have the potential to markedly alter drug bioavailability. Notably, data on developmental expression of the efflux transporter P-glycoprotein (MDR1) in humans is absent.

## DISTRIBUTION

Age-dependent changes in body composition (Figure 1)<sup>16</sup> alter the physiologic "spaces" into which a drug may distribute. Larger extracellular and total body water spaces in neonates and young infants, coupled with adipose stores that have a higher water/lipid ratio than in adults, produce lower plasma concentrations for drugs that distribute into these respective compartments when administered in a weight-based fashion. For lipophilic drugs that associate primarily with tissue, the influence of age on altering the apparent volume of distribution is not as readily apparent.

Changes in the composition and amount of circulating plasma proteins (e.g., albumin,  $\alpha_1$  acid-glycoprotein) can also influence the distribution of highly bound drugs. A reduction in the quantity of total plasma proteins (including albumin) in the neonate and young infant increases the free fraction of drug, thereby influencing the availability of the active moiety. Other factors associated with development and/or disease such as variability in regional blood flow, organ perfusion, permeability of cell membranes, changes in acid-base balance and cardiac output can also influence drug binding and/or distribution.



**Figure 1** Changes occurring in body fat and water stores along the continuum of age.<sup>16</sup> TBW – total body water; ECW – extracellular water.



## DRUG METABOLISM

Cardiovascular collapse associated with the "gray baby syndrome" in newborns treated with chloramphenicol is often cited as a clinically significant consequence of developmental deficiencies in drug metabolizing enzyme activities.<sup>17,18</sup> Multiple examples exist of clinically important developmental changes in drug biotransformation sufficient to produce the need for age-appropriate dose regimen selection in neonates and young infants (e.g., methylxanthines, nafcillin, 3rd generation cephalosporins, captopril, morphine). As reflected by recent reviews, distinct patterns of isoform-specific developmental changes in drug biotransformation are apparent for many Phase I (primarily oxidation) and Phase II (conjugation) drug metabolizing enzymes.<sup>19,20</sup>

## PHASE I ENZYMES

Development has a profound effect on the expression of Phase I enzymes such as the cytochromes P450 (CYPs). CYP3A7 is the predominant CYP isoform expressed in fetal liver where it may play a fetoprotective role by detoxifying dehydroepiandrosterone sulfate and potentially teratogenic retinoic acid derivatives.<sup>21</sup> CYP3A7 expression peaks shortly after birth and then declines rapidly to levels that are undetectable in most adults.<sup>22</sup> Distinct patterns of isoform-specific developmental CYP expression have been observed postnatally. Within hours of birth, CYP2E1 activity surges<sup>23</sup> followed closely by the onset of CYP2D6 expression.<sup>24</sup> CYP3A4 and CYP2C (CYP2C9 and 2C19) activities appear during the first week of life<sup>22,25</sup> whereas CYP1A2 is the last hepatic CYP to be acquired with significant expression being delayed until 1 - 3 months of life.<sup>26</sup>

Insight into the ontogeny of drug metabolism also can be derived from pharmacokinetic studies of drugs metabolized by specific CYP isoforms. Midazolam plasma clearance, which primarily reflects hepatic CYP3A4/5 activity after intravenous administration,<sup>27</sup> increases approximately 5-fold (1.2 to 9 ml/min/kg) over the first 3 months of life.<sup>28</sup> Carbamazepine plasma clearance, also largely dependent upon CYP3A4,<sup>29</sup> is greater in children relative to adults,<sup>30,31</sup> thereby necessitating higher weight-adjusted (i.e., mg/kg) doses of the drug to produce therapeutic plasma concentrations.

CYP2C9 and to a lesser extent, CYP2C19, are primarily responsible for phenytoin biotransformation.<sup>32</sup> Phenytoin apparent half life is prolonged (~ 75 h) in preterm infants but decreases to ~ 20 h in term infants less than one week postnatal age and to ~ 8 h after two weeks of age.<sup>33</sup> Saturable phenytoin metabolism does not appear until approximately 10 days of postnatal age, demonstrating the developmental acquisition of CYP2C9 activity.

Caffeine and theophylline are the most common CYP1A2 substrates used in pediatrics. Caffeine elimination *in vivo* mirrors that observed *in vitro* with full

3-demethylation activity (mediated by CYP1A2) observed by approximately four months of age.<sup>34</sup> Formation of CYP1A2-dependent theophylline metabolites reaches adult levels by approximately 4 - 5 months of postnatal age<sup>35</sup> and in older infants and young children, theophylline plasma clearance generally exceeds adult values.<sup>36</sup> Furthermore, caffeine 3-demethylation in adolescent females appears to decline to adult levels at Tanner stage II relative to males where it occurs at stages IV/V,<sup>37</sup> thus demonstrating an apparent sex difference in the ontogeny of CYP1A2.

## **PHASE II ENZYMES**

Phase II reactions generally result in pharmacological inactivation or detoxification by conjugating xenobiotics with small molecules such as UDP-glucuronic acid, glutathion, or acetyl coenzyme A. These reactions are catalyzed by a variety of enzymes, the activity of which appears to be associated with development. While the impact of ontogeny on Phase II enzymes has not been investigated to the same extent as for Phase I enzymes, a conceptual understanding of their known developmental profiles is important to understanding the acquisition of metabolic competence in the neonate and its potential therapeutic implications.<sup>20</sup>

### **UDP glucuronosyltransferase (UGT)**

The mammalian UGTs are responsible for the glucuronidation of hundreds of hydrophobic endogenous (e.g., bilirubin, bile acids, thyroxine, and steroids) and exogenous (e.g., morphine, acetaminophen, and NSAIDs) xenobiotics. Additionally, UGTs detoxify an extensive group of potentially carcinogenic or teratogenic xenobiotics that enter the body as components of the diet or as air-borne pollutants.<sup>38</sup>

The UGTs comprise a super family of enzymes that are subdivided into families based on sequence homology. In neonatology, serious clinical consequences of allelic variants in UGT isoforms are well known. Over 30 different perturbations of the UGT1A gene results in absent or reduced enzyme activity that can lead to lethal hyperbilirubinemia (Crigler-Najjar syndrome).<sup>39</sup> Mutations of the promoter region of the UGT1 gene have been associated with a milder form of congenital unconjugated hyperbilirubinemia (Gilbert's syndrome).<sup>40</sup> From a pharmacologic perspective, failure to recognize the impact of development on the glucuronidation of chloramphenicol and its implications for age-associated individualization of therapy lead to the historical catastrophe of the Grey Baby Syndrome.<sup>2</sup>

Low levels of immunoreactive UGT protein are found early in gestation in liver, spleen, and kidney. Relatively greater reactivity has been observed in red blood cells as early as 32 days post-conception.<sup>41,42</sup> Functional UGT activity, as assessed by bilirubin conjugation, is nearly undetectable in fetal liver with activity increasing immediately after birth in parallel with an increase in protein. The increase in

catalytic activity is not dependent upon gestational age,<sup>43-45</sup> thereby suggesting that postnatal events are essential for the expression and/or activation of the UGT gene.

The most complete information on the development of UGT activity as it relates to drug metabolism comes from studies of morphine glucuronidation by UGT2B7. Morphine is metabolized by UGT2B7 to morphine-6-glucuronide and morphine-3-glucuronide.<sup>46</sup> *In vitro* studies have shown that liver microsomes from fetuses aged 15 - 27 weeks glucuronidate morphine at a rate that is only 10 - 20% of that seen in adult microsomes.<sup>47,48</sup> No correlation was seen between gestational age and the rate of glucuronidation, again suggesting that birth related events play a role in the activation of this enzyme. In *in vivo* studies, the mean plasma clearance of morphine was 5-fold lower in premature infants (gestational age 24 - 37 weeks) when compared to children 1 to 16 years of age. Generally, morphine clearance reaches adult levels between 2 and 6 months, but may take as long as 30 months.<sup>49</sup> Since glucuronidation is the primary metabolic pathway for morphine metabolism, the impact of development on clearance appears to accurately reflect the ontogeny of UGT2B7.

The impact of development on the activity of other UGT isoforms can also be indirectly assessed using pharmacologic substrates for this enzyme. Acetaminophen is metabolized by UGT1A6, and to a lesser extent by UGT1A9.<sup>50</sup> Acetaminophen glucuronidation, as reflected by urinary metabolite data, appears negligible in the fetus, is low at birth and appears to approach full competence by 9 to 12 months of postnatal life.<sup>51</sup> Zidovudine plasma clearance in neonates less than 2 weeks of age is only half that of infants 2 weeks and older.<sup>52</sup> Collectively, the pharmacokinetic data for the aforementioned UGT substrates illustrate that the developmental profile for acquisition of enzyme activity is isoform and substrate specific.

### ***N*-acetyltransferase (NAT)**

Recognized initially for its ability to metabolize isoniazid, *N*-acetyltransferase (NAT) was one of the first drug metabolizing enzymes shown to possess genetic polymorphisms that dramatically affected the pharmacokinetics of its substrate.<sup>53</sup> Genetic variation, primarily in the NAT2 gene locus, is responsible for the division of the general population into rapid and slow acetylators. Subsequently, several studies have shown an association between polymorphisms at both the NAT1 and NAT2 loci and susceptibility to disease or drug reactions.<sup>54-56</sup>

Relatively sparse information on the ontogeny of human NAT1 and NAT2 is available. NAT1 mRNA has been identified in fetal, newborn, and adult skeletal muscle with relatively greater immunostaining in fetal tissue.<sup>57</sup> Using caffeine as a probe for NAT activity, a discordant relationship between genotype and phenotype in infants has been observed. Little difference in NAT activity is observed between premature infants and those 1 - 19 months of age.<sup>58</sup> In subsequent studies, the cumulative frequency of fast acetylators was shown to increase with age, reaching a plateau at around 4 years.<sup>59,60</sup> Thus, there are a relatively higher proportion of

phenotypically slow acetylators in children less than 2 years. A maturational process appears to occur, independent of genotype, that is not complete until about 4 years of age. This maturational process may reflect developmentally regulated expression of the NAT gene, or may arise secondarily from the maturation of pharmacokinetic parameters other than metabolism (i.e., absorption, distribution, elimination).<sup>61</sup>

Altered NAT expression and activity with age may have important clinical implications for drug dosing and toxicity and possibly, in the initiation and progression of certain disease processes. A higher incidence of adverse effects from co-trimoxazole has been demonstrated in children with a slow acetylator genotype.<sup>56</sup> A significantly greater portion of children with immunoglobulin E-mediated food allergies are homozygous slow acetylators when compared to normal children. In these studies, no homozygous fast acetylators were observed in the group of individuals with severe food allergies.<sup>55</sup> Collectively, our best evidence would indicate that all infants, particularly neonates, are essentially slow acetylators and would be subject to the consequences of decreased drug metabolism and increased disease susceptibilities inherent to this phenotype.

In contrast to the cytochromes P450, what is currently known concerning the developmental pattern for the acquisition of phase II enzyme activity is far less precise. As illustrated in Table 1, the presence or absence of activity for many phase II drug metabolizing enzymes has been investigated in the fetus, neonate and infant. While pharmacokinetic data for many drugs that are substrates for these enzymes (e.g., zidovudine, acetaminophen) suggests a pattern for the acquisition of function during postnatal life, the apparent absence of enzyme/isoform-specific quantitative data precludes reliable prediction of drug clearance based upon age-associated differences in enzyme activity at the present time.

## **RENAL ELIMINATION**

Maturation of renal function is a dynamic process that begins early during fetal organogenesis and is complete by early childhood. The developmental increase in glomerular filtration rate (GFR) involves active nephrogenesis, a process that begins at 9 weeks and is complete by 36 weeks of gestation, followed by postnatal changes in renal and intrarenal blood flow.<sup>62</sup> Following birth, the GFR is approximately 2 - 4 ml/min/1.73 m<sup>2</sup> in term neonates and as low as 0.6 - 0.8 ml/min/1.73 m<sup>2</sup> in preterm neonates. GFR increases rapidly during the first two weeks of life followed by a steady rise until adult values are reached by 8 - 12 months.<sup>63,64</sup> Similarly, tubular secretory pathways are immature at birth and gain adult capacity during the first year of life. Collectively, these changes dramatically alter the plasma clearance of compounds with extensive renal elimination and thus, provide a major determinant for age-appropriate dose regimen selection. Pharmacokinetic studies of drugs primarily excreted by glomerular filtration such as ceftazidime<sup>64</sup> and famotidine<sup>65</sup> have demonstrated significant correlations between plasma drug

clearance and normal, expected maturational changes in renal function. For example, tobramycin is eliminated predominantly by glomerular filtration, necessitating dosing intervals of 36 to 48 h in preterm and 24 h in term newborns.<sup>66</sup> Failure to account for the ontogeny of renal function and adjust aminoglycoside dosing regimens accordingly can result in exposure to potentially toxic serum concentrations.<sup>67</sup> Also, concomitant medications (e.g., betamethasone, indomethacin) may alter the normal pattern of renal maturation in the neonate.<sup>68</sup> Thus, for drugs with extensive renal elimination, both maturational and treatment associated changes in kidney function must be considered and used to individualize treatment regimens in an age-appropriate fashion.

## CONCLUSIONS

The advances in pediatric clinical pharmacology during the past decade reside with an enhanced understanding of the influence of growth and development on drug disposition and action. As this moves forward, it is essential that the ultimate goal be kept clearly in sight. Specifically, providing infants and children with safe and effective drug therapy made possible by including them in the process of development of medications essential to ensure their health.

The goal of rational drug therapy in neonates, infants, children and adolescents resides with the ability to individualize it based upon known developmental differences which impact drug disposition and action. The clinical challenge in this is accounting for the variability in all of the contravening factors that influence pharmacokinetics (e.g., polymorphic expression of drug metabolizing enzymes and efflux transporters, effect of disease and/or concomitant therapy on enzyme/transporter activity, altered drug delivery associated with drug formulations that may not be suited for accurate drug dosing) and pharmacodynamics (e.g., polymorphic expression of cellular transporters and/or receptors) between patients of a given age and developmental stage. Despite the fact that most therapeutic drugs are polyfunctional substrates for drug metabolizing enzymes (both Phase I and II) and that their activity (as well as transporters and potentially, drug receptors) is polygenically determined, knowledge of substrate specificity for a given drug and the patterns of expression for activity which are associated with age (development) can afford the clinician with an element of prediction, be it applied to projecting either the dose of a drug and/or its pharmacologic consequences in the pediatric patient.

## ACKNOWLEDGEMENTS

Supported in part by grants K12 RR017613 (N.Y.R.), K24 RR019729 (J.N.A.), National Center for Research Resources and 1U10HD45993 (J.N.A.), National Institute of Child Health and Development, Bethesda, MD.

**Table 1** Summary of developmental dependent changes in drug disposition

<b>Physiological System</b>	<b>Age-related trends</b>	<b>Pharmacokinetic implications</b>	<b>Clinical implications</b>
Gastrointestinal tract	<i>Neonates and young infants:</i> reduced and irregular peristalsis with prolonged gastric emptying time. <i>Neonates:</i> greater intragastric pH (> 4) relative to infants. <i>Infants:</i> enhanced lower GI motility.	Slower rate of drug absorption (e.g., increased T <sub>max</sub> ) without compensatory compromise in the extent of bioavailability. Reduced retention of suppository formulations.	Potential delay in the onset of drug action following oral administration. Potential for reduced extent of bioavailability from rectally administered drugs.
Integument	<i>Neonates and young infants:</i> thinner statum corneum (neonates only), greater cutaneous perfusion, enhanced hydration and greater ratio of total BSA to body mass.	Enhanced rate and extent of percutaneous drug absorption. Greater relative exposure of topically applied drugs as compared to adults.	Enhanced percutaneous bioavailability and potential for toxicity. Need to reduce amount of drugs applied to skin.
Body compartments	<i>Neonates and infants:</i> decreased fat, decreased muscle mass, increased extracellular and total body water spaces.	Increased apparent volume of distribution for drugs distributed to body water spaces and reduced apparent volume of distribution for drugs that bind to muscle and/or fat.	Requirement of higher weight-normalized (i.e., mg/kg) drug doses to achieve therapeutic plasma drug concentrations.
Plasma protein binding	<i>Neonates:</i> decreased concentrations of albumin and $\alpha_1$ -acid glycoprotein with reduced binding affinity for albumin bound weak acids.	Increased unbound concentrations for highly protein-bound drugs with increased apparent volume of distribution and potential for toxicity if the amount of free drug increases in the body.	For highly bound (i.e., > 70%) drugs, need to adjust dose to maintain plasma levels near the low end of the recommended "therapeutic range".
Drug metabolizing enzyme (DME) activity	<i>Neonates and young infants:</i> immature isoforms of cytochrome P450 and phase II enzymes with discordant patterns of developmental expression. <i>Children 1 - 6 years:</i> apparent increased activity for selected DMEs over adult normal values. <i>Adolescents:</i> attainment of adult activity after puberty.	<i>Neonates and young infants:</i> decreased plasma drug clearance early in life with an increase in apparent elimination half life. <i>Children 1 - 6 years:</i> increased plasma drug clearance (i.e., reduced elimination half life) for specific pharmacologic substrates of DMEs.	<i>Neonates and young infants:</i> increased drug dosing intervals and/or reduced maintenance doses. <i>Children 1 - 6 years:</i> for selected drugs, need to increase dose and/or shorten dose interval in comparison to usual adult dose.
Renal drug excretion	<i>Neonates and young infants:</i> decreased glomerular filtration rates (first 6 months) and active tubular secretion (first 12 months) with adult values attained by 24 months.	<i>Neonates and young infants:</i> accumulation of renally excreted drugs and/or active metabolites with reduced plasma clearance and increased elimination half life, greatest during first 3 months of life.	<i>Neonates and young infants:</i> increased drug dosing intervals and/or reduced maintenance doses during the first 3 months of life.

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## chapter

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### **PERSONALIZED THERAPEUTICS: HIV TREATMENT IN ADOLESCENTS**

Rakhmanina NY, Capparelli EV, van den Anker JN.

*Clin Pharmacol Ther.* 2008;84:734-740.

**ABSTRACT**

Adolescents infected with human immunodeficiency virus (HIV) represent a heterogeneous group of pubertal children and young adults. Antiretroviral therapy (ART) in adolescents is complex and depends on multiple factors. The continued use of higher (weight- or surface-based) pediatric doses can result in potentially toxic drug exposure, whereas early introduction of lower adult doses can lead to the development of drug resistance and virologic failure. The physiological and psychosocial changes during puberty create strong grounds for an individualized therapeutic approach in HIV-infected adolescents.

Despite significant progress in the prevention of mother-to-child transmission of human immunodeficiency virus (HIV) infection, it was estimated that there were 2.3 million children under the age of 15 years with HIV infection in 2006.<sup>1</sup> Among those, ~ 780,000 (600,000 - 1,000,000) were estimated to be in need of antiretroviral therapy (ART). In the United States and other developed countries, an increasing number of children with perinatally acquired HIV infection are now surviving into adolescence and adulthood. In addition, large numbers of American teenagers continue to acquire HIV infection through sexual contact and intravenous drug use; youths between 13 and 24 years of age account for 15% of the 40,000 new HIV cases per year.<sup>2</sup> Based on the strengthening global response to the problem of HIV/AIDS in recent years, the number of adolescents receiving ART worldwide is rapidly increasing.<sup>3</sup> Given the growing number of HIV-infected youth with access to ART, a better understanding of the disposition of antiretroviral (ARV) drugs during puberty is urgently needed. The selection of ART dosages for patients in the pubertal stages of maximal growth is left to the provider's discretion, and individual differences in the progression to sexual maturation are often not provided for. The recently published report by the Committee on Pediatric AIDS, dealing with increasing access to ARV drugs for pediatric patients, identifies the lack of appropriate dosing of ARV drugs in adolescents as one of the barriers to providing ART to HIV-infected children and youth globally.<sup>3</sup> The report underlines that, even with the availability of appropriate ART, the pharmacokinetic (PK) data may be insufficient to appropriately guide the dosing in adolescent patients, who may need higher than "maximum adult dose" to ensure adequate drug exposure.<sup>3,4</sup> Consequently, adolescents are identified as a separate study cohort when investigating the PK and pharmacodynamics (PD) of ARV agents in children, and the need for phase II and III studies in this cohort is underscored.

Several pediatric HIV research networks (Pediatric AIDS Clinical Trials Group (PACTG)/International Maternal Pediatric Adolescent AIDS Clinical Trials, Collaborative HIV Pediatric Study, Pediatric European Network for Treatment of AIDS, the HIV Netherlands Australia Thailand Research Collaboration, and Adolescent Medicine Trials Network) have addressed issues of developmental PK/PD of ART in HIV-infected pediatric patients. However, very few studies have investigated the PK/PD of ARV agents in adolescents and young adults. The PK of abacavir were reported from the PACTG protocols P1018 and P1052;<sup>5,6</sup> lamivudine and zidovudine data (including data on phosphorylation to the intracellular triphosphate metabolites) were collected in PACTG studies P1012 and P1052;<sup>7</sup> lopinavir/ritonavir and saquinavir were studied in PACTG protocol P1038;<sup>8</sup> and atazanavir was studied in PACTG protocol 1020A and in Adolescent Medicine Trials Network studies along with tenofovir.<sup>5,9</sup> The PACTG protocol P1038 and the author's study in children with experience of ARV suggested that the use of high doses of lopinavir/ritonavir might be required for salvage HIV therapy in adolescent patients.<sup>8,10</sup> The Adolescent Medicine Trials Network study by Kiser *et al.* evaluated the PK of the combined administration of atazanavir/ritonavir and tenofovir in young adults with HIV infection.<sup>9</sup> A higher level of tenofovir exposure was expected

on the basis of data from healthy volunteers, but this was not seen in HIV-infected subjects, most likely because of a faster tenofovir clearance, as apparent in the higher creatinine clearances observed in this age group.<sup>9</sup> None of the available studies in adolescents has investigated the effects of pubertal changes on the required dosage in ART, and information on failed ART or increased drug resistance in HIV-infected adolescents is very limited. No studies have addressed the important issue of the long-term effects of ART exposure during puberty. Such possible effects include ART-associated hyperlipidemia and a risk of developing cardiovascular disease. Finally, adolescent patients are frequently exposed to antidepressants, hormonal contraceptives, anabolic steroids, alcohol, and illicit drugs. No studies are available to date on the effects of psychotropic drugs, substance abuse, and exogenous sex hormones on ART drug disposition and efficacy in adolescence, although there is an abundance of pharmacological data pertaining to the adult population.

HIV-infected adolescents represent a heterogeneous group of pubertal children and young adults at different stages of psychosocial development, with vertically and horizontally transmitted HIV infection, varying demographic and socioeconomic statuses, and diverse histories of sexual and substance abuse. The choice of ART regimen must balance many of these factors in addition to the selection of the correct dosage of ARV drugs. Clinicians are faced with the dilemma of choosing a dosing regimen that falls somewhere between pediatric (weight- and surface-based) and adult (fixed-dose) guidelines. The World Health Organization guidelines and the US guidelines for ART in adolescent patients include adolescents within both categories: "pediatric" (infants and children) and "adults and adolescents." The World Health Organization defines the "adolescent" cohort as children aged between 10 and 19 years; the US guidelines use 12 years of age as the cutoff for transition to adolescence.<sup>5,11</sup> (Table 1) The appropriate dosing of ARV medications in adolescents is complex and depends on multiple factors, including developmental changes throughout puberty. Although current pediatric HIV treatment guidelines for older children recommend considering Tanner stages of puberty when prescribing ART, they do not provide data justifying those recommendations. For children in early puberty (Tanner stages I and II), the US guidelines recommended using pediatric schedules, whereas for adolescents in their growth spurt (Tanner stages III and IV), both adult and pediatric dosing schedules are recommended.<sup>5</sup> The World Health Organization pediatric guidelines extend pediatric dosing to Tanner stage III and recommend adult dosing in adolescents in Tanner stages IV and V.<sup>11</sup> Both guidelines recommend considering issues such as toxicity, adherence, and virologic and immunologic parameters when determining the timing of transition from pediatric to adult doses. The continued use of higher (weight- or surface-based) pediatric doses during adolescence can result in increased and potentially toxic drug exposure, whereas early introduction of lower adult doses can lead to suboptimal therapeutic exposure and development of drug resistance and subsequent virologic failure.

Few studies have addressed the effect of HIV infection and ART on puberty, and these were conducted in adolescents with perinatally acquired HIV infection in the era of limited therapeutic choices.<sup>12,13</sup> The study by De Martino *et al.* reported a delay in the onset of puberty in Caucasian HIV-infected children independent of clinical or immunological status.<sup>12</sup> A much larger prospective cohort study from the United States, carried out by the PACTG 219 Study Team among children of diverse ethnic and racial backgrounds, reported a direct association between immunosuppression and a delayed onset of puberty.<sup>13</sup> This study, however, simplified the classification of Tanner stages and did not record orchidometric data. This omission could lead to a potential misclassification of the Tanner stage. Recently, a much smaller longitudinal study of 10 HIV-infected children, involving a thorough puberty evaluation including serial quantitations of plasma growth hormones (GHs), gonadotropins, and sex steroids, has demonstrated that children with a good control of HIV infection showed growth and pubertal development within the physiological percentile for their age.<sup>14</sup> Further studies are necessary to understand the impact of perinatally and behaviorally acquired HIV infection and ART on the onset and progression of puberty.

Puberty is characterized by an increase in growth velocity, changes in body composition, and the appearance of striking somatic changes. The changes in body composition vary between the sexes, with a significant increase in lean body mass in boys and an accumulation of fat in girls.<sup>15,16</sup> Girls are generally a year or two more advanced in pubertal maturation than boys, and the African-American race has been associated with an earlier onset of menarche.<sup>16,17</sup> Peak height velocity in girls occurs before menarche (which in turn takes place ~ 2 - 2.5 years after the first signs of breast development), whereas significant changes in genital development usually precede peak height velocity in boys.<sup>18</sup> In each gender, it takes about 4.5 years (3 - 6 years) from the first appearance of secondary sex characteristics to adult body configuration. Significant hormonal changes occur during adrenarche just prior to the appearance of secondary sexual characteristics, and gonadotropins, sex steroids, and GHs show different secretory patterns during different pubertal stages in boys and girls. The increase in circulating GH and insulinlike GH (IGF-1) levels is seen in girls at Tanner stage II (Breast II), whereas in boys it is usually delayed until stages II to III (Genital II-III).<sup>19</sup> In boys, rapidly rising testosterone levels cause an increase in GH/IGF-1 secretion and growth velocity. In girls, mean plasma levels of estradiol have been shown to rise steadily throughout puberty, with wide individual fluctuations depending on the time relative to menarche and the ovulatory cycle.<sup>19</sup> A careful history and thorough physical examination by an experienced provider is required for accurately evaluating the onset and progress of puberty.

**Table 1** Recommended doses of antiretroviral medications in adolescent<sup>a</sup> patients according to WHO and US guidelines<sup>b</sup>

<b>Drug</b>	<b>WHO Guidelines</b>	<b>US Guidelines</b>
Abacavir (ABC, Ziagen <sup>®</sup> )	<p>Children &lt; 16 years or body weight &lt; 37.5 kg — 8 mg/kg/dose twice daily.</p> <p>Maximal dose — &gt; 16 years or ≥ 37.5 kg — 300 mg/dose twice daily.</p> <p>Once daily dosing is not yet approved in children but encouraging PK data is available.</p> <p>Adults — 300 mg/dose twice daily or 600 mg once daily.</p> <p>Combination ART:</p> <ul style="list-style-type: none"> <li>• 300 mg AZT/150 mg 3TC/300 mg ABC — 1 tablet/dose twice daily.</li> </ul>	<p>Pediatric: 8 mg/kg/dose (maximal dose, 300 mg) twice daily.</p> <p>Adolescent: There is limited ABC data for adolescents.</p> <p>Adolescent &gt; 16 years/adults: 300 mg/dose twice daily or 600 mg/dose once daily.</p> <p>Combination ART (adults):</p> <ul style="list-style-type: none"> <li>• Trizivir<sup>®</sup> (300 mg AZT/150 mg 3TC/300 mg ABC) — 1 tablet/dose twice daily.</li> <li>• Epzicom<sup>®</sup> (300 mg 3TC/600 mg ABC) — 1 tablet/dose once daily.</li> </ul>
Didanosine (dideoxinosine, ddI, Videx <sup>®</sup> )	<p>Children &lt; 13 years of age — 90 - 120 mg/m<sup>2</sup>/dose twice daily.</p> <p>Maximal dose — &gt; 16 years or body weight &gt; 60 kg - 200 mg/dose twice daily or 400 mg/dose once daily (enteric coated (EC)). Once daily dosing for chewable tablets is authorized in United Kingdom for children &gt; 6 years of age.</p> <p>Adults — body weight &gt; 60 kg — 400 mg once daily, body weight &lt; 60 kg — 250 mg/dose once daily.</p>	<p>Pediatric: 90 - 150 mg/m<sup>2</sup>/dose twice daily (average dose 120 mg/m<sup>2</sup>) or in <i>treatment-naïve</i> patients 3 - 21 years of age 240 mg/m<sup>2</sup>/dose once daily.</p> <p>Adolescent/adults: oral solution — body weight &gt; 60 kg — 200 mg/dose twice daily or 250 mg/dose, if co-administered with TDF. Body weight &lt; 60 kg — 125 mg/dose twice daily. The total daily dose may be administered once daily, twice daily dosing is preferred.</p>
Emtricitabine (FTC, Emtriva <sup>™</sup> )	<p>No dosing guidelines for children.</p> <p>Adults — 200 mg/dose once daily.</p>	<p>Pediatric: oral solution — 6 mg/kg/dose (maximal dose, 240 mg) once daily. Capsules (body weight &gt; 33 kg) — 200 mg/dose once daily.</p> <p>Adolescent &gt; 18 years/adults: oral solution — 240 mg/dose once daily, capsules — 200 mg/dose once daily.</p> <p>Combination ART (adults):</p> <ul style="list-style-type: none"> <li>• Truvada<sup>®</sup> (200 mg FTC/300 mg TDF) — 1 tablet/dose once daily.</li> <li>• Atripla<sup>™</sup> (200 mg FTC/300 mg TDF/600 mg EFV) — 1 tablet/dose once daily.</li> </ul>
Lamivudine (3TC, Epivir <sup>®</sup> , EpiVir HBV)	<p>Body weight &lt; 50 kg — 4 mg/kg/dose twice a day to a maximum of 150 mg/dose twice daily.</p> <p>Body weight &gt; 50 kg — 150 mg/dose twice daily.</p> <p>Adults — 150 mg/dose twice daily or 300 mg/dose once daily.</p> <p>Combination ART:</p> <ul style="list-style-type: none"> <li>• 300 mg AZT/150 mg 3TC — 1 tablet/dose twice daily.</li> <li>• 300 mg AZT/150 mg 3TC/300 mg ABC — 1 tablet/dose twice daily.</li> <li>• 30 mg d4T/150 mg 3TC/200 mg NVP — 1 tablet/dose twice daily.</li> </ul>	<p>Pediatric: 4 mg/kg/dose (maximal dose, 150 mg) twice daily.</p> <p>Adolescent ≥ 16 years/adults: body weight &gt; 50 kg — 150 mg/dose twice daily or 300 mg/dose once daily. Body weight &lt; 50 kg — 4 mg/kg/dose (maximal dose, 150 mg) twice daily.</p> <p>Combination ART (adolescents &gt; 12 years/adults):</p> <ul style="list-style-type: none"> <li>• Combivir<sup>®</sup> (300 mg AZT/150 mg 3TC) — 1 tablet/dose twice daily.</li> <li>• Trizivir<sup>®</sup> (300 mg AZT/150 mg 3TC/300 mg ABC) — 1 tablet/dose twice daily.</li> <li>• Epzicom<sup>®</sup> (300 mg 3TC/600 mg ABC) — 1 tablet/dose once daily.</li> </ul>
Stavudine (d4T, Zerit <sup>®</sup> )	<p>Body weight &lt; 30 kg — 1 mg/kg/dose twice daily.</p> <p>Body weight &gt; 30 kg — 30 mg/dose twice daily.</p> <p>Body weight &gt; 60 kg — 40 mg/dose twice daily (using 30 mg dosing</p>	<p>Pediatric: body weight &lt; 30 kg — 1 mg/kg/dose twice daily.</p> <p>Adolescent (body weight ≥ 30 kg)/adults: body weight 30 - &lt; 60 kg — 30 mg/dose twice a day.</p>



<p>leads to delay or reduction of toxicity, although limited data on efficacy is available).</p> <p>Adults — body weight &gt; 60 kg — 40 mg/dose twice daily, body weight &lt; 60 kg — 30 mg/dose twice daily.</p> <p>Combination ART:</p> <ul style="list-style-type: none"> <li>• 1 mg/kg/dose d4T plus 4 mg/kg/dose 3TC (oral solution) twice daily or 30 mg d4T/150 mg 3TC (1 tablet/dose) twice daily.</li> <li>• 30 mg d4T/150 mg 3TC/200 mg NVP — 1 tablet/dose twice daily.</li> </ul> <p>No dosing guidelines for children.</p> <p>Adults — 300 mg/dose once daily.</p>	<p>Body weight <math>\geq</math> 60 kg — 40 mg/dose twice daily.</p>
<p>Tenofovir (TDF, Viread<sup>®</sup>)</p>	<p>Pediatric: investigational dose — 210 mg/m<sup>2</sup>/dose (maximal dose, 300 mg) once daily.</p> <p>Adolescent &gt; 18 years/adults: 300 mg/dose once daily.</p> <p>Combination ART (adults):</p> <ul style="list-style-type: none"> <li>• Truvada<sup>®</sup> (200 mg FTC/300 mg TDF) — 1 tablet/dose once daily.</li> <li>• Atripla<sup>™</sup> (200 mg FTC/300 mg TDF/600 mg EFV) — 1 tablet/dose once daily.</li> </ul>
<p>Zidovudine (ZDV, AZT, Retrovir<sup>®</sup>)</p>	<p>Children — 180 - 240 mg/m<sup>2</sup>/dose (maximal dose, 300 mg) twice daily.</p> <p>Adults — 250 - 300 mg/dose twice daily.</p> <p>Combination ART:</p> <ul style="list-style-type: none"> <li>• 300 mg AZT/150 mg 3TC — 1 tablet/dose twice daily.</li> <li>• 300 mg AZT/150 mg 3TC/300 mg ABC (tablet) — 1 tablet/dose twice daily.</li> </ul>
<p>Efavirenz (EFV, DMP-266EFV, Sustiva<sup>™</sup>)</p>	<p>Body weight &lt; 40 kg — 19.5 mg/kg/dose (syrup) or 15 mg/kg/dose (capsule/tablet) once daily.</p> <p>Body weight &gt; 40 kg — 600 mg/dose once daily.</p> <p>Adults — 600 mg/dose once daily.</p>
	<p>Pediatric: 160 mg/m<sup>2</sup>/dose every 8 hours (three times daily) or 180 - 240 mg/m<sup>2</sup>/dose twice daily.</p> <p>Adolescent <math>\geq</math> 12 years/adults: 200 mg/dose three times daily or 300 mg/dose twice daily.</p> <p>Combination ART (adolescent/adults):</p> <ul style="list-style-type: none"> <li>• Combivir<sup>®</sup> (300 mg AZT/150 mg 3TC) — 1 tablet/dose once daily.</li> <li>• Trizivir<sup>®</sup> (300 mg AZT/150 mg 3TC/300 mg ABC) — 1 tablet/dose once daily.</li> </ul> <p>Pediatric: body weight &lt; 40 kg (20 - &lt; 25 kg — 300 mg; 25 - &lt; 32.5 kg — 350 mg; 32.5 - &lt; 40 kg — 400 mg) per dose once daily.</p> <p>Adolescent (body weight <math>\geq</math> 40 kg)/adults: 600 mg/dose once daily.</p> <p>Combination ART (adults):</p> <ul style="list-style-type: none"> <li>• Atripla<sup>™</sup> (200 mg FTC/300 mg TDF/600 mg EFV) — 1 tablet/dose once daily.</li> <li>• Co-administration with certain PIs requires following dose adjustments — 300 mg ATV plus 100 mg RTV with 600 mg EFV, all once daily; 1,000 mg IDV 3 times daily plus 600 mg EFV once daily; 700 mg f-AMP plus 100 mg RTV once daily or 1,400 mg f-APV plus 300 mg RTV with 600 mg EFV, all once daily.</li> <li>• Co-administration with MVC requires 600 mg MVC twice daily with 600 mg EFV once daily.</li> </ul> <p>Combination ART (adolescents &gt; 12 years/adults):</p> <ul style="list-style-type: none"> <li>• Co-administration with LPV/RTV requires 600 mg LPV/150 mg RTV (3 200/50 mg tablets) twice daily with 600 mg EFV once daily.</li> </ul>

Drug	WHO Guidelines	US Guidelines
Etravirine (ETR, ETV, Intelence™, TMC125)	No dosing guidelines for children. No dosing guidelines for adults.	Pediatric: not approved for use in children. Adolescent/adults: adult dose for ARV-experienced patients 200 mg/dose twice daily.
Nevirapine (NVP, Viramune®)	Children: 160 - 200 mg/m <sup>2</sup> /dose (maximal dose, 200 mg) twice daily. Scale up the dosing schedule at initiation starting at 160 mg/m <sup>2</sup> /dose once daily for the first 14 days, and moving up to full dose if no rash or untoward effects after 14 days. Adults — 200 mg/dose once daily for 14 days, followed by 200 mg/dose twice daily. Combination ART: • 30 mg d4T/150 mg 3TC/200 mg NVP — 1 tablet/dose twice daily.	Pediatric: 150 - 200 mg/m <sup>2</sup> /dose (maximal dose, 200 mg) twice daily. Scale up the dosing schedule at initiation starting at 150 mg/m <sup>2</sup> /dose once daily for the first 14 days, and moving up to full dose if no rash or untoward effects after 14 days. Adolescent/adults: 200 mg/dose twice daily. Combination ART (adolescents > 12 years/adults): • Co-administration with LPV/RTV requires dose adjustment for LPV/RTV. Combination ART (adults): • Co-administration with MVC requires 150 mg MVC dose with 200 mg NVP, all twice daily.
Atazanavir (ATV, Reyataz™)	No dosing guidelines for children. Adults — 300 mg ATV plus 100 mg RTV once daily.	Pediatric: Not approved for use in children. Currently under the study in PACTG/IMPAACT 1020 A. Adolescent ≥ 16 - 21 years/adults: in <i>treatment-naïve</i> patients — 400 mg daily (this dose may be inadequate). In <i>treatment-experienced</i> patients — 300 mg plus 100 mg RTV once daily. Combination ART(adults): • Only RTV-boosted ATV (300 mg ATV plus 100 mg RTV) should be used in combination with TDF, LPV/RTV. • Co-administration with MVC requires 150 mg MVC dose twice daily with 300 mg ATV plus 100 mg RTV.
Darunavir (DRV, TMC114, Prezista®)	No dosing guidelines for children. No dosing guidelines for adults.	Pediatric: Not approved for use in children < 18 years. Currently under the DELPHI (TMC 114-C2T2) study. Adolescent ≥ 18 years/adults: 600 mg/dose DRV plus 100 mg RTV twice daily. DRV should not be used without RTV. Combination ART (adults): • Co-administration with MVC requires 150 mg MVC dose with DRV 600 mg plus 100 mg RTV, all twice daily.
Fosamprenavir (f-AMP, Lexiva™)	No dosing guidelines for children. Adults — 700 mg f-AMP plus 100 mg RTV twice daily.	Pediatric > 6 and < 18 years: in <i>treatment-naïve</i> patients — 30 mg/kg/dose (maximal dose, 1,400 mg, can be used in patients with body weight ≥ 47 kg) twice daily without RTV or 18 mg/kg/dose (maximal dose, 700 mg, can be used in patients with body weight ≥ 39 kg) plus RTV 3 mg/kg/dose (maximal dose, 100 mg) twice daily

(can be used in patients with body weight  $\geq$  33 kg). In *treatment-experienced* patients — 18 mg/kg/dose (maximal dose, 700 mg, can be used in patients with body weight  $\geq$  39 kg) plus RTV 3 mg/kg/dose (maximal dose, 100 mg, can be used in patients with body weight  $\geq$  33 kg) twice daily.

Adults<sup>6</sup>: in *treatment-naïve* patients — 1,400 mg/dose twice daily without RTV or 700 mg plus 100 mg RTV both twice daily or 1,400 mg plus 200 mg RTV or 100 mg RTV both given once daily. In *treatment-experienced* patients — 700 mg plus 100 mg RTV twice daily. Only boosted f-AMP with RTV should be used in *treatment-experienced* patients.

Combination ART (adults):

- Co-administration with EFV requires f-AMP dose of 700 mg plus 100 mg RTV twice daily or 1,400 mg f-AMP plus 300 mg RTV once daily.
- Only boosted f-AMP should be used in combination with EFV.
- Co-administration with MVC requires 150 mg MVC dose twice daily in combination with 700 mg f-AMP plus 100 mg RTV twice daily.

Pediatric: Not approved for use in children. Investigational dose of 500 mg/m<sup>2</sup> of body surface area every 8 hours (three times daily) in children 4 - 15 years of age resulted adequate AUC and low plasma IDV trough.

Adolescent > 18 years/adults: 800 mg/dose every 8 hours. Adult dose in combination with RTV — 800 mg IDV plus 100 mg RTV twice daily.

Combination ART(adults):

- Co-administration with EFV requires 800 mg IDV plus 100 or 200 mg RTV twice daily.

Pediatric: body weight > 15 - < 40 kg — 10 mg/kg LPV/2.5 mg/kg RTV twice daily with food. Approximately equivalent to 230 mg/m<sup>2</sup> LPV/57.5 mg/m<sup>2</sup> RTV per dose. Body weight > 40 kg — 400 mg or 230 mg/m<sup>2</sup> LPV/57.5 mg/m<sup>2</sup> RTV per dose (maximal dose, 400 mg LPV/100 mg RTV). Use of 230 mg/m<sup>2</sup> LPV dose provides adequate AUC for LPV, but might produce lower trough, higher doses may be considered.

Adolescent > 12 years: 400 mg LPV/100 mg RTV per dose twice daily with food.

Adolescent > 18 years/adults: in antiretroviral naïve patients — 800 mg LPV/200 mg RTV per dose once daily.

No dosing guidelines for children.  
Adults — 800 mg IDV plus 100 mg RTV twice daily.

Indinavir  
(IDV, Crixivan<sup>®</sup>)

Lopinavir/Ritonavir  
(LPV/RTV,  
Kaletra<sup>®</sup>, ABT 378)

Body weight 14 - 39.9 kg — 10 mg LPV/kg/dose twice daily (equivalent to 300 LPV mg/m<sup>2</sup>). Body weight 15 - 40 kg — 2.5 mg RTV/kg/dose twice daily (equivalent to 75 mg/m<sup>2</sup>). Maximal dose — 400 mg LPV plus 100 mg RTV twice daily.

Adults/combination ART — capsules (133.3 mg LPV/33.3 mg RTV) 3 capsules twice daily or 4 capsules twice daily when co-administered with EFV (600 mg once daily) or NVP (150 mg twice daily); tablets (200 mg LPV/ 100 mg RTV) for *treatment-naïve patients* — 2 tablets twice daily, for *treatment-experienced patients* — 3 tablets twice daily when co-administered with EFV (600 mg once daily) or NVP (150 mg twice daily).

Drug	WHO Guidelines	US Guidelines
Lopinavir/Ritonavir (LPV/RTV, Kaletra <sup>®</sup> , ABT 378) (continued)		<p>Combination ART (adolescents &gt; 12 years/adults):</p> <ul style="list-style-type: none"> <li>Once daily dose should not be used in patients with concomitant therapy with EFV, NVP, f-AMP or NFV.</li> <li>Co-administration with NVP, EFV or f-AMP requires increase in LPV/RTV dose to 300 mg/m<sup>2</sup> LPV/75 mg/m<sup>2</sup> RTV per dose in children &lt; 12 years of age and 600 mg LPV/150 mg RTV per dose twice daily with food.</li> </ul> <p>Combination ART (adults):</p> <ul style="list-style-type: none"> <li>Co-administration with SQV requires 1,000 mg SQV dose without additional RTV twice daily with twice daily 400 mg LPV/100 mg RTV.</li> <li>Co-administration with MVC requires 150 mg MVC dose with 400 mg LPV/100 mg RTV, all twice daily.</li> </ul>
Nelfinavir (NFV, Viracept <sup>®</sup> )	<p>Body weight ≥ 20 kg — maximum recommended dose of 1,250 mg/dose twice daily. Adults — 1,250 mg/dose twice daily.</p>	<p>Pediatric &lt; 13 years: 45 - 55 mg/kg/dose twice daily or 25 - 35 mg/kg/dose three times daily. Adolescent /adults: 1,250 mg/dose twice daily or 750 mg/dose three times daily.</p>
Ritonavir (RTV, Norvir <sup>®</sup> )	<p>Children &lt; 16 years — 400 mg/m<sup>2</sup>/dose (maximal dose, 600 mg) twice daily. Scale up the dosing schedule at initiation starting at 250 mg/m<sup>2</sup>/dose of body surface area twice daily with increments by 50 mg/m<sup>2</sup>/dose every 2 - 3 day intervals to full dose as tolerated. As a booster for LPV for body weight 15 - 40 kg — 2.5 mg/kg/dose twice daily. No dosing guidelines for adults.</p>	<p>Pediatric: 350 - 450 mg/m<sup>2</sup>/dose (maximal dose, 600 mg) twice daily. Scale up the dosing schedule at initiation starting at 250 mg/m<sup>2</sup>/dose twice daily with increments by 50 mg/m<sup>2</sup> at 2- to 3-day intervals to full dose as tolerated. Adolescent/adults: 600 mg/dose twice daily. Scale up the dosing schedule at initiation starting at 300 mg/dose twice daily; and in stepwise increase until full dose is reached over 5 days as tolerated. Combination ART(adolescents/adults):</p> <ul style="list-style-type: none"> <li>RTV is used at lower doses as a pharmacokinetic enhancer to other PIs with doses ranging from 100 to 400 mg.</li> </ul>
Saquinavir (SQV, Invirase <sup>®</sup> )	<p>Not licensed for use in children &lt; 16 years of age or less than 25 kg. Never should be taken unboosted. Adults — 1,000 mg SQV plus 100 mg RTV twice daily.</p>	<p>Pediatric: Not approved for use in children. Investigational dose of 50 mg/kg/dose every 8 hours (three times daily) provided inadequate AUC and plasma trough. Co-administration with RTV, LPV/RTV and NFV is being investigated. Adolescent &gt; 16 years/adults: 1,000 mg plus 100 mg RTV twice daily. Never should be used unboosted. Combination ART (adults):</p> <ul style="list-style-type: none"> <li>Co-administered with LPV/RTV requires 1,000 mg SQV without additional RTV twice daily with twice daily 400 mg LPV/100 mg RTV.</li> <li>Co-administration with MVC requires 150 mg MVC dose with 1,000</li> </ul>

<p>mg SQV plus 100 mg RTV, all twice daily.</p> <p>Pediatric: Not approved for use in children. Currently under study in PACTG1051/BI1182.14. Adult dose<sup>d</sup>: 500 mg plus 200 mg RTV twice daily. Combination ART (adults):</p> <ul style="list-style-type: none"> <li>• Co-administration with MVC requires 300 mg MVC dose with 500 mg TPV plus 200 mg RTV, all twice daily.</li> </ul>	<p>No dosing guidelines for children. No dosing guidelines for adults.</p>	<p>Tipranavir (TPV, Aptivus<sup>®</sup>)</p>
<p>Pediatric: Not approved for use in children &lt; 16 years. No data currently available on dosage below this age. Combination ART (adolescents/adults): When given with CYP3A4 inhibitors (with or without CYP3A4 inducers) including all PIs (except TPV/RTV) – 150 mg/dose twice daily. When given with other drugs that are not strong inhibitors or inducers of CYP3A4, such as NRTIs, T-20, TPV/RTV, and NVP – 300 mg/dose twice daily. When given with CYP3A4 inducers including EFV – 600 mg/dose twice daily.<sup>e</sup></p> <p>Pediatric: 2 mg/kg (maximal dose, 90 mg (1 ml)) twice daily injected subcutaneously into the upper arm, anterior thigh, or abdomen. Adolescent &gt; 16 years/adults: 90 mg (1 ml) twice daily injected subcutaneously into the upper arm, anterior thigh, or abdomen.</p> <p>Pediatric: Not approved for children &lt; 16 years. Currently in phase I/II study in IMPAACT P1066 for children aged 2 - 18 years. Adolescent &gt; 16 years/adults: 400 mg/dose twice daily.</p>	<p>No dosing guidelines for children. No dosing guidelines for adults.</p>	<p>Maraviroc (MVC, Selzentry<sup>®</sup>)</p>
<p>Pediatric: 2 mg/kg (maximal dose, 90 mg (1 ml)) twice daily injected subcutaneously into the upper arm, anterior thigh, or abdomen. Adolescent &gt; 16 years/adults: 90 mg (1 ml) twice daily injected subcutaneously into the upper arm, anterior thigh, or abdomen.</p>	<p>No dosing guidelines for children. No dosing guidelines for adults.</p>	<p>Enfuvirtide (Fuzeon<sup>™</sup>, T-20) Fusion Inhibitor</p>
<p>Pediatric: Not approved for children &lt; 16 years. Currently in phase I/II study in IMPAACT P1066 for children aged 2 - 18 years. Adolescent &gt; 16 years/adults: 400 mg/dose twice daily.</p>	<p>No dosing guidelines for children. No dosing guidelines for adults.</p>	<p>Raltegravir (MK-0518, RGV, RAL, Isentress<sup>®</sup>)</p>

The recommended doses are taken verbatim from the public-domain guidelines available at <http://www.who.int/hiv/pub/guidelines/art/en/index.html> and <http://aidsinfo.nih.gov/Guidelines>, respectively.

ART – antiretroviral therapy; AUC – area under the curve; CYP3A4 – cytochrome P450 3A4; IMPAACT – International Maternal Pediatric Adolescent AIDS Clinical Trials; PACTG – Pediatric AIDS Clinical Trials Group; PI – protease inhibitor; PK – pharmacokinetic; WHO – World Health Organization.

<sup>a</sup> Both weight and age are used to define “adolescent” patient. In certain references neither is used and “adolescent” is referred to as a category.

<sup>b</sup> The table does not provide food requirements or the dosing recommendations for patients with hepatic and renal insufficiency. Please refer to the actual guidelines available online at: <http://www.who.int/hiv/pub/guidelines/art/en/index.html> and <http://aidsinfo.nih.gov/Guidelines>.

<sup>c</sup> Pediatric doses for younger children and neonates and infants are not represented in the table.

<sup>d</sup> No adolescent dose is referenced in the guidelines.

<sup>e</sup> The list of other than ARV drugs considered for the Maraviroc (MVC) dose adjustment is available under Pediatric HIV Guidelines at <http://aidsinfo.nih.gov/Guidelines>.

To date, little attention has been paid to the developmental processes of puberty, and drug PK/PD data in the adolescent population are remarkably limited.<sup>20</sup> The concept of adolescent developmental pharmacology was proposed by Hein in 1987.<sup>21</sup> Since then, few PK studies have been published in this field.<sup>22-24</sup> Changes in the effects of ABCB1 polymorphisms on the bioavailability of oral cyclosporine have been shown to occur in pediatric renal transplant patients > 8 years of age as compared to younger children.<sup>24</sup> The sexual maturation rate was shown to affect the net renal tubular secretion of digoxin.<sup>25</sup> Tanner stage and presence of sex steroids were shown to affect the clearance of antipyrine.<sup>22</sup> Puberty has also been shown to affect the activity of selected CYP450 isoforms. Using a caffeine breath test, Lambert *et al.* demonstrated an association between Tanner stage and age-dependent inducibility of the CYP1A2 pathway.<sup>23</sup> The correlation with Tanner stage appeared to be sex-dependent, with a decrease in clearance seen at an earlier Tanner stage in girls than in boys. Similar data on sex-associated differences were observed in studies on theophylline metabolism in adolescents.<sup>26</sup> A recent study by Saitoh *et al.* on the effect of CYP2B6 on efavirenz PK in HIV-infected children has demonstrated that age and CYP2B6 G516T genotype were independently and statistically associated with efavirenz clearance.<sup>27</sup> These results suggest that age-related changes in CYP2B6 activity may need to be considered when evaluating the impact of genetic variants on efavirenz PK in children. Developmental changes in CYP450 activity have the potential to affect disposition and clearance of non-nucleoside reverse transcriptase inhibitors and protease inhibitors during puberty and may produce new models of drug-drug interactions with CYP450 substrates, inducers, and suppressors such as those used in concomitant ARV and antimycobacterial therapy.

A high level of patient adherence to the regimen is required to achieve a successful outcome for ART.<sup>28</sup> It must be recognized that HIV-infected adolescents face multiple challenges in fulfilling the adherence requirements because of the dynamic period of transition from childhood to adulthood. In addition to the well-recognized pediatric adherence barriers such as dependence on a caregiver for obtaining medications, palatability, pill burden, and interference with lifestyle, many obstacles to adherence emerge in adolescence, and these are related to the psychosocial changes during puberty. Among these are changes in lifestyle involving growing independence and rebellion against parental involvement, increased peer pressure and fear of stigmatization, increased risk-taking behavior, denial and fear of HIV infection (particularly in recently diagnosed youth), long history of poor adherence and nondisclosure issues in perinatally infected adolescents, psychiatric problems (depression), and alcohol and substance abuse.<sup>29,30</sup> A comprehensive assessment of adherence through multiple methods (such as self-report, pill count, pharmacy refills, and therapeutic drug monitoring) should be incorporated into the ART of every adolescent HIV patient. Although strategies to promote long-term adherence to ART have not been rigorously evaluated in adolescents to date, preliminary data suggest that interventions based on intensive follow-up, involvement of family and peers, use of reminder systems, alternative dosing schedules, and directly observed therapy may facilitate adherence to the dosing regimen in this vulnerable population.<sup>30</sup>

The developmental physiological, psychological, and social changes during puberty create strong grounds for an individualized therapeutic approach to HIV-infected adolescents. The concept of developmental rather than chronological age needs to be considered in adolescents. As the use of ART continues to expand among an aging cohort of HIV-infected children and newly infected adolescents, large collaborative studies are urgently needed to evaluate ARV drug exposure in adolescents, and accurate growth curves and sexual maturation staging of HIV-infected children and adolescents of various ethnic and racial backgrounds need to be developed. Finally, better adherence interventions and simplified ART regimens with newer ARV agents are needed to improve the outcome of therapy in HIV-infected adolescents.

### **ACKNOWLEDGMENTS**

The clinical research of the authors is supported by the Department of Health and Human Services, National Institutes of Health Public Health Service grants NCRR K12 RR017613, NICHD 5U10 HD031318, NICHD 1U10 HD45993, and NCRR K24RR019729.

### **CONFLICT OF INTEREST**

E.V.C. is a DSMB Member of Pfizer Pharmaceuticals and a consultant for GlaxoSmithKline. The other authors declared no conflict of interest.

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# 4 chapter

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## **TREATING AN HIV-INFECTED PAEDIATRIC PATIENT: AN EASY TASK?**

Rakhmanina NY, van den Anker JN.

*Antivir Ther.* 2010;15:293-296.

**ABSTRACT**

HIV-infected children represent an incredibly diverse group of patients, ranging from neonates to young adults. With the change in age and maturity, paediatric highly active antiretroviral therapy (HAART) evolves from the meticulous twice-daily dosing of ml of liquid preparations to a choice of once-daily dosing of a single fixed-dose coformulated tablet. The use of simplified paediatric regimens could enhance the ability to achieve successful implementation of HAART in HIV-infected children. Pharmacokinetics and pharmacodynamic studies of antiretroviral drugs and their combinations need to be conducted in infants, children and adolescents to develop appropriate dosing in each of these specific groups.

It is well recognized that drug administration in children is more complex than in adults. A limited number of paediatric illnesses require long-term daily oral therapy, and HIV infection is one of these. HIV-infected children represent an incredibly diverse group of patients, ranging from neonates to young adults. With change in patient age and maturity, highly active antiretroviral therapy (HAART) evolves from the meticulous twice-daily dosing of ml of liquid preparations to a choice of single coformulated tablet administered once daily. Paediatric adherence barriers to HAART also evolve with growth and development, from spitting and vomiting of liquid preparations in infancy to the rejection of family involvement and increased peer pressure during puberty.

Currently recommended HAART requires daily and indefinite administration of antiretroviral (ARV) medications. Initiation of HAART is recommended for infants < 12 months of age, regardless of clinical status, CD4+ T-cell percentage or HIV RNA viral load.<sup>1-3</sup> In older children, the degree of immunological suppression and virological response, as well as the clinical course of the disease, guide the initiation of therapy. Administering HAART to infants and small children is particularly challenging, primarily because of the choice of liquid preparations, which often require refrigeration of large volumes. The Policy Statement by the Committee on Paediatric AIDS<sup>4</sup> provides a stunning estimate of 4.3 kg of liquids (approximately one-half the weight of the child) for a 3-month supply of three ARV drugs for a 10 kg infant. Getting liquids out of the container, measuring an appropriate dose and using different methods (spoon, bottle top or syringe) to dispense the medication to infants and small children are challenging tasks for the caregivers, many of whom are coping with their own disease. In addition, poor palatability of several liquid ARV drugs, particularly liquid protease inhibitors (PIs), has been cited as an additional barrier to adherence in infants and young children with HIV.<sup>5,6</sup>

The use of simplified regimens of better tasting medications and age-appropriate delivery mechanisms might enhance the ability to achieve successful implementation of HAART in paediatric patients.<sup>4</sup> Several studies have reported that caregivers and patients prefer less frequent dosing of long-term medications.<sup>7-9</sup> Continuous therapy with once-daily dosing of ARV drugs has been shown to improve adherence to HAART in adult studies without reducing drug exposure, increasing ARV drug-associated side effects or risking development of resistance.<sup>10,11</sup> An increasing number of adult studies evaluate the pharmacokinetics (PK), safety and efficacy of once-daily HAART, whereas the data in paediatrics are limited to a handful of studies in small groups of patients.

Several studies on the PK of once-daily HAART in children have been conducted recently. A once-daily regimen of emtricitabine/didanosine/efavirenz demonstrated safety and good immunological and virological efficacy over the period of 2 years in Pediatric AIDS Clinical Trials Group (PACTG) study P1021.<sup>12</sup> Another study of a once-daily efavirenz-based regimen with three nucleoside reverse transcriptase inhibitors (NRTIs) as a first-and second-line HAART in children older than 1 year of age

showed sustained CD4+ T-cell increase, irrespective of virological suppression, although the study had high rates of treatment discontinuation.<sup>13</sup> High levels of acceptability and adherence to once-daily administration of an abacavir (ABC) and lamivudine (3TC) regimen in combination with non-nucleoside reverse transcriptase inhibitor (NNRTI) in children > 2 years of age were demonstrated by the study through the Paediatric European Network for Treatment of AIDS (PENTA).<sup>9</sup> Even with the reduction in doses to once daily, palatability was reported to be one of the major barriers to adherence by over one-third of caregivers. Overall, the study showed a similar area under the concentration-time curve (AUC) and peak plasma concentrations ( $C_{max}$ ) in children between 2 and 13 years of age with sustained virological suppression.<sup>9,14</sup> In the current issue of Antiviral Therapy, PENTA<sup>15</sup> continue the important work of establishing the bioequivalence of once-daily liquid preparations of ABC and 3TC in HIV-infected infants through PENTA trial 15. The investigators report bioequivalence of once-daily ABC and near bioequivalence of once-daily 3TC in paediatric patients between 3 and 36 months of age. The need for the large volume of liquids required for the once-daily dosing of the current preparations of ARV medications in infants and small children is underscored. Although the study was conducted as a single arm crossover PK study, the authors report promising efficacy data with sustained virological suppression at 48 weeks and without major safety concerns.

For ARV drugs, the administration of once-daily dose carries the risk of lower trough plasma concentrations ( $C_{min}$ ) and higher  $C_{max}$  than the one observed with twice-daily dosing, requiring higher degree of drug 'forgiveness'.<sup>16</sup> In children receiving once-daily ABC and 3TC in combination with NNRTI, the subtherapeutic concentrations of the backbone regimen can lead to the quick development of NNRTI resistance because of low genetic barrier. The interest in more "forgiving" PIs prompted a series of paediatric investigations of once-daily administration of a single coformulated fixed-dose combination (FDC) of lopinavir/ritonavir (LPV/r; Kaletra®). The study by Rosso *et al.*<sup>17</sup> reported a median LPV  $C_{min}$  > 1.0 mg/l in paediatric patients of 3 - 15 years of age, which was considered inhibitory for wild-type virus. The high interindividual variability and low concentrations in some patients warrant caution and recommendation of therapeutic drug monitoring of once-daily LPV/r in children. The study by van der Lee *et al.*<sup>18</sup> of once-daily LPV/r in children between 6 months and 18 years of age reported that LPV PK were comparable to once-daily LPV exposure in adults, although the variability in  $C_{min}$  was much higher. The trend for younger children to have subtherapeutic  $C_{min}$  was more pronounced than in older children, which can be explained by the larger variability in LPV/r metabolism and absorption. The authors suggested that the higher dosage of LPV/r once-daily might allow the target LPV  $C_{min}$  to be achieved in children. A recent study by van der Flier *et al.*<sup>19</sup> reported that administration of a new tablet formulation of LPV/r resulted in greater LPV exposure and less variability compared with previously studied soft-gel capsule formulations. These findings suggest that administration of a tablet formulation of LPV/r could result in the feasibility of once-daily LPV/r therapy in children.<sup>19</sup> Finally, la Porte *et al.*<sup>20</sup> recently

reported similar values of LPV PK (AUC,  $C_{max}$  and  $C_{min}$ ) of once-daily LPV/r to twice-daily LPV dosing in patients between 5 and 15 years of age, suggesting to continue to consider once-daily administration of this FDC PI in children.

Lack of availability of appropriate paediatric drug formulations has been identified as major barrier in provision of efficient HAART in children and adolescents.<sup>4</sup> Alternative medication delivery systems, such as skin patches, are considered to be problematic in children because of high inter- and inpatient variability in absorption and metabolism, and developmental changes in PK. Urgent need for the development of tablets with smaller amounts of the drugs, soluble or dispersible tablets, sprinkles, sachets and paediatric FDC has been underscored by the World Health Organization and international experts on paediatric HIV.<sup>3,4</sup> Paediatric FDC are expected to improve access and adherence to HAART in children in resource-limited settings. They can also be of use in developed countries where the adherence levels among children and adolescents continue to present a major barrier to efficient HAART. In recent years, a number of inexpensive, generic paediatric FDC formulations, including combinations of zidovudine (AZT), ABC, stavudine (d4T), 3TC and nevirapine (NVP), in a variety of different ratios and formulations (regular tablets, dispersible tablets and suspension for reconstitution) for once- or twice-daily dosing, have been developed by generic suppliers.<sup>3</sup> Currently, only four paediatric FDC have been approved by the US Food and Drug Administration through the President's Emergency Plan For AIDS Relief (PEPFAR)<sup>21</sup>: 3TC/AZT, ABC/3TC, 3TC/d4T and 3TC/d4T/NVP. Questions about the ratio of ARV components and the bioequivalence of such generic formulations, particularly with NVP exposure, have been raised.<sup>22</sup> It is clear that the availability of ARV FDC requires more randomized, controlled studies to evaluate their therapeutic potential in paediatric patients. PK and pharmacodynamic studies of ARV drugs and their combinations need to be conducted in infants, children and adolescents to develop appropriate dosing in each of these specific age groups.<sup>4</sup> The World Health Organization has underscored the need for research on the efficacy of early PI- and NVP-based regimens in infancy, and further studies of PK, adherence and acceptability of the NRTI backbone regimen in children.<sup>3</sup>

As the access to HAART continues to expand among HIV-infected children, the need for simplified and feasible paediatric ARV regimens grows. There is no single solution to the successful delivery of HAART in children, and varying approaches need to be undertaken, depending on the available resources and clinical and social situations. Medical providers caring for an HIV-infected paediatric patient need to be prepared to adjust the treatment plan and interventions based on the developmental stage of the child and the degree of family and other support involved. The goal of paediatric HAART is to successfully navigate the patient through infancy, childhood and puberty, maintaining normal physical growth and neurocognitive development, and restoring and/or preserving their immune function and quality of life. The main task is to transition HIV-infected youth to long-term

adult care with maximal and durable virological suppression and preserved therapeutic options for the future management of their disease.

### **ACKNOWLEDGEMENTS**

The clinical research of the authors is supported by the Department of Health and Human Services, NIH PHS grants NICHD K23HD060452 (NYR), NICHD NO1-HD-3-3345 (NYR), NIBIB R01 EB005803 (NYR), NICHD 1U10 HD45993 (NYR, JNvdA) and NCRR K24RR019729 (JNvdA).

### **DISCLOSURE STATEMENT**

The authors declare no competing interests.



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**II**

**PART**

**PHARMACOKINETICS  
AND  
PHARMACODYNAMICS  
OF  
ANTIRETROVIRAL  
THERAPY IN  
PEDIATRIC PATIENTS**



# 5 chapter

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## **AGE-DEPENDENT PHARMACOKINETICS OF LAMIVUDINE IN HIV-INFECTED CHILDREN**

Burger DM, Verweel G, Rakhmanina N, Verwey-Van Wissen CPWGM, La Porte CJL, Bergshoeff AS, Lyall H, Hartwig NG, Green H, Soldin S, Gibb DM, de Groot R.

*Clin Pharmacol Ther.* 2007;81:517-520.

**ABSTRACT**

The recommended dose of lamivudine in children is higher when compared with adults: 4 mg/kg vs ~ 2 mg/kg (150 mg) and administered twice a day. Limited data are available to demonstrate that this increased dose results in adequate exposure to lamivudine in children with human immunodeficiency virus (HIV) infection. Data were selected from children who were using lamivudine for at least 2 weeks before a full pharmacokinetic (PK) study was conducted. Lamivudine PK parameters were significantly related to age. The age of 6 years appeared to be a cutoff for a change in PK parameters of lamivudine, with children < 6 years of age ( $n = 17$ ) having a median area under the curve 43% lower and a median peak plasma concentration 47% lower (both  $P < 0.001$ ) than older children ( $n = 34$ ). In conclusion, further investigation of the relationship between decreased lamivudine exposure and treatment outcome and long-term resistance development in younger children with HIV infection is warranted.

## INTRODUCTION

Lamivudine (3TC, Epivir®) has been licensed for the treatment of human immunodeficiency virus (HIV) infection in pediatric patients from 3 months to 16 years of age at a dose of 4 mg/kg twice daily (b.i.d.) (with a maximum of 150 mg per dose). When compared with the adult dose of 150 mg b.i.d. (approximately 4 mg/kg/day), this higher daily dose (8 mg/kg/day) in children was based on the reported increase in systemic clearance of lamivudine associated with younger age. The Epivir® Prescribing Information states that "total exposure to lamivudine, as reflected by mean area under the curve (AUC) values, was comparable between pediatric patients receiving an 8 mg/kg/day dose and adults receiving a 4 mg/kg/day dose."<sup>1</sup> However, validation of this higher dose in children is limited to a small number of patients and data are mostly on file with the manufacturer.<sup>2</sup> Recently, we reported a possible age effect on lamivudine pharmacokinetics (PKs) in a comparative study of once- and twice-daily administration of lamivudine in children.<sup>3</sup> The purpose of this study has been aimed at further investigation of the relationship between lamivudine PK and the age of children with HIV infection.

## METHODS

Plasma samples from children participating in a number of PK studies at our institutes were available for analysis.<sup>3-7</sup> All study protocols were approved by the local ethics committees. Children had to use the recommended lamivudine dose regimen of 4 mg/kg b.i.d. for at least 2 weeks before enrollment in this study. Lamivudine was administered as either oral solution (10 mg/ml) or tablets (150 mg). The adult dose of 150 mg b.i.d. was used as the maximum dose. Blood samples were taken at regular time intervals after observed drug intake with breakfast during 8 or 12 h (6 - 10 samples). In case of sampling up to 8 h ( $n = 12$ ),  $C_{12h}$  was calculated using the individual elimination rate constants ( $\beta$ ) by the equation:

$$C_{12h} = C_{8h} \times \exp^{-(\beta \times 4)}$$

A sampling schedule of 8 h contributed at least 90% of total AUC.

Lamivudine plasma concentrations were determined by high performance liquid chromatography with UV detection as previously reported.<sup>8</sup> The lower limit of quantification is 0.050 mg/l. Average accuracy ranged from 92 to 98% and precision ranged from 0.7 to 2.3%. PK parameters were calculated using non-compartmental methods using WinNonlin software version 4.1.<sup>9</sup>

## RESULTS

A total of 51 children participated in the study: 23 boys and 28 girls. Age at time of PK sampling ranged from 1.7 to 18 years (median: 8.4 years).  $AUC_{0-12}$  ( $r^2 = 0.180$ ;  $F = 10.757$ ;  $P = 0.002$ ), peak plasma concentration ( $C_{max}$ ) ( $r^2 = 0.158$ ;  $F = 9.171$ ;  $P = 0.004$ ), trough plasma concentration ( $C_{min}$ ) ( $r^2 = 0.092$ ;  $F = 4.971$ ;  $P = 0.030$ ),  $CL/F.kg$  ( $r^2 = 0.354$ ;  $F = 26.833$ ;  $P < 0.001$ ), and  $Vd/F.kg$  ( $r^2 = 0.156$ ;  $F = 9.072$ ;

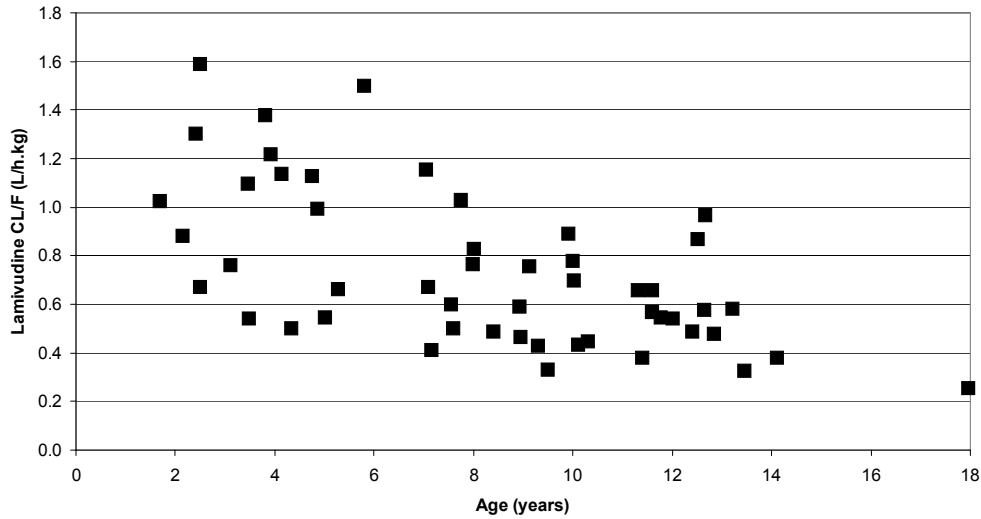
$P = 0.004$ ) were significantly related to age, with younger children having higher oral clearance of lamivudine (Figure 1 and Table 1). Lamivudine half-life was not significantly influenced by age. By visual inspection, the age of 6 years appeared to be a cutoff for a change in PK parameters of lamivudine in this dataset, with younger children ( $n = 17$ ) having a median AUC 43% lower and a median  $C_{\max}$  47% lower (both  $P < 0.001$ ) than older children ( $n = 34$ ) (Table 1). In line with these observations,  $CL/F.kg$  and  $Vd/F.kg$  were 79 and 89% higher in children 6 years and younger compared with children 7 years and younger ( $P < 0.001$  and  $P = 0.005$ , respectively; Figures 1 and 2). Sensitivity analyses showed that differences between age groups became less pronounced when other cutoff points were selected for age (for instance, the difference in median AUCs was 40% ( $P = 0.029$ ) or 27% ( $P = 0.006$ ) when 3 or 9 years of age were selected, respectively).

**Table 1** Plasma pharmacokinetic parameters of lamivudine in children aged  $\leq 6$  and 7 years or older (median + IQR)

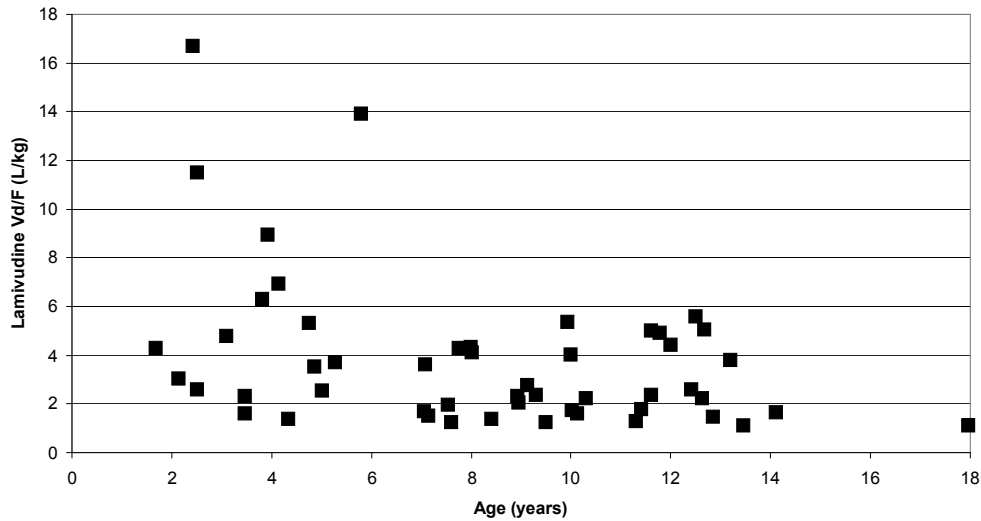
Parameter	Age $\leq 6$ years	Age 7 years or older
Number of children	17	34
Gender (F/M)	13/4	15/19
Age (years)	3.8 (2.5 - 4.8)	10.1 (8.5 - 12.3)
Weight (kg)	14.6 (13.8 - 16.7)	32.3 (27.9 - 42.4)
Height (cm)	98 (91 - 106)	138 (128 - 146)
Body surface area (m <sup>2</sup> )	0.63 (0.60 - 0.70)	1.12 (0.97 - 1.31)
Lamivudine dose		
In mg	60 (50 - 70)	150 (100 - 150)
In mg/kg	4.0 (3.8 - 4.1)	3.9 (3.2 - 4.2)
In mg/m <sup>2</sup>	97* (88 - 103)	111 (103 - 120)
AUC <sub>0-12</sub> (mg/l·h)	3.71* (3.44 - 6.17)	6.54 (5.21 - 8.58)
AUC corrected for dose in mg/kg	0.97* (0.82 - 1.49)	1.74 (1.35 - 2.22)
AUC corrected for dose in mg/m <sup>2</sup>	0.045** (0.034 - 0.061)	0.059 (0.049 - 0.072)
$C_{\max}$ (mg/l)	0.98* (0.82 - 1.43)	1.84 (1.21 - 2.28)
$C_{\max}$ corrected for dose in mg/kg	0.24* (0.20 - 0.35)	0.44 (0.34 - 0.68)
$C_{\max}$ corrected for dose in mg/m <sup>2</sup>	0.010*** (0.009 - 0.015)	0.016 (0.011 - 0.023)
$t_{\max}$ (h)	2.0 (1.0 - 2.0)	1.5 (1.0 - 2.0)
$C_{\min}$ (mg/l)	0.07 (0.06 - 0.08)	0.09 (0.07 - 0.11)
$CL/F$ (l/h/kg)	1.03* (0.67 - 1.22)	0.57 (0.45 - 0.74)
$CL/F$ (l/h/m <sup>2</sup> )	22.2 (16.4 - 29.0)	17.0 (13.8 - 20.3)
$Vd/F$ (l/kg)	4.31**** (2.59 - 6.93)	2.29 (1.64 - 4.10)
$Vd/F$ (l/m <sup>2</sup> )	93.2 (72.5 - 168.1)	67.9 (46.8 - 110.3)
$t_{1/2}$ (h)	3.27 (2.48-4.34)	3.05 (2.55 - 3.71)

F – female; IQR – interquartile range; M – male. \*  $P < 0.001$ ; \*\*  $P = 0.011$ ; \*\*\*  $P = 0.009$ ; all comparisons to children 7 years and older; \*\*\*\*  $P = 0.005$ .

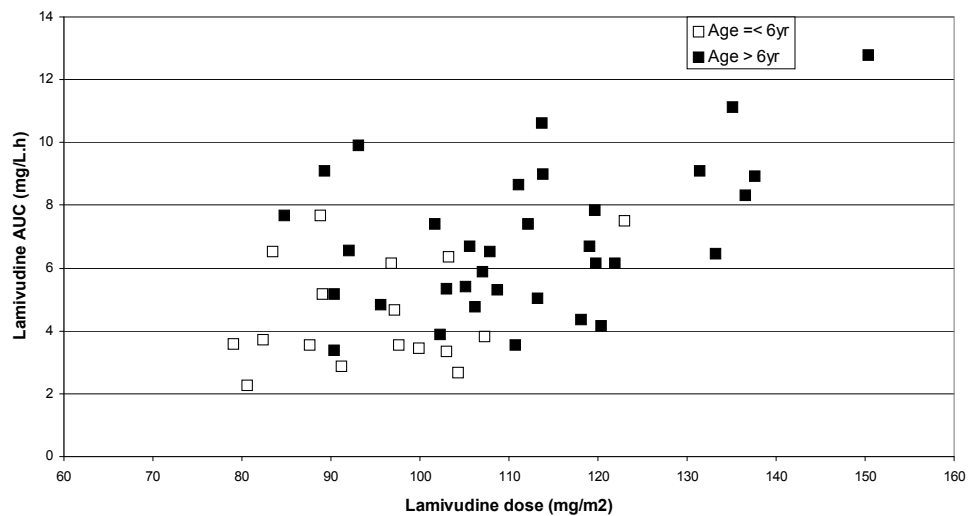




**Figure 1** Association of lamivudine body weight corrected CL/F and age.



**Figure 2** Association of lamivudine body weight corrected Vd/F and age.



**Figure 3** Association of lamivudine AUC and dose in mg/m<sup>2</sup>.

Lower exposure to lamivudine in younger children was not related to differences in lamivudine dosing in mg/kg, as the median dose in both age cohorts was 4 mg/kg b.i.d. (Table 1). Variability in lamivudine dose, however, was larger in children aged 7 years and older, probably because of the use of tablets instead of oral solution. In contrast to the lamivudine dose based on body weight, there was a significant difference in median lamivudine dose in mg/m<sup>2</sup> when comparing younger and older children: 97 vs 111 mg/m<sup>2</sup> ( $P < 0.001$ ). The higher dosing on body surface area was related to increased exposure to lamivudine ( $r^2 = 0.288$ ;  $F = 19.781$ ;  $P < 0.001$ ) (Figure 3). Normalization of AUC or  $C_{\max}$  for dose given per body weight or per body surface area did not markedly change the age dependency of the PK parameters (Table 1).

The mean  $C_{\max}$  and AUC in children aged 7 years and older were almost similar to average values observed in HIV-1-infected adults:<sup>10</sup>  $C_{\max}$  1.84 vs 2.08 mg/l, AUC 6.5 vs 8.5 mg/l·h, respectively. The reference data for adults were chosen as they represent the largest published dataset of adult PK parameters of lamivudine. Although numbers were small, there was no difference observed in mean AUC in children aged 7 - 12 years ( $n = 24$ ) vs children aged 12 years or older ( $n = 10$ ): 6.7 vs 5.9 mg/l·h ( $P = 0.91$ ) (Figure 1). Lamivudine AUC did not differ between boys (median value: 6.2 mg/l·h;  $n = 23$ ) and girls (median value: 5.4 mg/l·h;  $n = 28$ ) ( $P = 0.925$ ).

## DISCUSSION

Our data clearly describe the age dependency of lamivudine PKs. The Product Monograph of Epivir® describes this age dependency of lamivudine systemic clearance based on a cohort of children receiving lamivudine doses ranging from 1 - 20 mg/kg/day.<sup>1</sup> This includes a subset of nine children (aged 5 months to 12 years) who received the recommended dosing of 4 mg/kg b.i.d. Thus, our dataset of 51 children largely increases our knowledge on lamivudine PKs in HIV-infected children, not only because of the higher number of children but also because we describe age dependency in lamivudine as apparent oral clearance (vs systemic clearance in the Product Monograph). The increased dose of 4 mg/kg b.i.d. provides comparable exposure (in terms of AUC and  $C_{\max}$ ) in children aged 7 years and older compared with adults. However, for children 6 years and younger, the recommended dose of 4 mg/kg b.i.d. does not lead to comparable exposure, and this is the main finding of our study.

Lower bioavailability, increased clearance and/or increased volume of distribution are possible explanations for the differences observed in the study. Use of oral solution by younger children vs use of tablets in older children can be part of the explanation as bioavailability of the oral solution in children may be less than that of the tablets. Remarkably, although both formulations have been demonstrated to be bioequivalent in adults ( $87 \pm 13$  vs  $86 \pm 16\%$ ), bioavailability in children was only

tested for the oral solution and appeared to be less and more variable:  $66 \pm 26\%$ .<sup>1</sup> As we cannot exclude that there is a difference in bioavailability between the oral solution and the tablets in children, we strongly recommend performing bioequivalence studies in children with all available formulations in order to provide evidence-based support for dosing recommendations in children.

It is easily understood that there was no possible standardized breakfast for children aged 1.7 to 18 years. Because lamivudine PK is not dependent on food, variability in breakfast is not likely to have influenced our results. Lamivudine is eliminated primarily by renal excretion of nonmetabolized drug. It has been previously reported that younger children may have increased glomerular filtration and/or tubular secretion capacity.<sup>11</sup> As we have only studied lamivudine PK after oral administration, we cannot separate any age effect on drug bioavailability from an effect on renal clearance in our patients. However, it must be noted that the Epivir<sup>®</sup> Product Monograph describes age-dependent systemic clearance of lamivudine, suggesting that both bioavailability and renal clearance, independently, are influenced by age.

Finally, it must be acknowledged that we do not have information that this lower exposure to lamivudine is related to reduced virological activity of lamivudine-containing highly active antiretroviral treatment regimen in children younger than 6 years of age. First, we have not collected data on virological response as part of our study, as all the enrolled subjects were receiving various regimens for different periods of time and were both treatment-naïve and treatment-experienced. Furthermore, lamivudine plasma PK can only be considered as a limited marker of drug exposure as it is the intracellular lamivudine triphosphate metabolite that becomes pharmacologically active. Theoretically, increased plasma clearance of the lamivudine may be associated with both decreased and increased intracellular exposure to the lamivudine triphosphate,<sup>12</sup> and therefore, younger children with increased plasma clearance of lamivudine may reach lower or higher intracellular concentrations of the active drug. However, this hypothesis is very hard to prove as adequate sampling for determination of intracellular concentrations of nucleoside reverse transcriptase-inhibitor triphosphates is logistically and technically difficult and has been conducted in limited numbers by a limited number of centers.<sup>3</sup>

The only answer to the question of optimal dosing regimens of lamivudine in children should come from well-designed clinical studies with PK data collected from a sufficient number of children in each age group. Recently, Puthanakit *et al.*<sup>13</sup> presented data on 103 children, aged 2.1 - 13.8 years with a mean age of 7.7 years, who started with lamivudine-containing highly active antiretroviral treatment regimens within Thailand's National Access to Antiretroviral Program. In this study, age was not associated with the likelihood of having a virological response at week 72 of treatment ( $P = 0.92$ ), although it must be noted that any age effect may have been confounded by greater use of efavirenz vs nevirapine in older subjects (and efavirenz may be more efficacious than nevirapine<sup>14</sup>). In our PENTA-13 study, both

younger and older children did equally well after switching from a twice-daily (4 mg/kg/dose) to a once-daily (8 mg/kg/dose) regimen of lamivudine.<sup>3</sup> Finally, no age effect was observed in the clinical end point study of zidovudine plus lamivudine vs didanosine in PACTG 300 Study<sup>15</sup> (Glaxo-SmithKline, data on file). The data from these studies suggest that the reduced exposure to a lamivudine in younger children may not lead to a decrease in optimal efficacy of the medication. It is, however, unknown if the exposure to the subtherapeutic concentrations of lamivudine may play a role in accelerating the remote development of the resistance mutation for lamivudine in those children.

## **CONCLUSION**

Our study demonstrates that the recommended pediatric lamivudine dose of 4 mg/kg does not lead to similar plasma AUC and  $C_{\max}$  of lamivudine in children younger than 6 years of age when compared with older children or adults. It must be noted that the increased apparent clearance (corrected for body weight) of lamivudine in younger children has been previously reported.<sup>2,16,17</sup> Nevertheless, the dosing recommendations for lamivudine in children aged 3 months to 16 years remain unchanged, are similar for the oral solution and the tablets despite a potential difference in bioavailability between these two formulations in children, and all age groups continue to receive the same dose of lamivudine per kilogram body weight. Although, there is no evidence for reduced antiviral efficacy associated with this age effect on lamivudine PK, the question of lower lamivudine exposure and the long-term associated development of lamivudine resistance remains to be answered.

## **ACKNOWLEDGMENTS**

The work at the Children's National Medical Center, George Washington University, Washington DC was supported by NIH PHS Grants NCRR K12 RR017613, MO1-RR-020359, and NICHD 1U10 HD45993. Data have been presented partly at the 7th International Workshop on Clinical Pharmacology of HIV Therapy, Lisbon, Portugal, 20 - 22 April, 2006 (abstr. 20).

## **CONFLICT OF INTEREST**

The authors declared no conflict of interest.

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# 6

## chapter

**POPULATION  
PHARMACOKINETICS  
OF LOPINAVIR  
PREDICT SUBOPTIMAL  
THERAPEUTIC  
CONCENTRATIONS IN  
TREATMENT-  
EXPERIENCED HUMAN  
IMMUNODEFICIENCY  
VIRUS-INFECTED  
CHILDREN**

Rakhmanina N, van den Anker J, Baghdassarian A, Soldin S, Williams K, Neely MN.

*Antimicrob Agents Chemother.* 2009;53:2532-2538.

**ABSTRACT**

In adult protease inhibitor (PI)-experienced patients, a lopinavir (LPV) phenotypic inhibitory quotient (PIQ) of  $> 15$  has been associated with a higher likelihood of viral suppression. The aims of this study were to develop a population pharmacokinetic (PK) model of LPV in children and to estimate the probability of achieving a PIQ of  $> 15$ . HIV-infected, PI-experienced children receiving LPV were intensively sampled for 12 h to measure plasma LPV. The data were fitted to candidate PK models (using MM-USCPACK software), and the final model was used to simulate 1,000 children to determine the probability of achieving an LPV PIQ of  $> 15$ . In 50 patients (4 to 18 years old), the median LPV plasma 12-hour-postdose concentration was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter) lower than that reported in adults. After a delay, LPV was absorbed linearly into a central compartment whose size was dependent on the weight and age of the patient. Elimination was dependent on weight. The regression line of observed vs predicted LPV had an  $R^2$  of 0.99 and a slope of 1.0. Visual predictive checks against all available measured concentrations showed good predictive ability of the model. The probability of achieving an LPV PIQ of  $> 15$  was  $> 90\%$  for wild-type virus but  $< 10\%$  for even moderately resistant virus. The currently recommended dose of LPV/ritonavir appears to be adequate for children infected with wild-type virus but is unlikely to provide adequate inhibitory concentrations for even moderately resistant human immunodeficiency virus (HIV). PI-experienced HIV-infected children will likely benefit from longitudinal, repeated LPV measurement in plasma to ensure that drug exposure is most often near the maximal end of the observed safe range.



## INTRODUCTION

Lopinavir/ritonavir (LPV/RTV) (Kaletra) is the first and only coformulated RTV-boosted protease inhibitor (PI) approved for use in children. The recommended doses for children who weigh more than 15 kg are 10 mg/kg of body weight or 230 mg/m<sup>2</sup> (body surface area) twice daily, with a maximum of 400 mg per dose unless it is combined with drugs affecting cytochrome (CYP) P450 metabolism, which require LPV dose adjustment.<sup>1,2</sup> Introduced as a salvage agent,<sup>3</sup> LPV/RTV has become one of the preferred PI choices for first-line regimens in children > 6 months of age in the countries with access to the drug.

Despite evidence for good antiviral efficacy among children in a clinical trial setting,<sup>4-6</sup> the standard dose may not be adequate for every child. Noncompartmental analysis has shown that the average LPV plasma 12-hour-postdose concentration ( $C_{\text{trough}}$ ) in children given the currently recommended pediatric dose of LPV is 67% lower than in adults.<sup>7</sup> Recently, Jullien *et al.* published a population pharmacokinetics (PK) model of LPV in children aged 0 to 18 years.<sup>8</sup> The model was based on a retrospective analysis of LPV plasma measurements in 157 children, with a median of 3 samples (range, 1 to 14) per patient, obtained for monitoring purposes after self-reported LPV intake. In that model, clearance (CL) and volume were dependent on body weight, and there was an additional increase in CL in boys after the age of 12 relative to girls. The study suggested that a distinct possibility of LPV underdosing exists in young infants and adolescent males.

PK measures of LPV exposure, such as area under the concentration-time curve (AUC) or  $C_{\text{trough}}$ , are most relevant to clinical practice in the context of an estimated concentration-response relationship. Different LPV  $C_{\text{trough}}$  targets have been proposed for PI-naïve patients (1 mg/liter)<sup>9</sup> and for PI-experienced patients (3 to 5.7 mg/liter).<sup>10-13</sup> A more comprehensive target, the phenotypic inhibitory quotient (PIQ), incorporates viral drug susceptibility, which is measured as the concentration of drug required to achieve 50% *in vitro* inhibition of replication of the virus (IC<sub>50</sub>) relative to the replication of human immunodeficiency virus (HIV) in drugfree medium. The PIQ is the ratio of a patient's  $C_{\text{trough}}$  to the IC<sub>50</sub> of the dominant viral strain. For LPV, a PIQ target of 15 has been associated with more than 90% chance of achieving < 400 HIV copies/ml when combined with active background antiretroviral therapy (ART) comprising efavirenz<sup>14</sup> or two nucleoside reverse transcriptase inhibitors.<sup>15</sup>

We conducted a prospective study to develop a population PK model of LPV in a large cohort of HIV-infected children, with intense PK sampling after observed LPV intake. Additionally, we used Monte Carlo simulation to estimate the proportion of all children who would fail to achieve the LPV PIQ target of 15 when given the currently recommended dose of LPV.

## MATERIALS AND METHODS

### Patients

The participants were children with HIV type 1 infection who were receiving care at the large metropolitan pediatric HIV program at Children's National Medical Center, Washington, DC. The research protocol, parental consent, and assent documents (for children older than 7 years of age) were approved by the institutional review board.

All subjects in this study were on uninterrupted, twice-daily doses of LPV/RTV-based ART for at least 4 weeks prior to study entry. Blood samples for LPV measurement were obtained during a 12-hour admission to the Pediatric Clinical Research Center. The subjects were administered their standard prescribed LPV/RTV dose under direct observation with a standard light snack. Plasma samples were obtained before and 0.5, 1, 2, 4, 8, and 12 h after observed intake of LPV/RTV.

### Tandem mass spectrometry analysis

For each measurement of the plasma LPV concentration, 2 ml of venous blood was obtained. The blood was collected in a heparin tube and separated by centrifugation at 4,000 rpm for 10 min. The plasma was stored at  $-70^{\circ}\text{C}$  pending analysis. The plasma samples were incubated for 30 min at  $56^{\circ}\text{C}$  to deactivate the HIV particles. Heating of the samples has been proven not to produce degradation of LPV.<sup>16,17</sup>

The LPV plasma concentrations were determined by a tandem-mass spectrometric method using the Applied Biosystems/Sciex API-2000.<sup>16,17</sup> Within-run error was below 7%, and between-day error was below 10% for all analytes at the tested concentrations. The lower limit of quantification was 0.1 mg/liter. The laboratory at Children's National Medical Center that performed the LPV assays is an accredited member of the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection (University Medical Center, Nijmegen, The Netherlands).<sup>18,19</sup>

### PK modeling and simulation

MM-USCPACK, a parametric/nonparametric population-modeling software collection (available by license through the University of Southern California at <http://www.lapk.org>) was used to fit candidate PK models to the time-concentration data for LPV.<sup>20</sup> The models were evaluated on the basis of maximization of the log likelihood, visual inspection of observed vs predicted plots, visual predictive checks, and parsimony.

To minimize model bias from uncertainty in dose times,<sup>21-23</sup> for each patient, the initial amount of LPV in the PK dosing compartment was set equal to zero under the reasonable assumption that the previous LPV dose approximately 12 h before had completed its passage through the gut. In contrast, the initial amount in the central PK compartment was set equal to the measured LPV trough plasma concentration just prior to the observed dose multiplied by the estimated volume of the central compartment, which was updated after each iteration. Beyond these initial conditions,

the only drug input to the model was the observed dose. By restricting model inputs to observed or measured data, this technique eliminated misspecification associated with variable adherence prior to the PK sampling and potentially false assumptions of steady state.

In accordance with the allometric size scaling of PK parameters in children proposed by Anderson *et al.*,<sup>24</sup> among others, the model was initially parameterized with the volume of distribution ( $V$ ) proportional to weight and CL proportional to weight<sup>0.75</sup>. However,  $V$  and CL were strongly correlated ( $R = 0.76$ ;  $P < 0.0001$ ), leading to highly variable parameter estimates; therefore, the model was reparameterized in terms of  $V$  being proportional to weight and elimination ( $k_{el}$ ) being proportional to weight<sup>-0.25</sup>, which were independent of each other ( $R = 0.10$ ;  $P = 0.47$ ). By the relationship  $CL = k_{el} \cdot V$ , the net allometric power scaling on weight was the same in the CL-based and  $k_{el}$ -based models. Effects of age and sex were added to the allometric model and retained if they improved the  $2 \cdot \log$  likelihood by at least 4 ( $P < 0.05$ ; chi-square with 1 degree of freedom) and resulted in some qualitative improvement in either the observed vs predicted plots or the visual predictive check.

Data for the modeling were weighted by the reciprocal of the LPV assay variance. This error model was obtained from the MM-USCPACK software, which fits up to a third-order polynomial ( $C_0 + C_1 \cdot [\text{drug}] + C_2 \cdot [\text{drug}]^2 + C_3 \cdot [\text{drug}]^3$ ) to the entire data set. The initial values for the polynomial coefficients were set at 0, 0.1, 0, and 0 to reflect the standard deviation at each of the standard concentrations in the assay validation set. The final assay error coefficients were 0.385, 0.013, 0, and 0. A multiplicative scalar, gamma, on the polynomial was iteratively fitted to capture additional random error and approximate homoscedasticity in the residuals of predicted vs observed concentrations; gamma on the final run (cycle 1,290) was 1.78.

In order to compare the results of the present study with published LPV PK parameter values, CL and half-life ( $t_{1/2}$ ) were calculated using the following formulae:  $CL = \text{dose}/AUC$  and  $t_{1/2} = \ln(2)/k_{el}$ , where AUC is the AUC from dose time to infinity postdose as estimated by MM-USCPACK from model parameters, and  $k_{el}$  is the LPV elimination rate constant estimated by MM-USCPACK. As a numerical check, CL was also calculated using the formula  $CL = k_{el} \cdot V$ .

A visual predictive check was made to validate a model's ability to provide realistic predictions. Full-parameter mean and covariance data from the model, including weight, were passed to the simulation subroutine of the MM-USCPACK software so that LPV could be administered on the standard basis of body surface area (BSA). The BSA was calculated from weight (kg), which was also a simulated parameter, using the simplified formula  $BSA (m^2) = 0.111 \cdot \text{weight}^{0.65}$ .<sup>25</sup> BSA calculated using this formula in the real population was nearly perfectly correlated ( $R = 0.995$ ;  $P < 0.0001$ ) with BSA calculated with the more familiar height- and weight-based Mosteller formula.<sup>26</sup> The median dose in the study population was used for the

simulation, and parameters were log transformed. One thousand patients were simulated by Monte Carlo sampling, and LPV concentrations, which were calculated at the same sampling times as for the original (real) population, were compared to the measured, true LPV concentrations. The 25th, 50th, and 75th percentile concentrations of LPV from the 1,000 simulated patients were plotted vs time. Superimposed upon these plots were the real concentrations of each compound measured in the patients. The visual predictive check was acceptable if the distribution of the simulated population was similar to that of the real population.

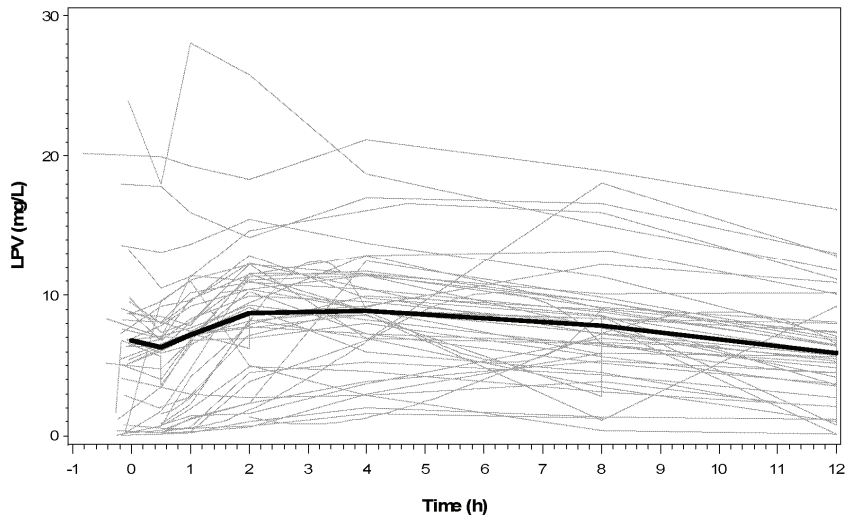
Using the final model, a second simulation was performed, identical to the first, except that the LPV dose used was the recommended dose of 230 mg/m<sup>2</sup>. This simulated population was used to define the increase in the LPV IC<sub>50</sub> that would result in 50% of children falling below a PIQ of > 15. Furthermore, since a target PIQ of 15 was simply derived as the observed PIQ that was associated with 100% virologic response,<sup>14</sup> a continuous model relating the PIQ to percent "clinical activity" of lopinavir could be made so that percent activity was equal to  $[(1 - E_{\min}) \cdot \text{PIQ}^H / \text{PIQ}_{50}^H + \text{PIQ}^H]$ , where PIQ is the PIQ calculated as before;  $E_{\min}$  is the virologic response rate at minimal LPV PIQ (i.e., the response due to other drugs in the regimen);  $H$  is the "Hill constant," which determines the shape of the sigmoid curve when plotted on semilog paper; and  $\text{PIQ}_{50}$  is the PIQ that results in a 50% loss of LPV activity, i.e.,  $(1 - E_{\min})/2$ .  $E_{\min}$ ,  $H$ , and  $\text{PIQ}_{50}$  were derived from the data of Hsu *et al.* as 0.66, 1.7, and 8.7, respectively.<sup>14</sup>

## RESULTS

Fifty-two full 12-hour PK studies were conducted with 50 PI-experienced children and adolescents on ART with LPV/RTV as a single PI, with 2 participants repeating the PK study after LPV dose adjustments made through the study protocol. The majority of the children were African-American (78%), the median age was 11.0 years (range, 5.3 to 17.5 years), and equal numbers of boys and girls were enrolled. The median LPV dose was 275 mg/m<sup>2</sup> (interquartile range, 246 to 287 mg/m<sup>2</sup>); 31 children were dosed with 200 mg LPV/50 mg RTV Kaletra capsules and the remainder with liquid LPV/RTV (80 mg LPV/20 mg RTV/ml). At the time of the PK studies, the new tablet formulation of LPV/RTV was not available.

### PK modeling

From 52 study visits, there were 359 possible PK samples, 6 (1.7%) of which were missing and 14 (3.9%) of which were reported as below the assay's limits of quantification. Treating the latter simply as missing data was unlikely to significantly bias the results.<sup>27</sup> The median plasma LPV post-dose  $C_{\text{trough}}$  was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter); the median peak was 10.3 mg/liter (range, 0.6 to 28.8 mg/liter), occurring a median of 3.1 h (range, 0 to 12 h) after the dose. The wide ranges in these values are reinforced by the variability in a plot of the individual LPV time-concentration curves, shown in Figure 1.



**Figure 1** Individual observed LPV time-concentration curves for each of the 52 patient visits (gray lines). The median observed time-concentration curve for the entire population is superimposed (black line).

The final model consisted of linear absorption, after a delay, into a single compartment. The volume was dependent on weight and inversely dependent on age, so that a 17-year-old adolescent would have an 80% smaller  $V$  per kilogram of body weight than a 4 year-old child. Elimination was inversely dependent on weight<sup>0.25</sup>. The net effect of the parameter dependence on age and weight implied a higher LPV  $C_{\text{trough}}$  on average in older children. Indeed, age was moderately correlated with the LPV  $C_{\text{trough}}$  in the study population ( $R = 0.21$ ;  $P = 0.16$ ) by the same magnitude as in the simulated population ( $R = 0.21$ ;  $P < 0.0001$ ), with the latter achieving statistical significance due to the large number of samples. Sex was not significantly related to any PK parameter estimate. The median and interquartile ranges of the individual Bayesian posterior parameter estimates, using the population parameter estimates as Bayesian priors, are reported in Table 1. The distributions of estimates for CL were similar whether calculated from the dose and AUC or  $k_{\text{el}}$  and  $V$ , and the paired values were tightly correlated ( $R = 1.0$ ;  $P < 0.0001$ ).

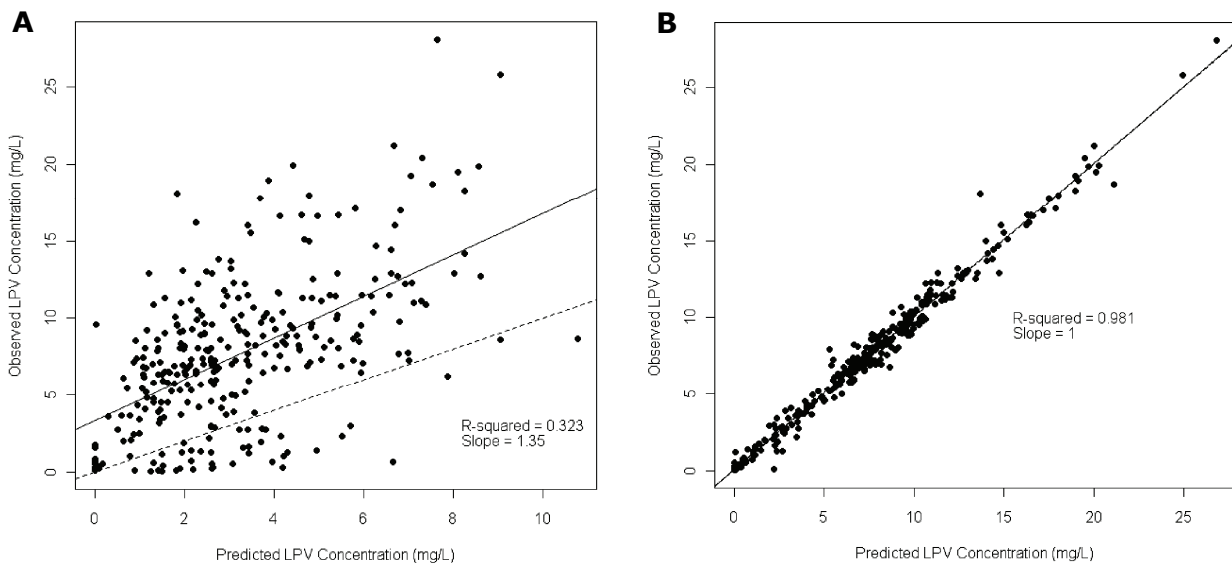
The quality of the fit for the model population, as measured by linear regression of observed vs predicted LPV concentrations, was poor for the model-based predictions (Figure 2A) but excellent for the Bayesian posterior predictions (Figure 2B), reinforcing the high degree of interindividual LPV PK variability. Plots of the residuals between observed and predicted concentrations with respect to either the Bayesian predicted concentration or time were homoscedastic and centered on zero (Figure 3). The visual predictive check showed that the model described the population well (Figure 4), despite the fact that the two  $C_{\text{trough}}$  values differed by more than 50% in half of the children (range, 5% to 9,000%), indicating that many were not at steady state. Finally, the advantage of the nonparametric approach is obvious in Figure 5, which shows that, for example, lag time is clearly not normally distributed and that, although the bulk of the children had delays in absorption of less than 30 min, a subgroup of 15 children had delays of up to approximately 2 h. This can be seen by careful inspection of Figure 1 for the trajectories that peak

much later than the majority. The LPV/RTV formulation was significantly associated with absorption lag time: children dosed with LPV/RTV liquid had a twofold-shorter median lag time than those dosed with capsules ( $P = 0.005$ ).

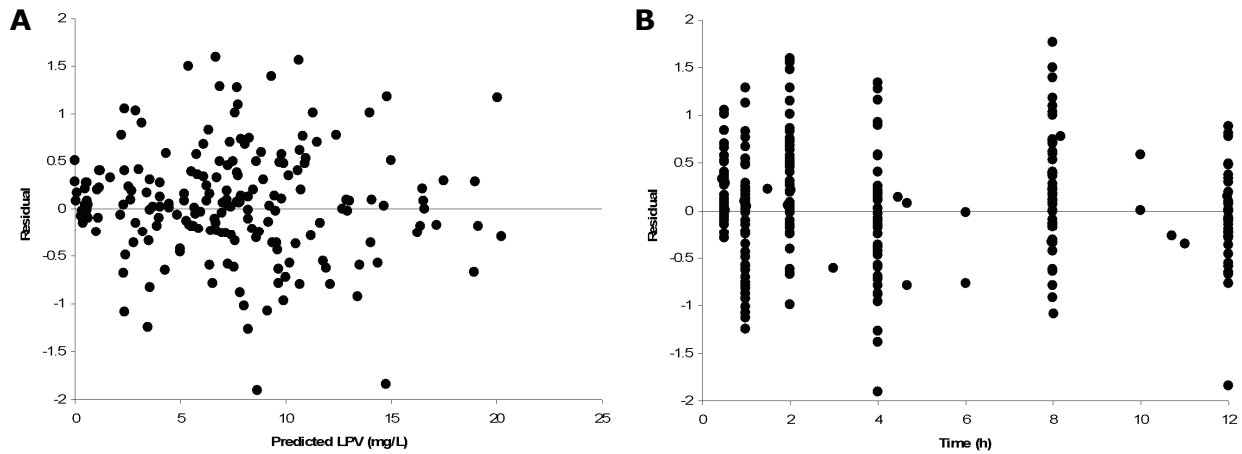
**Table 1** Estimated and calculated PK parameters in the final PK Model

Parameter <sup>a</sup>	Median (interquartile range)
Estimated	
$t_{lag}$ (h)	0.37 (0.12 - 0.86)
$k_a$ ( $h^{-1}$ )	0.64 (0.21 - 1.71)
$V$ (liters) = $\theta_1 \cdot wt/age^{\theta_2}$	25.15 (14.00 - 32.88)
liters/kg	0.69 (0.45 - 0.98)
$\theta_1$	15.04 (10.30 - 17.79)
$\theta_2$	11.44 (3.69 - 90.66)
$K_{el}$ ( $h^{-1}$ ) = $\theta_3 / wt^{0.25}$	0.09 (0.07 - 0.15)
$\theta_3$	0.22 (0.18 - 0.37)
$AUC_{0-12}$ (mg · h/liter)	96.09 (62.73 - 114.30)
$AUC_{\infty}$ (mg · h/liter)	190.40 (123.50 - 321.20)
Calculated	
$CL$ (liters/h) = dose/AUC (liters/kg <sup>0.75</sup> )	1.79 (1.00 - 2.57) 0.11 (0.07 - 0.20)
$CL$ (liters/h) = $k_{el} \cdot V$ (liters/kg <sup>0.75</sup> )	2.17 (1.39 - 3.18) 0.14 (0.09 - 0.24)
$t_{1/2}$ (h) = $\ln(2)/k_{el}$	7.69 (4.63 - 10.44)

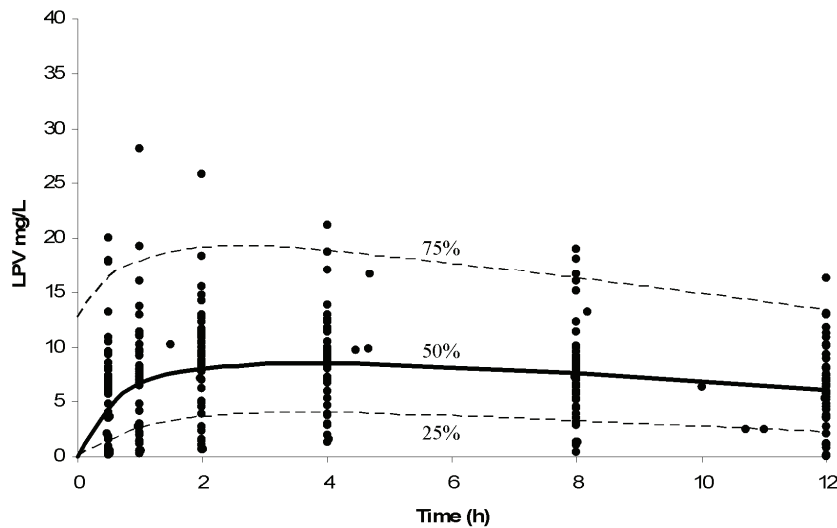
<sup>a</sup>  $t_{lag}$  – lag time;  $k_a$  – rate constant of absorption.



**Figure 2** Linear regression of individual observed vs predicted LPV concentrations. Predictions using mean model parameter values (A) and using the means of the individual Bayesian posterior parameter distributions (B) are shown. The solid line is the fitted regression line. The dashed line is the unity line, which is fully superimposed on the regression line in panel B.



**Figure 3** Model residual errors with respect to predicted LPV (A) and time (B).



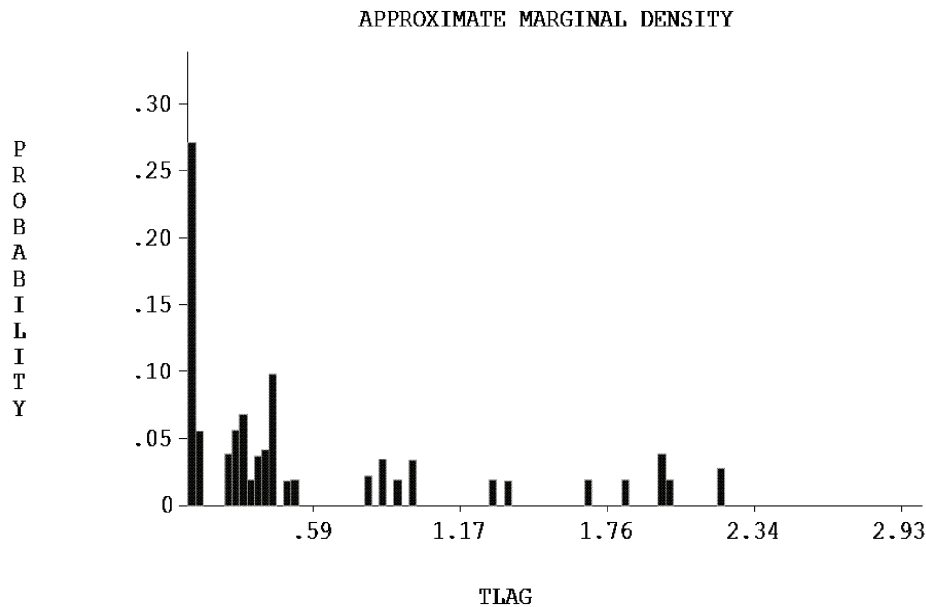
**Figure 4** Visual predictive check of concentration percentiles from model-simulated data (lines) superimposed on measured patient concentrations from the entire population (dots).

### Probability of achieving PIQ targets

As shown in Figure 6A, for wild-type virus, > 90% of children are predicted to achieve an LPV IQ of > 15. However, at the Phenosense (Monogram Biosciences, Inc., CA) or Vircotype (Virco Labs, Inc., NJ) cutoffs between fully sensitive and intermediate virus (ninefold and sixfold increases in  $IC_{50}$ , respectively), only 32% or 48% of children are likely to achieve a PIQ of > 15. If resistance increases to 20-fold, well within the "partially sensitive" range, which extends up to 55- and 51-fold, respectively, fewer than 10% of children are likely to achieve a PIQ of > 15, given the currently recommended LPV dose.

Considering the LPV contribution to virologic effect as a continuous function of the PIQ, it can be seen in Figure 6B that the standard dose of LPV will maintain > 90% LPV activity against wild-type virus in > 75% of children. Children whose LPV dose is sufficient to ensure a  $C_{trough}$  of 16.1 mg/liter (the 75th percentile for the population) will maintain at least 50% LPV effect until resistance increases to > 27

times that of the wild type; in contrast, children whose  $C_{\text{trough}}$  is only 2.9 mg/liter (the 25th percentile for the population) will lose 94% of their LPV effect at this same level of resistance. Alternatively, children whose LPV  $C_{\text{trough}}$  is at the 25th percentile will fall below a PIQ of 15 at a 2.8-fold increase in the  $IC_{50}$  compared to those at the 75th percentile, who will not drop below a PIQ of 15 until their viral  $IC_{50}$  is increased 15.4-fold relative to the wild type.



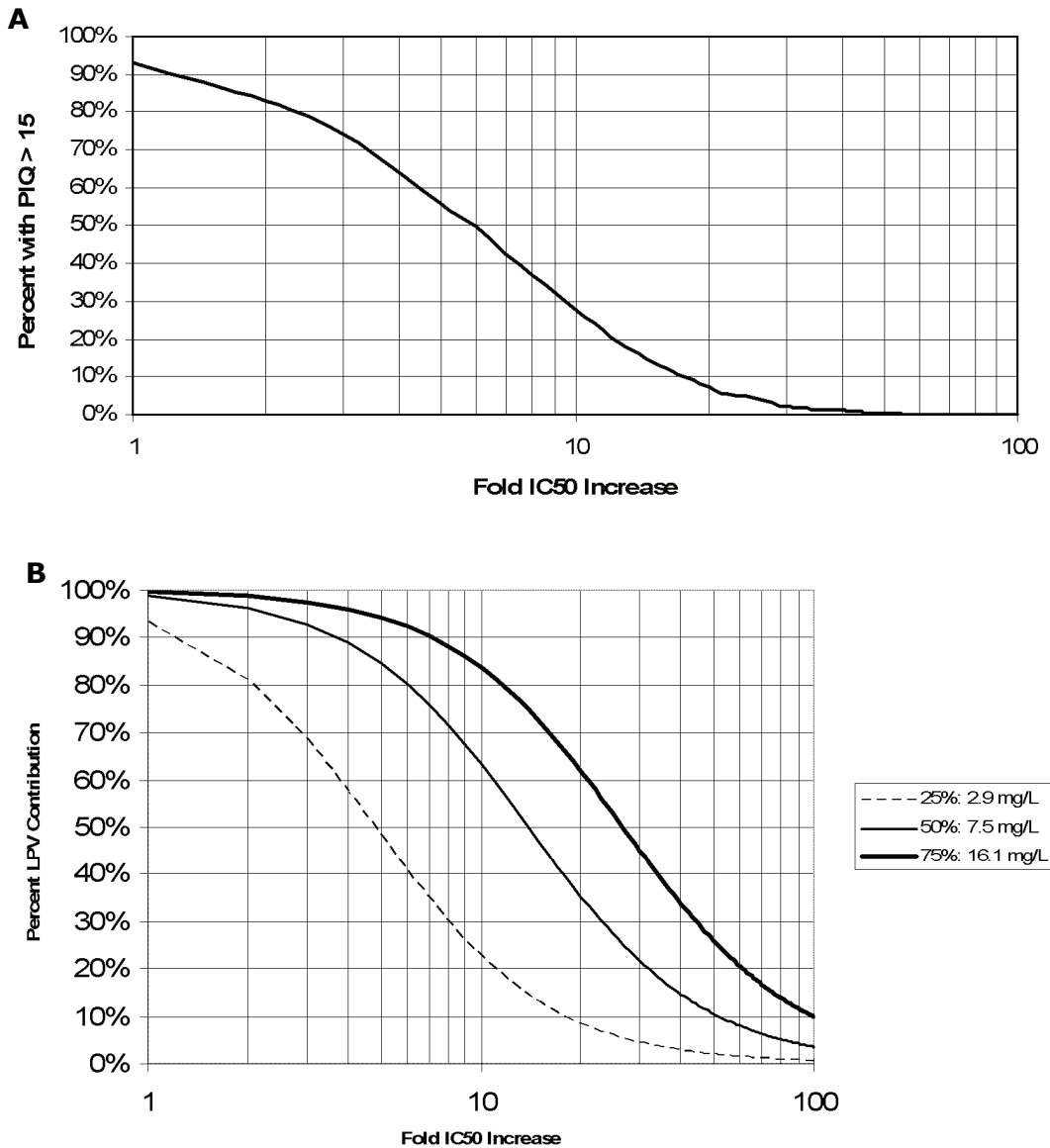
**Figure 5** Distribution of lag times in the population.

## DISCUSSION

In this study, a model of LPV disposition in children and adolescents 4 through 18 years of age was developed and validated by a visual predictive check of the distribution of LPV concentrations in 1,000 model-simulated patients compared with the distribution of LPV concentrations in the study population. In the final model, LPV was rapidly absorbed after an initial formulation-dependent delay, with an apparent  $V$  that increased nonlinearly as a function of weight over age. Overall, the drug CL varied according to weight<sup>0.75</sup>, in accordance with the allometric scaling principles in children suggested by Anderson *et al.*,<sup>24</sup> among others.

Because of high correlation between  $V$  and CL, the model was parameterized in terms of fractional elimination ( $k_{el}$ ) that declined with increasing weight. The CL- $V$  correlation indicates that in the model clearance of LPV was driven primarily by the apparent  $V$ , which raises the possibility that LPV bioavailability may have been variable. Due to the lack of an intravenous formulation, the absolute bioavailability of LPV has not been established. Close inspection of Figure 1 reinforces the fact that most of the LPV PK variability is in absorption and bioavailability, since after an individual trajectory peaks, it generally declines consistently thereafter. In other words, between- individual variability is larger than within-individual variability, which enables the close fit shown in Figure 2B vs that in Figure 2A.





**Figure 6** Relationship between LPV concentrations and antiviral effect. (A) Percentages of children who will achieve a steady-state PIQ target of at least 15 when given the standard dose of 230 mg/m<sup>2</sup> LPV/RTV twice daily for a given viral IC<sub>50</sub>. (B) Clinical activity of LPV plotted against increasingly resistant strains for the 25th, 50th, and 75th percentiles of LPV C<sub>trough</sub> concentrations in children given the standard dose.

In adults given the standard dose of 400/100 mg LPV/RTV twice daily, the Kaletra package insert reports a steady-state LPV AUC of 92.6 mg · h/liter, a C<sub>max</sub> of 9.8 mg/liter occurring 4 h after a dose, and a C<sub>trough</sub> of 7.1 mg/liter. For children given 230/57.5 mg/m<sup>2</sup> LPV/RTV twice daily, the package insert reports a steady-state LPV AUC of 72.6 mg · h/liter, a C<sub>max</sub> of 8.2 mg/liter occurring 4 h after a dose, and a C<sub>trough</sub> of 3.4 mg/liter. The children in our study received a median LPV dose of 275 mg/m<sup>2</sup>, which is 20% higher than the recommended dose. The median LPV AUC, C<sub>max</sub>, and C<sub>trough</sub> were correspondingly higher than those reported for children in the package insert and were similar to adult values, with the exception of the C<sub>trough</sub>, which was slightly lower than that of adults.

With large interpatient variability in LPV PK, it is very important to estimate the chance of a child on ART having suboptimal LPV exposure. Other studies have suggested that the currently recommended dose of LPV/RTV might result in suboptimal exposure in HIV-infected children.<sup>1,7,28</sup> Based on our PK model and the LPV pharmacodynamic models by Hsu *et al.* and Podzamczar *et al.*,<sup>14,15</sup> we have shown that the majority of children are unlikely to achieve therapeutic plasma LPV concentrations against virus that is moderately resistant to LPV, at degrees far below the clinical cutoffs suggested by current phenotypic resistance testing. Furthermore, patients with fewer than two active background antiretroviral agents would have even higher IQ targets<sup>14,15</sup> and would require correspondingly higher doses of LPV. It is this population of PI-experienced patients who would most likely derive direct benefit from measurement of plasma LPV concentrations and dose adjustment if necessary to avoid excessive dependence on the remaining drugs in the therapeutic regimen. Separate analysis in this cohort of HIV-infected children will compare the measured relationships of individual LPV PK and phenotypic and genotypic IQs to virologic responses.

## CONCLUSION

In this study, a model of LPV disposition in pediatric patients > 4 years of age was developed and validated by a visual predictive check of the distribution of LPV concentrations in 1,000 model-simulated patients compared with the distribution of LPV concentrations in the study population. Based on this PK population model, the currently recommended dose of LPV/RTV appears to be adequate for children infected with wild-type virus but is unlikely to provide adequate inhibitory concentrations for even moderately resistant HIV. PI-experienced HIV-infected children would likely benefit from longitudinal, repeated LPV drug measurement in plasma in combination with resistance evaluation to ensure that LPV dosing is sufficient to maximize the contribution of LPV to virologic control.

## ACKNOWLEDGMENTS

We thank the children who participated in this study, their families and caregivers, the clinic staff, and laboratory and Pediatric Clinical Research Center personnel for their dedication and support. This work was supported by Department of Health and Human Services, NIH, PHS grants NIBIB R01 EB005803-01A1 (M.N.N. and N.R.) and NIAID K23 AI076106-01 (M.N.N.), MO1-RR-020359 and NICHD 1U10 HD45993 (J.V.D.A.), and NCRR K12 RR017613 (N.R.).

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# 7

# chapter

***CYP3A5, MDR1 AND  
SLCO1B1  
POLYMORPHISMS  
AND THE  
PHARMACOKINETICS  
AND  
PHARMACODYNAMICS  
OF  
LOPINAVIR/RITONAVIR  
IN CHILDREN***

Rakhmanina N, Neely MN, van Schaik RH, Gordish-Dressman H, Williams K, Soldin S, van den Anker J.

*Submitted.*

## ABSTRACT

### Objective

*CYP3A5*, *MDR1* (*ABCB1*) and *OATP1* (*SLCO1B1*) polymorphisms have been associated with variability in the pharmacokinetics (PK) of protease inhibitors. The aim of this study was to investigate the influence of *CYP3A5* A6986G, *MDR1* (C3435T and G2677T), and *SLCO1B1* (T521C and A388AG) polymorphisms on the PK and pharmacodynamics (PD) of lopinavir/ritonavir (LPV/RTV) in HIV-infected children.

### Design and methods

Prospective cohort study in children (4 - 18 yrs old) on stable antiretroviral therapy with LPV/RTV. *CYP3A5*, *MDR1* and *SLCO1B1* genotypes were determined using PCR amplification with allelic discrimination assays. The 12 hr plasma area under the concentration-time curves (AUC) and clearances (CL) of LPV and RTV were estimated using non-compartmental models. Analysis of covariance models with adjustment for age and adherence were used to assess associations between studied polymorphisms and AUC, CL and HIV RNA.

### Results

50 children (median age 11.2 yrs) were enrolled. Allele frequencies were in Hardy-Weinberg equilibrium: for *CYP3A5* A6986G 0.42; for *MDR1* C3435T 0.31 and for G2677T 0.20; for *SLCO1B1* T521C 0.04 and for *SLCO1B1* A388G 0.70. There was no statistically significant association between LPV or RTV AUC or CL, and *CYP3A5*, *MDR1* or *SLCO1B1* A388G polymorphisms. There was a significant association between *SLCO1B1* T521C and LPV AUC ( $P = 0.042$ ) and a nearly significant association with LPV CL ( $P = 0.063$ ). Studied polymorphisms were not associated with virologic outcome.

### Conclusions

There was no influence of the *CYP3A5*, *MDR1* or *SLCO1B1* A388AG polymorphisms on the PK and PD of LPV/RTV in HIV-infected children. *SLCO1B1* T521C polymorphism was significantly associated with LPV AUC.

## INTRODUCTION

Lopinavir/ritonavir (LPV/RTV, Kaletra<sup>®</sup>, Abbott, Abbott Laboratories, North Chicago, Illinois) is a widely used protease inhibitor (PI) component of antiretroviral (ARV) therapy of paediatric patients due to co-formulation of LPV with RTV in liquid and tablet forms suitable for young patients. Limited data are available regarding the affect of genetic polymorphisms in drug metabolizing enzymes and drug transporters on the pharmacokinetics (PK) and pharmacodynamics (PD) of this boosted PI.

LPV and RTV are both essential substrates and inhibitors of the CYP3A enzymes, and variations in the CYP3A5 expression have been suggested to be potentially responsible for the inter-individual variability in the absorption and disposition of several PIs.<sup>1-4</sup> In addition to CYP3A enzymes, drug transporters may affect the disposition of PIs into tissues and cells.<sup>5</sup> P-glycoprotein (P-gp) expression and polymorphisms in the multidrug resistant (*MDR*) 1 (*ABCB1*) gene encoding for P-gp have been associated with variability in drug absorption and disposition, drug response, and toxicity of several PIs in HIV-infected adult patients.<sup>2,6-8</sup> Recent reports suggest an important role in the disposition of PIs of another major hepatic drug transporter: organic anion transporting polypeptide (OATP), coded by the *SLCO* genes.<sup>9,10</sup> The trough concentrations of LPV have been reported to be significantly increased in patients with the *SLCO1B1* 521T>C polymorphism.<sup>10</sup> Reduced uptake of LPV by hepatocytes in carriers of *SLCO1B1* T521T>C genotype has been suggested to be responsible for this effect, but the data on the clinical importance of *SLCO1B1* polymorphisms in LPV PK are lacking.<sup>9</sup>

While no association between the *MDR1* polymorphisms and LPV or RTV plasma trough concentrations has been reported in adult patients with HIV infection, in paediatric patients the *MDR1* G3435T polymorphism has been reported to affect plasma concentrations and virologic response to another PI, nelfinavir.<sup>11</sup> In addition, recent studies of immunosuppressive therapy in children have shown that the effect of *MDR1* polymorphisms on the PK of cyclosporine is related to age, and thus developmental stage.<sup>12,13</sup> These findings suggest that developmental changes in *MDR1* activity may need to be considered when evaluating the impact of genetic variants in children. To date no studies have evaluated the role of the CYP3A5, *MDR1* and OATP1B1 expression on the PK and PD parameters of LPV or RTV in HIV-infected children.

This study was originally designed to investigate whether genetic polymorphisms in *CYP3A5* A6986G, *MDR1* (C3435T and G2677T) affect the steady-state PK and PD of LPV and/or RTV in HIV-infected children. Following recent reports on the significance of *SLCO1B1* expression on the LPV PK in adults, we have also studied the effect of the genetic polymorphisms of *SLCO1B1* (T521C and A388G) on the steady-state PK of LPV/RTV and evaluated the effect of these polymorphisms on the

virologic outcome of LPV/RTV based therapy in paediatric patients in our initial study cohort.

## **PATIENTS AND METHODS**

Paediatric patients with HIV-1 infection (ages 4 - 18 years) receiving care at a large metropolitan paediatric HIV program at Children's National Medical Center (CNMC) and treated with ARV regimen based on Kaletra<sup>®</sup> as a single PI were eligible for study participation. The study protocol was reviewed and approved by the IRB at CNMC. Written informed consent was obtained from parents/legal guardians of children younger than 18 years of age. The subject's assent was obtained in children older than 7 years of age, unless the waiver of assent was required. Prior to study entry, all subjects had received uninterrupted, unchanged combination ARVs, which included twice daily LPV/RTV, for a minimum of 4 weeks. *CYP3A5*, *MDR1* and *SLCO1B1* genotypes were determined using PCR amplification followed by allelic discrimination assays based on the use of fluorogenic oligonucleotide probes (TaqMan) (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and direct sequencing analysis performed on ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Allele frequencies were identified and all three SNPs were determined to be in Hardy-Weinberg Equilibrium (HWE) using a 1 degree of freedom chi-square test.

The plasma PK samples were collected at times 0 (immediately before), 0.5, 1, 2, 4, 8, and 12 hrs after the observed dose. LPV and RTV concentrations were determined by a published validated tandem-mass spectrometric method using the Applied Biosystems/Sciex API-2000 (Foster City, California, USA).<sup>14</sup> Within-run precision (%CV) was below 7% and between-day precision was below 10% for all analytes at the tested concentrations. Accuracy ranged between 95% and 105%. Loss of assay precision (more than 15%) occurred at concentrations less than 10 ng/mL; hence, results below this limit were censored. The ARV therapeutic drug monitoring (TDM) laboratory at CNMC is an accredited member of the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection (University Medical Center Nijmegen, The Netherlands).

Patient adherence to LPV/RTV was assessed by an interactive 3-day-recall interview of the caregivers and children older than 10 years of age and was calculated as the percentage of the number of doses taken over the number of doses prescribed. HIV RNA viral load (Roche Amplicor, Branchburg, NJ, USA) and self-reported adherence were measured every 12 weeks for 52 weeks. The lower limit of quantitation of HIV RNA viral load (VL) was 400 copies/ml. The virological outcome was determined as Study Undetectable VL (SUVL) if ever achieving VL < 400 copies/ml during the study duration or Ever Undetectable VL (EUVL) if ever achieving VL < 400 copies/ml while on ARV therapy with LPV/RTV.



LPV and RTV area under the time-concentration curves from 0 to 12 hours (AUC) were estimated by the linear trapezoidal algorithm using the *R* statistical package (version 2.9.2, R Foundation, Vienna, Austria, available at <http://cran.r-project.org/>). Apparent oral clearance (CL) was estimated using the formula  $CL = F \cdot \text{Dose} / \text{AUC}$ , where *F* is bioavailability. Since *F* could not be estimated without intravenous dosing, it was fixed at a value of 1 (note that apparent oral clearance will be larger than clearance after intravenous dosing if *F* is less than 1).

Several statistical methods were used to measure the relationships between *CYP3A5*, *MDR1* and *SLCO1B1* polymorphisms and LPV/RTV AUC, CL and virologic outcome. Analysis of covariance models with adjustment for age was used to assess associations between polymorphisms and single parameters, multivariate analysis of covariance was used for associations between polymorphisms and multiple parameters, and logistic regression models were used to assess relationships between polymorphisms and virological outcome. LPV and RTV AUC and CL parameters were log-transformed to conform to normality and all analyses were performed using Stata V10 (College Station, TX, USA).

## RESULTS

The study enrolled 50 children (24 boys, 26 girls) with a median age of 11.2 years (4.3 - 17.2 years). The majority of the children were African American (43), with 3 patients of African origin, 1 Asian patient, and 3 Caucasian patients, 2 of whom were Hispanic. Median weight was 38.5 kg (15.4 - 90.5), median height was 139.4 cm (108.7 - 168.3) and a median BMI was 19.0 (12.8 - 33.0). Background regimens consisted of 2 NRTIs in 44 (88%) patients, 3 NRTIs in 2 patients, and 1 or 2 NRTIs plus NNRTI in 4 patients (LPV dose adjusted for NNRTI exposure). The median LPV/RTV dose was 275/69 mg/m<sup>2</sup> (246/62 - 287/72 mg/m<sup>2</sup>) and the median LPV exposure was 2.2 yrs (0.5 - 5.2 yrs). Mean self-reported adherence was 88% (41 - 100%). Of the 50 participants, 27 (54%) patients achieved VL < 400 copies/ml at least once during the study period (SUVL) and 36 (72%) achieved VL < 400 copies/ml at least once on LPV/RTV therapy (EUVL).

Of the 300 possible samples, 17 (5.6%) were missing or censored, and were equally distributed within the dosing interval. The median (range) LPV AUC and CL were 96.87 µg\*h/l (15.29 - 228.77) and 0.09 l/h/kg (0.04 - 0.44), respectively. For RTV, AUC and CL were 44.73 µg\*h/l (0.45 - 14.90) and 0.53 l/kg/h (0.15 - 3.74), respectively. As anticipated the AUC and CL of LPV had direct linear relationship with AUC and CL of RTV ( $r^2 = 0.755$ ;  $P < 0.0001$  and  $r^2 = 0.755$ ;  $P < 0.0001$ , respectively). The CL of LPV and RTV was statistically significantly associated with age ( $P = 0.008$  and  $P = 0.002$  respectively).

The allelic frequencies of the polymorphisms for *CYP3A5* A6986G and *MDR1* (C3435T and G2677T) and for *SLCO1B1* (T521C and A388G) were as follows:

*CYP3A5* (A6986G) - p(A) = 0.583, p(G) = 0.417 (HWE *P*-value = 0.113); *MDR1* (C3435T) - p(C) = 0.688, p(T) = 0.312 (HWE *P*-value = 0.834); *MDR1* (G2677T) - p(G) = 0.800, p(T) = 0.200 (HWE *P*-value = 0.693); *SLCO1B1* (T521C) - p(T) = 0.958, p(C) = 0.042 (HWE *P*-value = 0.763); *SLCO1B1* (A388G) - p(G) = 0.702, p(A) = 0.298 (HWE *P*-value = 0.595)

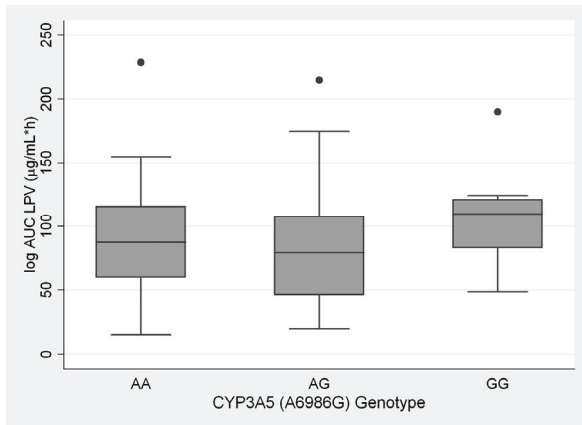
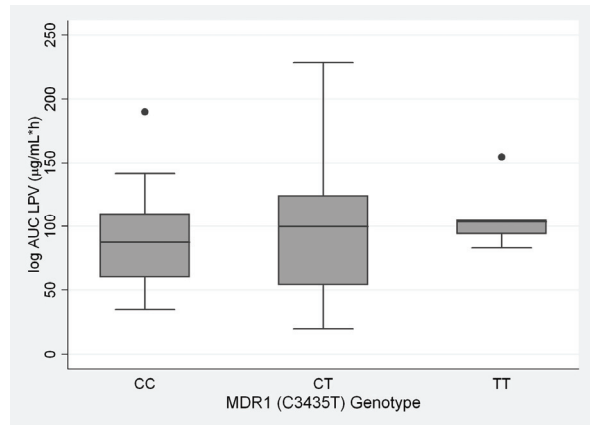
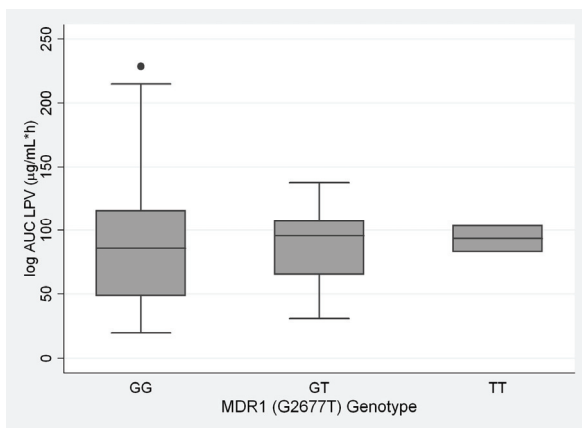
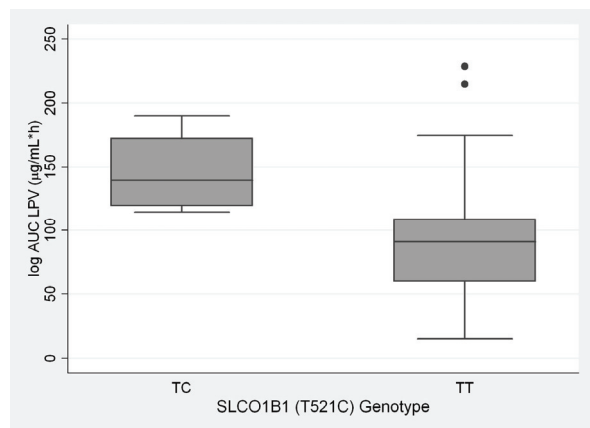
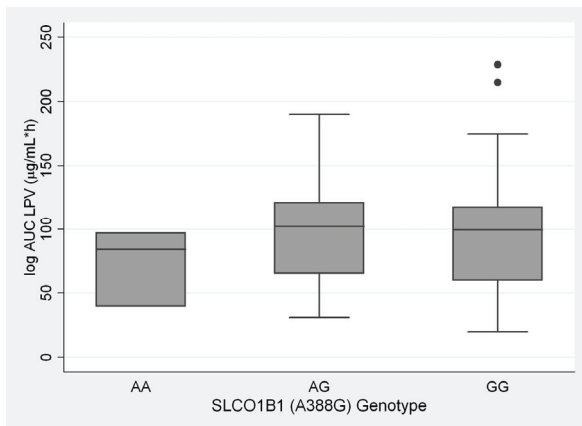
There was no statistically significant association between LPV or RTV AUC<sub>0-12</sub> and *CYP3A5*, *MDR1* C3435T and G2677T, and *SLCO1B1* A388G genotypes. (Figures 1, 2, 3 and 4) There was, however, a significant association between *SLCO1B1* T521C genotype and LPV AUC (*P* = 0.042), but no association between the same genotype and RTV AUC (*P* = 0.3453). (Figures 1 and 2) The *CYP3A5*, *MDR1* and *SLCO1B1* polymorphisms did not significantly influence the clearance of LPV and RTV in children, although *SLCO1B1* 521T>C genotype was nearly significantly associated with an increase in LPV CL (*P* = 0.063). (Figure 3 and 4) When linear models were adjusted by age no statistical difference was observed. The combined PK of LPV and RTV (RTV AUC + LPV CL) were equally not affected by the *CYP3A5*, *MDR1* and *SLCO1B1* genotypes. (Table 1)

*CYP3A5*, *MDR1* and *SLCO1B1* polymorphisms had no statistically significant association with virologic suppression (VL < 400 copies/ml) during the study period (SUVL) or ever achieved with LPV/RTV therapy (EUVL). (Table 2) The multiple SNP analysis did not reveal significant association between *CYP3A5*, *MDR1*, and *SLCO1B1* combined genotypes and PK and PD parameters of LPV/RTV therapy. (Table 3)

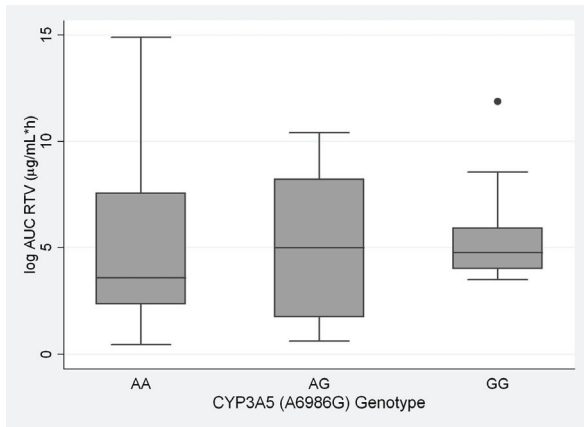
**Table 1** Analysis of combined PK parameters (LPV AUC and RTV CL) in association with each SNP

Covariates	Phenotypes	SNP	Wald's <i>P</i> -value for SNP effect
None*	RTV AUC <sup>§</sup> + LPV CL <sup>§§</sup>	<i>CYP3A5</i> A6986G	0.6638
	RTV AUC + LPV CL	<i>MDR1</i> C3435T	0.9317
	RTV AUC + LPV CL	<i>MDR1</i> G2677T	0.9626
	RTV AUC + LPV CL	<i>OATP1B1</i> A388G	0.8730
	RTV AUC + LPV CL	<i>OATP1B1</i> T521C	0.1017
Age	RTV AUC + LPV CL	<i>CYP3A5</i> A6986G	0.6580
	RTV AUC + LPV CL	<i>MDR1</i> C3435T	0.7367
	RTV AUC + LPV CL	<i>MDR1</i> G2677T	0.9316
	RTV AUC + LPV CL	<i>OATP1B1</i> A388G	0.8959
	RTV AUC + LPV CL	<i>OATP1B1</i> T521C	0.1209

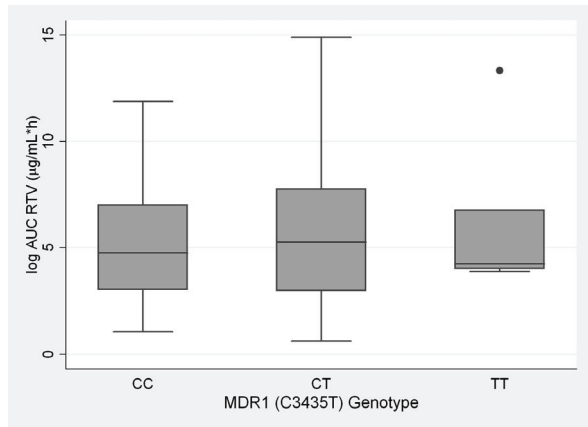
<sup>§</sup> AUC<sub>0-12</sub>; <sup>§§</sup> CL<sub>0-12</sub>; \* Unadjusted analysis.

**1a****1b****1c****1d****1e**

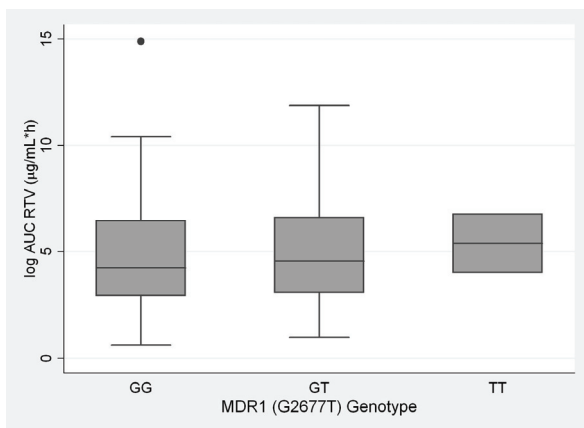
**Figure 1** Association between LPV AUC and CYP3A5, MDR1, and SLCO1B1 genotypes: 1a) CYP3A5 A6986G; 1b) MDR1 C3435T; 1c) MDR1 G2677T; 1d) SLCO1B1 T512C; 1e) SLCO1B1 A388G.



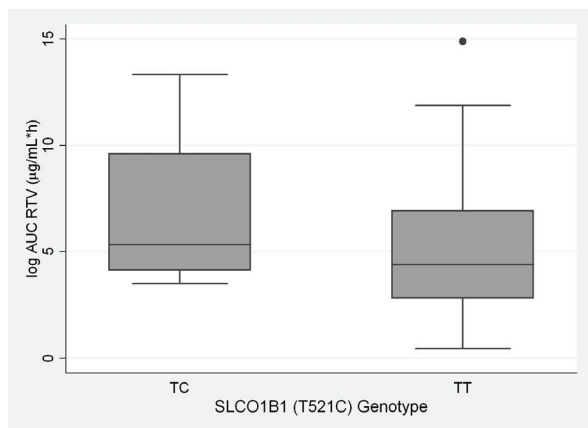
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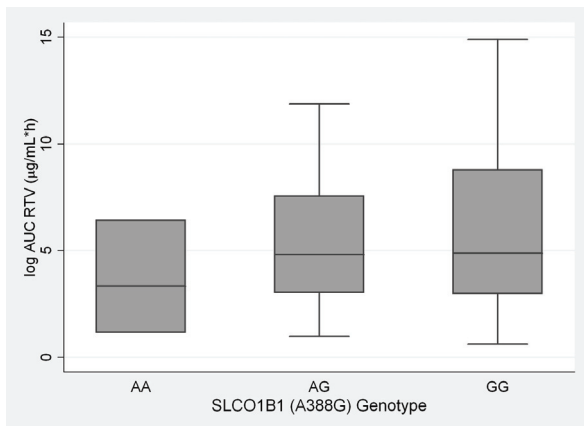
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2c

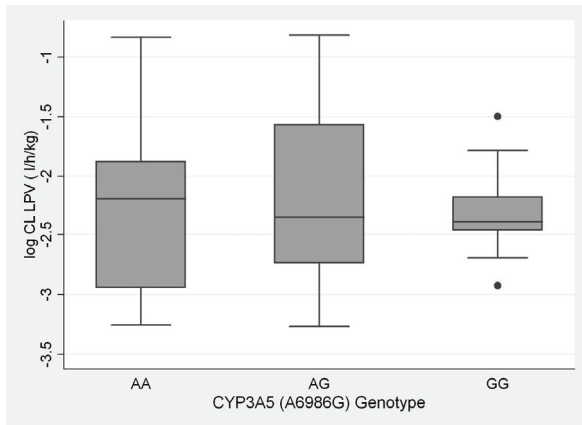
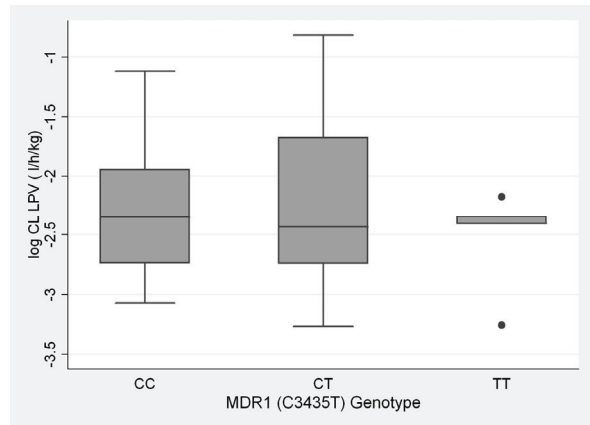
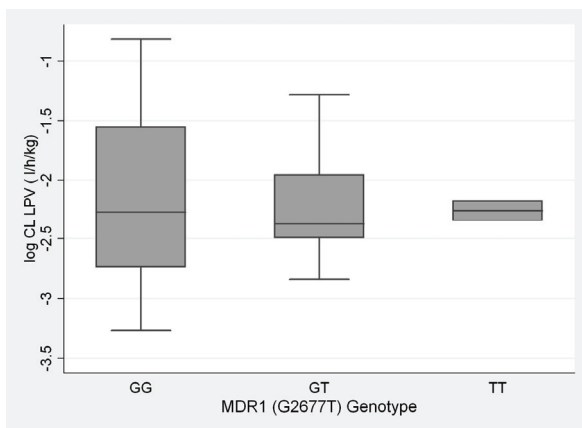
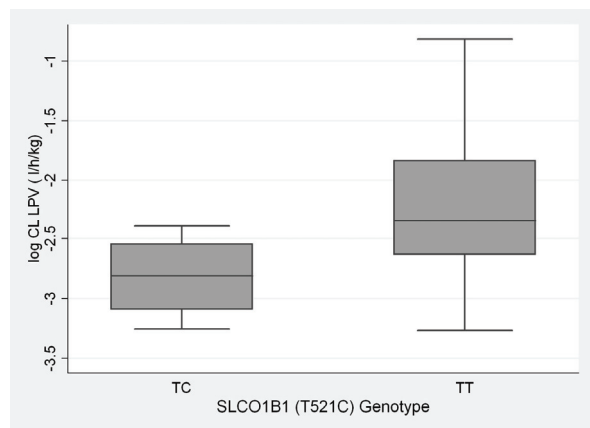
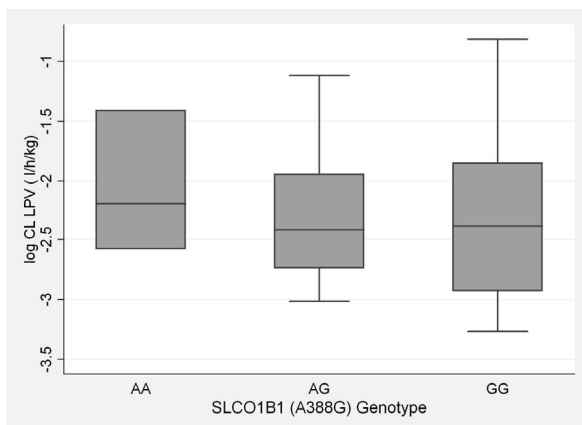


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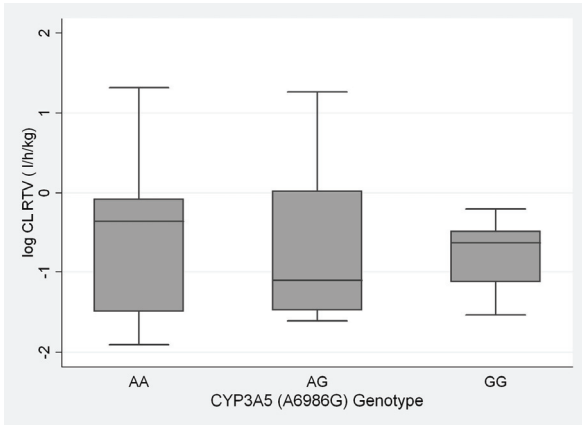


2e

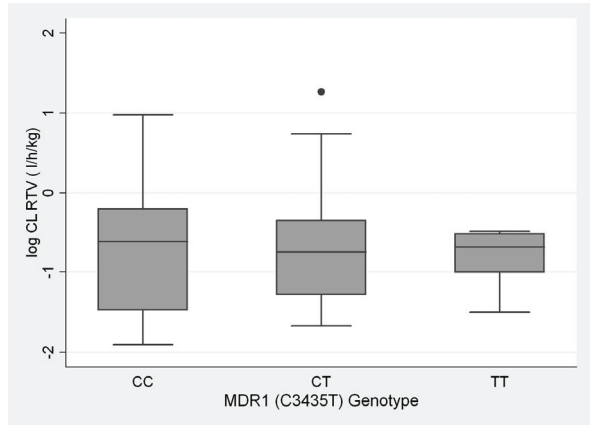
**Figure 2** Association between RTV AUC and CYP3A5, MDR1, and SLCO1B1 genotypes: 2a) CYP3A5 A6986G; 2b) MDR1 C3435T; 2c) MDR1 G2677T; 2d) SLCO1B1 T521C; 2e) SLCO1B1 A388G.

**3a****3b****3c****3d****3e**

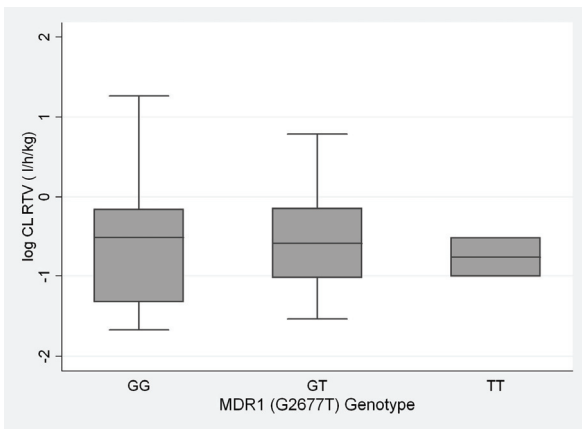
**Figure 3** Association between LPV CL and CYP3A5, MDR1, and SLCO1B1 genotypes: 3a) CYP3A5 A6986G; 3b) MDR1 C3435T; 3c) MDR1 G2677T; 3d) SLCO1B1 T521C; 3e) SLCO1B1 A388G.



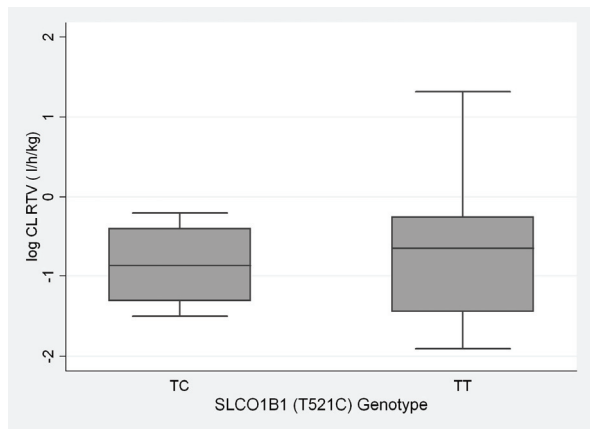
4a



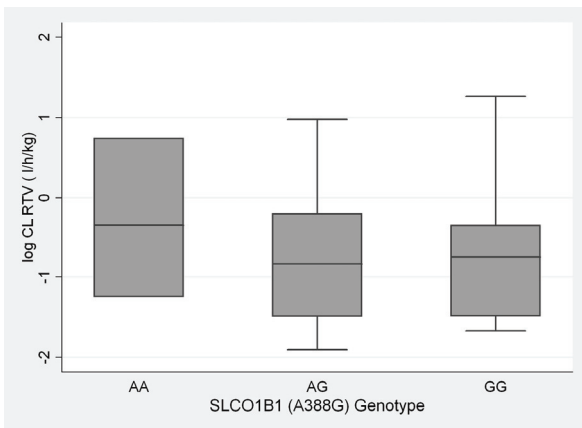
4b



4c



4d



4e

**Figure 4** Association between RTV CL and CYP3A5, MDR1, and SLCO1B1 genotypes: 4a) CYP3A5 A6986G; 4b) MDR1 C3435T; 4c) MDR1 G2677T; 4d) SLCO1B1 T521C; 4e) SLCO1B1 A388G.

**Table 2** Associations between virologic outcome and SNPs: analysis adjusted for study medical adherence and age

Phenotype	SNP	Genotype	Number of No	Number of Yes	OR*	P-value	95% CI	P-value**
SULV400 <sup>§</sup>	CYP3A5 A6986G	AA	9	10	1.00			
		AG	7	11	1.072	0.924	0.255 - 4.522	
		GG	6	5	2.119	0.366	0.416 - 10.790	0.3889
	MDR1 C3435T	CC	11	12	1.00			
		CT	9	11	0.955	0.942	0.275 - 3.312	
		TT	2	3	0.656	0.684	0.086 - 5.001	0.7216
	MDR1 G2677T	GG	15	11	1.00			
		GT	7	5	0.971	0.968	0.236 - 3.988	
		TT	0	2				
	SLCO1B1 A388G	GG	8	12	1.00			
		AG	9	10	1.281	0.714	0.341 - 4.816	
		AA	2	1	2.667	0.468	0.189 - 37.712	0.5921
TT		24	20	1.00				
SLCO1B1 T521C	TC	2	2	1.828	0.576	0.220 - 15.166		
	CC	0	0					
	AA	6	13	1.00				
EULV400 <sup>§§</sup>	CYP3A5 A6986G	AA	6	13	1.00			
		AG	4	14	0.762	0.736	0.157 - 3.702	
		GG	3	8	1.012	0.990	0.176 - 5.836	0.7488
	MDR1 C3435T	CC	6	17	1.00			
		CT	7	13	1.676	0.451	0.437 - 6.421	
		TT	0	5				
	MDR1 G2677T	GG	10	16	1.00			
		GT	2	10	0.280	0.156	0.048 - 1.623	
		TT	0	2				
	SLCO1B1 A388G	GG	4	16	1.00			
		AG	5	14	1.321	0.723	0.283 - 6.157	
		AA	1	2	1.862	0.653	0.123 - 28.042	0.8054
SLCO1B1 T521C	TT	13	31	1.00				
	TC	0	4					
	CC	0	0					

<sup>§</sup> Undetectable viral load during the study; <sup>§§</sup> Undetectable viral load ever on ARV therapy that included LPV/RTV; \* Yes is the reference group; \*\* P-value for comparison of homozygous and heterozygous mutants.

**Table 3** Multiple SNP analysis: for each phenotype as the outcome (dependent) variable, all 5 polymorphisms are included in the model, with and without age as an additional covariate. *P*-values are for the effect of each covariate

Phenotype	Age effect	CYP3A5 A6986G effect	MDR1 C3435T effect	MDR1 G2677 effect	OATP1B1 A388G effect	OATP1B1 T521C effect
	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value
LPV AUC <sup>§</sup>	None*	0.725	0.950	0.982	0.945	0.190
LPV CL <sup>§§</sup>		0.861	0.973	0.938	0.9075	0.328
RTV AUC <sup>§</sup>		0.705	0.935	0.908	0.750	0.716
RTV CL <sup>§§</sup>		0.952	0.952	0.902	0.797	0.658
SUVL400		0.594	0.803	0.401	0.894	
EUVL400		0.149	0.907	0.098	0.744	
LPV AUC	0.126	0.710	0.897	0.990	0.986	0.258
LPV CL 12	0.033	0.959	0.708	0.928	0.893	0.455
RTV AUC	0.027	0.499	0.947	0.952	0.967	0.474
RTV CL 12	0.008	0.764	0.746	0.931	0.999	0.372
SUVL400	0.803	0.577	0.816	0.410	0.876	
EUVL400	0.991	0.153	0.907	0.099	0.745	

<sup>§</sup> *AUC*<sub>0-12</sub>; <sup>§§</sup> *CL*<sub>0-12</sub>; \* *Unadjusted analysis*.

## DISCUSSION

The *CYP3A5* 6986A allelic frequency varies from approximately 50% in African American to 90% in Caucasian populations.<sup>15</sup> *CYP3A5* has been detected in measurable amounts in 10% - 30% of adult white population and in up to 60% of African American.<sup>16,17</sup> The allelic frequencies in our study of predominantly African American children demonstrate the higher prevalence of wild-type, active enzyme encoding allele of the *CYP3A5* gene in African American population. The allelic frequency of *MDR1* 3435C was seen in the majority of our patients in accordance with previously reported high frequency in the African American population.<sup>15,18</sup> The incidence of *MDR1* 2677G allele was predominant in our study cohort and was higher than reported in the literature for African American adults.<sup>15,19</sup> The allelic frequencies of *SLCO1B1* 521C polymorphisms are known to be rare in patients of African origin, and were very low in our study population of predominantly African American children (comparable with the data from Brazilian HIV-infected population).<sup>10,20,21</sup> The incidence of *SLCO1B1* 388G allele was predominant in our population in accordance with published distribution in African subjects.<sup>20,21</sup>

In our study neither LPV nor RTV AUC and CL were affected by the *CYP3A5* A6986G polymorphism. These data correlate with previously published data on the lack of effect of *CYP3A5* A6986G on LPV and RTV trough plasma concentrations in adults.<sup>22</sup> The study by Estrela *et al.* also did not find an association between another *CYP3A5* G14690A polymorphism and trough plasma concentrations of LPV/RTV and ascribed



the overall lack of the CYP3A5 influence on the PK of LPV/RTV to the inhibition of the CYP3A5 activity by RTV.<sup>22</sup> Both *MDR1* C3435T and G2677T polymorphisms and their haplotypes also had no effect on plasma AUC and CL of LPV/RTV as previously reported in adult studies.<sup>23-26</sup> The most recent adult study evaluating LPV plasma trough concentrations in plasma, semen and saliva of adult HIV-infected men found no association with *MDR1* C1236T, T2677A and C3435T genotypes and haplotypes.<sup>27</sup>

The reported effects of the *MDR1* SNPs on the outcome of LPV/RTV based antiretroviral therapy have been controversial. *MDR1* C3435T polymorphism has been associated with CD4 cell recovery in HIV-infected adult patients by Fellay *et al.*,<sup>24</sup> but not by other studies.<sup>23,28</sup> *MDR1* C3435T polymorphism has been associated with HIV viral load outcome with earlier virologic failure reported in adult HIV-infected patients with 3435CC genotype.<sup>29</sup> These data have not been confirmed by Haas *et al.*,<sup>23</sup> who reported that the allelic *MDR1* G2677T and C3435T variants were not associated with difference in the rate of phase 1 viral decay. Our study found no association between the *MDR1* polymorphisms and the virologic outcome in treatment-experienced HIV-infected children.

Our data on the significance of *SLCO1B1* T521C polymorphism on the trough concentrations of LPV are consistent with recently published reports from the adult data from Brazil and England.<sup>9,10</sup> The study by Hartkoorn *et al.* has evaluated the effect of *SLCO1A1*, *SLCO1B1* and *SLCO1B3* on the LPV plasma concentrations in adult subjects (> 18 years) at 10 - 14 hours post dose (326 patients) and 2 - 6 hours post dose (293 patients). No data on ethnic/racial background or virologic outcome were available. The study combined the data from a clinical database with *in vitro* research evaluating PIs as substrates to the OATP1A2, OATP1B1 and OATP1B3 in *Xenopus laevis* oocyte model. The data from the *X. laevis* oocyte model showed that the PIs saquinavir and LPV are substrates to OATP1A2, OATP1B1 and OATP1B3, while RTV had no inhibitory effect on OATP1B1. In human samples, however, no association was found between *SLCO1A2* and *SLCO1B3* and LPV plasma concentrations. In contrast, the data from human samples showed a significant association between *SLCO1B1* 521T to C polymorphism with higher LPV plasma concentrations at both time points studied.<sup>9</sup> Significantly higher median plasma LPV  $C_{min}$  and  $C_{2-6}$  were observed in patients that were homozygous for the C allele at position 521 of *SLCO1B1* compared with either T homozygotes or heterozygotes. The study by Kohlrausch *et al.* in Brazilian HIV-infected men showed similar association between *SLCO1B1* 521 t to C polymorphism with higher LPV  $C_{min}$  when compared with wild-type genotype.<sup>10</sup>

Similar findings of the significant effect of *SLCO1B1* polymorphisms (T521C and A388G) on the PK of other OATP substrates such as statins have been reported and the *SLCO1B1* polymorphism has been linked to be associated with statin-induced myopathy.<sup>30-33</sup> As a result of these findings, the speculation about the association of *SLCO1B1* polymorphism and increased risk of resistance to LPV and virologic failure has been made. Our study is the first to evaluate the clinical significance of the

*SLCO1B1* polymorphisms. In our study cohort of experienced HIV-infected paediatric patients, the *SLCO1B1* polymorphisms (A388G and T521C) had no statistically significant association with virologic outcome (undetectable virologic suppression < 400 copies/ml) during the study period or ever achieved with LPV/RTV based regimen. We recognize that CYP3A, MDR1 and *SLCO1B1* expression may be affected by HIV infection and concomitant ARV therapy, and hence may be a confounding variable in determining drug response.<sup>34-37</sup> The full spectrum of the substrates/suppressor/inducer interaction between CYP3A enzymes, P-gp, OATP and ARV therapy and HIV infection is complex and remains to be determined.

Finally, as we reported in our population PK analysis for LPV,<sup>38</sup> in this study we also found a significant association between RTV clearance and age, due to age related changes in apparent volume of distribution that were independent of weight. For both drugs, the weight-adjusted volume of distribution in l/kg on average diminishes with age, which is likely a reflection of the age-related reduction in extracellular body water as a percentage of total body weight.<sup>39</sup>

This study has several limitations. First, the sample size of this paediatric cohort from our single center is relatively small. Second, we did not evaluate the contribution of the resistance to ARV drugs to the virologic outcome. Finally, the adherence in the study was evaluated through self-report and is subject to recall bias.

## CONCLUSIONS

These data add to our understanding of the factors that contribute to variability in plasma concentrations of protease inhibitors. Our data showed the impact of the *SLCO1B1* T521C polymorphism on LPV AUC and CL. There was no influence of the *CYP3A5* A6986G, *MDR1* (C3435T and G2677T) and *SLCO1B1* A388AG polymorphisms on the PK of LPV and RTV in children. This is the first study to evaluate the clinical significance of *MDR1* and *SLCO1B1* polymorphisms on the outcome of HIV infection in paediatric patients. The virologic outcome of LPV/RTV based HAART was not affected by the *MDR1* or *SLCO1B1* polymorphisms in the treatment experienced HIV-infected children and adolescents. Age had a statistically significant effect on the clearance of LPV and RTV. The full spectrum of the substrates/suppressor/inducer interaction between CYP450 enzymes, P-gp, OATP and ARV therapy and HIV infection is complex and remains to be determined.

## ACKNOWLEDGMENTS

We would like to thank the children who participated in this study, their families and caregivers, the clinic staff, laboratory and Pediatric Clinical Research Center personnel for their dedication and support.

This work was supported by Department of Health and Human Services, NIH PHS grants NIBIB R01 EB005803-01A1(MN, NR), NCRR K12RR017613 (NR), NICHD K23HD060452 (NR), NIAID K23 AI076106-01 (MN), MO1-RR-020359 (NR, JNA), and NICHD 1U10 HD45993 (JNA).

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**III**  
**PART**

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**THE ROLE OF  
THERAPEUTIC DRUG  
MONITORING IN  
THE THERAPY OF  
PEDIATRIC HIV  
INFECTION**





# 8

## chapter

**NEVIRAPINE  
CONCENTRATION  
IN NONSTIMULATED  
SALIVA:  
AN ALTERNATIVE TO  
PLASMA SAMPLING IN  
CHILDREN WITH  
HUMAN  
IMMUNODEFICIENCY  
VIRUS INFECTION**

Rakhmanina NY, Capparelli EV, van den Anker JN, Williams K, Sever JL, Spiegel HM, Soldin SJ.

*Ther Drug Monit.* 2007;29:110-117.

## **ABSTRACT**

### **Background**

The monitoring of nevirapine (NVP) concentrations in pediatric patients has gained interest since the introduction of NVP as part of the preferred first-line antiretroviral regimen for human immunodeficiency virus (HIV)-infected children in resource-limited settings. Adequate trough concentrations of NVP predict successful therapy, whereas subtherapeutic levels are correlated with virologic failure and development of resistance. The aim of this study was to determine the extent of agreement between total and free plasma NVP concentrations and nonstimulated saliva NVP concentrations and to evaluate the feasibility of saliva sampling as an alternative tool for therapeutic drug monitoring of NVP in children.

### **Design and methods**

The study was designed as an observational cohort analysis. NVP concentrations were obtained in paired plasma and saliva samples of pediatric patients receiving antiretroviral therapy, including NVP. NVP plasma and saliva concentrations were determined by a tandem-mass spectrometric method. The intraclass correlation coefficient and Bland-Altman analysis were used to evaluate agreement and to assess pattern in any discrepancies between measurements.

### **Results**

For the random paired plasma and saliva NVP sampling, 19 African-American children (8 boys, 11 girls) with a median age of 8.0 years were enrolled. Two male subjects were recruited for the 12 hour NVP plasma and saliva pharmacokinetics study. The intraclass correlations between saliva and serum measurements of NVP concentrations indicated > 90% agreement between these two modes of measurement. The saliva concentrations reflected the free concentrations very closely but were on average 34% higher. The Bland-Altman plots indicated that the discrepancy between saliva and plasma measures is consistent across the range of average NVP concentrations.

### **Conclusions**

Our study results strongly indicate agreement between saliva and plasma NVP concentrations in pediatric patients with HIV infection, on the basis of Bland-Altman analysis. Nonstimulated NVP saliva concentrations can be used as an alternative noninvasive, reliable, cost-effective method for direct measurement of adherence and application of therapeutic drug monitoring in NVP therapy.

## INTRODUCTION

Treatment of HIV infection in pediatric patients is complicated by the need for multiple drug therapy and lack of palatable, easy-to-use pediatric formulations with reliable pharmacokinetics. Nevirapine (NVP) is one of the few antiretroviral drugs that is well suited for infants and children, and the use of NVP in children is associated with excellent clinical and immunological responses.<sup>1</sup> The administration of NVP is convenient, the taste of the liquid preparation is well tolerated, and the pill burden is low, which favors adherence to therapy. NVP-based highly active antiretroviral therapy (HAART) regimens are cost-effective, are available as generic formulations, and do not require refrigeration and for these reasons are the preferred first-line antiretroviral strategy for infants and children in resource-limited settings.<sup>2</sup> Single-dose NVP is also a highly cost-effective strategy to reduce perinatal HIV-1 transmission and has become an important component of many programs for preventing mother-to-child transmission (PMTCT) in resource-limited countries.<sup>3,4</sup>

Adequate levels of NVP predict successful therapy whereas subtherapeutic levels are correlated with virologic failure.<sup>5,6</sup> Higher values of NVP exposure are associated with greater initial clearance of HIV-1 RNA from plasma and higher probability of achieving undetectable levels of plasma HIV-1 RNA with more sustained suppression of viral replication.<sup>5</sup> The genetic threshold for development of NVP resistance is low, and cross-class non-nucleoside reverse transcriptase (NNRTI) resistance of HIV-1 can evolve after reverse transcriptase (RT) single amino-acid substitutions. Low NVP concentrations ( $\leq 3$  mg/l) are associated with a 5-fold increase in virologic failure.<sup>6</sup> Patients receiving NVP as part of their HAART regimen are also at high risk for developing treatment-limiting toxicity. During pregnancy, severe NVP-induced hepatotoxicity has been associated with CD4+ counts above  $350/\text{mm}^3$  and with the introduction of NVP in late gestation.<sup>7</sup> Whereas a definite relationship between hepatotoxicity and NVP concentrations is confounded by other risk factors, including pre-existing hepatic dysfunction, viral hepatitis and high CD4+ cell counts in several case reports indicate a causal relation between NVP concentrations and elevation of liver transaminase levels.<sup>8,9</sup> Therefore, optimal use of NVP may require maintaining concentrations within a narrow range.

NVP is metabolized by enzymes of the CYP3A and CYP2B6 families, creating a solid base for multiple drug-drug interactions.<sup>10,11</sup> Many drugs used for the treatment of infectious complications of HIV infection and acquired immunodeficiency syndrome (AIDS), such as ketoconazole, fluconazole, rifampin, and rifabutin, as well as herbal supplements (St. John's wort) have shown significant interactions with antiretroviral drugs, leading to toxicity-related complications and subtherapeutic concentrations.<sup>12,13</sup> For patients in developing countries, possible interactions of NVP with commonly used drugs or local herbal supplements are less well studied. It has been shown, however, that sulfadoxine-pyrimethamine, commonly used as prophylaxis for malaria, can lead to adverse drug reactions and overlapping toxicity

in pregnant women who receive daily nevirapine and/or zidovudine for the prevention of perinatal transmission of HIV.<sup>14</sup> The ability to determine NVP concentrations in the case of drug interaction-induced toxicity or treatment failure may lead to appropriate NVP dose adjustment with clinical and laboratory follow-up.

Because of the long half-life of NVP, a single NVP plasma concentration can be used for accurately estimating the area under the plasma concentration-time curve 12 hours after dosing ( $AUC_{0-12h}$ ). The data suggest that a pharmacokinetic assessment with a single, nontrough, clinic sample can be used to accurately monitor NVP therapy and allow optimization of therapy with dose modifications.<sup>15</sup> Samples for therapeutic drug monitoring (TDM) purposes may be taken without regard to time between intake and sampling. The minimal effective concentration (MEC) for NVP, based on clinical outcome data, has been reported to be 3400 ng/ml.<sup>5</sup> Although obtaining a single NVP plasma concentration sample is easier than determining full or partial pharmacokinetic profiles with multiple sampling, noninvasive methods for determining NVP concentration would gain greater acceptance in pediatric and resource-limited settings. Previous studies have suggested that saliva assessment can be used as a noninvasive method for TDM of several antiretroviral drugs.<sup>16-18</sup> The purpose of this study was to determine the extent of agreement between total and free plasma NVP concentrations and nonstimulated saliva NVP concentrations in order to evaluate the feasibility of saliva sampling as an alternative tool for TDM of NVP in children.

## **MATERIALS AND METHODS**

### **Patients**

The study represents a subgroup analysis of a pilot study of 50 subjects that was designed to assess the relationship between saliva and plasma levels of multi-HAART regimens. Pediatric patients with HIV-1 infection (aged 4 to 14 years) receiving care at a large metropolitan pediatric HIV program in the United States (Special Immunology Service at Children's National Medical Center) and receiving HAART including NVP were eligible for study participation. The research protocol, parental consent, and assent documents (for children older than 7 years of age) as well as the confidentiality documents for the protection of personal health information (HIPAA) were approved by the institutional review board.

Patient adherence to NVP was assessed by interviewing all caregivers as well as those children older than 10 years of age and able to self-report drug intake. Patients with reported adherence < 75 % (more than three missed drug doses during 1 week) were excluded. All subjects in this study had been on uninterrupted, unchanged, twice-daily dosed NVP-based HAART for at least 4 weeks.

Blood samples for the determination of NVP concentration were collected through venipuncture during routine clinical visits. An extra 2 ml of blood was obtained from

all participants, in addition to the samples for standard of care laboratory tests. Nonstimulated saliva samples were collected within 10 minutes of the blood samples. After rinsing the mouth with water [children of all ages swished and rinsed twice with clean tap water (100 cc) offered in a plastic cup], participants were asked to spit into a small plastic cup, and 0.25 ml of saliva was transferred into a clean tube with a syringe. The time after the last dose and dosing regimen were recorded.

In addition to single sample determinations, two patients who had been recruited for a separate institutional review board-approved pediatric study of 12 hour plasma pharmacokinetic profiles of antiretroviral agents received NVP as part of their HAART and consented to concurrent saliva sampling for NVP determination. Both subjects ingested NVP under direct observation with a light snack (apple juice and one cracker). Plasma and saliva samples were obtained before and 0.5, 1, 2, 4, 8, and 12 hours after observed ingestion of NVP in these subjects.

### **Tandem-mass spectrometry analysis**

For the determinations of total and free plasma NVP concentrations, 2 ml of venous blood was obtained. The blood was collected in a heparin tube and separated by centrifugation at 4000 rpm for 10 minutes. Plasma was stored at -70°C pending analysis. Saliva samples were directly frozen upon collection at -70°C pending analysis. They were thawed at room temperature for 20 minutes and centrifuged at 4000 rpm for 10 minutes at the laboratory at the time of the analysis to separate the mucus. The plasma and saliva samples were incubated for 30 minutes at 56°C to deactivate infectious HIV-1 particles. The heating of the sample has been proven not to produce degradation of NVP during heat incubation.<sup>19,20</sup>

Unbound NVP was separated with Centrifree YM-30 ultrafiltration devices (30,000 MW cut-off; Millipore, Bedford, MA). The drug plasma and saliva concentrations were determined by a tandem-mass spectrometric method with use of the Applied Biosystems/Sciex API-2000.<sup>19,20</sup> Within-run precision [% coefficient of variation (CV)] was below 7% and between-day precision (%CV) was below 10% for all analytes at the tested concentrations. Accuracy ranged between 95% and 105%. The lower limit of quantitation was at 10 ng/ml. No interference by concurrent medications has been reported to be associated with an assay. Cimetidine is used as the internal standard; therefore, cimetidine therapy was considered to be an exclusion criterion for participation in the study.<sup>19,20</sup>

The antiretroviral TDM laboratory at Children's National Medical Center, which performed the NVP assays, is an accredited member of the International Quality Control Program for Therapeutic Drug Monitoring in HIV Infection (University Medical Center Nijmegen, The Netherlands).<sup>21,22</sup>

### **Data analysis**

The analysis of the study employed two methods. The first was to use the intraclass correlation coefficient (ICC) to assess the level of agreement between the two

alternative measurements.<sup>23</sup> In deriving the ICC using analysis of variance, we treated the two methods, one based on saliva and the other on serum, as fixed effects and treated the subjects on whom the tests were run as random effects. This would allow the estimates of agreement to be generalized beyond the patient samples from which they were derived. We considered 80% or better ICC to constitute excellent agreement. The second method was the use the Bland-Altman procedure to graphically evaluate the relationship between the average of paired measures and their difference to reveal patterns in any discrepancies such as any discrepancies increasing as the level of the measured outcome increases.<sup>24</sup>

Plasma and salivary concentrations vs time data were analyzed by noncompartmental methods. Correlation and regression analyses were applied for analysis of the differences between NVP formulations. Descriptive statistics were used for analysis of the patient population.

**Table 1** Characteristics of patients who underwent random Nevirapine concentration sampling

Characteristic	Value
Female	11 (58)
Male	8 (42)
Ethnicity	
Non-Hispanic Black	19 (100)
Age in years	
Mean	8.5 ± 1.9
Median; range	8.0; 5.9 - 13.2
CDC clinical category	
N	6 (32)
A	4 (21)
B	9 (47)
DAIDS toxicity category	
Grade I hepatic	1 (16)
Grade I pancreatic	6 (37)
Combined grade I hepatic and pancreatic	1 (5)
CD4+ count, per mm <sup>3</sup>	
Mean	1,268 ± 596
Median; range	1,066; 315 - 2,334
Percentage of CD4+ cells/mm <sup>3</sup>	
Mean	38 ± 8
Median; range	37; 15 - 49
Viral load, copies/ml	
Mean	13,944 ± 45,256
Median; range	1,000; 1,000 - 196,518

*Note: Numbers in parentheses are percentages. CDC, Centers for Disease Control and Prevention; DAIDS, Division of Acquired Immunodeficiency Syndrome, NIAID, NIH.*

## RESULTS

For the random paired sampling of NVP in plasma and saliva, 19 pediatric African-American patients (8 boys, 11 girls) with a median age of 8.0 years receiving antiretroviral therapy including NVP were enrolled. The majority of the participants had a history of moderately symptomatic HIV infection (CDC clinical category B) with good immunologic function and mild viremia at the time of evaluation (Table 1). Nine patients had mild antiretroviral or HIV-associated hepatic and pancreatic toxicities, and one had a combination of both (Table 1).

Five subjects had evaluations on more than one occasion, several weeks apart. The median time from NVP intake to sample collection was 5 hours (Table 2). The mean NVP dose was 179 mg/m<sup>2</sup> (SD, ± 22), every 12 hours, and 11 subjects received the medication as the liquid formulation. The total plasma concentrations of NVP ranged from 240 to 13,900 ng/ml, with a median of 9,160 ng/ml, whereas the median free NVP concentration was 3,690 ng/ml (range, 110 to 11,500 ng/ml). The saliva NVP concentrations ranged from 120 to 13,400 ng/ml (median, 5,780 ng/ml). NVP saliva concentrations reflected more closely the free NVP plasma concentrations but were on average 34% higher.

**Table 2** Random Nevirapine (NVP) concentration assessment

Variable	Value
Time after NVP intake, hours	
Mean	5.4 ± 2.8
Median; range	5; 2.2 - 10
NVP dose, mg/kg/dose	
Mean	179 ± 22
Median; range	187; 135 - 199
NVP liquid	11 (58)
NVP solid	8 (42)
NVP total serum concentrations, ng/ml	
Mean	8,147 ± 4,290
Median; range	9,160; 240 - 13,900
NVP free serum concentrations, ng/ml	
Mean	4,218 ± 2,842
Median; range	3,690; 110 - 11,500
NVP saliva concentrations, ng/ml	
Mean	5,592 ± 3,268
Median; range	5,780; 120 - 13,400

*Note: Numbers in parentheses are percentages.*

In addition, two male subjects were recruited for the extended sampling for NVP pharmacokinetics over 12 hours (Table 3). The overall mean total and free plasma NVP concentrations were 5,722 ng/ml (SD,  $\pm$  4,328) and 2,818 ng/ml (SD,  $\pm$  2,608), respectively. The mean salivary concentration was 3,885 ng/ml (SD,  $\pm$  3,233). For the two subjects with complete profiles the total, free, and salivary mean AUCs were 30.6, 14.7, and 18.0  $\mu\text{g}\cdot\text{hr}/\text{ml}$ , respectively (Figures 1 and 2).

The study results indicated high levels of agreement between saliva and serum measurements. With very few exceptions, saliva results were basically synonymous with serum results across the full range of data. The intraclass correlations between saliva and serum measurements of NVP concentrations indicated  $> 90\%$  agreement between these two modes of measurement (Tables 4 and 5). The Bland-Altman plots indicated that the discrepancy between measures was consistent across the range of average NVP concentrations (Figures 3 and 4). The figures show the relationship between saliva and plasma NVP concentrations vs the average of the two. The plots also show 95% confidence intervals through shading as well as the number and percent outside of the 95% confidence interval.

Overall, there was no difference in the saliva to plasma ratios between formulations. Although subjects taking the liquid formulation were younger, the serum albumin concentrations were similar between the subjects receiving each formulation, and sample collection occurred predominantly after drug absorption was complete for both formulations. For the two subjects who provided multiple samples, the average within-subject variability was 10%. This was lower than the 11% to 27% among repeated samples on separate visits.

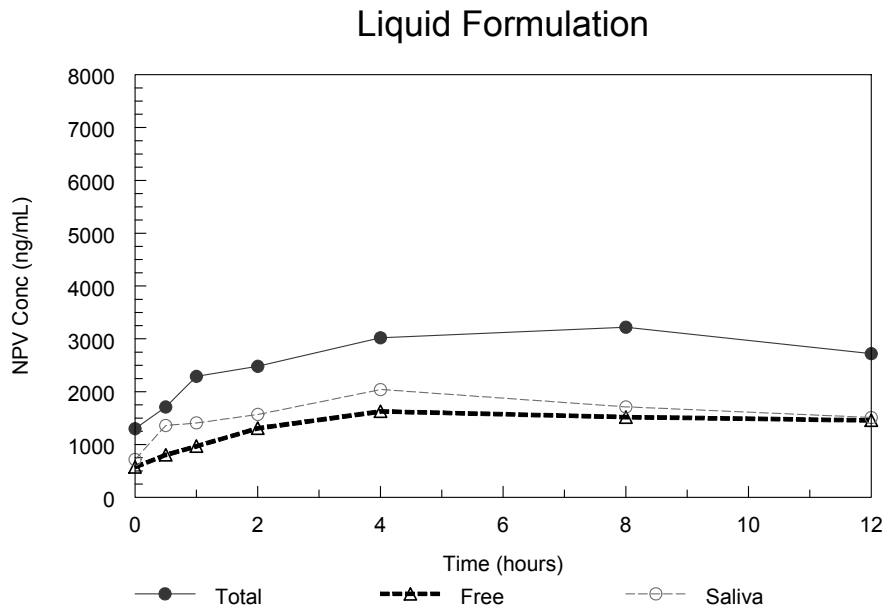
**Table 3** Characteristics of patients in the 12 hour pharmacokinetics study of Nevirapine (NVP) concentrations in saliva and plasma

Characteristic	Subject 44	Subject 6
Sex	Male	Male
Age, years	11.5	10.7
Ethnicity	Non-Hispanic Black	Non-Hispanic Black
CDC clinical category	C	C
DAIDS toxicity category	Normal toxicity values	Grade I Pancreatic
CD4+ cell count, per $\text{mm}^3$	1,056	695
CD4+ cell count, %	32	32
Viral load, copies/ml	15,800	457
HAART combination	3TC+NVP+LPV/rtv	TNF+NVP+LPV/rtv
NVP dose, mg/kg/dose	198	188

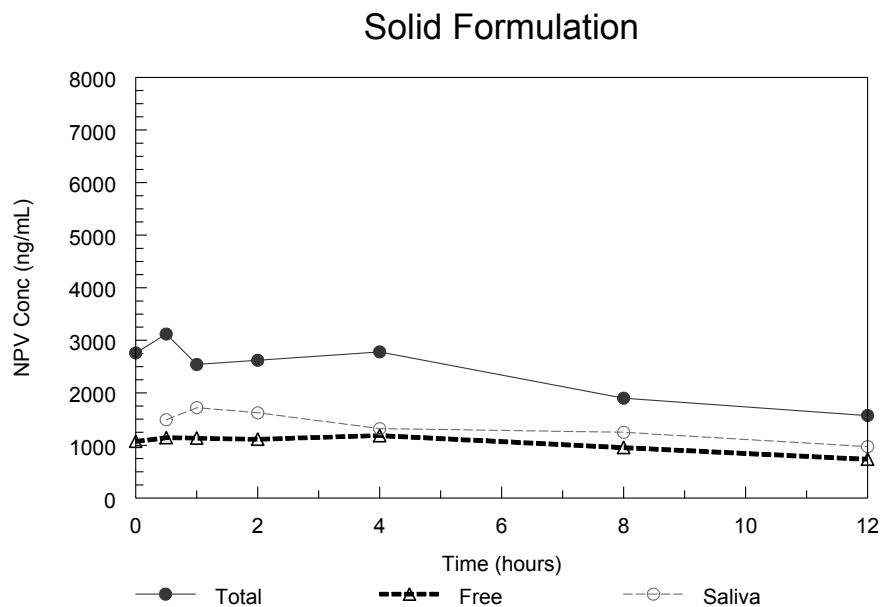
*CDC, Centers for Disease Control and Prevention; DAIDS, Division of Acquired Immunodeficiency Syndrome, NIAID, NIH; HAART, highly active antiretroviral treatment; 3TC, lamivudine; LPV/rtv, lopinavir/ritonavir; TNF, tenofovir.*



Sample collection time had minimal impact on the relationship of saliva and total plasma NVP concentrations. The saliva/total plasma ratio was within 0.45 to 0.9 for all subjects, and although there was a trend toward lower ratios later in the dose interval, this did not reach statistical significance and was small in magnitude ( $r = 0.30$ ,  $P = 0.12$ ) (Figure 5).



**Figure 1** Pharmacokinetics of nevirapine (NVP) in study subject number 6 (total, free plasma, and saliva concentrations).



**Figure 2** Pharmacokinetics of nevirapine (NVP) in study subject number 44 (total, free plasma, and saliva concentrations).

**Table 4** Intraclass correlation coefficients between saliva and total plasma nevirapine (NVP) concentrations

Measures	Intraclass correlation	95% Confidence interval	
		Lower limit	Upper limit
Single	0.913	0.830	0.957
Average	0.955	0.907	0.978

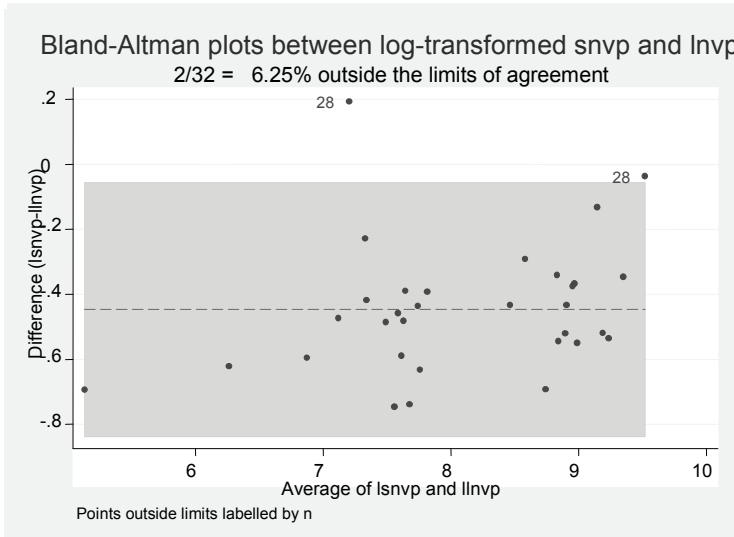
**Table 5** Intraclass correlation coefficients between saliva and free plasma nevirapine (NVP) concentrations

Measures	Intraclass correlation	95% Confidence interval	
		Lower limit	Upper limit
Single	0.954	0.905	0.978
Average	0.976	0.950	0.989

## DISCUSSION

The use of saliva for TDM has distinct advantages for children because saliva sampling is painless, prevents extra blood loss, and eliminates patient and caregiver stress as well as the infection risk associated with venipuncture. Obtaining saliva instead of blood reduces the cost by eliminating the need for venipuncture and for the involvement of skilled nursing personnel.<sup>25</sup> It requires fewer medical supplies and equipment for collection and initial processing, making it particularly suitable for low-resource environments. Prior studies involving children led to recommendations that saliva be used for TDM of certain medications, including phenytoin, carbamazepine, phenobarbital, and caffeine.<sup>26-28</sup>

In a previous study of the NVP concentrations in stimulated saliva of adult patients with HIV infection, by Heeswijk *et al.*, patients chewed for 1 minute a dental cotton roll impregnated with citric acid.<sup>29</sup> The study did not estimate the final volume of saliva extracted through centrifugation of the cotton roll and did not specify the centrifugation process. Because our laboratory method required a very small saliva volume (0.25 ml) and was aimed at the application in pediatrics, we chose to evaluate nonstimulated saliva sampling. We enrolled children older than 4 years of age and did not encounter significant obstacles in obtaining nonstimulated saliva samples. Collection of samples from younger participants (the youngest was 5.9 years of age) did take slightly longer (2 to 3 minutes, vs 1 to 2 minutes for older children) because these patients were less able to concentrate on the task. Thorough rinsing of the mouth is required prior to saliva sampling, because remnants of orally administered medicines may contaminate saliva specimens and yield spuriously high values. The similar saliva to plasma ratios between formulations indicate that our procedure was adequate to prevent contamination. Stimulation of saliva secretion with a chemical stimulus such as citric acid applied to the tongue facilitates sampling from younger patients and might be necessary for collecting samples from neonates and infants.<sup>28</sup>

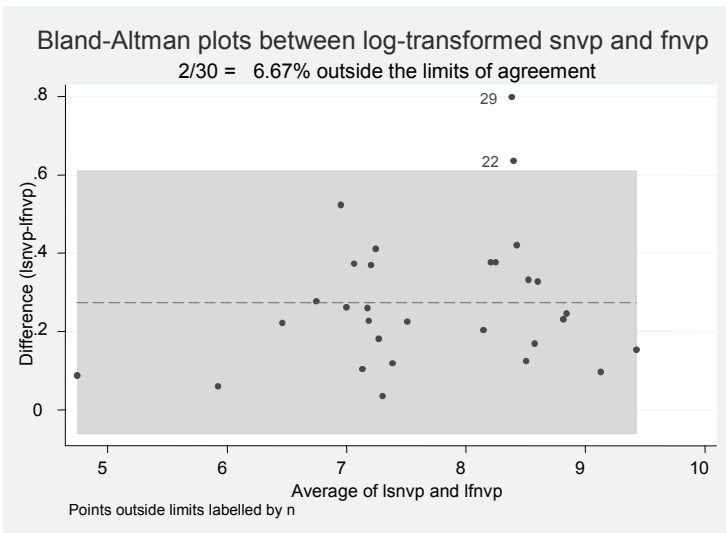


95% CI for Bland-Altman limits of agreement:

Difference = lnvp - lnvp		95% Limits Of Agreement (Bland & Altman, 1986)	
Average	Std Dev.		
-0.447	0.200	-0.838	-0.056

snvp – saliva NVP concentrations  
lnvp – total plasma NVP concentrations

**Figure 3** Total nevirapine (NVP) plasma concentration vs saliva concentration.

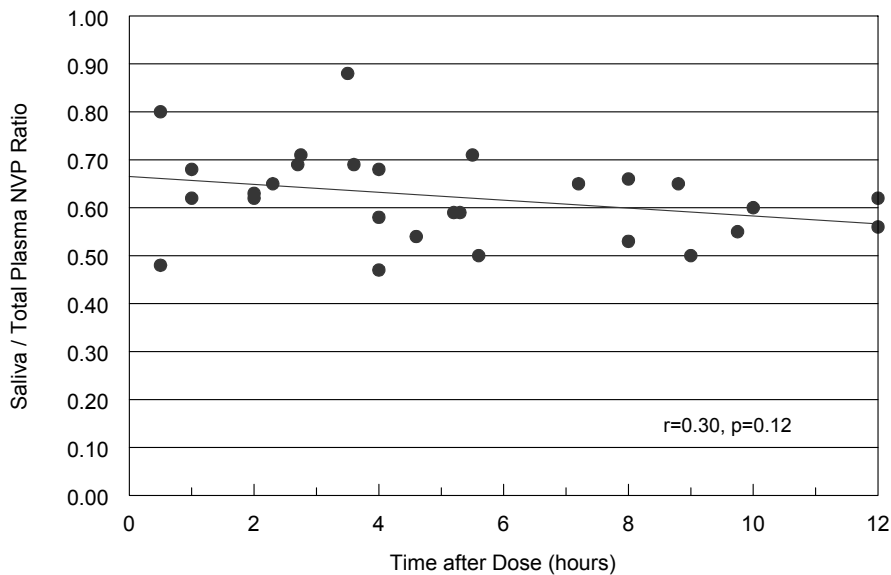


95% CI for Bland-Altman limits of agreement:

Difference = lnvp - lnvp		95% Limits Of Agreement (Bland & Altman, 1986)	
Average	Std Dev.		
0.274	0.171	-0.062	0.610

snvp – saliva NVP concentrations  
fnvp – free plasma NVP concentrations

**Figure 4** Free nevirapine (NVP) plasma concentration vs saliva concentration.



**Figure 5** Correlation between saliva/total plasma nevirapine (NVP) ratio and time after NVP dose.

Since drug passage into saliva follows the general principles of movement of drugs across biological membranes, only the unbound fraction of the drug in plasma is available for diffusion into saliva. However, a pH gradient exists between plasma and saliva that may alter the saliva/plasma concentration ratio of many polar drugs through ion trapping. The use of stimulated rather than resting saliva is advocated by some authors, because of the benefits of larger sample volume, smaller pH gradient between plasma and saliva, and smaller variability in saliva/plasma concentration ratios.<sup>26,27</sup> However, the acid dissociation constant (pKa) for NVP (2.8) is far from the physiologic pH, and thus the transfer of the drug is less sensitive to the plasma/saliva pH gradient. The saliva concentrations in our study reflected the free concentrations very closely but on average were 34% higher. Though we did not measure the saliva/plasma pH ratios, the pH gradient theory appears to be supported by the previously published data from van Heeswijk *et al.*<sup>29</sup> The study yielded similar findings in saliva-to-plasma ratio, with greater differences (60% higher salivary concentration over plasma) when saliva was collected with use of citric acid, which induced a decrease in salivary pH. Overall, the nonstimulated saliva to total and free plasma concentration ratios in our study show small variability, with a trend toward a statistically nonsignificant decrease over the dose interval. The 34% value indicates that the saliva measurement overestimates the free plasma level by an average of 34%. This difference between the saliva and plasma NVP concentrations does not preclude using saliva concentrations as a predictor of plasma concentrations, especially if a consistent regression model can be developed that accounts for most of the variability in plasma levels. In order to evaluate the utility of such a model, it will be necessary to collect data from a larger sample of HIV-infected children with a range of plasma values to provide a more complete assessment of the relationship.

Our study population demonstrates a wide range of concentrations of NVP in plasma and saliva. Even with exclusion of the samples with total concentrations < 1000

ng/ml (which may have been associated with suboptimal adherence), the concentrations varied more than 10-fold in all 3 matrices. The high interpatient variability in plasma concentrations, narrow therapeutic window, strong correlation between drug concentration and therapeutic effect, and failure of the longterm outcome with inadequate target concentrations make NVP an excellent candidate for successful TDM. Timely dosage adjustments based on individual plasma concentrations can optimize the therapeutic effect of the medication and prevent exposure to toxic or subtherapeutic concentrations of the drug and therefore limit the development of resistance mutations.<sup>30</sup>

Standard NVP dosage as part of HAART may have reduced virological effect due to poor compliance, poor absorption, increased metabolism and/or elimination, and drug-drug and drug-food interactions. Although the rates of adherence among pediatric patients are similar to those among adults with chronic diseases, averaging only about 50%, achieving full adherence in pediatric patients is a particularly difficult task. It requires not only the child's cooperation but also a devoted, persistent, adherent parent or caregiver. Measurement of the concentration of drug or its metabolite in blood and body fluids is considered one of the direct methods for measurement of adherence. Despite the cost and increased level of awareness by healthcare providers, measuring drug levels became a commonly used adherence assessment tool for certain drugs.<sup>17,31</sup> Although the acceptance of TDM for antiretroviral drugs as the standard of care is regional, many publications support its role in identifying adherence problems and optimizing treatment in vulnerable populations such as pregnant women and children.<sup>5,30,32,33</sup>

As pediatric fixed-dose combinations become available in the developing world, the ability to evaluate the steady-state pharmacokinetics of NVP in HIV-infected children of various ethnic and cultural backgrounds will become necessary. Children are at increased risk for subtherapeutic or toxic drug concentrations due to dependency on the continuous dose adjustment, palatability, and pill burden of HAART, the caregiver's ability to administer the medications in a proper way, and changes in drug pharmacokinetics during childhood development. The majority of current investigations involve abbreviated pharmacokinetic studies in children involving a minimum of 3 blood samples, requiring placement of an intravenous catheter or 3 separate venipunctures.<sup>34</sup> The splitting of adult-dose solid formulation antiretrovirals, although suboptimal, is the best currently available option for treatment of children in resource-limited areas of the world. Although satisfactory virological and immunological benefits in children receiving such formulations were reported from Thailand and Uganda, the use of tablets that require splitting can result in underdosing or overdosing of children, which can lead to increased risk of resistance or toxicity.<sup>34,35</sup>

The implementation of PMTCT programs in developing countries has revealed the risk for development of high rates of resistance for different HIV subtypes after single-dose NVP prophylaxis in both the mother and the infected newborn.<sup>36</sup> Recent

studies have yielded detectable NVP plasma concentrations after more than 2 weeks following single-dose exposure to NVP.<sup>37,38</sup> The results of both of those studies suggest that prolonged exposure to subtherapeutic drug concentrations of NVP might be responsible for development of NVP resistance in women and newborns exposed to single-dose PMTCT prophylaxis. These data highlight the need to know the interpatient variability in NVP half-life in women receiving a single dose of NVP in order to develop interventional strategies. Recently reported high rates of adherence to single-dose NVP for PMTCT have created excellent grounds for evaluation of maternal and neonatal exposure to NVP and its variability.<sup>39</sup> The application of a noninvasive method to conduct these studies would not only provide increased acceptance and enrollment but also would significantly reduce the risks associated with blood draws during pregnancy and reduce the costs associated with sample collection.

The potential ability to apply TDM for NVP in newborns is an area of particular interest because of the need for different NVP dosing regimens for infants, based on the length of maternal history of NVP therapy and time between the last NVP intake and delivery. An infant's NVP exposure may vary from 1 to 2 doses postpartum.<sup>40,41</sup> More data are needed on the excretion of NVP in saliva in infants and neonates.

The Current Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection in the USA mention the usefulness of direct plasma concentration monitoring only for assessment of adherence to therapy with protease inhibitors, on the basis of a study published by van Rossum in 2002.<sup>42,43</sup> Although our study was not aimed at evaluating the usefulness of NVP TDM for measuring adherence and optimizing therapy to improve outcomes for children with HIV infection receiving NVP-based HAART, it offers a valuable noninvasive tool to further address these important questions.

## **CONCLUSION**

Our study results indicate strong agreement between saliva and plasma NVP concentrations in pediatric patients with HIV-1 infection, on the basis of Bland-Altman analysis. The analysis has demonstrated that the two sampling methods are interchangeable and that nonstimulated saliva sampling for NVP concentrations may be used as a substitute for plasma sampling in clinical practice. Saliva sampling for NVP concentrations may be used as a noninvasive, reliable, cost-effective method for direct adherence measurement and application of TDM for NVP therapy. We believe that TDM of NVP can benefit many children and adults with HIV infection and can also serve as a tool in the direct measurement of adherence to NVP, particularly in newborns, young infants, and pregnant women, by preventing unnecessary blood loss, decreasing costs, and lowering the risks of infections associated with the venipuncture. This noninvasive measurement of NVP concentrations may prove useful as adapted technology for TDM of HAART in the developing world.

**ACKNOWLEDGMENT**

The data analysis was completed under the direction and guidance of Dr. Robert McCarter, RM, ScD, Associate Professor, Department of Epidemiology and Biostatistics, Department of Pediatrics, at George Washington University, School of Medicine, Washington, DC.

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# 9 chapter

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## **CAN THERAPEUTIC DRUG MONITORING IMPROVE PHARMACOTHERAPY OF HIV INFECTION IN ADOLESCENTS?**

Rakhmanina NY, van den Anker JN, Soldin SJ, van Schaik RH, Mordwinkin N, Neely MN.

*Ther Drug Monitor. 2010;32:273-281.*

**ABSTRACT**

Currently, therapeutic drug monitoring (TDM) of antiretroviral therapy (ART) is not performed in the United States as part of routine clinical care of an HIV-infected adolescent patient. TDM is recommended to rule out subtherapeutic drug concentrations and to differentiate among malabsorption, drug interactions, poor adherence, or increased drug metabolism or clearance as possible causes of decreased drug exposure. The use of TDM is also considered to assist in finding the optimal dose of a drug in patients whose virus has shown reduced susceptibility to that drug. The dosing of antiretroviral (ARV) drugs in adolescent patients with HIV infection depends on the chronologic age, weight, height, and the stage of sexual maturation. As a result of the limited data on the pharmacokinetics of ART during puberty, the transition of a dosing regimen from higher pediatric (weight and surface-based) to adult (fixed) range is not well defined. Developmental pharmacokinetic differences contribute to high variability in pediatric and adolescent patients and an increased frequency of suboptimal ARV exposure as compared to in adults. Individualized, concentration-targeted optimal dosing of ARV medications can be beneficial to patients for whom only limited dosing guidelines are available. This article describes three cases of the application of TDM in treatment-experienced adolescent patients whose ART was optimized using ARV TDM. TDM of ARV drugs is useful in managing the pharmacotherapy of HIV in adolescent patients and is well received by the adolescent patients with HIV and their families. Among others, the benefits of TDM provide evidence for adherence interventions and create grounds for enhanced education of the adolescent patient and involved adult caregivers about ART. Finally, TDM in adolescents provides valuable information about the clinical pharmacology of ART during puberty.

## INTRODUCTION

Currently, therapeutic drug monitoring (TDM) of antiretroviral therapy (ART) is not routine in the clinical care of HIV-infected adolescents in the United States. Nonetheless, in the recently updated Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection, TDM is considered to be useful in a pediatric patient with ART failure.<sup>1</sup> TDM is recommended to rule out subtherapeutic drug concentrations and to differentiate among malabsorption, drug interactions, poor adherence, or increased drug metabolism or clearance as possible causes of decreased drug exposure. The use of TDM is also considered to assist in finding the optimal dose of a drug in a patient whose virus has shown a reduced susceptibility to that drug.<sup>2,3</sup>

The dosing of antiretroviral (ARV) drugs in an adolescent patient with HIV infection depends on the chronologic age, weight, height, and the stage of sexual maturation.<sup>4</sup> As a result of the limited data on the pharmacokinetics (PK) of ART during puberty, the transition of a dosing regimen from higher pediatric (weight and surface-based) to adult (fixed) range is not well defined. Developmental PK differences contribute to high variability in pediatric and adolescent patients and a greater frequency of suboptimal ARV exposure as compared to in adults.<sup>5-8</sup> Although multiple adherence barriers represent one of the major challenges to the successful ART of pediatric HIV infection, the information on failed ART or increased resistance in the HIV-infected adolescents is very limited.<sup>9,10</sup> Equally limited are the data on the short- and longterm effects of potentially toxic ARV exposures during growth and development in early childhood and puberty.

In the absence of TDM, HIV-infected adolescents and young adults are placed at a significant risk of developing resistance from subtherapeutic drug exposure or toxicity from suprathreshold dosing.<sup>5</sup> This article describes three cases of the application of TDM in treatment-experienced HIV-infected adolescent patients whose therapy was ultimately optimized through the use of ARV TDM.

## PATIENTS AND METHODS

All patients were adolescents with perinatally acquired HIV-1 infection receiving care at the large (greater than 300 patients) metropolitan pediatric HIV program at Children's National Medical Center, Washington, DC. TDM of ART has been routinely implemented into clinical practice within the program at Children's National Medical Center since 2002. In addition, in the period between 2004 and 2008, the program conducted a study on the optimization of ART in children and adolescents with HIV infection, which involved the evaluation of CYP450 and MDR1 genotypes, extensive (up to 12 hours) PK studies, and intense TDM follow-up.<sup>11</sup> The research protocol, parental consent, and assent documents (for children older than 7 years of age) were approved by the Institutional Review Board. All subjects in this study were on uninterrupted, protease inhibitor-based ART for at least 4 weeks before study entry. Blood samples for ARV drug measurement were obtained during routine clinical visits

(for random TDM samples) and during admission to the General Clinical Research Center for an extensive PK study after an observed dose. The subjects were administered their standard prescribed dose under direct observation with a standard light snack. Plasma and paired saliva samples were obtained before and at 0.5, 1, 2, 4, 8, and 12 hours after the observed intake of ARV for the 12-hour PK study or on the alternative schedule for a shortened PK (less than 12 hours) study.

The ARV plasma and saliva concentrations of all ARV drugs except tenofovir (TFV) were measured in the laboratory at Children's National Medical Center, which is an accredited member of the of the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection (University Medical Center Nijmegen, Nijmegen, The Netherlands).<sup>12,13</sup> A published tandem-mass spectrometric method with an Applied Biosystems/Sciex API-2000 (Foster City, CA) was used.<sup>14-16</sup> The lower limit of quantification was 10 ng/ml. TFV was measured at the University of Southern California using an API 3+ tandem-mass spectrometer coupled to an Agilent 1100 liquid chromatography system (Santa Clara, CA). Samples were extracted using methanol. The lower limit for TFV quantification was 10 ng/ml. For both assays, within-run error was below 7% and between-day error was below 10% for all analytes at the tested concentrations.

Noncompartmental PK techniques were used to analyze individual concentration data. Area under the drug time-concentration curve (AUC) was calculated using the standard trapezoidal approximation implemented in the freely available statistical package "R" (Version 2.10, available at [www.rproject.org](http://www.rproject.org)). Drug clearance was calculated using the formula  $\text{clearance} = \text{dose}/\text{AUC}_{\infty}$ , where  $\text{AUC}_{\infty}$  is the sum of the observed AUC up to the last sample time,  $t$ , and an extrapolated AUC from time  $t$  to infinity. The latter is calculated from the last two or three observed concentrations in the terminal elimination phase after drug absorption is largely complete. Adult reference values for all drugs included in this report are in Table 1. All plots were generated using R.

**Table 1** Reference adult PK ARV values

Drug	Dose (mg)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hours)	C <sub>min</sub> (ng/ml)	AUC (ng*h/ml)	CL (l/h)	Reference
Abacavir	300	3090	0.75	18	6080	51	50, 51, PI
Tenofovir	300	326	2.3	41	3020	131*	52, 53, PI
Efavirenz	600	4072	3 - 5	1640	58,085	10*	54, PI
Atazanavir	400	2298	2	120	14,874	25	55, PI
Lopinavir	400	9800	4	5500	92,600	6	PI
Amprenavir	1200	7600	1	1480	17,700	20	PI
Ritonavir	100	500	4	320	3580	19	56,57, PI

\* Calculated

PK – pharmacokinetic; ARV – antiretroviral; C<sub>max</sub> – maximum drug concentration; T<sub>max</sub> – time of maximum concentration; C<sub>min</sub> – minimum drug concentration; AUC – area under the concentration-time curve; CL – clearance; PI – package insert. Values for atazanavir are those for unboosted dose, i.e., without ritonavir; values for lopinavir are when given with ritonavir as a coformulation.

*CYP2B6* genotypes were determined using polymerase chain reaction amplification followed by allelic discrimination assays based on the use of fluorogenic oligonucleotide probes (TaqMan) and direct sequencing analysis performed on ABI Prism 3130 Genetic Analyzer at the Department of Clinical Chemistry, Erasmus Medical Centrum, Rotterdam, The Netherlands.

## RESULTS

### **Scenario 1: Therapeutic drug monitoring in a patient with treatment failure**

Patient A was a 13-year-old obese black girl (Tanner Stage IV) with perinatally acquired HIV infection, Centers for Disease Control and Prevention category B3. Her medical history was significant for poststreptococcal glomerular nephropathy (resolved), familial obesity, and mild lipodystrophy with slightly elevated cholesterol and triglycerides. Her mother died from AIDS, and the patient was living with her maternal grandmother and two siblings, both of whom were also HIV-infected. She has been disclosed about her HIV status since the age of 12 years. She had a history of multiple nucleoside reverse transcriptase and nelfinavir resistance mutations by the age of 11.5 years. At the age of 12 years, she was placed on a new regimen of atazanavir (ATV) boosted with low-dose ritonavir (RTV), nevirapine (NVP), abacavir (ABC) and tenofovir disoproxil fumarate (TDF). Throughout the next 12 months, a progressive decline in CD4+ cell count and an increase in HIV RNA viral load were observed.

Despite the immunologic and virologic evidence of treatment failure, both the patient and her caregiver consistently reported 100% adherence on joint and separate interviews. However, all random plasma ARV drug concentrations obtained during routine clinic visits were undetectable. The patient enrolled in our optimization of ART study with the CD4+ count of 18% (292 cells/mm<sup>3</sup>) and HIV RNA of 50,600 copies/ml.<sup>11</sup> During the first 12-hour study visit, the patient again reported 100% adherence at home and was observed by staff to take all five medications with a glass of water after a light standardized snack. All ARVs (ABC, TFV, ATV, RTV, and NVP) were undetectable in the plasma and paired saliva (TFV not measured in saliva) predose sample and in six paired samples obtained over 12 hours after the observed intake. Potential sample processing and laboratory errors were thoroughly investigated and not found. The patient denied the use of herbal preparations or concomitant medications except for the use of loratadine (Claritin, Merck & Co., Inc., Whitehouse Station, NJ) on an as-needed basis, which was not administered in the 24 hours before study. The findings were discussed with the family and the patient and it was mutually agreed to repeat the 12-hour PK study, which was performed 24 weeks later. During this period, the NVP therapy was discontinued as a result of a newly identified resistance mutation (Y181C).

As previously, during the second 12-hour PK study, 100% adherence to ARV medications was reported by the caregiver and the patient. The concentrations of all ARVs were again undetectable in the plasma, whereas the saliva concentrations of

ABC were detectable at 1, 2, 4, and 8 hours (757, 545, 365, and 18 ng/ml, respectively). The study and clinical teams, including a treatment adherence specialist, social worker, nutritionist, and psychologist, reviewed the results and concluded that the evidence overwhelmingly supported either self-induced emesis or oral retention of the medications without swallowing. With the patient and caregiver's agreement, the TDM after an observed intake of the medications followed by 2 hours of close supervision (the patient was never left alone by the clinic nurse) was conducted during a routine clinic visit. The plasma concentrations of ABC (2105 ng/ml) and ATV (290 ng/ml) were detectable 2 hours after observed intake with supervision and yet RTV concentrations were still undetectable. The results of all PK and TDM studies were discussed with the patient and the caregiver, and they both denied the possibility of self-induced emesis while continuing to insist on 100% adherence. The family agreed, however, to a psychology evaluation and additional adherence interventions, which included phone call reminders, pill boxes, partial directly observed therapy (DOT), and financial rewards. During psychologic counseling, the adolescent girl admitted that she was very concerned with a potential ART-associated increase in her obesity and did not like swallowing the pills.

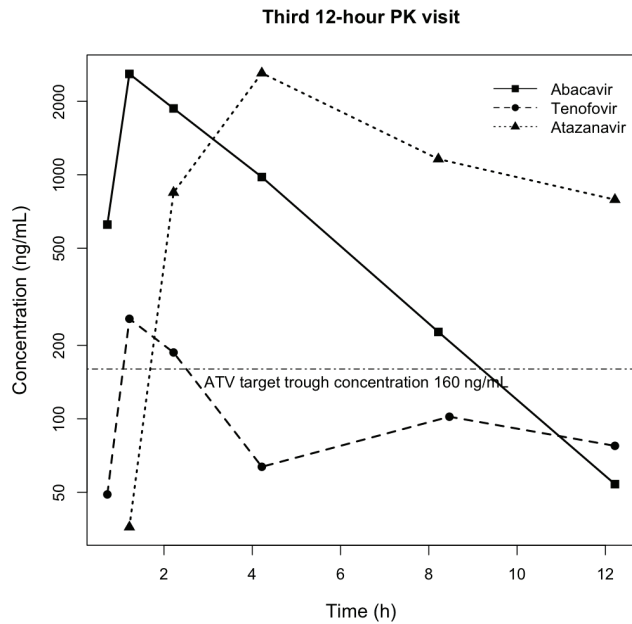
After a further 24 weeks of counseling and adherence interventions, the positive feedback from the family and team assessments suggested that adherence to the ARV regimen had improved. Based on the low ATV/RTV concentrations at 2 hours postdose (references are shown in Table 1), the ATV/RTV dose had been increased to 600/200 mg and a third 12-hour PK study was conducted 5 weeks after ATV/RTV dose adjustment. (Figure 1). Although all administered ARVs were measured in plasma, RTV was not detected in plasma or saliva (Table 2).

**Table 2** The PK parameters of ARV medications in Patient A

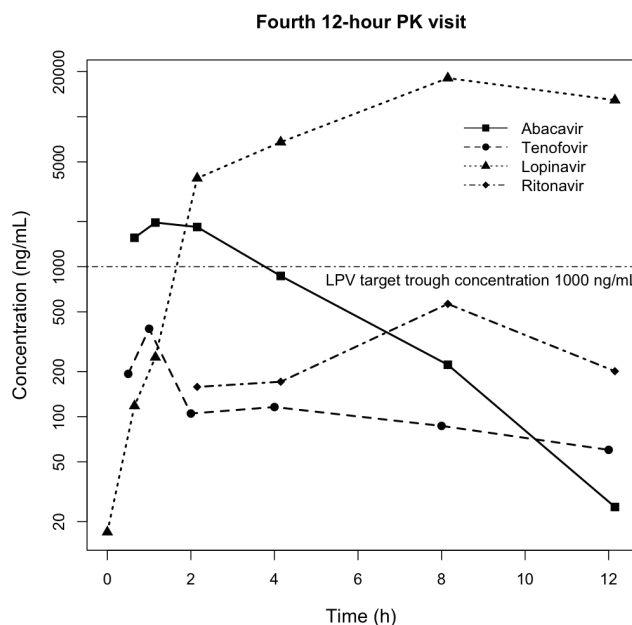
Drug	Dose (mg)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hours)	AUC <sub>∞</sub> (ng*h/ml)	CL (l/h)
12-hour PK study with increased dose ATV/RTV					
Abacavir	300	2590	1.2	9231	32.5
Tenofovir	300	257	1.2	2313	129.7
Atazanavir	600	2610	4.2	23,611	25.4
Ritonavir	200	0	-	0	-
12-hour PK study with standard dose LPV/RTV					
Abacavir	300	1970	1.1	8722	34.4
Tenofovir	300	386	1.1	2013	149.0
Lopinavir	400	18,100	8.2	276,959	1.4
Ritonavir	100	565	8.2	4190	23.9

PK – pharmacokinetic; ARV – antiretroviral; C<sub>max</sub> – maximum drug concentration; T<sub>max</sub> – time of maximum concentration; AUC – area under the concentration–time curve; CL – clearance; RTV – ritonavir; LPV – lopinavir.





**Figure 1** Area under the concentration–time curve of the standard dose abacavir, tenofovir, and high-dose atazanavir/ritonavir (600 mg/200 mg) in Patient A.



**Figure 2** Area under the concentration–time curve of the standard dose abacavir, tenofovir, and lopinavir/ritonavir in Patient A.

As a result of persistent virologic failure and concerns for nonadherence to RTV as a separate pill, after the PK study, the patient was placed on a new regimen with ABC, TDF, and lopinavir/ritonavir (LPV/RTV; Kaletra<sup>®</sup>, Abbott, Abbott Park, IL), and the fourth 12-hour PK study after 10 weeks was performed (Figure 2). This PK study demonstrated normal PK parameters for all measured ARV medications including RTV (Table 2). Nonetheless, during routine clinic follow-up, the undetectable random LPV/RTV plasma concentrations (on two separate measurements), small (less than 1 log) decline in HIV RNA and continued decline in CD4+ cells prompted the arrangement of the full-time DOT with 30 minutes close

observation after the dose intake. With DOT, the patient maintained a detectable random plasma concentration of LPV (10,760 ng/ml) and achieved undetectable HIV RNA (less than 400 copies/ml) within 12 weeks.

Unfortunately, after 6 months of vigorous DOT, the family grew tired of the effort and the ART intake again became unsupervised, which led to undetectable LPV plasma concentrations and recurrent treatment failure. During our recent discussion of adherence, the patient (now 17 years old) openly admitted that she finds 200-mg Kaletra® tablets too difficult to swallow and was switched to 100-mg tablets. Further considerations are given to the return to DOT, repeat psychological counseling, and new classes of ARV drugs.

### **Scenario 2: Therapeutic drug monitoring in patients with excellent virologic suppression**

Patient B was a 13-year-old black boy (Tanner Stage III-IV) with perinatally acquired HIV, Centers for Disease Control and Prevention category B3, and history of excellent adherence. His medical history included lymphocytic interstitial pneumonitis, herpes zoster, and failure to thrive. At the age of 6 years, he was placed on the ART regimen with stavudine, efavirenz (EFV), and amprenavir, which remained unchanged for more than 7 years. Since the initiation of this regimen, he had maintained an undetectable HIV viral load (less than 400 copies/ml and later less than 48 copies/ml) and stable CD4+ cell counts greater than 500 cells/mm<sup>3</sup> (25% - 30%).

Random TDM plasma samples obtained during routine clinic visits demonstrated high concentrations of EFV (21,000 ng/ml) and low concentrations of amprenavir (29 ng/ml) (references are shown in Table 1). The patient enrolled in the optimization of ART study<sup>11</sup> and had the 12-hour PK study. Before the release of the results of the PK study, his medical provider increased his EFV dose from 400 mg to 600 mg based on his weight. Subsequently, when the plasma EFV exposure was found to be high on the first PK study, he underwent a second confirmatory PK study at the higher EFV dose (Table 3). Despite the extremely high EFV exposure (Figure 3), at no time did the patient demonstrate any evidence of clinical or laboratory toxicity, including sleep patterns, energy levels, liver enzymes, amylase, lipase, and lipids. Nonetheless, concern for possible long-term adverse effects of continued high EFV exposure and a documented *CYP2B6 516 TT* "slow metabolizer" polymorphism prompted reduction of his EFV dose to 200 mg daily, 33% of the recommended dose for his weight. The final, abbreviated PK study on 200 mg EFV once daily is also shown in Figure 3, whereas the summary of PK values for all three doses of EFV are represented in Table 3. Subsequent random plasma concentrations at 9 to 11 hours after unobserved 200-mg doses at home ranged between 2790 to 4200 ng/ml. In addition to high EFV exposure, at his first PK visit, the patient was found to have negligible concentrations of amprenavir ( $C_0 = 20$  ng/ml;  $C_{0.5} = 20$  ng/ml;  $C_1 = 25$  ng/ml;  $C_2 = 42$  ng/ml;  $C_4 = 15$  ng/ml;  $C_8 = 11$  ng/ml;  $C_{12}$  less than 10 ng/ml). The results of the PK study prompted a change of his ART to lamivudine

and ABC as Epzicom<sup>®</sup> (GlaxoSmithKline, Research Triangle Park, NC), TDF, and EFV at doses discussed previously. The patient maintained full virologic suppression (HIV RNA less than 48 copies) at 52 weeks after the change in EFV dose.

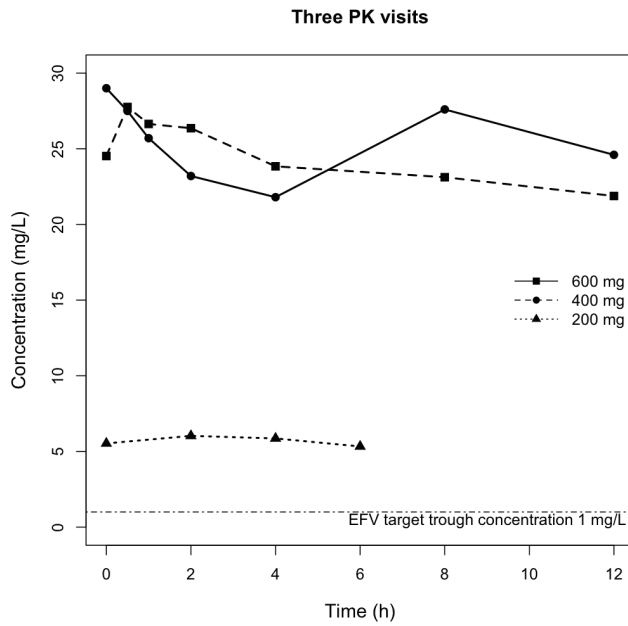
Patient C was a 14-year-old black boy with perinatally acquired HIV, Centers for Disease Control and Prevention category N-2, and a history of excellent adherence and virologic suppression. From 4 to 12 years of age, he was treated with stavudine, lamivudine, and NVP. At the age of 12 years, secondary to the request for once-daily dosing of ART, his regimen was switched to a fixed-dose combination tablet containing 600 mg EFV, 300 mg TDF, and 200 mg emtricitabine (Atripla<sup>™</sup>, Bristol-Meyers Squibb, Princeton, NJ). Three months after starting this new regimen, the patient reported headache and diarrhea, and also reported a rash, but all symptoms resolved within another 3 months. No toxicities on laboratory findings, including liver enzymes, amylase, lipase, and lipids, were detected.

TDM during routine clinic visits revealed high plasma EFV concentrations ranging from 22,400 to 23,400 ng/ml at 10 to 11 hours after reported intake (references are shown in Table 1). Interestingly, high random NVP plasma concentrations (up to 15,120 ng/ml) were also found during routine TDM in the past but were used only as a proof of adherence at that time. Based on these results, the *CYP2B6* genotype was ordered and a 12-hour PK study was conducted in the clinic after unobserved dose intake, which was documented on the phone by the clinic nurse the night before the clinic visit. The EFV plasma concentrations were measured at 12, 14, 16, 20, and 24 hours after the intake and confirmed high EFV exposure (Figure 4), whereas the patient genotype confirmed *CYP2B6 516 TT* polymorphism. Based on the results of the pharmacogenetic analysis and PK data, the therapy with fixed-dose TDF/emtricitabine/EFV was discontinued and was replaced with the smaller dose of EFV (200 mg) in combination with TDF/emtricitabine (Epzicom<sup>®</sup>). Full virologic suppression (HIV RNA less than 48 copies) was maintained at 52 weeks after the change in EFV dose and two random concentrations measured on different days at 22 and 24 hours after unobserved dose intake at home were 1.8 and 1.3 mg/l, respectively (references are shown in Table 1).

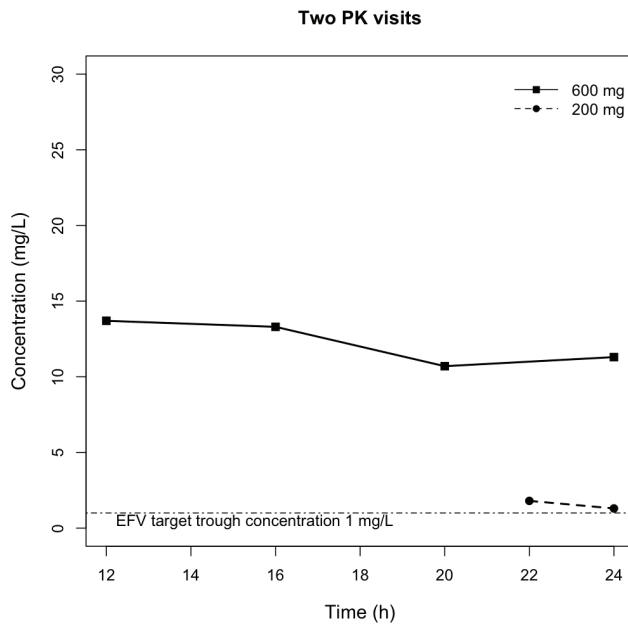
**Table 3** The PK parameters of three different doses of efavirenz in Patient B

Drug	Dose (mg)	C <sub>max</sub> (mg/l)	T <sub>max</sub> (h)	AUC <sub>∞</sub> (mg*h/l)	CL (l/h)
Efavirenz	200	6.03	2	147	1.4
Efavirenz	400	27.76	0.5	1875	0.2
Efavirenz	600	27.50	0.5	1155	0.5

*C<sub>max</sub>* – maximum drug concentration; *T<sub>max</sub>* – time of maximum concentration; *AUC* – area under the concentration-time curve; *CL* – clearance.



**Figure 3** The summary of three 12-hour pharmacokinetic studies with three different doses of efavirenz (EFV) in Patient B.



**Figure 4** The summary of the pharmacokinetic parameters with two different doses of efavirenz (EFV) in Patient C.

## DISCUSSION

TDM in HIV-infected adolescents has similar indications to adults and is primarily used to monitor the adherence to ART, evaluate the cause of treatment failure, and to manage drug–drug interactions. Depending on the availability of a TDM consultant and the acceptance of TDM in clinical practice, the use of ART TDM may extend to optimization of the ARV dose by targeting specific trough concentrations or ratios of trough concentration and viral susceptibility (i.e., inhibitory quotients). Several investigations have demonstrated the benefits of incorporating TDM into the

clinical management of HIV infection.<sup>2,3</sup> Very few studies, however, have evaluated the benefits of measuring ARV concentrations in HIV-infected adolescents and young adults.<sup>17</sup> Our cases represent a summary of some of the diverse applications of TDM in the management of the pharmacotherapy of ART in adolescent patients. We have selected three patients, one of whom represented currently accepted clinical indications for the use of TDM, whereas two others would have not been considered for the application of TDM under current standard of care.

The first scenario describes a challenging case of an adolescent patient with consistently reported 100% adherence despite the evidence of treatment failure. The high level of adherence was not only stated by the patient, but was also confirmed by the dedicated adult primary caregiver who also provided ART to twin siblings with HIV infection who are 3 years younger than Patient A. Both of these siblings have consistently demonstrated high levels of medical adherence, which has been confirmed by TDM and clinical outcome. In addition, Patient A had been fully aware of her HIV status since 12 years of age and had regularly participated in the treatment discussions. Despite the fact that some form of nonadherence had been long suspected by the clinical team, none of the involved home and medical providers foresaw her practice of withholding medications in the mouth and spitting them up until documented through TDM. The first and second PK studies confirmed the absence of the ARV drugs in plasma samples after an observed intake. The absence of the ARV saliva concentrations in the first study was likely related to prompt spitting up of the medications after intake. During the second PK study, the patient was detained for approximately 10 minutes by the nurse despite her urge to use the bathroom right after taking the medications, which could be responsible for the detectable concentrations of ABC in saliva. We speculate that the patient either swallowed the medications and induced subsequent emesis or retained the medications in her mouth without the staff noticing and discarded them after a 10-minute delay. It is plausible that capsule preparations of ATV and RTV were less likely to start dissolving in saliva than ABC tablets, and therefore the detectable concentrations of ABC in saliva were found after holding the medications in the mouth before spitting them up (TFV was not measured in saliva).

The results of the first two PK studies prompted more open, evidence-based discussion than previously possible with Patient A and family members involved in her care. An assumption of the behaviorally motivated nonadherence without the evidence would have had the potential to disrupt the patient and caregiver liaison with the medical team. Even after psychologic counseling, Patient A clearly had selective acceptance of ARV drugs, as demonstrated by the repeat PK study with increased dose of ATV/RTV. The only 12-hour PK study, which documented the presence of ATV, demonstrated adequate concentrations of ATV with increased dose (ATV/RTV = 600 mg/200 mg) and the absence of RTV in plasma samples. The PK parameters of ATV perfectly fit the PK of the unboosted ATV and, together with absent RTV in plasma, provide strong evidence for continued oral withholding of RTV dose by the patient despite close observation by the medical staff at the

General Clinical Research Center. The therapy with an unboosted ATV regimen is not recommended in treatment-experienced pediatric patients with pre-existing protease inhibitor mutations, because ATV resistance can develop through mutations associated with resistance to other protease inhibitors instead of through the ATV associated I50L mutation.<sup>1</sup> Once placed on a fixed-dose coformulated boosted protease inhibitor (Kaletra<sup>®</sup>), Patient A demonstrated normal absorption and PK parameters for RTV.

HIV-infected adolescents face multiple adherence challenges during their transition to adulthood: in addition to palatability issues, pill burden, and interference of ART with lifestyle, adolescent patients with HIV experience growing independence, increased peer pressure and fear of stigmatization, increased risk-taking behavior (including substance abuse), denial and fear of HIV infection (particularly after witnessing the death from HIV like in our Patient A who lost her mother to AIDS), a long history of poor adherence and nondisclosure issues in perinatally infected patients, and psychiatric problems (depression, anorexia).<sup>9,18-21</sup> In our recent study, we have shown that adolescents (13 - 18 years old) were significantly less likely to reach undetectable HIV RNA than younger children (younger than 13 years old) (odds ratio = 0.38; 95% confidence interval: 0.16 - 0.89).<sup>22</sup> For every year increase in age, the odds of reaching undetectable viral load (VL) decreased by 10% after controlling for self-reported adherence and medications refill mechanism. Although considered to be ready to assume the responsibility for adherence to an appropriate administration of their ARV medication, many adolescents lack social and financial autonomy, privacy, and mobility and generally will decrease their adherence to ART.<sup>23</sup> A comprehensive assessment of adherence through multiple indirect methods (self-report, caregiver report, pill count, pharmacy refills) should be incorporated into the management of every adolescent patient with HIV infection. Although patient and caregiver reports are the main adherence measurement used in the majority of clinical setting,<sup>20,24</sup> TDM is the only direct measure of verifying the patient compliance because all other methods do not prove the actual intake of ARV drugs.

Studies have shown that it is crucial to take the evolutionary nature of the caregiver's and the child's coping process into account when integrating adherence to ART into children's daily lives.<sup>25</sup> The care team should work continuously and concomitantly on three factors: knowledge, capacity, and motivation. In the case of Patient A, TDM has allowed us to identify a cause of nonadherence in a form of a very complex behavioral pattern with selective acceptance of ARV medications. The TDM evidence created grounds for the motivation of the patient and the family and their cooperation with the implementation of DOT. Although the adherence problems in this young woman are far from being solved, the success of the previous TDM-based interventions allows us to continue our work with her and her family to explore further strategies to increase her ART adherence.

According to the current TDM guidelines, the measurement of the ARV drug concentrations would have not been indicated in the second scenario with Patients B

and C as a result of the excellent virologic suppression and immunologic status with EFV-based ART. Doses that result in excessive plasma drug concentrations are unlikely to be detected unless and until clinical toxicity develops, and without TDM, dose-dependent vs dose-independent toxicity cannot be distinguished.<sup>5</sup> EFV is extensively metabolized by CYP2B6 with partial involvement of CYP3A4 and CYP2A6.<sup>26-29</sup> The *CYP2B6* G to T polymorphism at position 516 has been associated with elevated EFV plasma concentrations and an increase in neurotoxicity in adults and children.<sup>30-33</sup> Most recently, the *CYP2B6* 983T>C and *CYP2A6* genotypes have also been reported to affect EFV plasma concentrations.<sup>34-36</sup> High EFV plasma concentrations and successful *CYP2B6* genotype-based EFV dose reduction were demonstrated in adults with the haplotypes *CYP2B6* \*6/\*6 (516G>T, 785A>G) and \*6/\*26 (499C>G, 516G>T, 785A>G).<sup>37</sup> Genotype *CYP2B6*-based dose reduction has also been proposed in several population PK models.<sup>38,39</sup>

The identification of the high EFV exposure in our patients led to pharmacogenetic evaluation and confirmation of the "slow metabolizer" type of *CYP2B6* polymorphism. Moreover, the lack of dose proportionality in EFV AUC observed in Patient C indicates that at the two higher doses, the PK behavior of EFV could best be described as Michaelis-Menten or zero-order elimination. Michaelis-Menten PK occurs when clearance mechanisms become saturated and a constant amount of drug, rather than a constant fraction, is eliminated per unit time. The implications of Michaelis-Menten PK are that clearance becomes dose-dependent (Table 3) and small changes in dose can result in large changes in plasma concentration, as was observed when the dose was reduced by 50% from 400 mg to 200 mg daily, yet the AUC dropped by more than 90% (Table 3). Note that at all doses, the observed clearance was still well below the referenced adult values (Table 1). To our knowledge, this is the first description of Michaelis-Menten EFV PK associated with *CYP2B6* "slow metabolizer" polymorphisms.

Although the successful dose reduction of EFV has been described in adults and a single report in an adolescent patient, to our knowledge,<sup>40,41</sup> this is the first report of successful reduction of the EFV dose in two black adolescent patients based on the *CYP2B6* genotype in combination with PK evaluations. The *CYP 2B6 516 G>T* polymorphism is significantly higher in sub-Saharan Africans (45.5%) and blacks (46.7%) as compared with Hispanic (27.3%), European (21.4%), and Asian (17.4%) populations.<sup>42-44</sup> In addition to the *CYP2B6 516 G>T* polymorphisms, the DNA samples for both patients were analyzed for the presence of *CYP 2B6 415>G*, *785A>G*, *983 T>C* and *1459 C>T* polymorphisms. Patient C had *CYP2B6 785GG* polymorphism in addition to the *CYP 516 TT* genotype, suggesting the presence of the haplotype *CYP2B6* \*6/\*6 (516G>T, 785A>G) associated with the "slow metabolizer" profile for EFV.<sup>37</sup> Patient C had also a history of high NVP exposure caused by his *CYP2B6* polymorphism.<sup>45</sup> However, the plasma NVP concentrations were used only for the confirmation of adherence at that time. Interestingly, although both patients and their families recalled that children experienced transient sleep problems shortly after the initiation of EFV therapy, their high EFV

exposure did not prompt treatment discontinuation. This is consistent with recently published data on the lack of association between *CYP2B6* genotype and EFV plasma concentrations and the risk of EFV discontinuations because of neurotoxicity.<sup>46,47</sup> Equally, no other EFV-associated toxicities were identified in both patients, particularly in Patient B with more than 7 years of high EFV exposure. This patient was placed on the combination ART with EFV coadministered with APV before the wide acceptance of boosted fos-APV into pediatric practice. EFV has been reported to decrease the  $C_{max}$ , AUC, and  $C_{min}$  of unboosted APV by approximately 40% in adults; however, this effect of EFV is compensated by the PK booster effect of RTV when APV is combined with RTV.<sup>48</sup> Although the treatment with EFV in combination with unboosted APV is not recommended, Patient B continued his regimen as a result of the excellent virologic and immunologic outcome. In reality, his high EFV concentrations produced an APV exposure so negligible that he can be considered to have been treated with dual (stavudine and EFV) therapy for a prolonged period of time (greater than 7 years). We would like to speculate that such a high degree of EFV exposure allowed him to avoid development of the K103N, Y181C, and other multinucleoside reverse transcriptase resistance mutations on the ART regimen with a single efficient nucleoside reverse transcriptase backbone.

In summary, our experience suggests that TDM evaluation (when available) should be considered in HIV-infected adolescent patients on ART independently of the degree of virologic suppression and immunologic outcome. We recognize that many of the currently identified barriers to the routine application of TDM in pediatric ART (prolonged time for laboratory processing, difficulties in coordinating sample collections at appropriate times, limited availability of certified laboratories for ARV drug concentrations, lack of third party reimbursement of costs) were not experienced at our site. The extended PK analyses were provided through grant funding. However, routine TDM measurements during clinic follow-up are a well-accepted standard of care in our program and we have not encountered difficulties in the reimbursement process. Clearly, through the availability of a dedicated pediatric General Clinical Research Center and inhouse laboratory, we were able to repeat the studies to eliminate significant limitations of ARV TDM such as high inpatient variability from single drug concentration measurement.<sup>2,49</sup> Finally, we hope that similar reports and randomized controlled studies will help to eliminate the most significant barrier to the successful TDM of ART such as inadequate information on safety and effectiveness of dose adjustment strategies in children and adolescents.

## CONCLUSIONS

The physiological and psychosocial changes during puberty create strong grounds for an individualized therapeutic approach to an HIV-infected adolescent. Individualized, concentration-targeted optimal dosing of ARV medications can be



beneficial to patients for whom there are limited dosing guidelines. TDM of ARV drugs is useful in managing the pharmacotherapy of HIV in adolescent patients and is well received by the adolescent patients with HIV and their families. Among others, the benefits of TDM provide evidence for adherence interventions and create grounds for enhanced education of the adolescent patient and involved adult caregivers about ART. Finally, TDM in adolescents provides valuable information about the clinical pharmacology of ART during puberty.

#### **ACKNOWLEDGMENTS**

We thank the children who participated in this study, their families and caregivers, the clinic staff, laboratory, and Pediatric Clinical Research Center personnel for their dedication and support.

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# IV PART

**INDIVIDUALIZED  
APPROACH TO THE  
TREATMENT OF  
HIV-INFECTED  
CHILDREN AND  
ADOLESCENTS**



# 10

# chapter

## **EVALUATING ADHERENCE TO MEDICATION IN CHILDREN AND ADOLESCENTS WITH HIV**

Khan M, Song X, Williams K, Bright K, Sill A, Rakhmanina NY.

*Arch Dis Child.* 2009;94:970-973.

## **ABSTRACT**

### **Objective**

The study was aimed to evaluate the relationship between pharmacy supply, self-reported treatment adherence and HIV viral load in HIV-infected children.

### **Methods**

A retrospective (52 weeks) cohort study was conducted through the review of the existing databases. Pharmacy supply was classified as "home delivery" when the medications were delivered home and as "in pharmacy pick-up" when they were picked up at the pharmacy. Adherence was assessed through retrospective (3 days recall) self-report. Fisher's exact model, univariate and multivariate logistic regression analyses were used.

### **Settings**

The study collected data on 140 HIV-infected children (< 18 years). Adherence, pharmacy supply information and HIV viral loads were obtained from clinical and research databases.

### **Patients**

The data from 127 HIV-infected children (60 boys and 67 girls; mean age 9.9 years) were collected.

### **Main outcome measures**

Complete adherence (100%) was reported in only 24% of patients. With 40% of patients being rarely or never completely adherent, 64% of children achieved undetectable viral loads during the study period.

### **Results**

No association between pharmacy supply and self-reported adherence was found ( $P = 0.605$ ). Self-reported adherence ( $P = 0.0328$ ) and age ( $P = 0.025$ ) were the significant predictors of reaching undetectable viral loads. Adolescents (> 13 years) were significantly less likely to reach undetectable viral loads than children under 13 years (odds ratio 0.38; 95% CI 0.16 to 0.89).

### **Conclusion**

In our study, pharmacy supply was not associated with self-reported adherence. Most importantly, adherence and age were significant predictors of reaching undetectable viral loads.



## INTRODUCTION

Combination antiretroviral therapy (ART) has led to dramatic changes in the clinical course of HIV infection in paediatric patients.<sup>1,2</sup> High ART adherence is required to achieve a complete and sustained suppression of viral replication.<sup>3-5</sup> Poor adherence leads to treatment failure, the development of viral resistance with subsequent reduction in treatment options, increased morbidity and mortality.<sup>3-6</sup> In adult patients, the mean ART adherence has been reported to be between 80% and 91%, and multiple adherence barriers have been identified.<sup>6,7</sup> In paediatric HIV-infected patients, ART adherence has been reported to be lower (58%),<sup>8,9</sup> due to the combination of patient or caregiver factors including age and maturity-related tolerability and parental involvement.<sup>10-13</sup> Several population-specific ART adherence barriers have been established in paediatric HIV such as dependence on a caregiver, poor palatability (particularly of liquid preparations), large pill size and pill swallowing capacity relative to the child's developmental stage.<sup>9,11</sup>

While some of the barriers related to the patients' ability to tolerate and receive ART are difficult to change, others such as pharmacy supply can be modified through thoughtful evaluation and interventions by the providers, pharmacies and insurance companies. The examination of the relationship between pharmacy factors and treatment adherence in adults has shown the significance of pharmacy supply in patient adherence.<sup>14,15</sup> Among pharmacy-related adherence barriers patients reported that either the pharmacy was out of the medication or the medications were not refilled in a timely manner.<sup>14</sup> As children's access to ART is dependent on their caregivers, the efficient mechanism of medications supply and positive experience of the caregiver with the pharmacy may lead to increased ART adherence and improved outcome in paediatric patients with HIV.<sup>16,17</sup> To date, no studies have been published on the effects of pharmacy factors on treatment adherence and outcome in HIV-infected children and adolescents.

This study was conducted to characterise the methods of obtaining antiretroviral medications by caregivers and to evaluate the relationship between pharmacy supply, self-reported ART adherence and virological outcome in children and adolescents with HIV.

## PATIENTS AND METHODS

This was a retrospective (52 weeks) cohort study of 140 HIV-infected paediatric patients (0 - 18 years old) treated at the Special Immunology Program at the Children's National Medical Center, Washington DC. The Special Immunology Program provides care to perinatally HIV-infected children and adolescents from Washington DC metropolitan area including suburban areas, and is a site of multiple clinical studies including the National Institutes of Health and industry-sponsored research. Paediatric HIV-infected patients are seen in the clinic every 3 months as routine follow-up. Patients are given verbal and written instructions on antiretroviral

medications at each clinic visit. The adherence assessment, pharmacy supply information and HIV-RNA viral load are routinely obtained as part of standard of care and clinical research studies. The majority of patients (95%) are black with approximately equal gender distribution. More than 90% of patients receive ART consisting of two nucleoside reverse transcriptase inhibitors and a single protease inhibitor (60%) or non-nucleoside reverse transcriptase inhibitor (30%).

The records of self-reported adherence, HIV-RNA viral load, and pharmacy use were reviewed in a retrospective follow-up of 52 weeks of clinic and research records. Patients without ART, with incomplete pharmacy and adherence information in the clinical and research databases, were not included. Parents/legal guardians enrolled in the research studies have consented to personal health information storage in a database for future HIV research. The protocol and the Health Insurance Portability and Accountability Act/Institutional Review Board (IRB) authorisation for waiver of consent were reviewed and approved by the IRB at the Children's National Medical Center.

Self-reported adherence was assessed through interactive 3 days recall questionnaire-based interview by the clinic staff with the caregiver and older children (> 10 years of age) at routine clinic visit (every 3 months). During the interview caregivers and older children were asked about the medications names, dosing schedule, time of last dose, and the number of missed doses in the 3 days preceding the clinic visit (yesterday, 2 days ago and 3 days ago). They were also asked to identify adherence difficulties such as "forgetting", "running out of medications", "having difficulties taking the medications", "missing medications due to pharmacy-related factors" and "others". Three days recall was used since the available data suggest that patients or caregivers cannot accurately recall missed doses beyond a few dates.<sup>18</sup> The history of pharmacy use was obtained from the clinical database, which contained information on the pharmacies used in the previous 12 months (including a history of problems or switching pharmacy), patient satisfaction with the pharmacy and the history of missing antiretroviral doses due to pharmacy errors or delays. Adherence was calculated as the number of all antiretroviral doses taken divided by the number of doses prescribed x 100(%) during the 72 h preceding the visit. The patients were graded into four categories based on the percentage of the 100% adherence reports (complete adherence) during the 52 weeks of follow-up: (1) always adherent (96 - 100% of visits completely adherent); (2) mostly adherent (50 - 95% of visits completely adherent); (3) rarely adherent (25 - 49% of visits completely adherent) and (4) never adherent (< 25% of visits completely adherent). The pharmacy supply was classified as "home delivery" (HD) when the caregiver received antiretroviral medications at home (through mail or personal delivery) and as "in pharmacy pick-up" (IPP), when the caregiver picked up the medications at the pharmacy. HIV-RNA viral load (Roche Amplicor; Roche, Molecular Systems, Inc, Branchburg, New Jersey, USA) was considered to be undetectable with less than 400 copies/ml (the lowest limit of quantitation during the study period). The demographic

characteristics including race, sex and age of the subjects were collected. All analyses were performed using SAS version 8.2.

Descriptive statistics, such as means and standard deviations were calculated for each continuous variable, and frequency distributions were generated for each categorical variable as appropriate. Frequency distribution characterised the pharmacy supply of obtaining antiretroviral medications (IPP vs HD). Fisher's exact tests were used to estimate the association between pharmacy supply and self-reported adherence. Univariate and multivariate logistic regression analyses were used to estimate the association between pharmacy supply, self-reported adherence, age and virological outcome.

## RESULTS

Data were collected on 127 paediatric patients with perinatally acquired HIV. There were 60 (47%) girls and 67 (53%) boys with a mean age of 9.9 years (SD 4.3) and the majority ( $n = 117$ ; 93%) of patients was black. The mean number of visits per patient was 4.5 during the 52 weeks. Seventy-seven (61%) patients were children under 13 years, whereas 50 (39%) were adolescents (13 - 18 years old). Eighty-one patients (64%) have achieved an HIV-RNA viral load of less than 400 copies/ml during the study period.

A significant proportion of the children and adolescents reported suboptimal adherence during the study period (table 1). The majority of caregivers of adolescents ( $n = 29$ ; 58%) used HD, whereas among the caregivers of younger children 36 (47%) used IPP. The caregivers of younger children were more likely to use the combination (IPP plus HD) supply than the caregivers of adolescents (8% vs 2%, respectively). Interestingly, pharmacy factors as a barrier to adherence were reported more often among HD ( $n = 8$ ; 67%) than IPP ( $n = 4$ ; 33%) patients. A history of missing antiretroviral doses due to the pharmacy was more frequently reported among the patients who used HD ( $n = 14$ ; 70%) than those who used IPP ( $n = 6$ ; 30%).

There was no association between pharmacy supply (IPP vs HD) and self-reported adherence (Fisher's exact test  $P = 0.605$ ). Examination of the multivariate logistic regression to determine the association between pharmacy supply, self-reported adherence and virological outcome revealed the overall model as significant ( $P < 0.001$ ). Moreover, adherence ( $P = 0.0328$ ) and age ( $P = 0.025$ ) were the only significant predictors of ever reaching an undetectable viral load during the study period (table 2). Adolescents (13 - 18 years old) were significantly less likely to reach an undetectable viral load than younger children (< 13 years old) (odds ratio (OR) 0.38; 95% CI 0.16 to 0.89). For every year increase in age, the odds of reaching an undetectable viral load decreased by 10% after controlling for self-reported adherence and refill mechanism.

Those patients who chose a combination of IPP and HD were four times more likely to become undetectable (OR 3.85; 95% CI 0.38 to 38.58) than those who used HD; however, due to small numbers ( $n = 7$ ), this association was not statistically significant. For every percentage increase in adherence, the odds of reaching an undetectable viral load increased by 3% after controlling for age and pharmacy supply.

**Table 1** Adherence to ART and characteristics of pharmacies and pharmacy supply used to obtain antiretroviral medications for HIV-infected paediatric patients

	<b>N = 127 (%)</b>
<i>Self-reported adherence to ART</i>	
Always adherent (96 - 100%)	30 (24)
Mostly adherent (50 - 95%)	47 (37)
Rarely adherent (25 - 49%)	24 (19)
Never adherent (< 25%)	26 (21)
<i>Barriers</i>	
None	73 (58)
Forgetting	14 (11)
Pharmacy issues	12 (9)
Difficulties taking the drug	9 (7)
Running out of medication	8 (6)
Other	11 (9)
<i>Pharmacy Use</i>	
General pharmacy stores	69 (54)
Supermarket pharmacies	17 (13)
Specialty pharmacy	9 (7)
Provider pharmacy	7 (6)
Other pharmacies	25 (20)
<i>Pharmacy supply</i>	
IPP	56 (44)
HD	64 (50)
Combination IPP and HD	7 (6)
<i>History of pharmacy use</i>	
History of problem with pharmacy	7 (6)
Switched pharmacy in past year	12 (9)
(Switched due to problems with pharmacy)	3 (25)*
Missed medication due to pharmacy	20 (16)
Satisfied with pharmacy	115 (91)

\* Percentage of patients from those who switched pharmacy (out of 12 patients).  
ART – antiretroviral therapy; HD – home delivery; IPP – in-pharmacy pick-up.

**Table 2** Association between age, self-reported adherence, pharmacy supply and the odds of achieving undetectable HIV-RNA viral load (< 400 copies/ml)

Variable	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Age	0.92 (0.84, 1.00)	0.90 (0.81, 0.99) <sup>†</sup>
Children (< 13 yrs old)	1.00	1.00
Adolescent (13 - 18 yrs old)	0.39 (0.18, 0.83) <sup>†</sup>	0.38 (0.16, 0.89) <sup>†</sup>
Self-reported adherence	1.02 (1.01, 1.04) <sup>†</sup>	1.03 (1.01, 1.04) <sup>†</sup>
Never adherent (< 25%)	1.00	1.00
Rarely adherent (25 - 49%)	1.15 (0.38, 3.53)	1.01 (0.30, 3.40)
Mostly adherent (50 - 95%)	4.46 (1.59, 12.5) <sup>†</sup>	4.80 (1.58, 14.59) <sup>†</sup>
Always adherent (96 - 100%)	5.23 (1.59, 17.16) <sup>†</sup>	6.39 (1.81, 22.56) <sup>†</sup>
Pharmacy supply		
HD	1.00	1.00
IPP	2.00 (0.93, 4.29)	2.37 (1.00, 5.63)

\* Adjusted for all other variables in the model. <sup>†</sup> Statistically significant. HD - home delivery; IPP - in-pharmacy pick-up; OR - odds ratio.

## DISCUSSION

Whereas the overall model of association between pharmacy supply, self-reported adherence and virological outcome in our study was shown to be significant, self-reported adherence and age were the only significant predictors of ever reaching an undetectable viral load during the 52 weeks of the study period. Previous studies have demonstrated the complexity of adherence assessment in children and adolescents with HIV.<sup>19</sup> Direct measures of adherence, such as therapeutic drug monitoring, are expensive and difficult to interpret due to limited knowledge of the pharmacodynamics of paediatric ART. Indirect methods (self-reports, electronic drug monitoring, refill verification) have disadvantages ranging from the accuracy of the data to cost and practicality issues.<sup>19</sup> Our data support the use of interactive self-report as an efficient tool in assessing adherence in paediatric patients, despite a well-recognised potential for overreporting.<sup>19,20</sup> We must acknowledge that the study was conducted within a well-established paediatric HIV program with vigorous adherence interventions, and adherence reports were collected through interactive interview with the caregivers and older children by familiar clinic personnel in a non-biased nonjudgmental style that has the potential to decrease the overestimation of adherence. The study also used repeated adherence assessments, with an average number of reports of 4.5 per study period instead of a single report, which increased the reliability of self-reports.

In accordance with previously published studies, the higher level of adherence was significantly associated with the likelihood of reaching virological suppression when compared with a lower level of adherence. Rarely adherent (25 - 49%) and never

adherent (< 25%) patients were equally unlikely to achieve a viral load of less than 400 copies/ml.

The finding that adolescents were significantly less likely to reach an undetectable viral load than younger children in our study correlates with previously published data on the lower rates of ART adherence among HIV-infected youth.<sup>3,10</sup> Although we acknowledge that the adolescents in the study had perinatally acquired HIV, were highly treatment experienced, and therefore had higher chances of HIV resistance and virological failure, we must recognise many additional obstacles to ART adherence emerging during puberty. Transition to adolescence by children with perinatally acquired infection leads to changes in lifestyle involving growing independence, separation from parental involvement, increased peer pressure and fear of stigmatisation, increased risk-taking behaviour, psychiatric problems and substance abuse.<sup>6,19-22</sup> For providers and caregivers loss of adherence during puberty in adolescents with perinatally acquired HIV represents a difficult and emotional challenge that requires a team approach and close collaboration. While several strategies (directly observed therapy, regimen-related, education and counselling interventions) have been suggested to maximise ART adherence during this transition period,<sup>6,19-22</sup> clinicians frequently seek guidance from research and practice in other paediatric chronic illnesses such as asthma, juvenile rheumatoid arthritis and type I diabetes mellitus. Given the lack of a well-defined adherence intervention model in adolescents with HIV, more research on adherence among HIV-infected youth with interdisciplinary collaboration is warranted.

We recognise limitations of our study such as the retrospective study design, small sample size and lack of direct patient/caregiver interviews. In addition, all of the pharmacy information gathered from self-reports was not confirmed by the verification of refill histories. Other factors, such as drug resistance and treatment history, could account for the differences in reaching an undetectable viral load. In summary, self-reported adherence and age were the only significant predictors of ever reaching an undetectable HIV viral load during the 52 weeks of follow-up.

## **ACKNOWLEDGMENTS**

The authors express sincere gratitude to Drs Veronica Miller, PhD, Research Professor, Department of Prevention and Community Health, The George Washington University (GWU) School of Public Health and Health Services (SPHHS) and Ann Goldman, MA, MPH, Research Instructor, Department of Epidemiology and Biostatistics, GWU SPHHS for their guidance and critical review of the study. The data analysis was completed with the participation of Dante Verme, PhD, MS, Professor of Epidemiology and Biostatistics, and Gregory Phillips II, MS, PhD, Department of Epidemiology and Biostatistics, GWU SPHHS.

The authors were supported by Department of Health and Human Services, National Institutes of Health Public Health Service grants NCRR K12 RR017613 and NICHD 1U10 HD45993 (NR).

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# 11

# chapter

## **A URINARY BIOMARKER PROFILE FOR CHILDREN WITH HIV-ASSOCIATED RENAL DISEASES**

Soler-García AA, Rakhmanina NY, Mattison PC, Ray PE.

*Kidney Int.* 2009;76:207-214.

**ABSTRACT**

Human immunodeficiency virus (HIV)-infected children are at risk of developing several types of renal diseases, including HIV-associated nephropathy (HIVAN), which is usually seen during late stages of infection in children with a high viral load. This disease is defined by the presence of proteinuria associated with mesangial hyperplasia and/or global-focal segmental glomerulosclerosis combined with microcystic transformation of the renal tubules. Because HIVAN can have an insidious clinical onset, renal biopsy is the only definitive way of establishing a diagnosis. Given the risk of performing this procedure in HIV-infected children with other AIDS-defining illness, we sought to identify informative biomarkers such as growth factors in the urine of 55 HIV-infected children that might be predictive of the extent and activity of the renal lesions characteristic of HIVAN. We found that the levels of epidermal growth factor were lower in the urine of children with renal disease, whereas levels of fibroblast growth factor-2 and metalloproteinase-2 were higher as compared with those levels in infected children without renal disease. Similar changes were observed in HIV-Tg<sub>26</sub> mice correlating with the progression of renal disease in this model of HIVAN. Our findings suggest that this urinary growth factor profile may be useful in facilitating the diagnosis of HIV-infected children at risk of developing HIVAN when interpreted in the appropriate clinical setting.

## INTRODUCTION

Childhood human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) is a renal disease (RD) defined by the presence of persistent proteinuria associated with mesangial hyperplasia and/or global-focal segmental glomerulosclerosis in combination with microcystic transformation of renal tubules and rapid progression to chronic renal failure.<sup>1-3</sup> It is usually seen during the late stages of HIV infection in children with a high viral load. Black children show a unique susceptibility to develop this RD, and if the US data from the pre-HAART (highly active antiretroviral therapy) era were extrapolated to Africa,<sup>4</sup> we speculate that approximately 300,000 children without access to antiretroviral therapy could develop HIVAN. In addition, HIV-infected children can develop other RD or tubular toxicity secondary to HAART and other drugs.<sup>1-3</sup> Thus, to prevent the severe complications associated with these RD, it is necessary to perform an early diagnosis, and the only way of establishing a definitive diagnosis of HIVAN is to make a renal biopsy. Given the risk of performing this procedure in HIV-infected children, it is necessary to find new biomarkers to identify children at high risk of developing HIVAN.

In a recent study using a proteomic approach, we were unable to identify a single urine protein biomarker to identify children with biopsy-proven HIVAN.<sup>5</sup> Here, we sought to determine whether growth factors released into the urine of children with HIV-RD reflect the extent and activity of the renal lesions characteristic of this disease. This approach is based on earlier studies showing a progressive accumulation of heparin-binding growth factors in correlation with the development of the renal microcystic tubular lesions that are a characteristic feature of childhood HIVAN.<sup>6-9</sup> We found elevated urine levels of fibroblast growth factor-2 (FGF-2) and matrix metalloproteinase-2 (MMP-2), in association with reduced urine levels of epidermal growth factor (EGF) in children with HIV-RD. As these growth factors are involved in the processes of renal tubular regeneration and cyst formation,<sup>10-20</sup> our findings suggest that this urine profile might be useful in identifying children undergoing the tubular interstitial and microcystic lesions characteristic of HIVAN.

## MATERIALS AND METHODS

### Patients

Urine samples were collected from HIV-negative children (n = 28) and HIV-infected children (n = 56) who received regular clinical care at the Children's National Medical Center (CNMC) during the period between January 1995 and December 2008. The study protocol and consenting documents were approved by the CNMC's IRB. All HIV-infected patients acquired HIV-1 from their mothers through vertical transmission, with the exception of one child who was infected through a blood transfusion in the perinatal period. Children ranged in age from 3 months to 19 years with an approximately equal rate between male and females. Approximately 94% of all children were African Americans, whereas the remaining patients were Caucasians, Asians, and Hispanics. The diagnosis of HIV-infection and AIDS was

based on the criteria established by the Center for Disease Control (CDC). The HIV-infected patients were in the following CDC clinical categories: asymptomatic-A (~ 4%); mildly symptomatic-B (~ 52%), and severely symptomatic-C (~ 44%). All children received standard pediatric doses of antiretroviral therapy under the supervision of attending physicians with expertise in the treatment of HIV-infected children. The viral load (HIV-1 RNA PCR) was measured using standard laboratory assays at least every 6 months as part of routine clinical care.

In seven patients (one child < 2 years, and six children > 2 years of age), the diagnosis of HIVAN was confirmed by renal biopsy. In two additional patients, the diagnosis of HIVAN was based on the following clinical findings: (1) presence of protein creatinine ratios > 0.2 mg/mg for more than 6 months; (2) enlarged echogenic kidneys with a typical clinical history of HIVAN; and (3) development of chronic renal failure during the longitudinal follow-up period. With the exception of one child, all urine samples harvested from children with HIVAN were collected when the blood urea nitrogen and serum creatinine levels were within the normal range. All samples were stored immediately at -70 °C, and processed whenever indicated.

### **Urinary markers of renal injury**

The urinary protein and creatinine levels were confirmed using colorimetric assays from Wako Diagnostics (Richmond, VA) and R&D Systems (Minneapolis, MN), respectively. Cystatin C was measured using an enzyme-linked immunoabsorbent assay according to the manufacturer's instructions (R&D Systems).  $\gamma$ -Glutamyl transpeptidase and alkaline phosphatase were assayed following the procedures described by Chung *et al.*<sup>21</sup> and adapted to microtiter plates. Lysozyme was measured as described by Shugar.<sup>22</sup> Units of lysozyme/ml were determined following the Sigma quality control test for lysozyme.

### **Urinary growth factors and metalloproteinases**

Urinary levels of EGF, FGF-2, MMP-2 and -9 were measured by enzyme-linked immunoabsorbent assay kits following the instructions of the manufacturer (R&D Systems). Gelatin zymography was performed with 10% SDS-polyacrylamide gels containing 1 mg/ml gelatine as described earlier.<sup>23</sup> Urine samples were diluted 3:1 with substrate gel buffer (10% SDS, 4% sucrose, 0.25M Tris-HCl, pH 6.8, and 0.1% bromophenol blue) and loaded immediately onto the gel without boiling.<sup>23</sup> After staining the gels with Commassie Blue R-250, the gelatinolytic activity appeared as white bands of the corresponding molecular weight. Additional urine samples were incubated with an MMPs inhibitor 20 mM 1, 10-phenanthroline to inhibit the activity of MMPs.<sup>24</sup> The zymography gels were then scanned with an Epson EP1670 scanner, Long Beach, CA, USA, using Adobe Photo Shop and results were expressed in arbitrary optical density (OD) units as OD/urinary creatinine ratios.

### **Growth of cultured CV-1 cells**

CV-1 cells (American Type Culture Collection, Manassas, VA)<sup>25</sup> were seeded at a density of  $2.0 \times 10^2$  on 96-well plates, and exposed to urine samples harvested

from HIV-infected children with and without RD (10% urine diluted in Dulbecco's modified Eagle's medium supplemented with 1% fetal bovine serum and antibiotics (penicillin G, streptomycin sulfate, and amphotericin B) (Invitrogen, Grand Island, NY). To neutralize the activity of EGF in the urine, selected urine samples were preincubated with 30 µg/ml of EGF antibody for 1 h at 37 °C (R&D Systems). After 96 h, cell growth was measured colorimetrically by absorbance at 490 nm using the Cell Titer 96 reagent (Promega; Madison, WI).

### **HIV-Tg<sub>26</sub> mice**

Human immunodeficiency virus-Tg<sub>26</sub> mice carrying a defective HIV-1 proviral DNA<sup>26</sup> were used to confirm our findings. Urine and kidney samples were harvested in a sequential manner at 10, 25, and 60 days of life (n = 4 in each group) from WT littermate and HIV-Tg<sub>26</sub> mice with and without RD. Urinary EGF-like activity was determined by doing binding displacement assays in cultured A431 cells exposed to <sup>125</sup>I-EGF (0.1 µCi/ng, DuPont-New England Nuclear, Wilmington, DE, USA) as described by Mesri *et al.*<sup>27</sup>

### **Northern blots EGF analysis**

Total RNA was harvested from the renal medulla of mice at specific time points using guanidine-thiocyanate as described by Chirgwin *et al.*<sup>28</sup> Total RNA (10 µg) was electrophoresed through formaldehyde-agarose gels and transferred to nitrocellulose filters. The filters were hybridized with the 754 bp (SmaI-PvuII fragment (bases 2886–3639)) or preproEGF cDNA clone (pmeg10)<sup>17</sup> as described by Horikoshi *et al.*<sup>14</sup> Blots were normalized by probing for the small ribosomal protein S14 (American Type Culture Collection, plasmid no. 59247) as described earlier.<sup>29</sup> The collagenolytic activity of urine samples harvested from mice of 10, 25, and 60 days of age was assessed by gelatin zymography and quantified as described above.

### **Statistical analysis**

Comparison between two groups was done with the Student t-test. *P*-values less than 0.05 were considered significant. When more than two means were compared, significance was determined by one way analysis of variance followed by multiple comparisons using the Student-Neuman-Keul's *post hoc* test. Non-normal data were either logtransformed before analysis or analyzed by the Kruskal-Wallis analysis of variance followed by Dunn's multiple comparisons post-test.

## **RESULTS**

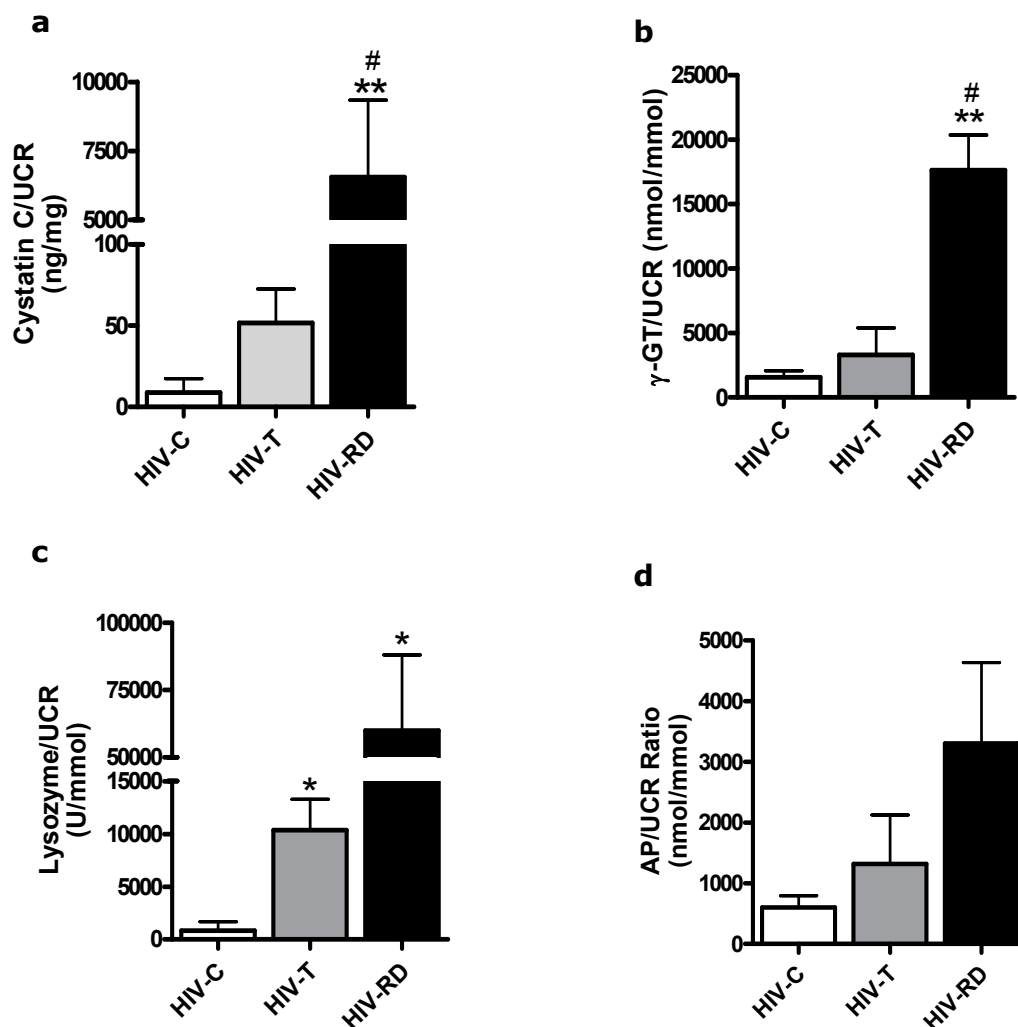
### **Renal tubular injury in HIV-RD**

As shown in Figure 1, we found elevated levels of cystatin C, γ-glutamyl transpeptidase (γ-GT), and lysozyme in a group of children with established HIV-RD. The urinary lysozyme levels were also significantly elevated in HIV-infected children with trace proteinuria (*P* < 0.05) (Figure 1a), suggesting that the brush border of renal proximal tubular cells was injured in these patients. On the basis of these

findings, we hypothesized that the urinary excretion of EGF, which is synthesized mainly by renal epithelial cells, would be reduced in children with HIVAN.

### Decreased urinary levels of EGF in children with HIV-RD

To determine the urinary levels of EGF in HIV-positive and -negative children of different ages without RD, EGF was measured in the following groups of control children: (1) < 2 years of age, with functionally immature kidneys with respect to glomerular function; (2) 2 - 12 years of age, with functionally mature pre-pubescent kidneys; and (3) > 12 years, with mature pubescent kidneys. No significant differences in urinary EGF levels were found between HIV-positive or -negative children of similar age without RD (data not shown). However, children < 2 years showed the highest urinary EGF levels when compared with all other age control groups (Table 1).



**Figure 1** Increased levels of urinary biomarkers of renal tubular injury in HIV-infected children with renal disease. Panels **a-d** show mean  $\pm$  SEM values for cystatin C (**a**),  $\gamma$ -glutamyl-transpeptidase ( $\gamma$ -GT) (**b**), lysozyme (**c**), and alkaline phosphatase (AP) (**d**) in urine samples harvested simultaneously from HIV-infected children without proteinuria (HIV-C;  $n = 10$ ); trace proteinuria (HIV-T;  $n = 8$ ); and established renal disease (HIV-RD;  $n = 9$ ). Urine values were expressed as a urinary creatinine (UCR) ratio.  $P$ -values less than 0.05 were considered statistically significant. (\* and # indicates a  $P < 0.05$ ; \*\* indicates a  $P < 0.001$  when compared to the HIV-C, and HIV-T groups, respectively).

**Table 1** Average urinary levels of EGF, FGF-2 and MMP-2 in children without renal disease

Biomarker	Control group age (years)*		
	< 2	2 - 12	> 12
EGF/UCR (pg/mg)	96,045 ± 7751 *	40,238 ± 2,967	29,485 ± 6,468
FGF-2/UCR (pg/mg)	5.86 ± 1.32	4.9 ± 1.14	2.33 ± 0.39
MMP-2 (ng/mg)	2.93 ± 0.86	0.93 ± 0.23	1.10 ± 0.22

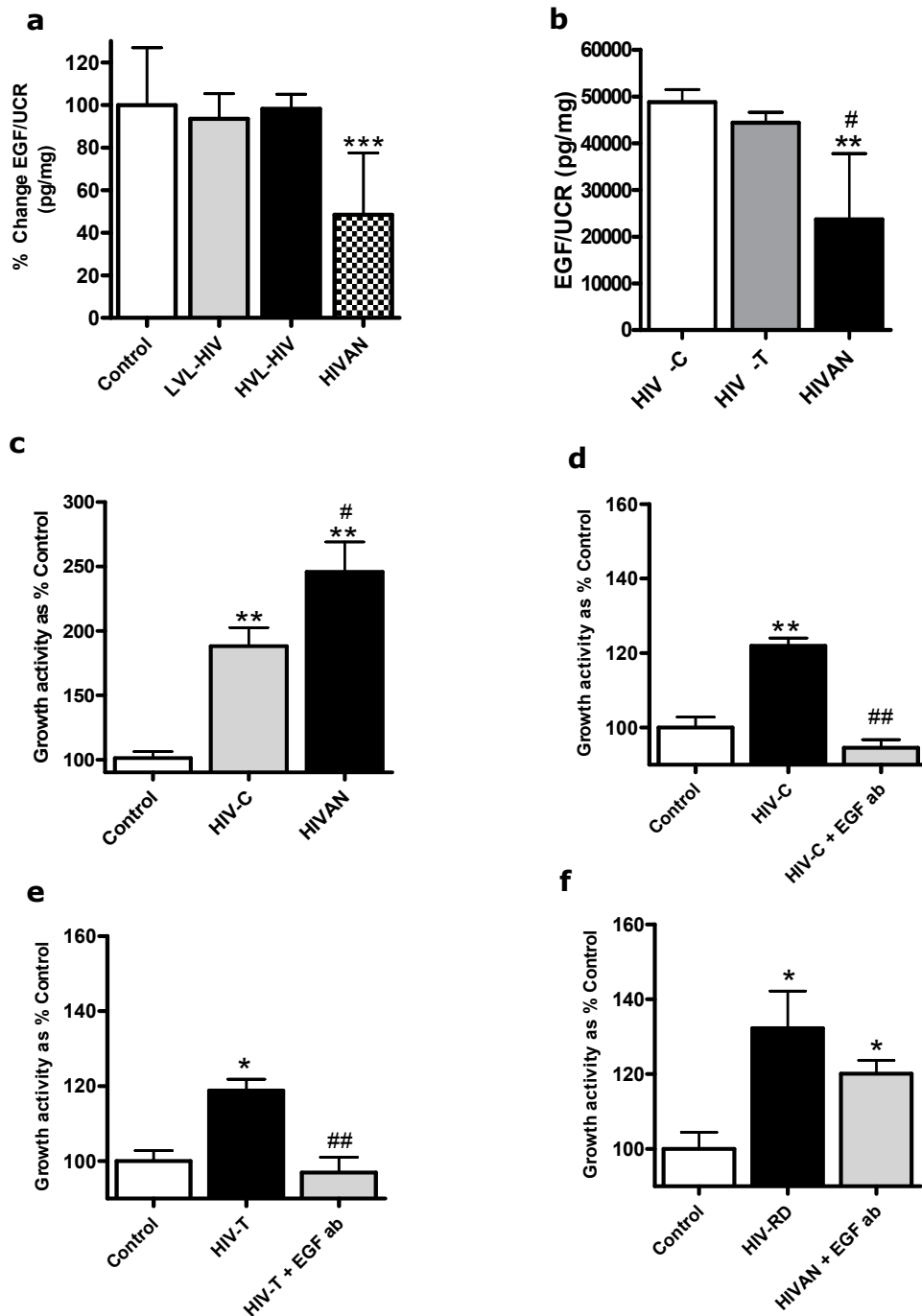
EGF – epidermal growth factor; FGF-2 – fibroblast growth factor-2; MMP-2 – matrix metalloproteinase-2; UCR – urinary creatinine ratio.

All values are expressed as the mean ± s.e. of the mean, and expressed as a ratio of the urinary creatinine value. (n = 8 - 24 children per group). Non-parametric analysis of variance \* P < 0.05 and 0.001 when compared with the EGF groups of children of 2 - 12 years and > 12 years of age, respectively. No statistically significant differences were found between the groups of children > 2 years of age for EGF, FGF-2, and MMP-2 (P = 0.0648; 0.1716; and 0.5668, respectively).

The urinary EGF concentration was significantly lower in children > 2 years with biopsy-proven HIVAN (n = 6), when compared with their corresponding age-matched HIV controls and trace proteinuria groups (Figure 2a, P < 0.0001 and Figure 2b, P < 0.05). Moreover, two children < 2 years with HIVAN also showed a reduction in their urinary EGF levels of approximately 32 and 45%, relative to the mean values of age-matched controls. These changes were similar to the low values reported in children of similar age with congenital obstructive nephropathy.<sup>10</sup> In summary, only one child within six children > 2 years with biopsy-proven HIVAN showed normal urinary EGF levels.

### Growth-promoting activity of EGF in urine samples harvested from HIV-infected children

As shown in Figure 2c, urine samples harvested from HIV-infected children with and without RD stimulated the growth of cultured renal tubular epithelial CV-1 cells by an average 88 and 145% (P < 0.001), respectively, when compared with the cells exposed to control media containing 1% fetal bovine serum. Moreover, urine samples harvested from children with HIVAN increased the growth of CV-1 cells by an average of 57.6% when compared with the control samples (P < 0.05) (Figure 2c). EGF-neutralizing antibodies were able to suppress the growth-promoting activity of urine samples harvested from the HIV-infected controls (HIV-C; P < 0.001) and trace proteinuria (HIV-T; P < 0.01) groups, respectively (Figure 2d and e). In contrast, EGF antibodies failed to exert a complete growth inhibition of CV-1 cells exposed to urine supernatants harvested from children with HIVAN (P > 0.05) (Figure 2f). These data suggest that other growth factors that are present in the urine of children with HIVAN (that is, FGF-2), may act in synergy with EGF to enhance the proliferation of tubular epithelial cells in these patients.<sup>6-9</sup>



**Figure 2** Decrease EGF levels in the urine of HIV-infected children > 2 years of age with HIVAN. Panel **a** shows the urinary EGF levels in controls (HIV-negative children without renal disease;  $n = 8$ ); HIV-infected children without renal disease and low viral load (< 5000 copies/ml; HIV-LVC,  $n = 15$ ); HIV-infected children without renal disease and high viral load (> 10,000 copies/ml; HIV-HVC,  $n = 26$ ); and children with biopsy-proven HIVAN ( $n = 6$ ). Urine values are expressed as % change relative to the control group. Panel **b** shows changes in urinary EGF levels in HIV-infected children > 2 years of age without proteinuria (HIV-C;  $n = 10$ ); trace proteinuria (HIV-T;  $n = 8$ ); and biopsy-proven HIVAN ( $n = 6$ ). Panels **c - f** show the urinary EGF-induced growth promoting activity in cultured CV-1 renal tubular epithelial cells. CV-1 cells were exposed for 96 h to urine samples harvested from HIV-infected children without proteinuria (HIV-C), trace proteinuria (HIV-T), and HIVAN as described in the Materials and Methods section. CV-1 cells exposed to 1% FBS media were used as controls. These experiments were made in the presence or absence of an anti-EGF-neutralizing antibodies. Bars represent the mean  $\pm$  s.e.m.  $P$ -values less than 0.05 were considered statistically significant. (\* and # indicate a  $P < 0.05$ ; \*\* and ## indicate a  $P < 0.001$ ; \*\*\* indicates  $P < 0.0001$  when compared to all other groups. when compared with the control alone and cells exposed to urine, respectively). All the experiments were performed in triplicate.



### **Increased urinary FGF-2 in children with HIVAN**

Children < 2 years show the highest urinary levels of FGF-2, but these changes were not statistically significant when compared with all other age control groups (Table 1). All children > 2 years with biopsy-proven HIVAN (n = 6) showed high abnormal urinary FGF-2 levels, when compared with their corresponding age-matched HIV controls and trace proteinuria groups (Figure 3a and b). Similarly, in both children < 2 years with HIVAN, the urinary FGF-2 levels increased by an average of 2.5- to 11-fold over their age-matched controls, and above the normal values reported for children of similar age.<sup>18</sup>

### **Increase urinary MMP-2 in children with HIVAN**

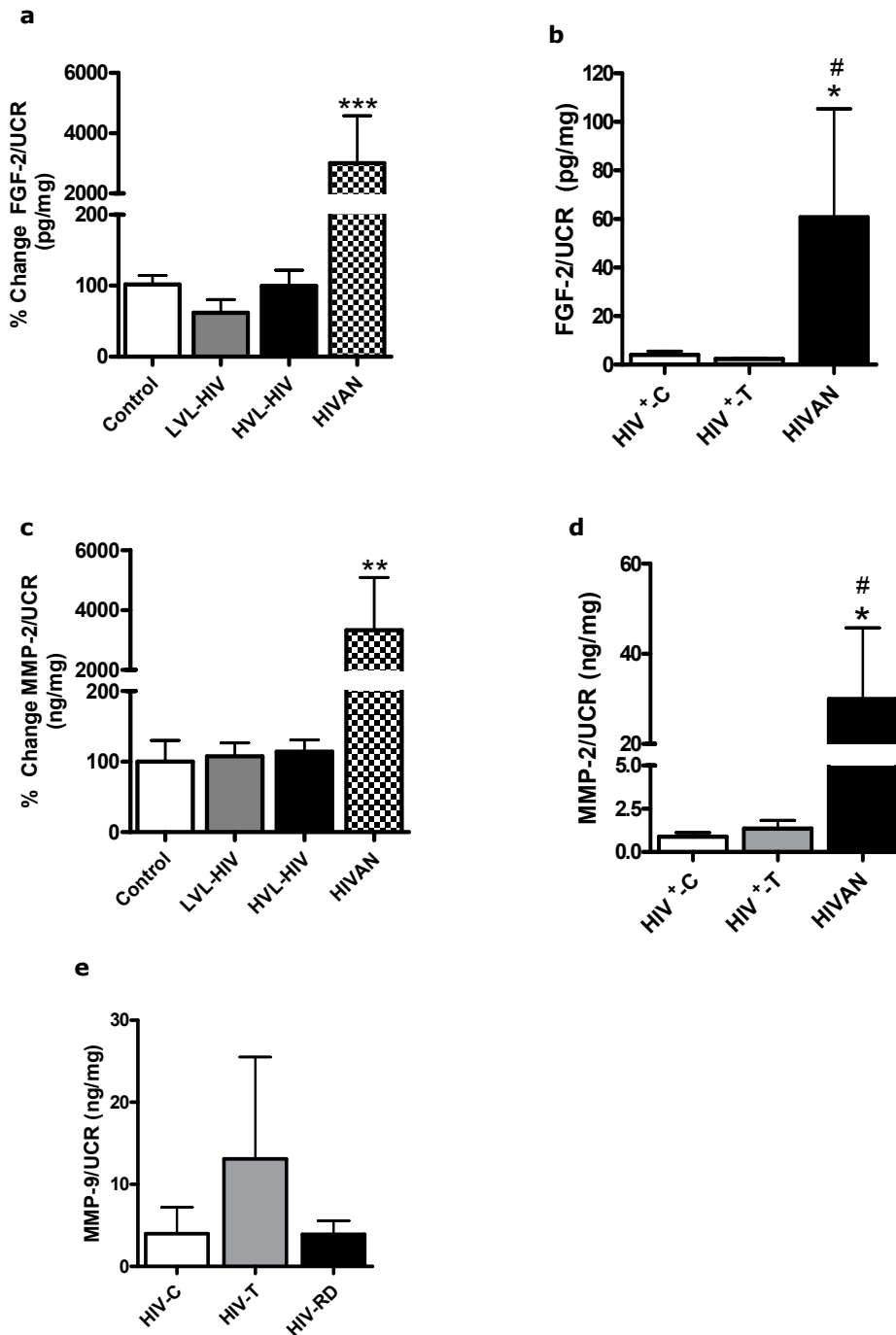
Children < 2 years showed the highest urinary levels of MMP-2, when compared with all other age control groups (Table 1). MMP-2 was elevated in the urine of children > 2 years with biopsy-proven HIVAN (n = 6) in comparison with their respective age-matched control ( $P < 0.001$ ) and trace proteinuria ( $P < 0.05$ ) groups (Figure 3c and d). In a similar way, urinary MMP-2 increased in both children < 2 years with HIVAN by an average of 0.5- to 68-fold over their age-matched controls and above the normal values reported by others.<sup>13</sup> Finally, no significant differences in the urinary levels of MMP-9 were detected between HIV-infected children in the control, trace proteinuria, and HIVAN groups, respectively ( $> 0.05$ ) (Figure 3e). In summary, all children with biopsy-proven HIVAN showed abnormally elevated urinary levels of both MMP-2 and FGF-2 relative to their corresponding age-matched control groups.

### **Gelatin zymographic analysis**

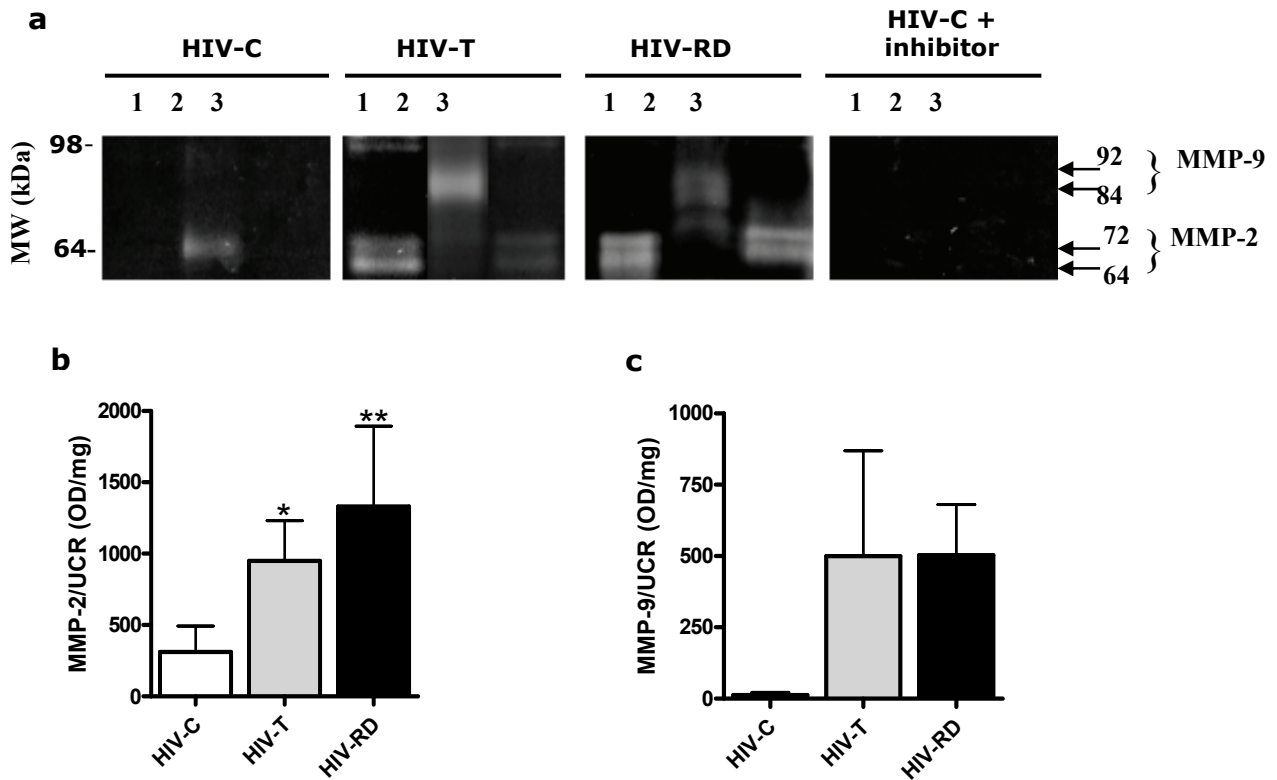
Figure 4a shows a representative gelatin zymographic analysis of urine samples harvested from HIV-infected children with and without RD, including seven children with HIVAN. The collagenolytic activity in these urine samples was inhibited in the presence of 20 mM 1, 10-phenanthroline, confirming the presence of metalloproteinases activity (Figure 4a). MMP-2 was detected as 72 and 64 kDa collagenolytic bands corresponding to the latent and active forms of this metalloproteinase.<sup>13</sup> The activity of MMP-2 was increased in the urine of children with established RD relative to the control group (Figure 4b). MMP-9 was detected as 92 and 82 kDa collagenolytic bands corresponding to the latent and active forms of this metalloproteinase (Figure 4c). HIV-infected children with trace proteinuria and established RD showed an increased urinary MMP-9 activity; however, these changes were not statistically significant (Figure 4c).

### **Progressive changes in urinary EGF, FGF-2, and MMP-2 levels in a child with biopsy-proven HIVAN**

As shown in Figure 5, the urinary levels of EGF in a child with HIVAN increased in correlation with the recovery of renal function in response to antiretroviral therapy (Figure 5a and b). In contrast, the urinary concentration of FGF-2 and MMP-2 decreased in correlation with the recovery of renal function. However, the urinary levels of EGF, FGF-2, and MMP-2 remained within abnormal values throughout the follow-up period (Figure 5c and d).



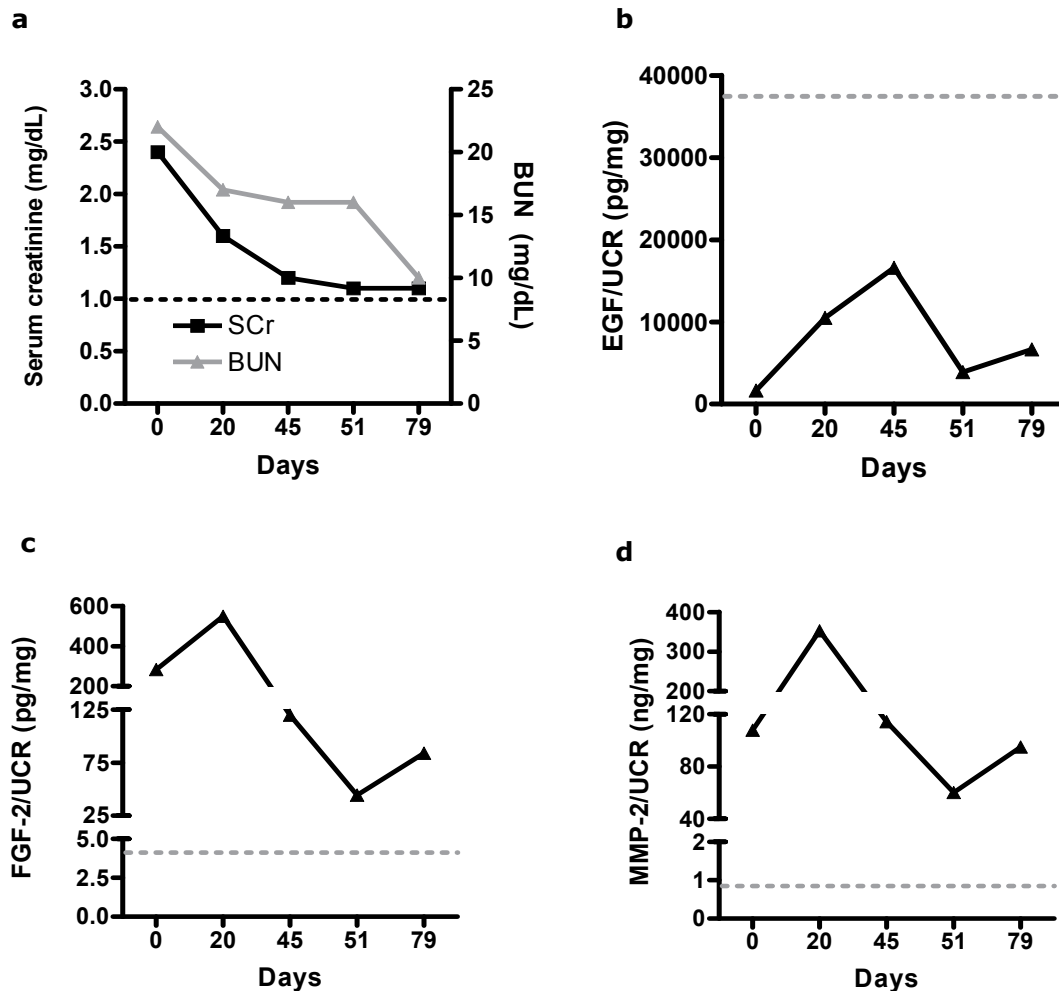
**Figure 3** Increase FGF-2 and metalloproteinase 2 (MMP-2) levels in the urine of HIV-infected children > 2 years of age with HIVAN. Panels **a** and **c** show changes in FGF-2 and MMP-2 urine values corresponding to controls (HIV-negative children without renal disease;  $n = 8$ ); HIV-infected children without renal disease and low viral load (< 5000 copies/ml; HIV-LVC,  $n = 15$ ); HIV-infected children without renal disease and high viral load (> 10,000 copies/ml; HIV-HVC,  $n = 26$ ); and children > 2 years of age with biopsy-proven HIVAN ( $n = 6$ ). Urine values are expressed as a % change relative to the control group. Panels **b** and **d** show changes in FGF-2 (**b**) and MMP-2 (**d**) urine values in HIV-infected children > 2 years of age without proteinuria (HIV-C;  $n = 10$ ); HIV-infected children with trace proteinuria (HIV-T;  $n = 8$ ); and HIV-infected children > 2 years of age with biopsy-proven HIVAN ( $n = 6$ ). Panel **e** shows urinary levels of MMP-9 (**e**) in HIV-infected children of all ages without proteinuria (HIV-C;  $n = 10$ ); trace proteinuria (HIV-T;  $n = 8$ ); or established renal disease (HIV-RD;  $n = 9$ ). This last group included seven children of all ages with biopsy-proven HIVAN.  $P$ -values less than 0.05 were considered statistically significant. (\* and # indicate a  $P < 0.05$  when compared with HIV-C, and HIV-T groups, respectively; \*\* and \*\*\* indicate  $P < 0.001$  and  $P < 0.0001$ , respectively, when compared to all the other groups).



**Figure 4** Representative zymographic analysis of urine samples harvested from HIV-infected children without proteinuria (HIV-C), trace proteinuria (HIV-T), and established renal disease (HIV-RD). Duplicate gels were subjected to incubation in the presence of 20 mM 1, 10-phenanthroline, an MMP inhibitor. Panel **a** shows urinary zymograms of HIV-infected children with and without RD. Panels **b** and **c** show the mean  $\pm$  s.e.m. collagenolytic activity values corresponding to MMP-2 (**b**) and MMP-9 (**c**). The results are expressed in arbitrary optical density units (OD) as a urinary creatinine (UCR) ratio (OD/UCR). HIV-C,  $n = 10$ ; HIV-T,  $n = 8$ ; and HIV-RD,  $n = 9$  (\* and \*\* indicate  $P < 0.05$  and  $0.001$  when compared with the HIV-C, respectively).

### Changes in EGF and MMPs expression in correlation with the development of RD in HIV-Tg<sub>26</sub> mice

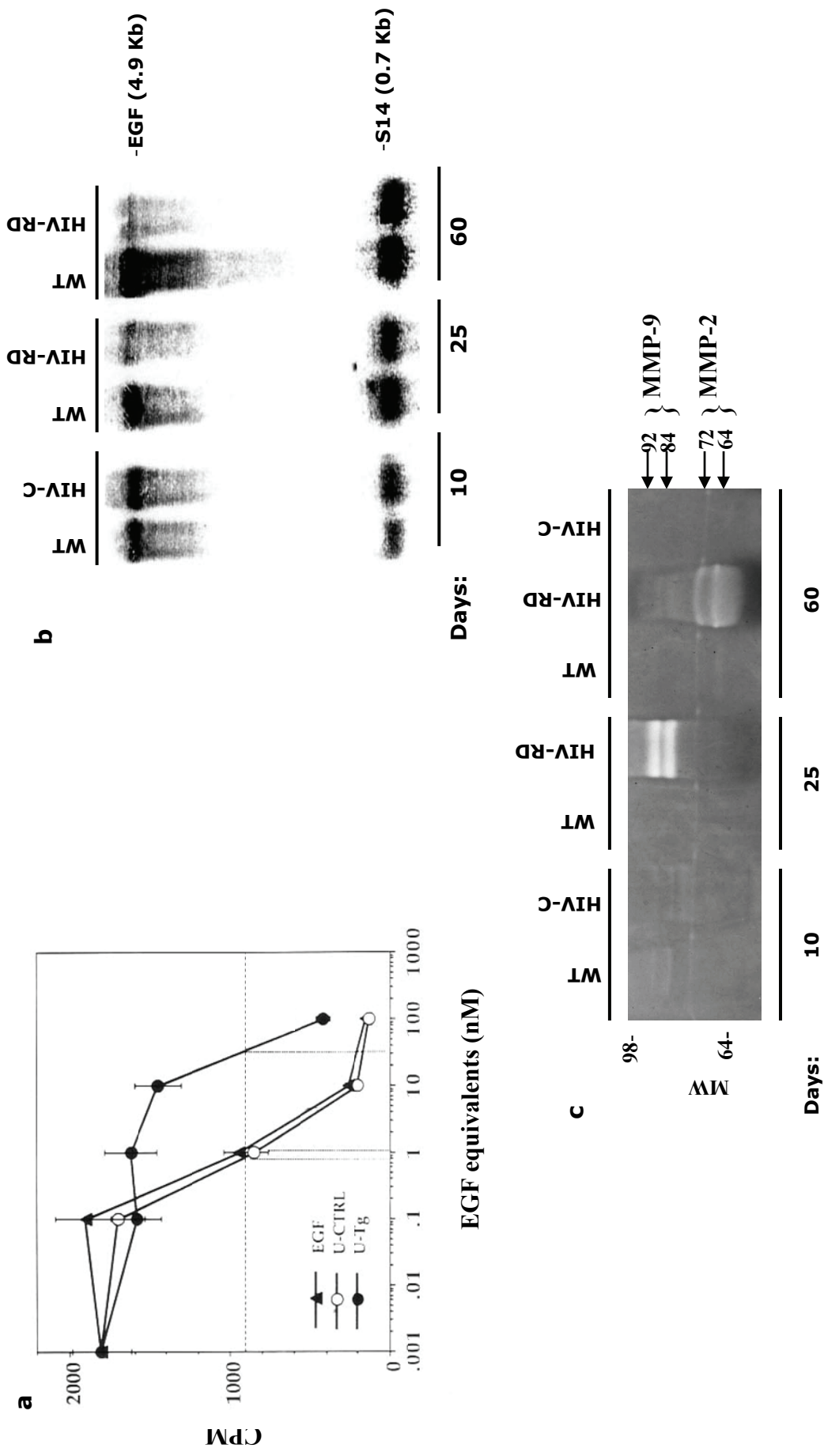
As shown in Figure 6, representative urine samples harvested from wild-type (WT) littermate control mice showed an increased EGF-binding activity when compared with the samples corresponding HIV-Tg mice with severe RD (Figure 6a). When adjusted for the urinary creatinine concentration, EGF activity was approximately fivefold greater in the urine of WT mice relative to the urine of HIV-Tg mice with RD. In support of these findings, northern blots analysis showed a significant reduction of EGF mRNA expression of approximately one- to twofold in the renal medulla of HIV-Tg<sub>26</sub> mice with early and late RD when compared with WT control littermate mice (Figure 6b). In a similar manner, the collagenolytic activity of MMP-9 and MMP-2 was increased in the urine of HIV-Tg<sub>26</sub> mice with RD by an average of 10- to 50-fold, relative to WT and HIV-Tg<sub>26</sub> mice without RD (Figure 6c). Finally, in an earlier study, we found a significant accumulation of FGF-2 in the kidney of HIV-Tg<sub>26</sub> mice with RD in association with the presence of the tubular microcystic lesions characteristic of HIVAN.<sup>9</sup> Taken together, all these findings strongly suggest that the urinary profile of EGF, MMP-2, and FGF-2 changes in correlation to the progression of the renal tubulointerstitial and microcystic lesions characteristic of HIVAN.



**Figure 5** Progressive changes in the urinary biomarker profile in a child with biopsy-proven HIVAN. Progressive changes in serum creatinine (SCr) and blood urea nitrogen (BUN) (**a**), EGF (**b**), FGF-2, (**c**) and MMP-2 (**d**) in urine samples collected from a child with biopsy-proven HIVAN treated successfully with antiretroviral therapy. Dotted lines indicate the expected average normal values for each measurement.

## DISCUSSION

Childhood HIV-associated nephropathy is characterized by the presence of mesangial hyperplasia and/or focal segmental glomerulosclerosis in association with the microcystic transformation of renal tubules and rapid progression of the RD.<sup>1-3</sup> Earlier studies have shown that cytokines play a role in the development of these renal lesions.<sup>30-33</sup> In the present study, we found a reduced excretion of EGF in the urine of children with HIV-RD in association with increased urinary levels of FGF-2 and MMP-2, when compared with HIV-positive or -negative children without RD. Overall, six out of seven children of all ages with biopsy-proven HIVAN showed abnormal urinary values of all three candidate biomarkers in samples collected when their blood urea nitrogen and serum creatinine levels were still within the normal range. In contrast, none of the HIV-positive or -negative control children without RD or trace proteinuria showed abnormal urinary levels for all three candidate biomarkers. These findings suggest that the urinary profile of EGF, FGF-2, and MMP-2 may allow the identification of children at high risk of developing HIVAN.



**Figure 6** Representative changes in EGF and MMPs activity in HIV-Tg<sub>26</sub> mice. Urine and kidney samples were harvested in a sequential manner at 10, 25, and 60 days of life from wild-type (WT) and HIV-Tg<sub>26</sub> (HIV) mice with and without renal disease (n = 4 per group). Panel **a** shows a representative EGF displacement binding assay in cultured A431 cells exposed to <sup>125</sup>I-EGF in the presence of one of the following: recombinant EGF, urine from WT control mice (U-CTRL), or urine from HIV-Tg mice with renal disease (U-Tg). Panel **b** shows a representative northern blot analysis corresponding to the EGF and the S14 control probes in the renal medulla from WT and HIV-Tg<sub>26</sub> control mice (HIV-C), and HIV-Tg<sub>26</sub> mice with renal disease (HIV-RD). Panel **c** shows a representative experiment showing the collagenolytic activity of urine samples harvested from WT and HIV-Tg<sub>26</sub> control mice (HIV-C), and HIV-Tg<sub>26</sub> mice with RD (HIV-RD). The collagenolytic activity was assessed by gelatin zymography as described in the Materials and Methods section (**c**). These experiments were repeated three times with similar results.

Both EGF and FGF-2 are powerful mitogenic growth factors for renal tubular epithelial cells.<sup>14-20</sup> They play important roles during renal development and in the pathogenesis of renal cystic disorders. EGF is predominately synthesized by renal tubular epithelial cells,<sup>14-17</sup> and in the presence of renal tubular injury, its synthesis and release into the tubular lumen is expected to decrease. This notion is supported by the findings in HIV-Tg<sub>26</sub> mice with RD, which showed a significant reduction in EGF mRNA expression in correlation with the presence of renal tubular injury and progression of the RD. A similar global reduction of mRNA and protein EGF expression was found by Horikoshi *et al.*<sup>14</sup> in mice with polycystic kidney disease (cpk). EGF is needed for the normal growth and regeneration of renal epithelial cells,<sup>14-17</sup> and we have confirmed that EGF present in the urine of HIV-infected children can stimulate the growth of cultured human renal tubular epithelial cells. However, as antibodies against EGF were unable to suppress the complete mitogenic activity of the urine samples harvested from children with HIVAN, other growth factors present in these urine samples (that is, FGF-2) might play a synergistic role with EGF in this process.<sup>6-9</sup> In summary, taken in consideration all these findings, we propose that the progressive reduction in the urinary levels of EGF in HIV-infected children with RD reflect the presence of diffuse renal tubular injury characteristic of childhood HIVAN. In support of this notion, we have found that at least three markers of tubular injury, cystatin C,  $\gamma$ -glutamyl transpeptidase, and lysozyme, were elevated in the urine of patients with RD in correlation with the decreased EGF levels.

Fibroblast growth factor-2 is a heparin-binding growth factor that lacks a classic signal peptide for secretion, but is released into the circulation by non-conventional pathways and injured endothelial cells.<sup>3,6-9,34</sup> In an earlier study, we found elevated levels of FGF-2 in the plasma of children with HIVAN.<sup>6</sup> In addition, we found an upregulation of heparan sulfate proteoglycans in the kidney of HIV-infected children and HIV-Tg<sub>26</sub> mice with RD.<sup>6-9</sup> Thus, renal heparan sulfate proteoglycans may act as a sink trapping FGF-2 and other heparin-binding growth factors from the circulation. In addition, renal tubular epithelial cells harvested from the urine of children with HIVAN produce and release high levels of FGF-2, as well as an FGF-binding protein, that facilitates the release of several members of the FGF's family, including FGF-2.<sup>7,8</sup> These findings may explain the elevated levels of urinary FGF-2 in children with HIVAN. In support of this notion, HIV-Tg<sub>26</sub> kidneys with RD show a significant accumulation of FGF-2 and FGF binding protein-1 in parallel binding protein1 in parallel with the decreased renal production of EGF mRNA and development of renal microcysts.<sup>8,9</sup> We have earlier found that FGF-2 induces the proliferation of human renal epithelial and mesangial cells harvested from HIV-infected children,<sup>6</sup> and systemic infusions of FGF-2 into rodents and monkeys induce mesangial hyperplasia, FGFS and/or microcystic transformation of renal tubules.<sup>35-40</sup> In summary, these findings suggest that the accumulation of FGF-2 in the kidney of HIV-infected children may constitute a risk factor for the development of HIVAN.

Matrix metalloproteinases are metal-dependent enzymes that participate in the remodeling of extracellular matrix.<sup>12,13</sup> In the kidney, the migration and transdifferentiation of renal tubular epithelial cells and fibroblasts is at least partially mediated by the proteolytic activity of MMPs, which degrade the renal basement membranes allowing the release of FGF-2 and the activation of growth factors bound to the extracellular matrix.<sup>11,12,39,40</sup> The activity and excretion of MMP-2 is increased in a murine model of infantile-type polycystic kidney disease.<sup>12</sup> Moreover, the expression of MMP-2 in cells harvested from patients with AIDS-Kaposi's sarcoma is induced by FGF-2,<sup>34</sup> and increased levels of MMP-2 and FGF-2 are found in the urine of patients with AIDS-Kaposi's sarcoma or polycystic kidney diseases.<sup>40-42</sup> Given the extent of renal tissue remodeling and microcystic changes in children with HIVAN, we speculate that FGF-2 and MMPs may play a similar role in the pathogenesis of HIV-RD. In support of this notion, we have found a consistent elevation of MMP-2 protein and activity in the urine of HIV-infected children with HIVAN and HIV-Tg<sub>26</sub> mice in correlation with the accumulation of FGF-2 and progression of the RD. Taken together, these findings may explain the synergistic increase in urinary FGF-2 and MMP-2 in children with HIVAN.

We also found elevated levels of MMP-2 and/or MMP-9 in a few HIV-infected children without proteinuria. These findings are consistent with the results of other studies showing that both MMP-2 and MMP-9 can be detected in the urine of healthy children, probably in connection to the process of normal tissue growth and remodeling.<sup>43</sup> However, the changes in MMP-2 activity seen in children with HIVAN can be clearly differentiated from all the other seen in HIV-infected patients. Of interest, earlier studies have shown that protease inhibitors can inhibit the activity of MMP-2 by blocking the conversion from its latent to the active form.<sup>44-46</sup> On the basis of these data, we speculate that these drugs could have a potential beneficial effect in children with HIV-RD by blocking the activation of MMP-2.

In conclusion, urinary levels of EGF, FGF-2, and MMP-2 might be useful to identify HIV-infected children undergoing the renal tubular interstitial and microcystic lesions characteristic of childhood HIVAN. It remains to be determined whether this urinary growth factor profile is specific for childhood HIVAN, or could be also found in HIV-infected children with other RD. A large multicenter clinical study will be needed to answer this question. Nevertheless, urinary biomarkers acquire their clinical value when interpreted in the appropriate clinical context. African-American children with HIVAN typically show enlarged echogenic kidneys and isolated proteinuria, which is responsive to HAART, in the absence of early-onset hypertension, hematuria, generalized edema, or acute renal failure.<sup>1-3</sup> We have presented evidence that the urinary levels of EGF, FGF-2, and MMP-2 can also change in response to HAART. In contrast, other RD seen in HIV-infected children are less responsive to HAART and can be suspected by their clinical history and symptoms, the urinalysis, ultrasound findings, and their response to antihypertensive drugs or steroids.<sup>2</sup> In summary, we propose that if the urinary

EGF, FGF-2, and MMP-2 profile is interpreted in the appropriate clinical context, it will facilitate the early identification of children at high risk of developing HIVAN.

### **DISCLOSURE**

All the authors declared no competing interests.

### **ACKNOWLEDGMENTS**

We thank Dr Enrique A. Mesri, University of Miami School of Medicine, for his advice on performing the EGF-binding assays. This study was supported in part by the Department of Health and Human Services, National Institutes of Health grants: R01-HL55605; R01-DK049419; and NCRR K12 RR017613, R21-AT002278.



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# 12

# chapter

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**DISCUSSION**

## **EVOLUTION OF ANTIRETROVIRAL THERAPY IN PEDIATRIC HIV INFECTION**

Following the development of the first antiretroviral (ARV) drugs in the late 1980s, the treatment of pediatric HIV infection has evolved from monotherapy with zidovudine (ZDV), to dual therapy with nucleoside reverse transcriptase inhibitors (NRTIs) and subsequently to multi-drug therapy involving a combination of three or more ARV agents.<sup>1,2</sup> This therapeutic evolution has allowed to decrease significantly the incidence of opportunistic infections and HIV-associated complications, prevent the detrimental effect of the HIV disease on growth and development, and achieve highly functional survival and improved quality of life in HIV-infected children and adolescents.<sup>3-5</sup> The success of pediatric antiretroviral therapy (ART), however, comes with the price of managing chronic multi-drug therapy throughout the period of transition from infancy through early school and teenage years into young adulthood. With increased ART exposure among pediatric patients worldwide, the concerns for long-term ART adherence, ARV drug resistance and long-term ART associated toxicities, some of which are only now beginning to be investigated in children, have arisen. The choice of the most efficient and least toxic ART and optimal ARV drugs dosing at different stages of growth and maturation in children of diverse ethnic and racial background is challenging and requires ongoing efforts in producing high quality pharmacological pediatric data.

### **Approval of pediatric ARV drugs**

Out of 22 ARV medications currently marketed for therapy of HIV infection, 16 are licensed in the United States for use in children younger than 16 years of age.<sup>2</sup> The approval of ARV drugs in children by the FDA requires the submission of 24 week safety and tolerability data and the antiviral activity is assessed from non-comparative data. A significantly smaller number of ARV drugs is approved for use in resource limited settings, primarily due to cost and availability considerations.<sup>6</sup> As with any new therapeutic modality, the data guiding the dosing, efficacy and safety of ARV drugs for children have lagged behind the information for adults. With the lag of approval of new medications for children, off-label prescribing of ART in pediatric HIV-infected patients became a necessary and accepted practice. Off-label use of ARV medications for unapproved age groups at unapproved dosages and frequencies of administration has the potential to expose children to treatment failure and ARV associated toxicity. Fortunately, the era of ARV drug development coincided with significant changes in the legislative and regulatory initiatives for the pediatric drug approval process. While the majority of the NRTIs and first non-nucleoside reverse transcriptase inhibitor (NNRTI) were evaluated prior to the Food and Drug Modernization and Administration Act (FDAMA) from 1997, the evaluation of the majority of the protease inhibitors (PIs) took place following the enacting of the Best Pharmaceutical for Children Act (BPCA) in 2002 and the Pediatric Research Equity Act (PREA) in 2003. The approval of pediatric dosing of the newer PIs, entry and fusion inhibitors, integrase inhibitors and C-C chemokine receptor type-5 (CCR5) inhibitors is happening in the era after the Food and Drug Administration Amendments Act (FDAAA) of 2007 and EU Pediatric Regulation of 2007. In addition,

strong international advocacy efforts driven by the universal nature of the HIV epidemic, support by large international organizations such as the World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS), quick availability of extensive federal and private funding, and industry interest in world size marketing, have contributed to early incorporation of pediatric trials into the development of ARV drugs.<sup>7</sup> As a result, the evaluation and approval of age-appropriate doses and regimens of ARV drugs in children made a relatively fast progress unlike many other therapeutic modalities such as pediatric tuberculosis drug development.<sup>8</sup>

### **Principles of the development of pediatric ART**

The pediatric ARV dose development is aimed primarily to meet the HIV therapeutic exposure targets from adults while adjusting to the developmental changes in drug metabolism and disposition in children. A different dose strategy to match adult peak ( $C_{max}$ ), trough ( $C_{min}$ ) and area under the curve (AUC) exposure is required in pediatric populations.<sup>9</sup> Acceptable mean/median differences and size of extreme values for the pharmacokinetic (PK) parameters need to be defined and optimized based on PK and pharmacodynamic (PD) knowledge. Most importantly, the differences in pediatric HIV infection need to be taken into consideration. Children are prone to rapid progression of failure to thrive and gastrointestinal illness with dehydration, both scenarios having the potential to affect the absorption and distribution of ARV drugs. The high impact of HIV viremia on neurocognitive development in young children makes central nervous system (CNS) penetration of ARV drugs more relevant in pediatric patients than in adults. Moreover, the HIV disease progression is more rapid in younger children and has different criteria for the initiation of ART.

Developmental and disease specific parameters determine the design and development of pediatric ARV clinical trials.<sup>9</sup> The most important considerations for pediatric ART study design are reflected in Figure 1. In very young infants, the difference in the HIV diagnostic tests (virologic vs antibody tests) between young infants and older children needs to be considered. PK evaluations through sparse blood sampling and limited blood volumes are particularly important in small children, especially in resource limited settings where co-morbidity and malnutrition are frequent causes of anemia. The exposure to maternal ART during the time of breastfeeding and its effect on drug metabolism and development of viral resistance in resource limited settings needs to be addressed. Finally, the inclusion of the pediatric expertise in the development of ARV clinical trials in children is crucial and can contribute significantly to the success of the study.

### **Clinical trials of ART in children**

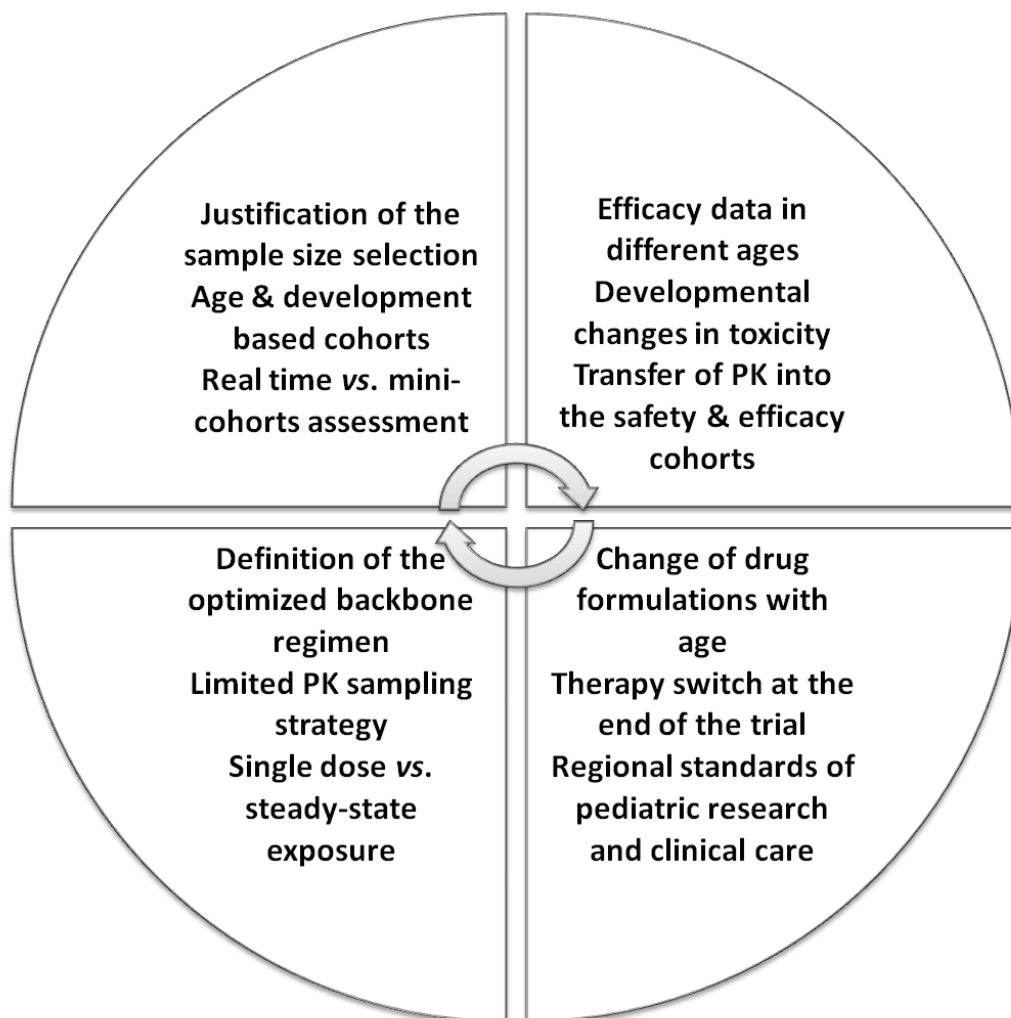
A significant number of clinical trials in HIV-infected children have been conducted by the pediatric HIV research networks and pharmaceutical industry since the late 1980s. In the early era of the HIV epidemic and ARV development, when the treatment options were extremely limited, the design of ARV drug trials in adult and

pediatric patients was driven by the model of oncology studies with the concept of the maximally tolerated dosing. Single agent studies were conducted for the first NRTIs such as ZDV, didanosine (ddI) and lamivudine (3TC) in the late 1980s and early 1990s.<sup>9</sup> (Table 1) NRTIs are subject to intracellular phosphorylation and have linear PK without cytochrome P450 (CYP450) associated induction or inhibition. The drugs from this class have a short plasma half-life and high inter- and intra-subject variability. Single dose PK usually serves as a good predictor of steady-state concentrations, however due to the intracellular metabolic pathway and relatively weak antiviral activity, the plasma concentrations serve as a poor biomarker of the clinical outcome. During the first stage investigations of ZDV monotherapy the limited understanding of the PD of HIV infection has led to high and frequent (5 times a day) dosing which resulted in significant toxicity. The high rates of NRTI dose associated clinical and laboratory toxicities pushed the clinical trials toward the consideration of significantly lower doses which resulted in subtherapeutic exposure and increased HIV resistance. As a consequence, the pediatric trials of NRTIs have conducted most of the PK evaluation after a single dose and had a more than 10 times dose range within the dose escalation module. As a result of those trials, the "effective doses" of NRTIs entered pediatric practice by mid 1990s, and only a handful of studies have re-evaluated those doses and dosing schedules since then. Among those is our study of the developmental changes in 3TC PK in a cohort of 51 children with a median age of 8.4 years (1.7 to 18 years) receiving a standard recommended dose of 3TC.<sup>10</sup> The age of 6 years appeared to be a cut-off for a change in PK of 3TC in this study, with children < 6 years of age having a median AUC 43% lower and a median  $C_{max}$  47% lower than older children. In line with these observations, clearance (CL/kg) and volume of distribution (Vd/kg) were 79% and 89% higher in children aged 6 years or less when compared to children aged 7 years and older. The mean  $C_{max}$  and AUC in children  $\geq 7$  years were almost similar to the adult PK values. Moreover, the decreased 3TC exposure in younger children is potentially related to the development of M184V HIV mutation and resistance to 3TC, and deserves further investigation.

The majority of highly potent second generation classes of ARV drugs (NNRTIs and PIs) have been evaluated using concentration controlled clinical trials (PACTG P1039 for lopinavir (LPV), P382 for efavirenz (EFV), and P1020 for atazanavir (ATV)).<sup>9</sup> (Table 1) Drugs from both classes of the medications are substrates/inducers/suppressors of the CYP450 enzymes and have complex drug interaction profiles. The relationship between plasma concentrations and viral efficacy is strong, and for some drugs (EFV) the relationship between plasma exposure and drug associated toxicity has been reported. The pediatric NNRTIs and PIs studies have used 1 - 2 dose levels and assessed the PK at steady state. These trials were the first to use combination ART with NRTI backbone and to address the optimization of the backbone regimen in patients with NRTI resistance. Therapeutic drug monitoring (TDM) was included in the majority of these studies. In recent years, the clinical trials have been evaluating the new classes of highly potent ARV drugs such as second generation PIs and NNRTIs, fusion inhibitors, integrase inhibitors and CCR5 inhibitors.<sup>9</sup> (Table 1) These drugs are equally



substrates/inducers/suppressors of CYP450 and uridine-glucuronosyltransferase (UGT) enzymes and are prone to multi-drug interactions. The plasma concentration-effect relationship of these drugs is associated with a flat portion of the concentration-effect curve. Since ART is started in all infants and at earlier stages of HIV disease in children > 12 months of age, the patient population in clinical trials is more heterogenic than ever before in terms of previous ART exposure and HIV resistances. As a result, the newest pediatric ARV clinical trials are designed to evaluate single dose concentrations in mini cohorts of highly treatment experienced children. TDM is included in some of the newer ARV drug trials. Early rapid assessment of PK generated from "mini-cohort" ensures limiting the number of subjects exposed to potentially inadequate ART.



**Figure 1** Considerations for pediatric ARV drugs study design.

**Table 1** Summary of the pediatric ART development<sup>9</sup>

	<b>Early Agents</b>	<b>Second Wave Agents</b>	<b>Newer Agents</b>
Individual ARVs*	ZDV** ddI 3TC	LPV/RTV NFV EFV ATV/RTV	RAL MCV ETV
Metabolic Pathway	Intracellular Phosphorylation	CYP450***	CYP450+UGT
Induction/Inhibition	- / -	+++ / +++++	++ / +
Potency	+	++ / +++	+++
Plasma Concentrations – Effect Relationship	Weak	Strong	Flat Portion of Concentration-Effect Curve
Dose Selection	10 Fold Dose Range	1 - 2 Dose Levels	Single Dose Level
Antiretroviral Therapy	Mono-therapy	Combination Therapy	Combination Therapy
Timing of PK Evaluation	First Dose	Steady State	Steady State
Study Design	Dose Escalation	Concentration Control	Mini Cohorts
Therapeutic Drug Monitoring	-	+	+/-

\* the full listing of all studied agents is not provided; only examples of antiretroviral agents are included in the table.

\*\* ZDV – zidovudine; ddI – didanosine; 3TC – lamivudine; LPV/RTV – lopinavir/ritonavir; NFV – nelfinavir; EFV – efavirenz;

ATV/RTV – atazanavir/ritonavir; RAL – raltegravir; MCV – maraviroc; ETV – etravirine

\*\*\* CYP450 – cytochrome P450, UGT – uridine-glucuronosyltransferase.

Throughout the course of the evolution of clinical trials of ARV drugs in children, new elements of ART study design have emerged. With a larger number of more potent agents in practice our definition of "optimal background" has changed, it has become more difficult to define the control arm and the concept of "functional mono-therapy" has emerged in patients with multi-drug ARV resistance. Moreover, the proper tools of measuring the adherence component has become of crucial importance in the evaluation of short and long term efficacy and toxicity of ART in children. Although many pediatric PK studies describe variability in ARV concentrations or PK parameters such as clearance, typically derived from non-compartmental analytic techniques, variability within and between individuals is not distinguished.<sup>11</sup> In contrast, population PK techniques estimate distributions of PK parameter values globally across the entire study population as well as for the individual subjects within the population, and more accurately partition sources of variability such as age and/or developmental stage. There are several other advantages to population analyses that are relevant to ART studies in children. Homogeneous data is not a pre-requisite; therefore, strict adherence to a sampling schedule is not required.<sup>11</sup> Population and individual parameter values may be estimated with sparse data, although accuracy, precision, and predictive power of population PK models will suffer with increasingly sparse data. Most importantly, population analysis offers a formal method to incorporate what is known about the PK of a drug, e.g. in adults, so that estimation of pediatric PK does not begin from a zero-knowledge state. Most importantly, because population models are distributions of parameter estimates, they may be used to predict PK parameter values, with measures of statistical confidence, and thus ARV concentrations in children who were not part of the study population.

In recent years, the population PK/PD modeling approach to the development of dosing strategy of pediatric ART became highly supported by the US and European regulatory authorities. Pediatric Model Based Drug Development (MBDD) often have existing information on drug and disease processes and can be focused to fill knowledge gaps. Since pediatric labeling is based on a limited number of studies the use of modeling and simulation (M&S) might allow the development of age appropriate dosing and maximize the information gained from a successful trial. The M&S part of the FDA Conceptual Framework for the Pediatric Initiative has also the potential to fill in the gaps in communication between pharmacologists, statisticians and clinicians.

In this thesis we present several of our studies of pediatric HIV therapy that contributed to the evolution of pediatric ART. These studies addressed the developmental changes in PK and PD of ARV drugs, ontogeny of ARV metabolizing enzymes and drug transporters, the application of TDM, and sustainability and complications of pediatric ART in children with perinatally acquired HIV infection. (Table 2) The descriptions of these studies and their results are included in the discussion.

**Table 2** Summary of the studies from this thesis**Developmental changes in PK and PD of ARV drugs**

- Age-dependent pharmacokinetics of lamivudine in HIV-infected children
- Population pharmacokinetics of lopinavir in children

**Ontogeny of ARV drug metabolizing and transporter enzymes**

- Association between *CYP3A5*, *MDR1* and *OATP1B1* polymorphisms with the PK/PD of lopinavir in children

**Therapeutic drug monitoring of pediatric ART**

- The use of nevirapine concentration in non-stimulated saliva as an alternative for plasma sampling in children
- The role of therapeutic drug monitoring in the pharmacotherapy of HIV infection in adolescents

**Sustainability and complications of ART in children**

- Evaluation of adherence to medication in children and adolescents with HIV infection
- Urinary biomarker growth factor profile for children with HIV-associated nephropathy

*PK* – pharmacokinetics; *PD* – pharmacodynamics; *ARV* – antiretroviral; *ART* – antiretroviral therapy; *CYP3A5* – cytochrome 3A5; *MDR1* – multidrug resistant transporter 1; *OATP1B1* – organic anion transporting polypeptide 1B1.

**THERAPEUTIC TARGETS OF ANTIRETROVIRAL DRUGS FROM INFANCY TO ADOLESCENCE**

The primary outcomes of ARV studies are typically surrogate markers that include the virologic response (e.g. undetectable HIV RNA or  $> 1 \log_{10}$  drop in HIV RNA viral load) and/or immunologic response (e.g. change in CD4+ lymphocyte count or percentage), all measured at baseline and after a defined duration of therapy. The immunologic surrogates are associated with the occurrence of opportunistic infections and survival,<sup>12-14</sup> and virologic surrogates define treatment success or failure.<sup>6</sup> Since in the majority of ART trials these virologic and immunologic surrogates are not measured typically until after 12, 24 or 48 weeks of therapy, several strategies have been proposed to predict outcomes of ART even before the first dose, including target therapeutic concentrations and inhibitory quotients (IQ). The IQ incorporates both patient-specific drug exposure and ARV susceptibility of the dominant strain as a ratio of  $C_{\min}$  to  $IC_{50}$ , with  $IC_{50}$  being equal to the concentration of drug required for 50% inhibition of viral replication *in vitro*. This is more specifically termed the phenotypic IQ, or pIQ. Alternatives to the pIQ include the virtual IQ (vIQ), normalized IQ (nIQ) and genotypic IQ (gIQ). The vIQ uses the fold-change in virtual  $IC_{50}$  (derived from the genotype),<sup>12</sup> multiplied by a reference wild-type protein-adjusted  $IC_{50}$  as the measure of viral susceptibility. The nIQ is the patient-specific IQ divided by a reference IQ calculated as the ratio of typical  $C_{\min}$  for a given dose and wild type viral  $IC_{50}$ , which normalizes the IQ target across ARVs to a ratio of  $> 1$ . The gIQ is the ratio of  $C_{\min}$  to the number of resistance-associated mutations (RAMs) as defined in one of several large databases (e.g. Stanford, International AIDS Society [IAS], or Agence Nationale de Recherche sur le Sida [ANRS]). When applying any form of IQ, it is important to recognize that the resistance to the first-generation NNRTIs, NVP and EFV, can be high-level with a

single mutation, and it is not considered possible to overcome such resistance by increasing the dose. In contrast, for all PIs resistance develops cumulatively with successive mutations, and therefore can, at least theoretically, be overcome by higher doses if they can be tolerated. For adults, various IQ targets have been proposed for the majority of the PIs. Although the second generation NNRTI ETV is similar to PIs in this regard, there is currently no data on the significance of ETV IQ.

Because all ARV drugs except CCR5 inhibitors have viral rather than human molecular targets, a major assumption for pediatric therapy has been that ARV PK/PD targets should be the same in children as in adults. Indeed, all ARV PK studies in children have been designed to find the dose which is associated with exposures (e.g.,  $C_{max}$ , AUC, and  $C_{min}$ ) similar to those already known to be safe and effective in adults. This section describes what is known of the relationships between ARV drug concentrations and antiviral efficacy and toxicity in pediatric patients with HIV.

### **Therapeutic targets of NRTIs**

The relationship between plasma NRTIs concentrations and virologic and immunological outcomes are not well correlated. Nonetheless, improved virologic suppression has been demonstrated in adults dosed with NRTIs to achieve specific plasma targets vs standard dosing.<sup>13</sup> The relationship between ZDV and ddI exposure and virologic outcome was also reported in infants and children.<sup>14,15</sup> Low tenofovir diphosphate (TDF) exposure (single-dose and steady-state tenofovir (TFV) AUC) in younger pediatric patients has also been associated with virologic outcome.<sup>16</sup> The relationship between the intracellular NRTI concentrations and virologic and immunologic outcome has been studied in adult population only. Intracellular concentrations of ZDV and 3TC were both strongly and significantly related to virologic replication and immunologic recovery, while neither outcome was related to plasma concentrations of either drug.<sup>17</sup> Correlation between plasma and intracellular drug concentrations was also weak. Moreover, the intracellular concentrations of NRTIs are unlikely to become therapeutic targets for ART, since the measurement of active intracellular triphosphate metabolites is extremely expensive and labor-intensive, and is limited to highly specialized centers. In addition, the methodology requires larger blood volumes than typical plasma samples which make it unsuitable for the pediatric populations. Overall, due to the limited exposure–efficacy data for NRTIs no therapeutic targets for NRTIs have been defined to date in adult or pediatric populations except those used in specific ARV clinical trials. The relationship of the NRTI exposure and toxicity is not well defined. Chronic anemia has been associated with the degree of ZDV exposure where the decrease in hemoglobin was associated with elevated mean ZDV plasma concentrations.<sup>18</sup> The ddI induced pancreatitis appears to be dose related, but no relationship with plasma exposure has been found.<sup>19</sup> Increased frequency of TDF associated nephrotoxicity and osteoporosis in patients with concomitant administration of the boosted PIs has been reported, however, no significant relationship with TFV plasma concentrations has been demonstrated.<sup>20</sup>

### Therapeutic targets of NNRTIs

For the NNRTIs, the relationship between plasma drug concentrations and efficacy and toxicity has been well established in adult studies.<sup>21</sup> Long-term virologic suppression of HIV has been associated with maintenance of trough plasma concentrations above 3000 ng/ml for NVP, in and above 1000 ng/ml for EFV in adult patients with HIV infection.<sup>22</sup> Higher NVP plasma trough concentrations (> 4300 ng/ml) have been associated with reduced emergence of resistance mutations relative to lower troughs (3000 - 4300 ng/ml) in adults.<sup>23</sup> However, no similar pediatric NVP PD data have been published. In children earlier HIV RNA suppression has been associated with higher EFV concentrations with significant differences in virologic suppression rates between plasma trough concentrations of 1.9 mcg/ml vs 1.3 mcg/ml, respectively ( $P = 0.01$ ).<sup>24</sup> Recent studies report a correlation between suboptimal EFV plasma concentrations and suboptimal virologic suppression and fast development of NNRTI resistance in pediatric patients in West Africa.<sup>25</sup> Suboptimal exposure in this study occurred in 19% of children (44% of children weighing less than 15 kg) with the use of FDA-approved pediatric dosing. Most importantly, similar findings of subtherapeutic EFV exposure in pediatric populations from the different regions using the standard EFV dose have been reported.<sup>26,27</sup> As a result of these findings, consideration is being given to a potential increase in the recommended dose of EFV in pediatric patients. Current pediatric ART guidelines recommend EFV dose adjustment with TDM in selected clinical situations, such as virologic rebound or lack of response in an adherent patient.<sup>2</sup> EFV is the only NNRTI with a well established relationship between EFV induced CNS toxicity associated with the  $C_{min}$  plasma concentrations > 4 mcg/ml in both adults and children.<sup>24,28,29</sup> Finally, for the second generation NNRTI ETV adult data have not shown a relationship between ETV plasma concentrations and 48-week efficacy.<sup>30</sup> No pediatric data on therapeutic targets of ETV are available, but the Phase II study of efficacy and tolerability is in progress.

### Therapeutic targets of PIs

For the PIs, efficacy plasma trough concentrations have been established for atazanavir (ATV), fosamprenavir (f-APV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV) and tipranavir (TPV) in treatment-naïve adults.<sup>22</sup> The expected trough concentrations for all PIs are reported in combination with low-dose, boosting RTV, except for ATV, where the  $C_{min}$  for both unboosted and RTV-boosted ATV have been reported. Pediatric ATV PD data were derived from an ongoing PACTG 1020A study with TDM component, which used a PK target of  $AUC > 45 \text{ mcg}\cdot\text{h}/\text{ml}$ .<sup>31</sup> Dosing without RTV required higher ATV doses which resulted in a very high ATV  $C_{max}$ , particularly in younger children, and as a result is generally not recommended for children.<sup>2</sup> If dosing unboosted ATV in pediatric patients, TDM is recommended to achieve adequate exposure. Similar to adult data, a minimum target plasma trough concentration of ATV in children (150 ng/ml) is currently recommended and higher target trough concentrations may be required in PI-experienced pediatric patients.<sup>32</sup> ATV does not have a well identified window of

toxicity, although ATV induced hyperbilirubinemia has been associated with increased ATV plasma trough concentrations in adult patients.<sup>33,34</sup>

IDV is one of very few ARV drugs that has been studied in HIV-infected children both as monotherapy and in combination with other ARV drugs. Decreased IDV exposure ( $AUC_{0-8}$  of  $< 15$  mg\*h/l and  $C_{min} < 100$  ng/ml) was associated with virologic failure.<sup>35</sup> The administration of IDV in combination with RTV booster has generated AUC and  $C_{min}$  concentrations similar to those observed with standard IDV/RTV doses in adults (with the  $C_{max}$  slightly decreased), and resulted in good virologic efficacy.<sup>36,37</sup> The data on the efficacy concentrations of IDV in children is limited due to the low usage of this drug in pediatric practice associated with high rates of drug associated nephrotoxicity in children.<sup>38</sup> IDV is the only PI with a well defined therapeutic window with a high frequency of nephrolithiasis associated with an IDV  $C_{max} > 10000$  ng/ml<sup>39</sup> and  $C_{min} > 500$  ng/ml<sup>40</sup> in adults. For LPV the association of plasma trough concentration with the degree of susceptibility of HIV (half maximal effective concentration = EC50) has been shown to be significant in children. We prospectively developed a population PK model of LPV PI-experienced children and showed that, based on simulations from the model, approximately more than two-thirds of children with standard FDA approved dose of LPV/RTV would be unlikely to achieve a pIQ  $\geq 15$  for the wild type virus,<sup>41</sup> a target which has been recommended in adults.<sup>42</sup> However, for even moderately resistant viral strains, well within the partially sensitive range of commercially available tests, fewer than 10% of children will achieve therapeutic target concentrations. This conclusion was supported by our results from another prospective study in a cohort of PI-experienced children treated with high-dose LPV.<sup>43</sup> Subsequently, we showed that at a pIQ of  $> 25$ , 69% of PI-experienced pediatric patients 4 - 18 years of age had undetectable HIV RNA vs only 20% of those with a pIQ  $< 25$  ( $P = 0.01$ ).<sup>44</sup> In a multivariate logistic regression that included duration of LPV therapy, self-reported adherence, age, and gender, only pIQ was a significant predictor of virologic suppression in our cohort of treatment experienced pediatric patients.<sup>44</sup> A recent study of reduced-dose LPV/RTV (70% of the standard recommended) in PI-naïve Thai children demonstrated adequate LPV/RTV exposure and virologic suppression in 83% of children, compared with 50% of children on the standard dose.<sup>45</sup> These findings reinforce the conclusions from our LPV population PK study that the majority of PI-naïve children are more likely to achieve adequate LPV exposure, but up to 10% may not do so. Moreover, low-dose LPV/RTV would not be appropriate for PI-experienced children, who, on the contrary, may benefit from augmentation of the LPV exposure near the maximal end of the observed safe range.

Among other PIs, NFV has been extensively studied in HIV-infected children in combination with other ARV drugs. Although NFV has a major metabolite, M8, with virologic activity, the contribution of M8 to overall activity of NFV is unclear; target troughs have largely been proposed for NFV.<sup>46,47</sup> An increased risk of virologic failure was associated with low NFV drug exposure, particularly with a NFV  $C_{min} < 1000$  ng/ml, in adults.<sup>48</sup> In a study of children treated with NFV, NFV  $C_{min}$  plasma

concentration > 800 ng/ml was significantly associated with virologic outcome.<sup>47</sup> Significant differences in virologic responses between the two age groups (3.8 and 8.3 years of age) were observed, which reinforces the fact that drug clearance and therefore dosing does not generally vary linearly with weight. For SQV a significant correlation between average trough plasma concentration and viral suppression was reported in pediatric patients, with an apparent threshold mean trough SQV concentration > 200 ng/ml correlating with sustained HIV RNA suppression.<sup>49</sup> SQV-based ART has been reported to produce higher SQV and LPV exposure and stronger virologic response in treatment-experienced children when used in combination with RTV.<sup>50-52</sup> Although both SQV/RTV and SQV/LPV/RTV regimens appeared to be promising, the appropriate dosing in children and adolescents for the different possible PI combinations is not known, and the strategy of "double-boosted" PIs has been deemed by the development of new ARV therapeutic agents. TPV efficacy and PK in HIV-infected children was evaluated in an open-label, multicenter, randomized trial, PACTG 1051/BI-1182.14, among treatment-experienced children (2 - 18 years of age) comparing two dose regimens with an optimized backbone ART.<sup>53</sup> For TPV virologic outcome was strongly predicted by gIQ in the high dose group. Finally, the efficacy data for DRV has not been published to date.

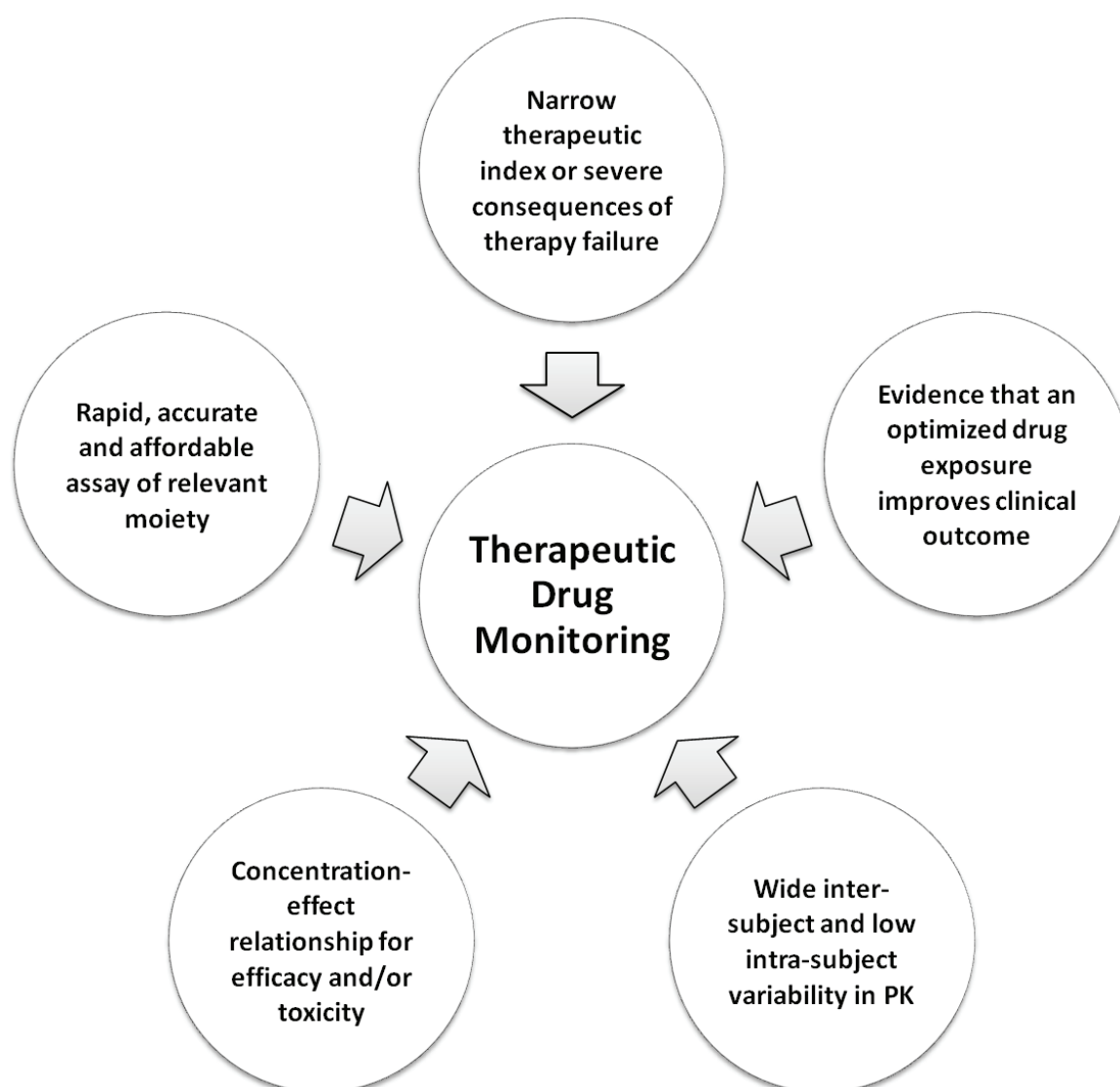
### **Therapeutic targets of entry and fusion inhibitors and CCR5 inhibitors**

For the entry inhibitor enfuvirtide (T-20) no statistical relationship between T-20 drug exposure and virologic benefit was observed in pediatric studies.<sup>54-56</sup> A minimally effective plasma concentration relative to *in vitro* viral susceptibility has not been established for the integrase inhibitor raltegravir (RAL). In early dose ranging studies in treatment-naïve patients, identical 48-week virologic and immunologic efficacy was seen with doses ranging from 100 to 600 mg administered twice daily.<sup>57</sup> In triple-class experienced patients, 24 week virologic efficacy was the same in the 200 mg twice daily group as in the 600 mg twice daily group.<sup>58</sup> RAL is currently being evaluated in IMPAACT 1066, a Phase I/II study in HIV-infected children. To date, there are no reports of the PD of CCR5 antagonist maraviroc (MVC) in children or adolescents < 16 years of age.

### **THERAPEUTIC DRUG MONITORING OF ANTIRETROVIRAL DRUGS IN CHILDREN**

The main goal of TDM is to individualize the dose/regimen of the therapeutic agent(s) in order to achieve maximal benefit and avoid or minimize drug associated toxicity. In order to apply TDM into practice a number of important components need to be present. (Figure 2) Among ARV drugs, NRTIs do not meet the majority of the described TDM requirements, while NNRTIs and PIs have been identified as most suitable candidates for TDM in HIV therapy. Given the physiologic and maturational differences between children and adults, numerous reviews, position papers and guidelines suggest children as a target population for ARV TDM, while routine ARV TDM in adults is considered of questionable benefit.<sup>59-63</sup>





**Figure 2** *Components of therapeutic drug monitoring.*

Despite the almost universal recognition of the potential benefits of ART TDM in children, substantial barriers exist that limit its routine use in clinical practice.<sup>63</sup> The expertise in pediatric PK samples collection and data transfer (accurate dose/time/demographic/adherence information) is limited to a few clinical centers with experience in pediatric clinical trials. The current matrices for TDM of ARV drugs in children include plasma/serum, saliva, urine, cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMC). The collection of blood samples is the most precise and common way to assess ARV exposure, but it is invasive, requires separation via centrifugation and cryopreservation. If transformed into the dried spot heat resistant methodology, it has the potential to become an attractive choice for future use, particularly in resource-limited settings. Dried blood and plasma spot ARV TDM studies are underway in several TDM laboratories with preliminary data showing limited processing issues with recovery.[personal communications] The collection of saliva represents an attractive, affordable and, most importantly, non-invasive alternative for the potential replacement of blood sampling in children.<sup>64,65</sup> The proper sample collection, however, is challenging and the saliva samples have

the potential for a large discordance with blood matrices due to protein binding and pH effects. Clearly, CSF and PBMC sampling are limited to research or rare clinical scenario settings due to the highly invasive nature of the first one and labor and resource involvement of the second one, respectively.

In addition to matrix consideration, the selection of TDM sampling strategy requires thorough evaluation in pediatric patients. The trough or pre-dose plasma concentrations ( $C_{\min}$ ) have a higher parental and child's acceptance, smaller blood volumes, are easier to collect, and can be linked to PD evaluation through of genotypic and phenotypic IQ. The interpretation of plasma  $C_{\min}$  concentrations, however, is quite sensitive to dose/sample time variations and is therefore subject to a greater degree of intra- and inter-patient variability. In contrast, a full AUC evaluation is much more reliable in predicting the average drug exposure-response relationship and is less dependent on the dose/sample time and patient adherence. The clear disadvantages of conducting a full PK study in children involve a higher degree of parental and patient inconvenience, higher volumes of blood samples and higher cost and time commitment involved. Several adult studies have evaluated limited sampling (LS) strategies to provide reasonable estimates of AUC for ARV drugs, but they have not been validated in children.<sup>66-69</sup> Among pediatric patients, LS strategies have been reported for ZDV, ddI, 3TC, d4T and NVP.<sup>70-72</sup>

LS approaches in children can be best used in PK/PD models, which have the potential to reduce the number of study subjects for the drug/dose/schedule approval and evaluation. While, LS data suffer from the same problem as traditional TDM of pre-defined sampling times, the full individual Bayesian adaptive control or forecasting allows for prediction of plasma drug concentrations at any time after a dose, given all information known about a patient, such as dose amount, frequency, estimated times of drug administration and known time(s) of blood sampling, regardless of the elapsed time.<sup>63,73</sup> Information not specific to the patient, but nonetheless relevant, such as PK behavior in other children or in adults, may also be included in the model to improve the quality of the predictions. The PK modeling techniques have been applied to a limited number of pediatric ARV PK studies to date.<sup>63,74-76</sup>

To date, the concentration controlled ARV dosing strategy has been confirmed to be efficient for EFV, NFV, and LPV/RTV/SQV in a series of pediatric clinical trials and case reports.<sup>2,24,41,63,77</sup> The inclusion of TDM in the pediatric study design has been limited by guaranteed access to validated ARV assays, extensive PK laboratory with real time capabilities, and analysis experience. Inability to rapidly adjust ARV dose based on early clinical response has become one of the barriers to include TDM into new drugs study design. In addition, the lack of healthy volunteer information to guide dosing, high inter-subject variability of many ARV drugs, formulation and food interactions constraints, limit the application of TDM in research and clinical practice.

The current practice of TDM in pediatric ART is limited to clinical trials and special indications such as adherence, ART failure in the presence of adherence, and drug-drug interactions. While smaller, well-conducted trials and case reports (including our data) have shown the benefit of TDM in the pediatric population,<sup>2,24,41,63,77,78</sup> data on clinical utility of pediatric ART TDM are lacking. The available pediatric data support the use of TDM in infants or adolescents that may "outgrow" ARV doses. It may also help determine the route of discordance between expected response and viral genotype/phenotype and identify drug-drug interactions in untested drug combinations. New, more potent ARV drugs with well defined PK data have the potential to limit the justification of TDM in treatment-naïve patient populations. However, the development of HIV resistance will most likely outpace the ability to design drugs for resistant virus making TDM more relevant for consideration among fusion and integrase inhibitors, and CCR5 inhibitors. Larger trials of TDM in children are needed to evaluate the cost-benefit analysis of TDM in pediatric populations, particularly in infants and adolescents. In order to conduct larger trials of TDM in children, newer, cheaper and patient friendly TDM matrices and LS sampling strategies need to be developed. Finally, the development and incorporation of modeling and simulation in pediatric clinical trials is critical in evaluating the exposure-response relationship of ARV drugs in children.<sup>79</sup>

## **GLOBALIZATION AND INDIVIDUALIZATION OF THERAPY OF PEDIATRIC HIV INFECTION**

Treatment of HIV infection in infants, children, and adolescents is rapidly evolving and becoming increasingly complex. On the global scale of the HIV epidemic, individualization of pediatric ART is restricted by the drug development process geared towards an economically sustainable and standardized weight band based doses suitable for resource-limited settings. Nevertheless, we must not forget that HIV-infected pediatric patients represent a highly heterogeneous group of infants, young and school age children, and adolescents with vertically and horizontally transmitted HIV infection. The maturational process does not proceed at a regular and predictable pace in all children, and PK and PD variability throughout different stages of growth and development needs to be addressed in pediatric patients on ART.

Valuable data on the PK and PD of ARV drugs in children have been generated and many lessons have been learned, yet many questions remain unanswered. To date very limited data have been collected on individualizing ARV exposure in HIV-infected children. Within the pediatric ART studies the concentration/efficacy/toxicity dose adjustment has been difficult to implement due to many constraints including:

- sample and dose time variability
- study and expert dependent data interpretation
- non-adherence confounder

- difficulties in defining target concentrations
- limited time window for benefit
- timely integration of viral phenotype/genotype information
- additional resources and cost considerations

Few data are available on the dosing of ARV drugs in very young infants and the data are lacking on the efficacy and toxicity of PI and NNRTI based regimens in early infancy. Little attention has been paid to the effects of developmental processes of puberty on the PK/PD targets in the adolescent population. The ARV dose selection remains challenging during puberty, where the continued use of higher (weight- or surface-based) pediatric doses can result in potentially toxic drug exposure and early introduction of lower adult doses can lead to the development of drug resistance and virologic failure.<sup>77</sup>

### **Pharmacogenetics of ART in children**

The impact of pharmacogenetics (PG) on the PK and PD parameters in children is poorly understood, as little knowledge is available on the ontogeny of drug metabolizing enzymes and transporters in children. Similar to the data from adults,<sup>80,81</sup> *CYP2B6* G516T polymorphisms and age were shown to significantly change the clearance of EFV in children and have been associated with increased CNS toxicity, but failed to show significant association between virologic and immunologic responses to date.<sup>82</sup> This polymorphism is highly prevalent in patients of African descent who currently represent the majority of the HIV-infected population worldwide.<sup>83,84</sup> In this thesis we describe successful EFV dose reduction in two African American adolescent patients with extremely high EFV exposure due to the *CYP2B6* G516T polymorphism.<sup>78</sup> Interestingly, although both patients and their families recalled that the children experienced transient sleep problems shortly after the initiation of EFV therapy, their high EFV exposure did not prompt treatment discontinuation. Similar studies of EFV dose reduction in patients with *CYP2B6* genotype have recently emerged in the adult and pediatric literature.<sup>63,85</sup> While low EFV exposure has been reported to be associated with virologic failure,<sup>28</sup> no studies have evaluated the long term consequences of high EFV exposure to date, and no cost analysis of EFV dose adjustment has been conducted. We believe that selective genotyping of populations with high prevalence of *CYP2B6* polymorphism deserves strong consideration and future studies need to be conducted to evaluate the validity of *CYP2B6* genotyping as a guide to EFV dose adjustment. Another drug metabolizing *CYP2C19* G681A polymorphism was found to be strongly associated with metabolism of NFV in pediatric HIV-infected patients.<sup>86</sup> In addition to the *CYP2C19*, the drug transporter *MDR1* C3435T polymorphism has also been shown to affect plasma NFV concentrations and clearance, and was associated with virologic outcome in pediatric patients.<sup>87</sup> Finally, the impact of growth and development on the activity of other UGT isoforms is well recognized and has been reported in the relationship to many drugs, including ZDV.<sup>88</sup> Collectively, these data suggest that developmental changes in drug metabolizing enzymes and drug transporter activity have the potential to affect

disposition and clearance of NNRTIs, PIs entry and fusion inhibitors, integrase inhibitors and CCR5 inhibitors.

### **Malnutrition and pediatric ART**

In addition to the developmental changes in drug absorption and disposition, the potential effects of malnutrition and concomitant infections on the PK/PD of ARV drugs in pediatric patients complicate the dosing of ART in resource-limited settings where decreased weight and delayed growth among HIV-infected children are common.<sup>5,89,90</sup> The high prevalence of co-morbidity with gastrointestinal illnesses, hepatitis, malaria and tuberculosis creates additional challenges in delivering efficient ART to pediatric HIV-infected patients. The presence of emesis, diarrhea, malabsorption, concomitant infections or malignancy may lead to altered absorption of ARV drugs. The loss of fat and lean body mass, and associated metabolic and endocrine abnormalities have the potential to influence the volume of distribution and the total body clearance of ARV agents, particularly of lipophilic drugs, such as PIs.<sup>91,92</sup> The changes in plasma albumin and  $\alpha$ 1-acid glycoprotein concentrations have been associated with the potential to alter the plasma exposure to total and free concentrations of the PIs.<sup>93,94</sup> Moreover, severe malnourishment during puberty when the transition to an adult dosing regimen is based on the sexual maturation might compromise ARV dosing in adolescents, whose body weight and development lags significantly behind the average growth parameters of a similarly aged cohort in developed countries. The data on the PK and PD of ARV drugs in children have been mostly generated in developed countries, though more data on regional, racial and ethnic pediatric PK and PD diversity have started emerging in the literature during recent years.<sup>95,96</sup> Limited pediatric data suggest that underlying malnutrition does not adversely affect immunologic and virologic response to ART.<sup>97,98</sup> While these PK immunologic and virologic outcome data in malnourished children is encouraging, more studies are necessary to evaluate the full effect of nutritional status and co-morbidities on the PK and PD of ART in pediatric patients.

### **Drug-drug interactions with ARV drugs**

The presence of co-morbidities such as **malaria** and **tuberculosis** (TB), which are most commonly associated with HIV infection,<sup>99</sup> further complicates pediatric ART in the resource-limited setting.<sup>100-102</sup> Co-administration of anti-TB and ART is common and results in potent drug-drug interactions affecting PK of ARV drugs caused by the induction of the CYP450 system. Significant reduction of NVP and EFV concentrations with concomitant rifampicin has been reported, particularly during the lead-in dose period when subtherapeutic concentrations occur in the majority of patients. Even more significant are the drug-drug interactions between rifampicin and RTV-boosted PIs, when the therapeutic concentrations can only be achieved with adjusted doses of LPV/RTV or with SQV/RTV. However, such dose adjustment has been reported to produce high rates of hepatotoxicity in healthy volunteers.<sup>103</sup> A few studies have evaluated the ARV and anti-TB drug interactions in pediatric HIV- and TB-co-infected patients.<sup>104,105</sup> In the studies of South African pediatric HIV-infected patients, attempts were made to compensate for the rifampicin generated

induction of LPV/RTV metabolism by increasing LPV/RTV ratio from 4:1 to 1:1.<sup>104,105</sup> Despite this change in boosting, the median  $C_{\max}$  and  $AUC_{0-12}$  were lowered by 26% and 31%, respectively,<sup>104</sup> and LPV oral clearance (CL/F) was decreased by 30%, and plasma  $C_{\min}$  greater than 1000 ng/ml were observed in most children in both studies. These data highlight the need to define optimal combination dosing regimens both for ARV and anti-TB drugs, and is particularly relevant today when several large international trials evaluating the new treatment modalities for pediatric TB in HIV-infected children are under way.

Another potential factor that can influence PK/PD of ARV drugs in children is the use of **traditional herbal medicines** (THM), which frequently represent primary treatment for HIV/AIDS and HIV-related problems.<sup>106,107</sup> Several drug interactions between herbal remedies and ART have been shown to affect the serum concentrations of ARV drugs resulting in clinical symptoms of toxicity in adults, and no studies are published in children.<sup>108</sup> Herbal remedies have the capacity to affect the metabolism of ARV drugs, especially NNRTI, PIs, CCR5 inhibitors and integrase inhibitors through the induction/suppression of CYP450 enzymes. It is equally important to consider the potential of herbal remedies to have an effect on ARV drug efficacy, as well as the potential for ARV drugs to increase the toxicity of THM. To date, no pediatric studies on ARV and THM interactions have been published.

### **Complications of pediatric ART**

Recent greater awareness and understanding of metabolic and cardiovascular ART complications in adults have generated renewed attention to long term consequences of HIV disease and ART exposure in children.<sup>109,110</sup> The data on the prevalence of pediatric ART associated metabolic complications in children start emerging from the studies conducted in developed countries, while in resource-limited settings, where the majority of HIV-infected children live, the prevalence and risk factors of metabolic complications are largely unknown. The significance of childhood ART associated lipodystrophy, dyslipidemia, insulin resistance, hyperlactatemia, renal insufficiency and osteopenia in the development of the cardiovascular, renal and bone disease of adulthood is not known and the management of these complications during childhood is under investigation.<sup>111</sup> Very few studies (including ours) have evaluated the impact of ARV drug exposure on the development of those complications.<sup>112-115</sup> A recent study in adolescents and young adults with HIV acquired perinatally or early in life reported a high rate of coronary artery abnormalities on cardiac magnetic resonance imaging (MRI), suggesting possible early atherosclerosis in this population.<sup>116</sup> Although not associated with coronary artery disease in adults, coronary irregularities were seen in youth with increased cumulative exposure to TDF and emtricitabine.<sup>116</sup> Given the potential risk of such condition and delayed onset of their clinical presentations, it is necessary to find new biomarkers to facilitate early identification of children at high risk of developing ARV associated toxicities.

### **Long-term sustainability of ART in children**

A limited number of studies has investigated the long-term sustainability of ART regimens in children. The ability to retain the first and second line ART regimens is highly dependent on the degree of patient's adherence. It is well recognized that HIV-infected children face multiple adherence challenges such as dependence on a caregiver for obtaining medications, palatability of liquid preparations, daily pill burden, food-drug and drug-drug interactions and interference with lifestyle of both the child and caregiver. As the child continues to grow, many additional obstacles related to the psychosocial changes during puberty emerge. Among these are changes in lifestyle involving growing independence and rebellion against parental involvement, increased peer pressure and fear of stigmatization, increased risk-taking behavior, denial and fear of HIV infection (particularly in recently diagnosed youth), long history of poor adherence and nondisclosure issues in perinatally infected adolescents, psychiatric problems (depression), and alcohol and substance abuse.<sup>117,118</sup> In our study of adherence in a cohort of 127 perinatally HIV-infected children complete adherence (100%) was reported in only 24% of the patients.<sup>118</sup> Self-reported adherence and age were significant predictors of reaching undetectable viral load. Most importantly, our data showed that adolescents (13 - 18 yrs old) were significantly less likely to reach undetectable viral load than younger children (< 13 yrs old).<sup>118</sup> For every year increase in age, the odds of reaching undetectable viral load decreased by 10% after controlling for self-reported adherence and refill mechanism.<sup>118</sup> A few pediatric studies have conducted a comprehensive assessment of adherence through different methods (self-report, pill count, pharmacy refills, and therapeutic drug monitoring) in children.<sup>119,120</sup> Although strategies to promote long-term adherence to ART have not been rigorously evaluated in adolescents to date, preliminary data suggest that interventions based on intensive follow-up, involvement of family and peers, use of reminder systems, alternative dosing schedules, and modified directly observed therapy may facilitate adherence to the dosing regimen in this vulnerable population.<sup>121,122</sup> Moreover, developmental and psychosocial factors affecting ART adherence in children and adolescents from different cultural and ethnic regions of the world are not well described and need to be evaluated. Culturally sensitive and acceptable adherence evaluation tools and interventions need to be developed.

### **Challenges in formulations of ARV drugs**

Due to the global need for pediatric ART, the ARV drugs face significant challenges in formulations of heat resistant small size solid preparations suitable for children. Following international efforts in ART delivery, it became quickly evident that the availability of liquid pediatric ARV formulations, which met the need of pediatric ART in developed countries, could not assure the delivery of ART in a large majority of resource limited settings with the highest prevalence of pediatric HIV disease. Alternative medication delivery systems, such as skin patches, have been investigated and are considered to be problematic in children due to the high inter- and intra-patient variability in absorption and metabolism and developmental changes in PK. As a result, in recent years a substantial effort has been made to

manufacture sustainable small size tablets and fixed dose co-formulations (FDC) of first line ARV drugs for pediatric patients in resource limited settings. To date, generic suppliers have developed a number of inexpensive, generic pediatric FDC formulations for reconstitution of once- or twice-daily dosing (i.e. combinations of ZDV, ABC, d4T, 3TC and NVP) in a variety of different ratios in formulations of regular and dispersible tablets, and suspension for reconstitution. The development of these formulations requires a ratio of active drugs to be in balance to allow dosing across a wide range of ages and body sizes. The bioequivalence and exposure-response for all FDC components needs to be considered and evaluated in Phase III trials in pediatric cohorts.<sup>123</sup> A few recent studies have addressed the PK and PD of FDC in children and raised concern for underdosing of NVP in pediatric patients.<sup>123,124</sup> It is clear that the availability of ARV FDC will generate more randomized, controlled studies to evaluate their therapeutic potential in pediatric patients.

## CONCLUSION

Multiple factors, including **drug** (formulations, PK, PD, drug-drug interactions), **patient** (developmental stage, PG, co-morbidities, malnutrition, adherence), and **viral** (genotypic/phenotypic profile, exposure targets) characteristics need to be combined in order to achieve sustained ART success at the minimal price of short and long-term complications in pediatric HIV-infected patients. Many excellent research and clinical data have been generated to address the optimization of pediatric ART, while ongoing ARV drug developments in the era of more complex therapies continue to generate new questions to be answered. In our center and through multiple collaborations, we will continue ongoing pediatric studies aimed to investigate: 1) the application of the gIQ and pIQ in LPV/rtv based ART in children; 2) the effect of puberty on the metabolism of EFV and ontogeny of CYP2B6 and UGT; 3) the development of pediatric population PK/PD models for TDF and EFV; 4) the relationship between childhood PI and NNRTI exposure and metabolic and hematologic complications of ART; and 5) the impact of incentive awards on pediatric ART adherence.

Modern pediatric combination ART faces a difficult challenge of preserving the focus on an individual child while addressing the globalization of ARV exposure. Future trials of ARV drugs in children will need to address the individualization of ART in the pediatric HIV-infected patient. Current pediatric ART research priority focuses on the most important areas with the least knowledge such as developmental changes in drug metabolizing enzymes and drug transporter activity (with particular emphasis on puberty), long term metabolic complications of ART in children, and the impact of pharmacogenetics on ARV drug disposition and response to ART in children of diverse racial/ethnic background. Finally, better adherence interventions and assessment tools need to be studied in large international trials. I hope that my thesis and future research in this field can contribute to the quest for optimization of the outcome of pediatric HIV infection in children worldwide.



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**SUMMARY**

**SAMENVATTING**

## SUMMARY

Despite the significant progress of the prevention of mother-to-child transmission of HIV, nearly 1,200 new infections in children younger than 15 years of age continue to occur daily. With an estimated > 3 million children and adolescents living with HIV infection, the delivery of efficient antiretroviral therapy (ART) to the world's pediatric population is of crucial importance. Given the growing number of HIV-infected children and adolescents with access to treatment, a better understanding of the therapeutic targets of antiretroviral (ARV) drugs in pediatric HIV infection is urgently needed.

The important etiological, physiological, psychological and social differences between children and adults create a unique consideration for HIV-infected pediatric patients, and require a population specific approach to ART. The global picture of pediatric HIV infections and pediatric ARV drug development is presented in the Introduction (**Chapter 1**). While acknowledging the important work of many international investigators, the chapter outlines the most significant gaps of knowledge in pediatric ART.

**PART I** focuses on the developmental changes from infancy to adolescence and its impact on ARV drug disposition and efficacy. The data guiding the dosing, efficacy and safety of medicines for children have lagged substantially behind as compared to the information available for adults. As a consequence, pediatricians who are faced with the prospect of confining their practice to medicines with adequate information have frequently resorted to prescribing medicines for unapproved uses (different dose, frequency, age group, route, indication or formulation). **Chapter 2** reviews the history and current progress in pediatric pharmacological research and pediatric drug development. The basic principles of drug absorption, distribution, metabolism and elimination from neonates to adolescents are summarized. Subsequently, **Chapter 3** focuses on the developmental changes in drug disposition during puberty. The study reviews physiological and psychological changes associated with physical and sexual maturation, and analyzes the potential impact of these changes on the pharmacokinetics (PK) and pharmacodynamics (PD) of ARV drugs in adolescent HIV-infected patients. The chapter reveals the paucity of data on the pharmacology of ARV drugs in puberty and provides a comprehensive summary of current adolescent ART guidelines. The lack of clear understanding of the PK and PD of ARV drugs during puberty leads to the continued use of higher (weight- or surface-based) pediatric doses with potential toxic drug exposure or early introduction of lower adult doses with the subsequent development of drug resistance and virologic failure.

HIV-infected children represent an incredibly diverse group of patients, ranging from neonates to young adults. With the change in age and maturity, pediatric ART evolves from the meticulous twice-daily dosing of milliliters of liquid preparations to a choice of once-daily dosing of a single fixed dose co-formulated tablet. The use of

simplified pediatric regimens may enhance the ability to achieve successful implementation of ART in HIV-infected children. PK/PD studies of ARV drugs and their combinations need to be conducted in infants, children, and adolescents to develop appropriate dosing in each of these specific groups. The current challenges in the availability, formulations and regimens of the pediatric ART are discussed in **Chapter 4**.

**PART II** of the thesis is dedicated to the analysis of developmental changes in the PK and PD of specific ARV drugs in children and adolescents. **Chapter 5** reports the data of a study conducted in collaboration with the University of Nijmegen, the Netherlands on the PK of lamivudine (3TC) in HIV-infected pediatric patients. 3TC is frequently used as NRTI backbone of treatment for HIV infection in children. Previous studies have indicated a higher oral clearance of 3TC in children than in adults. Therefore, the recommended dose of 3TC in children is approximately two times higher when compared to adults. In our study, the PK data were collected from 51 HIV-1 infected children (median age of 8.4 years) using a standard pediatric dose of 3TC and were compared with adult PK parameters. AUC,  $C_{max}$ ,  $C_{min}$ , CL/kg and Vd/kg were significantly related to age with younger children having lower exposure to 3TC. The age of 6 years appeared to be a cut-off for a change in 3TC PK parameters, with children  $\leq 6$  years of age having a median AUC 43% lower and a median  $C_{max}$  47% lower than older children. In line with these observations, CL/kg and Vd/kg were 79% and 89% higher in children  $\leq 6$  years of age when compared to older children. The mean  $C_{max}$  and AUC in children  $\geq 7$  years of age were almost similar to adult PK parameters. Most importantly, the decreased 3TC exposure in younger children is potentially related to the development of M184V HIV mutation and resistance to 3TC, and deserves further investigation.

The development of a population PK model for the most widely used inhibitor (PI) lopinavir (LPV) in pediatric practice is presented in **Chapter 6**. The data were obtained from HIV-infected, PI-experienced children receiving LPV through intensive PK sampling and were fitted to candidate PK models (using MM-USCPACK software). The final model was used to simulate 1,000 children to determine the probability of achieving an LPV phenotypic inhibitory quotient (PIQ) of  $> 15$ , which has been associated with a higher likelihood of viral suppression in adult studies. The probability of achieving an LPV PIQ of  $> 15$  was  $> 90\%$  for wild-type virus but  $< 10\%$  for even moderately resistant virus. The currently recommended dose of lopinavir/ritonavir appears to be adequate for children infected with wild-type virus but is unlikely to provide adequate inhibitory concentrations for even moderately resistant HIV. PI-experienced HIV-infected children will likely benefit from longitudinal, repeated LPV measurement in plasma to ensure that drug exposure is most often near the maximal end of the observed safe range.

The ontogeny of ARV metabolizing enzymes and drug transporters is investigated in the study of the association between the *CYP3A5*, *MDR1 (ABCB1)* and *OATP1 (SLCO1B1)* polymorphisms on the PK and PD of LPV/RTV therapy in children

(**Chapter 7**). *CYP3A5*, *MDR1* and *SLCO1B1* polymorphisms have been associated with variability in the PK of PIs. In this study we investigated the influence of *CYP3A5* A6986G, *MDR1* (C3435T and G2677T), and *SLCO1B1* (T521C and A388G) polymorphisms on the PK/PD of LPV/RTV in a prospective cohort of 50 HIV-infected children (median age 11.2 yrs). The results showed no statistically significant association between LPV or RTV AUC or CL, and *CYP3A5*, *MDR1* or *SLCO1B1* A388G polymorphisms. There was a significant association between *SLCO1B1* T521C and LPV AUC ( $P = 0.042$ ) and a nearly significant association with LPV CL ( $P = 0.063$ ). Studied polymorphisms, however, were not associated with virologic outcome within the study period. Larger pediatric studies of the developmental changes in drug distribution and metabolism are needed to better understand the role of pharmacogenetic factors in the response to ART in children of diverse racial/ethnic background.

**PART III** consists of two studies focusing on the role of the therapeutic drug monitoring (TDM) in the management of ART of pediatric HIV infection. **Chapter 8** investigates the non-invasive matrix of salivary concentrations of non-nucleoside reverse transcriptase inhibitor nevirapine (NVP) as an alternative to the plasma sampling in pediatric HIV-infected children. Adequate trough concentrations of NVP predict successful therapy while subtherapeutic levels are correlated with virologic failure and development of resistance. Our study examined the extent of agreement between total and free plasma NVP concentrations and non-stimulated saliva NVP concentrations and evaluated the feasibility of saliva sampling as an alternative tool for TDM of NVP in children. NVP concentrations were obtained in paired plasma and saliva samples of pediatric patients receiving ART including NVP. The saliva NVP concentrations reflected the free concentrations very closely, but were on average 34% higher. The Bland-Altman plots indicated that the discrepancy between saliva and plasma measures is consistent across the range of average NVP concentrations. Our study results strongly indicate the agreement between saliva and plasma NVP concentrations in pediatric patients with HIV infection. Non-stimulated NVP saliva concentrations can be used as an interchangeable non-invasive, reliable, cost-effective method for the application of TDM of NVP therapy.

Currently, TDM of ART is recommended to rule out subtherapeutic drug concentrations and to differentiate among malabsorption, drug interactions, poor adherence, or increased drug metabolism or clearance as possible causes of decreased drug exposure. The use of TDM is also considered to assist in finding the optimal dose of a drug in patients whose virus has shown reduced susceptibility to that drug. As outlined in Chapter 3, the dosing of ARV drugs in adolescent patients shows the transition of a dosing regimen from higher pediatric (weight and surface-based) to adult (fixed) range but this is not well defined. Developmental PK differences contribute to high variability in pediatric and adolescent patients and an increased frequency of suboptimal ARV exposure as compared to in adults. Individualized, concentration-targeted optimal dosing of ARV medications can be beneficial to patients for whom only limited dosing guidelines are available. The role

of TDM in the optimization of pharmacotherapy of HIV infection in adolescent patients is discussed in **Chapter 9**. In this chapter we describe two scenarios of the application of TDM in treatment-experienced adolescent patients whose ART was optimized using ARV TDM. In the first scenario TDM served as an efficient tool in the evaluation of the treatment failure in light of self-reported excellent medical adherence. The second scenario represents the first report of successful reduction of the efavirenz (EFV) dose in adolescent patients based on the *CYP2B6* genotype in combination with PK evaluation. TDM of ARV drugs is useful in managing the pharmacotherapy of HIV in adolescent patients and is well received by the adolescent patients with HIV and their families. Among others, TDM in adolescents provides valuable information about the clinical pharmacology of ART during puberty.

**PART IV** addressed the issues associated with long-term sustainability and complications of ART in children and adolescents. **Chapter 10** investigates the relationship between self-reported adherence to ART and the virologic and immunologic outcomes of HIV infection in 127 perinatally infected adolescents (mean age 9.9 yrs). In addition the relationship between the pharmacy supply of ARV drugs and self-reported adherence and HIV viral load (VL) in HIV-infected children was evaluated. The complete adherence (100%) was reported in only 24% of the patients. With 40% of the patients being rarely or never completely adherent, 64% of children achieved undetectable VL during the study period. In our study, we did not find an association between pharmacy supply and self-reported adherence. Self-reported adherence ( $P = 0.0328$ ) and age ( $P = 0.025$ ) were the significant predictors of reaching undetectable VL. Most importantly, adolescents ( $> 13$  yrs) were significantly less likely to reach undetectable VL than children  $< 13$  yrs (OR = 0.38; 95% CI: 0.16, 0.89). For every year increase in age, the odds of reaching undetectable VL decreased by 10% after controlling for self-reported adherence and refill mechanism. While we acknowledge that the adolescents in the study had perinatally acquired HIV, were highly treatment experienced, and therefore had higher chances for HIV resistance and virologic failure, we must recognize many additional obstacles to ART adherence emerging during puberty. For the providers and caregivers loss of adherence during puberty in adolescents with perinatally acquired HIV represents a difficult and emotional challenge that requires team approach and close collaboration. Given the lack of a well defined adherence intervention model in adolescents with HIV, more research on adherence among HIV-infected youth with interdisciplinary collaboration is warranted.

The development and application of biomarkers for the early identification of HIV-associated nephropathy are presented in **Chapter 11**. HIV-infected children are at risk of developing several types of renal diseases, including HIV-associated nephropathy (HIVAN). Childhood HIVAN is defined by the presence of proteinuria associated with mesangial hyperplasia and/or global-focal segmental glomerulosclerosis (FSGS), in combination with microcystic transformation of renal tubules. HIVAN can have an insidious clinical onset and the only way of establishing

a definitive diagnosis is to perform a renal biopsy. Given the risk of performing this procedure in HIV-infected children with other AIDS-defining illness, it is necessary to find new biomarkers to identify children at high risk of developing HIVAN. In this study, we sought to determine whether growth factors released into the urine of HIV-infected children with renal disease parallel the extent and activity of the renal lesions characteristic of HIVAN in fifty five HIV-infected children. We found reduced levels of Epidermal Growth Factor (EGF) in the urine of children with HIVAN in association with increased levels of Fibroblast Growth Factor- 2 (FGF-2) and metalloproteinase-2 (MMP-2), when compared to HIV-infected children without renal disease. Similar changes were found in HIV-Tg<sub>26</sub> mice in correlation with the progression of the renal disease. These findings suggest that the urinary growth factor profile of EGF, FGF-2 and MMP-2 may be a useful candidate biomarker to identify HIV-infected children at risk of developing HIVAN. Of interest, previous studies have shown that PIs can inhibit the activity of MMP-2 by blocking the conversion from its latent to the active form. Based on these data, we speculate that PIs could have a potential beneficial effect in children with HIVAN by blocking the activation of MMP-2.

Finally, the general discussion in **Chapter 12** focuses on the thorough review of the development and approval of ARV drugs in pediatric HIV disease. The paper summarizes the available data on the developmental PK and PD of ART and identifies current gaps in the knowledge of ART in children. This final chapter reviews the need for the globalization and individualization of pediatric ART and outlines the roadmap of future studies to optimize ART in HIV-infected infants, children and adolescents worldwide.

Current pediatric ART research priority focuses on the most important areas with the least knowledge such as developmental changes in drug metabolizing enzymes and drug transporter activity (with particular emphasis on puberty), long term metabolic complications of ART in children, the impact of pharmacogenetics on ARV drug disposition and response to ART in children of diverse racial/ethnic background. Moreover, better adherence assessment tools and interventions need to be studied in large international trials. This thesis represents collaborations of several academic and clinical programs in the Netherlands and USA. The global nature of the pediatric HIV infection requires global research efforts in developing better treatment modalities for pediatric HIV disease.

## SAMENVATTING

Het voorkomen van de overdracht van HIV van moeder naar kind is de laatste jaren aanzienlijk verbeterd. Desondanks openbaren zich dagelijks nog bijna 1200 nieuwe infecties bij kinderen jonger dan 15 jaar. Met een geschat aantal van meer dan 3 miljoen kinderen en adolescenten die met een HIV-infectie leven, is het van doorslaggevend belang om kinderen, waar ook ter wereld, efficiënte antiretrovirale therapie (ART) te geven. Het feit dat een groeiend aantal HIV-geïnfecteerde kinderen en adolescenten toegang hebben tot een behandeling maakt het beter begrijpen van de therapeutische doelen van antiretrovirale geneesmiddelen bij de behandeling van HIV bij kinderen dringend noodzakelijk.

De belangrijke etiologische, fysiologische, psychologische en sociale verschillen tussen kinderen en volwassenen vereisen een unieke overweging voor HIV-geïnfecteerde kinderen en vragen om een populatiespecifieke aanpak van ART. Het wereldbeeld van HIV-infecties en antiretrovirale geneesmiddelontwikkeling bij kinderen wordt besproken in de introductie (**Hoofdstuk 1**). Dit hoofdstuk geeft, naast erkenning van het belangrijke werk van vele internationale onderzoekers, de meest belangrijke tekorten in kennis van ART bij kinderen aan.

**Deel 1** richt zich op de veranderingen die plaatsvinden tijdens de ontwikkeling van kind tot adolescent en de invloed van deze veranderingen op het verwerken en de effectiviteit van antiretrovirale geneesmiddelen. De gegevens die als richtlijn dienen bij het doseren van het geneesmiddel, evenals de gegevens betreffende effectiviteit en veiligheid van geneesmiddelen bij kinderen, zijn duidelijk van een lagere kwaliteit in vergelijking met die voor volwassenen. Kinderartsen worden daardoor geconfronteerd met het feit dat er nauwelijks geneesmiddelen voorhanden zijn wanneer ze zich zouden beperken tot het voorschrijven van geneesmiddelen waarvan voldoende bekend is. Hierdoor zijn deze artsen genoodzaakt tot het voorschrijven van geneesmiddelen waarvoor geen toestemming voorhanden is, zoals het gebruik van een andere dosis, frequentie, leeftijdsgroep, wijze van toediening, indicatie of toedieningsvorm. **Hoofdstuk 2** geeft een overzicht van de geschiedenis en huidige vooruitgang van kinderfarmacologisch onderzoek en geneesmiddelontwikkeling. De basisprincipes van geneesmiddelabsorptie, distributie, metabolisme en eliminatie bij pasgeborenen tot aan adolescenten worden hier samengevat. **Hoofdstuk 3** richt zich vervolgens op het veranderende effect van geneesmiddelen tijdens de puberteit. Dit deel geeft een overzicht van de fysiologische en psychologische veranderingen die geassocieerd zijn met de lichamelijke en seksuele rijping. Verder wordt ingegaan op de mogelijke invloed van deze veranderingen op de farmacokinetiek en farmacodynamiek van antiretrovirale geneesmiddelen bij HIV-geïnfecteerde adolescenten. Dit hoofdstuk laat het gebrek aan gegevens betreffende de farmacologie van antiretrovirale geneesmiddelen tijdens de puberteit zien en geeft een uitgebreide samenvatting van de huidige richtlijnen voor het gebruik van antiretrovirale middelen bij adolescenten. Het gebrek aan kennis van de farmacokinetiek en farmacodynamiek van antiretrovirale

middelen tijdens de puberteit heeft enerzijds geresulteerd in het gebruik van hoge (gebaseerd op gewicht of lichaamsoppervlakte) kinderdoseringen met het risico op overdosering. Anderzijds heeft het toedienen van lagere volwassen doseringen geresulteerd in geneesmiddelresistentie en daardoor virologisch falen.

HIV-geïnfekteerde kinderen vertegenwoordigen een ongelofelijk diverse groep van patiënten, variërend van pasgeborenen tot jonge volwassenen. Gedurende de groei en ontwikkeling verandert ART op de kinderleeftijd van het preciese doseren van tweemaal daags een drankje, naar eenmaaldaags een tablet met een vaste dosis. Het gebruik van vereenvoudigde kindergeneeskundige doseringsschema's verbetert mogelijk het succesvol implementeren van ART bij HIV-geïnfekteerde kinderen. PK/PD studies van antiretrovirale geneesmiddelen en combinaties van deze geneesmiddelen moeten plaatsvinden bij zuigelingen, kinderen en adolescenten om de juiste doseringen voor elke leeftijdsgroep te bepalen. De beschikbaarheid, de toedieningsvormen en de doseringsschema's van antiretrovirale middelen bij kinderen worden besproken in **hoofdstuk 4**.

**Deel 2** van dit proefschrift is gewijd aan de analyse van de ontwikkelingsafhankelijke veranderingen in de PK en PD van specifieke antiretrovirale geneesmiddelen bij kinderen en adolescenten. **Hoofdstuk 5** beschrijft de uitkomsten van een studie naar de PK van lamivudine (3TC) bij HIV-geïnfekteerde kinderen, welke is verricht in samenwerking met de Universiteit van Nijmegen. 3TC wordt vaak gebruikt als onderdeel van de nucleoside reverse transcriptase remmers (NRTI) component bij de behandeling van HIV-geïnfekteerde kinderen. Eerdere studies laten een hogere orale klaring zien van 3TC bij kinderen in vergelijking met de klaring bij volwassenen. Derhalve is de geadviseerde dosering van 3TC voor kinderen twee keer zo hoog als de dosering bij volwassenen. In onze studie werden PK-gegevens verzameld bij 51 HIV-1-geïnfekteerde kinderen (mediane leeftijd 8,4 jaar), gebruikmakend van een standaard kindergeneeskundige dosering van 3TC. Deze PK-gegevens werden vergeleken met PK-gegevens bij volwassenen. AUC,  $C_{max}$ ,  $C_{min}$ , CL/kg en Vd/kg toonden een significante relatie met leeftijd, waarbij jongere kinderen een lagere blootstelling aan 3TC hadden. De leeftijd van 6 jaar lijkt een duidelijke grens te zijn voor een verandering in 3TC PK-waarden, waarbij kinderen jonger dan 6 jaar een 43% lagere mediane waarde voor AUC en een 47% lagere mediane waarde voor  $C_{max}$  laten zien vergeleken met oudere kinderen. In overeenstemming met deze observaties zijn CL/kg en Vd/kg 79% en 89% hoger bij kinderen van 6 jaar of jonger vergeleken met oudere kinderen. De gemiddelde  $C_{max}$  en AUC bij kinderen van 7 jaar en ouder waren bijna identiek aan de PK-waarden bij volwassenen. Het belangrijkste is echter dat verlaagde 3TC-blootstelling bij jongere kinderen mogelijk gerelateerd is aan het ontwikkelen van de M184V HIV-mutatie en resistentie tegen 3TC. Dit verdient nader onderzoek.

De ontwikkeling van een populatie PK-model voor de meest gebruikte proteaseremmer lopinavir (LPV) voor de kindergeneeskundige praktijk wordt beschreven in **hoofdstuk 6**. De gegevens werden verkregen door een intensieve



PK-studie van HIV-geïnficeerde kinderen die al behandeld werden met proteaseremmers en LPV kregen. Deze gegevens werden getoetst aan verschillende kandidaat PK-modellen, gebruikmakend van MM-USCPACK programmatuur. Het uiteindelijke model werd gebruikt om voor 1000 fictieve kinderen de kans te berekenen op het bereiken van een LPV-fenotypisch remmingsquotiënt van meer dan 15. Want een dergelijk quotiënt is bij volwassenen geassocieerd met een grote kans op virusonderdrukking. De kans op het bereiken van een LPV PIQ van meer dan 15 was groter dan 90% voor het wild-type virus maar minder dan 10% voor zelfs een matig resistent virus. De huidige geadviseerde dosis van lopinavir/ritonavir lijkt voldoende voor kinderen geïnficeerd met het wild-type virus, maar het is onwaarschijnlijk dat het voldoende remming geeft van zelfs een matig resistent virus. HIV-geïnficeerde kinderen die al blootgesteld zijn aan proteaseremmers, zullen waarschijnlijk profiteren van herhaalde bepalingen van LPV-spiegels om te garanderen dat de blootstelling aan LPV resulteert in spiegels die hoog en toch nog veilig zijn.

De ontogenie van enzymen en transporters betrokken bij het metabolisme en transport van antiretrovirale geneesmiddelen werd onderzocht in een studie waarin gekeken werd naar de associatie tussen *CYP3A5*, *MDR1 (ABCB1)* en *OATP1 (SLCO1B1)* polymorfismen en de PK en PD van LPV/RTV therapie bij kinderen (**hoofdstuk 7**). *CYP3A5*, *MDR1* en *SLCO1B1* polymorfismen zijn geassocieerd met variatie in de PK van proteaseremmers. In dit onderzoek hebben we het effect van *CYP3A5* A6986G, *MDR1* (C3435T and G2677T), en *SLCO1B1* (T521C and A388AG) polymorfismen op de PK/PD van LPV/RTV in een prospectieve cohortstudie van 50 HIV-geïnficeerde kinderen (mediane leeftijd 11,2 jaar) nagegaan. De resultaten lieten geen statistisch significante associatie zien tussen LPV of RTV AUC of CL, en *CYP3A5*, *MDR1* of *SLCO1B1* A388G polymorfismen. Er was een significante associatie tussen *SLCO1B1* T521C en LPV AUC ( $P = 0.042$ ) en een bijna significante associatie met de klaring van LPV ( $P = 0.063$ ). De onderzochte polymorfismen waren echter niet geassocieerd met de virologische uitkomst tijdens de studieperiode. Grotere studies bij kinderen naar de veranderingen in geneesmiddeldistributie en metabolisme tijdens groei en ontwikkeling zijn noodzakelijk om de rol van farmacogenetica op het effect van ART bij kinderen met verschillende raciale/etnische achtergronden beter te begrijpen.

**Deel 3** bestaat uit twee studies die zich richten op de rol van therapeutische geneesmiddelbewaking (TDM) bij de behandeling van HIV-infectie bij kinderen met ART. **Hoofdstuk 8** beschrijft het onderzoek naar de rol van het meten van de non-nucleoside reverse transcriptaseremmer nevirapine (NVP) in speeksel als een niet-belastend alternatief voor het meten van NVP in plasma bij HIV-geïnficeerde kinderen. Adequate dalspiegels van NVP voorspellen een goede uitkomst van de behandeling, terwijl subtherapeutische spiegels gecorreleerd zijn met virologisch falen en resistentie tot gevolg heeft. Onze studie onderzocht de verhouding tussen totaal en vrij (niet eiwitgebonden) plasma NVP spiegels versus NVP spiegels in niet-gestimuleerd speeksel en onderzocht tevens of speeksel bruikbaar is als een

alternatief voor TDM bij kinderen. NVP-spiegels werden bepaald in gepaarde plasma- en speekselmonsters van kinderen die met ART en NVP werden behandeld. De NVP-spiegels gemeten in het speeksel kwamen sterk overeen met de vrije NVP-spiegels in plasma, maar waren gemiddeld 34% hoger. De Bland-Altman figuren lieten zien dat de verschillen tussen speeksel- en plasmawaarden consistent zijn over de het gehele gebied van de gemeten spiegels. Onze resultaten tonen derhalve een sterke overeenkomst tussen speeksel- en plasma-NVP-spiegels bij HIV-geïnfekteerde kinderen. NVP speekselspiegels zonder speekselstimulatie kunnen dus gebruikt worden als een uitwisselbare, niet-belastende, betrouwbare en kosteneffectieve methode voor de toepassing van TDM van NVP-therapie.

Op dit moment wordt TDM van ART geadviseerd om subtherapeutische geneesmiddelconcentraties uit te sluiten en om onderscheid te maken tussen malabsorptie, geneesmiddelinteracties, lage therapietrouw, of verhoogd metabolisme en/of klaring van het geneesmiddel als mogelijke oorzaken van verlaagde geneesmiddelblootstelling. Het gebruik van TDM wordt ook overwogen om te helpen bij het vinden van een optimale dosering van een geneesmiddel bij patiënten waarbij het virus minder gevoelig blijkt jegens het geneesmiddel. Zoals beschreven in hoofdstuk 3 laat het doseren van antiretrovirale geneesmiddelen bij adolescenten de overgang zien van een doseringsregime met hoge kinderdoseringen (op gewicht en lichaamsoppervlakte gebaseerd) tot een relatief lage volwassendosering (vaste dosis). Dit is echter niet duidelijk gedefinieerd. PK-verschillen gebaseerd op de ontwikkeling van het individu dragen bij tot de sterke variabiliteit bij kinderen en adolescenten en een toegenomen frequentie van suboptimale blootstelling aan antiretrovirale geneesmiddelen vergeleken met volwassenen. Geïndividualiseerde, concentratie-gericht optimaal doseren van antiretrovirale geneesmiddelen kan gunstig voor patiënten zijn voor wie alleen beperkte doseringsrichtlijnen voorhanden zijn. De rol van TDM voor het optimaliseren van de farmacotherapie van HIV-infectie bij adolescentie patiënten wordt in **hoofdstuk 9** bediscussieerd. In dit hoofdstuk beschreven we twee scenario's van het toepassen van TDM bij adolescentie patiënten die al behandeld zijn met antiretrovirale geneesmiddelen waarbij ART verbeterd werd door TDM van deze middelen. TDM bleek een goede methode om, in het eerste scenario waarbij de patiënt zelf beweerde therapietrouw te zijn, het falen van de behandeling te evalueren. Het tweede scenario geeft een eerste verslag van een succesvolle verlaging van de dosering van efavirenz (EFV) bij adolescentie patiënten gebaseerd op *CYP2B6* genotype in combinatie met PK-evaluatie. TDM van antiretrovirale geneesmiddelen is bruikbaar bij het regelen van de farmacotherapie van HIV bij adolescentie patiënten en wordt door adolescentie patiënten met HIV en hun families als waardevol ervaren. TDM geeft ook bij adolescenten waardevolle informatie over de klinische farmacologie van ART tijdens de puberteit.

**Deel 4** beschrijft kwesties geassocieerd met langetermijn houdbaarheid en complicaties van ART bij kinderen en adolescenten. **Hoofdstuk 10** beschrijft de relatie tussen zelfgerapporteerde therapietrouw met ART en de virologische en

immunologische resultaten van HIV-infectie bij 127 perinataal geïnfecteerde adolescenten (gemiddelde leeftijd 9,9 jaar). Tevens werd de relatie tussen de manier van antiretrovirale geneesmiddelvoorziening door de apotheek, zelfgerapporteerde therapietrouw en HIV-virale belasting bij HIV-geïnfecteerde kinderen geëvalueerd. Complete therapietrouw (100%) werd slechts door 24% van de patiënten gerapporteerd. Bij de 40% van de patiënten die zelden of nooit helemaal therapietrouw waren, werd bij 64% daarvan een niet-detecteerbare virale belasting gevonden tijdens de duur van de studie. In onze studie vonden we geen associatie tussen de manier van geneesmiddelvoorziening door de apotheek en de zelfgerapporteerde therapietrouw. Zelfgerapporteerde therapietrouw ( $P = 0.0328$ ) en leeftijd ( $P = 0.025$ ) waren de belangrijke voorspellers voor het bereiken van een niet detecteerbare virale belasting. Het meest belangrijk was dat adolescenten (> 13 jaar) veel minder in staat waren niet-detecteerbare virale belasting te bereiken dan kinderen jonger dan 13 jaar (OR = 0.38; 95% CI: 0.16, 0.89). Voor elk jaar ouder verminderde de kans op het bereiken van niet-detecteerbare virale belasting met 10% rekeninghoudend met zelfgerapporteerde therapietrouw en de wijze van geneesmiddelvoorziening door de apotheek. Hoewel we toegeven dat de adolescenten in deze studie HIV perinataal hadden gekregen en vele behandelingen hadden ondergaan, waardoor ze een verhoogde kans op HIV-resistentie en virologisch falen hadden, zagen we toch extra belemmeringen voor optimale therapietrouw tijdens de puberteit. Voor zowel artsen als verzorgers betekent afname van therapietrouw tijdens de puberteit bij adolescenten met perinataal verworven HIV, een grote en emotionele uitdaging, dat goed teamwerk vereist. Aangezien een goed gedefinieerd model voor interventies gericht op therapietrouw voor adolescenten met HIV ontbreekt, is meer onderzoek naar therapietrouw bij HIV-geïnfecteerde jongeren met een interdisciplinaire samenwerking noodzakelijk.

De ontwikkeling en toepassing van biomarkers voor vroege detectie van HIV-geassocieerde nefropathie wordt besproken in **hoofdstuk 11**. HIV-geïnfecteerde kinderen lopen het risico allerlei nierziekten te krijgen, onder andere HIV-geassocieerde nefropathie (HIVAN). HIVAN tijdens de kinderleeftijd wordt gedefinieerd door de aanwezigheid van proteïnurie-geassocieerd met mesangiale hyperplasie en/of globale-focale segmentale glomerulosclerose (FSGS), in combinatie met microcysttransformatie van de niertubuli. HIVAN kan een verraderlijk klinisch beloop hebben. Een nierbiopsie is de enige manier om de diagnose te stellen. Het risico van een dergelijke ingreep bij HIV-geïnfecteerde kinderen met andere HIV-gerelateerde aandoeningen, maakt het vinden nieuwe biomarkers noodzakelijk. Kinderen die risico lopen op het ontwikkelen van HIVAN zijn dan gemakkelijker op te sporen. In deze studie keken we bij 55 HIV-geïnfecteerde kinderen of groeifactoren die HIV-geïnfecteerde kinderen met nierziekte in de urine uitscheiden, overeenkomen met de uitgebreidheid en activiteit van de karakteristieke lesies van HIVAN. We vonden verminderde concentraties van epidermale groeifactor (EGF) in de urine van kinderen met HIVAN in associatie met verhoogde concentraties van fibroblast groeifactor-2 (FGF-2) en metalloproteïnase-2 (MMP-2) vergeleken met HIV-geïnfecteerde kinderen zonder nierziekte.

Overeenkomstige veranderingen werden gevonden in HIV-Tg<sub>26</sub>-muizen, gecorreleerd aan de progressie van hun nierziekte. Deze bevindingen suggereren dat het groeifactorprofiel van EGF, FGF-2 and MMP-2 in urine mogelijk een nieuwe biomarker is om HIV-geïnfecteerde kinderen met een kans om HIVAN te ontwikkelen te identificeren. Eerdere studies hebben al laten zien dat proteaseremmers de activiteit van MMP-2 kunnen remmen door het blokkeren van de omzetting van MMP-2 van de latente naar de actieve vorm. Gebaseerd op deze gegevens speculeren we dat proteaseremmers een positief effect bij kinderen met HIVAN kunnen hebben door het blokkeren van de activatie van MMP-2.

Tot slot richt de algemene discussie in **hoofdstuk 12** zich op een grondig overzicht van de ontwikkeling en goedkeuring van antiretrovirale geneesmiddelen voor HIV-ziekte bij kinderen. Het vat de beschikbare gegevens samen van ontwikkelingsfarmacologie (PK en PD) van ART en wijst op de leemtes in de kennis van ART bij kinderen. Dit laatste hoofdstuk pleit voor een wereldwijde samenwerking voor toekomstige studies om ART te optimaliseren, zodat HIV-geïnfecteerde zuigelingen, kinderen en adolescenten waar ook ter wereld een individuele aanpak kunnen krijgen.

De prioriteiten voor ART-onderzoek liggen bij gebieden waarvan nauwelijks iets bekend is zoals:

- de veranderingen door groei en ontwikkeling van geneesmiddelmetaboliserende enzymen en geneesmiddeltransportactiviteit (met speciale aandacht voor de puberteit),
- langetermijn metabole complicaties van ART bij kinderen,
- de rol van farmacogenetica bij kinderen met verschillende raciale en etnische achtergrond wat betreft het verwerken van antiretrovirale geneesmiddelen en de reactie op ART,
- grote internationale studies voor het verbeteren van methoden om therapietrouw vast te stellen en te vergroten.

Dit proefschrift is tot stand gekomen dankzij samenwerking tussen verscheidene academische en klinische programma's in Nederland en de Verenigde Staten. Het mondiale karakter van HIV-infectie bij kinderen vereist mondiaal onderzoek om de behandelingsmogelijkheden voor kinderen met HIV te verbeteren.

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**LIST OF  
ABBREVIATIONS**

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
AMP	Amprenavir
ART	Antiretroviral therapy
ARV	Antiretroviral
ATV	Atazanavir
AUC	Area under the (concentration-time) curve
AZT	Zidovudine
BSA	Body surface area
CCR5	C-C chemokine receptor 5
CDC	Centers for Disease Control
CL	Clearance
$C_{\max}$	Maximum (peak) concentration
$C_{\min}$	Minimum (trough) concentration
CV	Coefficient of variation
CYP450	Cytochrome P450
d4T	Stavudine
ddI	Didanosine
DME(s)	Drug metabolizing enzyme(s)
DOT	Directly observed therapy
DRV	Darunavir
EFV	Efavirenz
EGF	Epidermal growth factor
ETR	Etravirine
ETV	Etravirine
f-AMP	Fosamprenavir
FDA	Food and Drug Administration
FDC	Fixed dose combination
FGF	Fibroblast growth factor
FTC	Emtricitabine
GFR	Glomerular filtration rate
GH	Growth hormone
GIQ	Genotypic inhibitory quotient
HAART	Highly active antiretroviral therapy
HD	Home delivery
HIV	Human immunodeficiency virus

HIVAN	HIV-associated nephropathy
IC	Inhibitory concentration
ICC	Interclass correlation coefficient
IDV	Indinavir
IGF-1	Insulin-like growth hormone
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Group
IPP	In pharmacy pick up
IQ	Inhibitory quotient
IRB	Institutional Review Board
LPV	Lopinavir
LPV/RTV	Lopinavir/ritonavir (Kaletra <sup>®</sup> )
MCTC	Mother-to-child transmission
MDR1	Multidrug resistance gene
MEC	Minimal effective concentration
MMP	Matrix metalloproteinase
MVC	Maraviroc
NAT	N-acetyltransferase
NCRR	National Center for Research Resources
NICHD	<i>Eunice Kennedy Shriver</i> National Institute of Child Health & Development
NIH	National Institutes of Health
NIQ	Normalized inhibitory quotient
NMEs	New molecular entities
NNRTI(s)	Non-nucleoside reverse transcriptase inhibitor(s)
NRTI(s)	Nucleoside reverse transcriptase inhibitor(s)
NVP	Nevirapine
OATP	Organic anion transporting polypeptide
PACTG	Pediatric AIDS Clinical Trials Group
PD	Pharmacodynamic(s)
PEPFAR	The United States President's Emergency Plan for AIDS Relief
PG	Pharmacogenetic(s)
P-gp	P-glycoprotein
PI(s)	Protease inhibitor(s)
PIQ	Phenotypic inhibitory quotient
PK	Pharmacokinetic (s)
PMTCT	Prevention of mother-to-child transmission
RAL	Raltegravir
RD	Renal disease
RTV	Ritonavir

SD	Standard deviation
SLC	Solute carrier
SQV	Saquinavir
TB	Tuberculosis
TDF	Tenofovir diproxil fumarate
TDM	Therapeutic drug monitoring
TFV	Tenofovir
THM	Traditional herbal medicines
TPV	Tipranavir
UDP	Uridine diphosphate
UGT	Uridine 5'-diphospho-glucuronosyltransferase
Vd	Volume of distribution
VL	Viral load
WHO	World Health Organization
WT	Wild type
$\gamma$ -GT	$\gamma$ -glutamyl transpeptidase
ZDV	Zidovudine



**LIST OF  
PUBLICATIONS**

**PHD PORTFOLIO**

**ABOUT THE AUTHOR**

**LIST OF PUBLICATIONS**

Rakhmanina NY, Kearns GL, Farrar HC 3rd.  
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Rakhmanina NY, van den Anker JN, Soldin SJ.  
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Therapeutic drug monitoring for patients with HIV infection: Children's National Medical Center, Washington DC experience.  
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Therapeutic drug monitoring in children with HIV/AIDS.  
*Ther Drug Monit. 2004;26:122-126.*

Capparelli E, Rakhmanina N, Mirochnick M.  
Pharmacotherapy of perinatal HIV.  
*Semin Fetal Neonatal Med. 2005;10:161-175.*

Rakhmanina NY, van den Anker JN.  
Pharmacological research in pediatrics: from neonates to adolescents.  
*Adv Drug Deliv Rev. 2006;58:4-14.*

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Age-dependant pharmacokinetics of lamivudine in HIV-infected children.  
*Clin Pharmacol Ther. 2007;81:517-520.*

Rakhmanina NY, Sill A, Baghdassarian A, Bruce K, Williams K, Castel AD, Rakusan T, Singh N, Spiegel HM.

Epidemiology of new cases of HIV-1 infection in children referred to the metropolitan pediatric hospital in Washington, DC.

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Soler-García AA, Rakhmanina NY, Mattison PC, Ray PE.

A urinary biomarker profile for children with HIV-associated renal diseases.

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Khan M, Song X, Williams K, Bright K, Sill A, Rakhmanina N.

Evaluating adherence to medication in children and adolescents with HIV.

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Rakhmanina NY, van den Anker JN.

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Rakhmanina NY, van den Anker JN, Soldin SJ, van Schaik RH, Mordwinkin N, Neely MN. Can therapeutic drug monitoring improve pharmacotherapy of HIV infection in adolescents?

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Pharmacokinetic optimization of antiretroviral therapy in children and adolescents.

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*CYP3A5*, *MDR1* and *SLCO1B1* polymorphisms and the pharmacokinetics and pharmacodynamics of lopinavir/ritonavir in children.

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Rakhmanina N, van den Anker JN, Baghdassarian A, Soldin SJ, Williams K, Neely MN. The phenotypic and genotypic susceptibility lopinavir scores and virologic response in treatment-experienced children with HIV.

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Ivy W, Dominguez K, Rakhmanina N, Iuliano D, Danner S, Gaur A, Mitchell C, Henderson S, Paul M, Barton T, Herbert-Grant M, Perez A, Van Dyke R, Nesheim S. Prevalence of and factors associated with pre-mastication of food among caregivers of human immunodeficiency virus-exposed children — United States, 2009–2010.

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Diep H, Best B, Capparelli E, Rossi S, Farrell M, Williams E, van den Anker J, Rakhmanina N.

Pharmacokinetics of lopinavir/ritonavir crushed versus whole tablets in children.

*Submitted.*

Rakhmanina NY, Capparelli EV.

Current research issues in pharmacokinetics of antiretroviral drugs in children.

*Submitted.*

**PHD PORTFOLIO SUMMARY****Summary of PhD training and teaching**

Name PhD student	Natella Yurievna Rakhmanina
Erasmus MC Department	Pediatrics
PhD period	July 2006 - September 2010
Promotors	Prof. dr. D. Tibboel Prof. dr. R. de Groot
Research School	Children's National Medical Center George Washington University Washington, DC, USA
Supervisors	Prof. dr. C. Flexner Prof. dr. E. Capparelli

<b>PhD training</b>	<b>Year</b>	<b>Workload (hours)</b>
<b>Courses</b>		
DC Clinical Research Training Consortium, Washington, DC, USA	2006 - 2008	360
Fundamentals of Genomics Core Class, George Washington University, Washington, DC, USA	2009 - 2010	16
<b>Seminars and Workshops</b>		
National Steering Committee of the Pediatric, Pharmacology Research Units, NIH, Bethesda, MD, USA	2007	8
Research Seminars in Clinical Pharmacology, John Hopkins University, Division of Clinical Pharmacology, Baltimore, MD, USA	2008 - 2009	8
Workshop on Pediatric Antiretroviral Pharmacology, Boston Medical Center, Boston, MA, USA	2008	8
Clinical and Translational Research and Education Meeting, Washington, DC, USA	2010	10
Mid-Atlantic Forum on HIV/AIDS Therapeutics, Washington, DC, USA	2008	8
Conference on Antiretroviral Dose Optimization, Clinton Health Initiative, Bill & Melinda Gates Foundation and John Hopkins University, Alexandria, VA, USA	2010	8
Practical Experience in Population PK, NONMEM analysis (direct supervision by Dr. Capparelli), University of California in San Diego, CA, USA	2010	12
Introduction to Population PK/PD Analysis Using NONMEM 7: Special Emphasis on New EM Algorithms, 39 <sup>th</sup> Annual Meeting of the American College of Clinical Pharmacology, Baltimore, MD, USA	2010	8

	Year	Workload (hours)
<b>Presentations (national and international)</b>		
8 <sup>th</sup> International Workshop on Clinical Pharmacology of HIV Therapy, Budapest, Hungary - <i>The distribution of the CYP3A5 6986A&gt;G polymorphisms among geographically diverse African ethnic groups: potential for population-specific dosing of antiretroviral drugs (poster)</i>	2007	10
Annual Meeting of the Pediatric Academic Society/Society for Pediatric Research, Toronto, Canada - <i>Novel urinary biomarkers to follow the progression of childhood HIV-1 associated nephropathy (poster)</i>	2007	36
47 <sup>th</sup> Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, IL, USA - <i>Lopinavir (LPV) pharmacokinetics (PK) and pharmacodynamics (PD) in HIV-infected children (poster)</i>	2007	24
10 <sup>th</sup> International Congress of Therapeutic Drug Monitoring and Clinical Toxicology, Nice, France - <i>Saliva concentrations of NRTIs and PIs are better predictors of free than total plasma concentrations in children with HIV infection (oral)</i>	2007	20
15 <sup>th</sup> Conference on Retroviruses and Opportunistic Infection, Boston, MA, USA - <i>Recommended dose of Lopinavir/ritonavir is sub-optimal in protease inhibitor-experienced children (poster)</i>	2008	36
11 <sup>th</sup> Congress of the European Society for Developmental, Perinatal and Paediatric Pharmacology, Rotterdam, the Netherlands - <i>CYP3A5 (A6986G) and MDR1 (G2677T &amp; G3435T) polymorphisms and the pharmacokinetics of Lopinavir/ritonavir in HIV-infected children (poster)</i>	2008	12
10 <sup>th</sup> International Workshop on Clinical Pharmacology of HIV Therapy, Amsterdam, the Netherlands - <i>The phenotypic and genotypic susceptibility Lopinavir scores and virologic response in treatment-experienced children with HIV (oral)</i>	2009	10
11 <sup>th</sup> International Congress of Therapeutic Drug Monitoring and Clinical Toxicology, Montreal, Canada - <i>Can TDM Improve Pharmacotherapy of HIV Infection in Adolescents? (oral)</i>	2009	20
17 <sup>th</sup> Conference on Retroviruses and Opportunistic Infection San Francisco, CA, USA - <i>Effect of Lopinavir/Ritonavir on Lipids in HIV-infected Children (poster)</i> - <i>Pharmacokinetics of Lopinavir/Ritonavir Crushed Versus Whole Tablets in Children (poster)</i>	2010	35
11 <sup>th</sup> International Workshop on Clinical Pharmacology of HIV Therapy, Sorrento, Italy - <i>Plasma Protease Inhibitor Concentrations and Fasting Lipid Profiles in HIV-infected Children (poster &amp; oral)</i>	2010	14

	<b>Year</b>	<b>Workload (hours)</b>
2 <sup>nd</sup> International Workshop on HIV Pediatrics, Vienna, Austria - <i>Serum Selenium Concentrations and Virologic and Immunologic Outcomes in HIV-infected Adolescents (poster)</i> - <i>Exposure to Nucleoside-Reverse Transcriptase Inhibitors and Fasting Lipid Profiles in HIV-infected Children (poster)</i>	2010	16
<b>Teaching Activities</b>		
<b><i>Didactic teaching</i></b>		
Practical Course on HIV Pharmacology for Pharmacy Residents, CNMC, Washington, DC, USA	2006 - 2007	40
MPH Course PUBH 209-13 "Issues in Pediatric HIV Care & Treatment", GWU School of Public Health and Health Services, Washington, DC, USA	2006 - 2008	16
Anthropology Course 150: "Human Rights and Ethics: HIV and Human Rights", Elliott School of International Affairs at GWU, Washington, DC, USA	2007	4
Professorial Rounds, "Perinatal HIV", Children's National Medical Center, Washington, DC, USA	2007 - 2008	8
Invited lecturer, "Epidemiology, Diagnostics and Treatment of Pediatric HIV infection", People's Friendship University, Moscow, Russia	2008	16
Lecturer at the Infectious Diseases Fellowship Core Education Program, Children's National Medical Center, Washington, DC	2008 - 2010	16
<b><i>Individual mentorship</i></b>		
Masters in Forensic Nursing, Clinical Nursing Student (Chelle Young-Anderson) Duquesne University School of Medicine, Pittsburg, PA, USA	2006	40
Postgraduate Student/Resident (Aline Baghdassarian) GCRC scholarship, Children's National Medical Center, Washington, DC, USA	2006 - 2010	160
Masters in Public Health (Ernest Igwacho) George Washington University School of Public Health, Washington, DC, USA	2007 - 2008	80
Masters in Public Health (Domonique Charles) George Washington University School of Public Health, Washington, DC, USA	2007 - 2008	80
Masters in Public Health (Munziba Khan) George Washington University School of Public Health, Washington, DC, USA	2007 - 2008	80
Postgraduate/Medical Student (Lara Walkoff) George Washington University, Washington, DC, USA	2008 - 2009	80
Masters in Public Health (Kathryn Swink) University of Florida, Gainesville, FL, USA	2008 - 2009	80
Masters in Public Health (Kimberly Saylor) George Washington University School of Public Health, Washington, DC, USA	2008 - 2009	80

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	<b>Year</b>	<b>Workload (hours)</b>
Medical Student (Mary Katherine Scott), Loyola University Chicago, Stritch School of Medicine, Chicago, IL, USA	2009	24
Pharmacy Student (Anne van Rongen) Utrecht University, School of Pharmacy, Utrecht, the Netherlands	2009 - 2010	40
Medical Student (Marjolein Miltenburg) Utrecht University, Utrecht, the Netherlands	2010	40
Medical Student (Nikita Rijgersberg) Utrecht University, Utrecht, the Netherlands	2010	40
Medical Student/Masters in Public Health (Kam Lam) Lazarus Family Fellowship, George Washington University, Washington, DC, USA	2010	28

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## **ABOUT THE AUTHOR**

Natella Yurievna Rakhmanina was born on August 17, 1964 in Moscow, Russia. In 1981 she graduated with a gold medal of excellence from the secondary Romain Rolland School in Moscow. The same year she started her studies at the Medical Faculty of the People's Friendship University in Moscow, Russia. After obtaining her medical degree with excellence, she completed two years of residency in pediatrics at the Moscow Children's Hospital N° 1. She subsequently became a fellow in pediatric academic medicine at the People's Friendship University in 1990. Her interest in clinical pharmacology led her to initiate a research project on the pharmacology of leukotriene inhibitors in children with bronchial asthma. Soon after beginning this project, she had a unique opportunity to receive one year of postgraduate research experience in the US. Although her fellowship position was considered among the best in Russia at that time, she wanted to obtain training and research experience in the US. In 1991 she became a visiting scientist at the National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration, in Jefferson, Arkansas, where she conducted a basic research project in toxicology. Following the postgraduate research practice at NCTR, she worked as a research assistant at the Arkansas Children's Hospital (ACH)/University of Arkansas for Medical Sciences (UAMS). In 1993 she entered the Residency Program in Pediatrics at ACH/UAMS. She successfully completed her US residency training in 1996, and entered the Fellowship Program in Allergy/Immunology at the Children's Mercy Hospital/University of Missouri, Kansas City School of Medicine, in Missouri. In 1997, after one year of fellowship training, she moved to the Netherlands with her husband John van den Anker. While in the Netherlands, she participated in several clinical trials conducted at the Division of Neonatology, Sophia Children's Hospital, and worked as a pediatrician in the Department of Pediatrics of St. Clara Hospital, one of the teaching hospitals in Rotterdam.

In 2000 Dr. Rakhmanina joined the Division of Infectious Diseases at the Columbus Children's Hospital/Ohio State University, Columbus, Ohio as the Director of the Pediatric HIV clinic and was appointed as Assistant Professor of Pediatrics at the Ohio State University School of Medicine. This was a pivotal year that directed her career interests toward the field of pediatric HIV infection. In 2002 she was appointed as Assistant Professor of Pediatrics at the George Washington University (GWU) School of Medicine and Health Sciences, and joined Children's National Medical Center (CNMC) as the Director of the Special Immunology Services, caring for a much larger cohort of HIV-infected pediatric patients in Washington, DC. Since that time her research has focused on the pharmacology of antiretroviral therapy in pediatric patients. She successfully completed a four year National Institutes of Health (NIH)-funded Pediatric Clinical Research Scholar (K-12) sponsored research project about the role of pharmacogenetics and pharmacokinetics in the treatment and outcome of children with pediatric HIV infection. From 2006 to 2008 she completed her training in science and practice of clinical research within the District of Columbia Clinical Research Training Consortium Program. In 2009 she became

the recipient of another four year competitive NIH Mentored Patient-Oriented Research Career Development Award (K-23) aimed to study the effect of puberty on therapeutic targets of HIV infection. In addition to her pharmacological research, she initiated universal HIV screening of adolescents in Washington DC metropolitan area, and leads several program development and research projects in the field of epidemiology of pediatric HIV sponsored by the DC Department of Health and NIH. She is certified by the American Academy of Pediatrics and the American Academy of HIV Medicine, and is an elected chair of the HIV Drugs Committee at the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. In 2008 she was promoted to the rank of the Associate Professor of Pediatrics at GWU/CNMC. She is married to John van den Anker and has a son John van den Anker (1998).

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**DANKWOORD**

As I approach the most moving part of my thesis, the Dankwoord, I realize that I am spending hours remembering the people who have crossed my life and supported me in my growth and endeavors. I am remembering those who have helped and inspired me during so many changes of countries, languages, cultures and professional environments. With the deepest love I look at my motherland Russia – such a rich and beautiful country with so many wonderful people! No matter where I live, that love has never faded away and will never leave my heart and soul. In the United States and the Netherlands, I can't account for all the people who opened their homes and offices for me, who listened and saw future in me and gave me advice and support. There are so many faces and eyes, names, words, letters, cards and phone calls, short and long e-mails, pictures and small gestures to which I owe a word of gratitude, that it would be impossible for me to ever finish this book if I attempted to list them all. With my deepest recognition of all people who helped me to make this thesis a reality, I am addressing those who are most directly related to me and my work below:

Dear **patients** and **families**, this work is done to help us to treat you better and to alleviate the burden of the chronic illness you carry in your lives. Without your dedication, your willingness to commit to the studies to help other children and families, and your discipline for the study protocols this work would have never been possible. The biggest dream of clinicians and researchers like me is to find the cure for HIV disease and many excellent scientists are diligently working to achieve this goal. Until now, we were able to make progress in treating this disease to transform it from a "death sentence" into a chronic illness which requires lifelong therapy. In my daily practice and research, I remain deeply humbled by your courage, determination and strength. You have taught me many unforgivable lessons and asked many brilliant questions. Together we will continue our task of building a better future for us and our children.

**Prof. dr. D. Tibboel**, beste Dick, I have had the privilege to know you for quite a few years now, first as John's colleague, then as a dear friend and afterwards as my promotor. You have one of the highest personal and professional standards and discipline I have encountered in my whole life. Greatly enthusiastic, you provided me with the guidance and support only a true Mentor can give to his student. Over the years I have seen you providing similar encouragement, directives and inspiration to many other PhD candidates. But it took me to become one of them to truly measure the depth of your commitment to the mentored person and their project. You helped me to see my work as one large project and because of that I was able to see a new meaning in my research and open new horizons for my future work. You are also a wonderful husband, father and friend and I hope to cherish our friendship for many years to come.

**Prof. dr. R. de Groot**, beste Ronald, I met you for the first time in the US during my fellowship training in 1996. I remember your great discussion with the trainees which left a deep impression among us. Your reputation of an excellent academic

physician and expert in infectious diseases has crossed many borders and has been well recognized. Your sharp, precise and witty remarks are a true manifest of your ever active and achieving personality. It is my honor and privilege to have you as my promotor and to have your judgment and guidance upon my work. I look forward to continue striving to achieve the standards you set for yourself and for those you mentor. And after all, I look very much forward to discuss with you Russian poetry, in knowledge of which you have also achieved a remarkable excellence as one might have expected from you.

**Prof. dr. C. Flexner**, dear Charles, you have provided me with a continued dedicated mentorship in my academic career since 2002. I am thankful to the proximity with which Baltimore and Washington were designed as US cities. The road between your office at John Hopkins University and my office in downtown DC has been driven many times in these years and you have always found the time for me in your busy academic schedule. You have provided me with invaluable advice in my research design and development. Your vision of clinical pharmacology of HIV therapy has opened a completely new level of understanding of this field for me. You have introduced me to and have helped me to establish multiple national and international research collaborations, several of which are presented in this thesis.

**Prof. dr. G. Kearns**, dear Greg, during my first years in the US I found a most wonderful mentor in you, and this mentorship has supported me through the most difficult years of establishing myself as a "Russian Doctor" in a completely new and unfamiliar surrounding. Only a few months into my residency I understood the high stature you have within the field of pediatric pharmacology. You tried to understand me through my accent and asked me questions, offered to support my interest in pharmacology and guided me in the preparation of my first paper. You supported me through residency and fellowship training and remained my mentor and advisor throughout the rest of my career development. You have revised many of my grant applications and papers including this thesis and always provided me with the most constructive feedback and advice for future development. Most importantly, you have become more than just a mentor for me and more than a friend for my family.

**Dr. D. Burger**, beste David, I can't think of any HIV related meeting where I have not seen you personally, or have seen the excellent work of your pupils and collaborators. Your name has become synergistic with the term "HIV Pharmacology". Your open and collaborative approach to the research aimed to optimize the antiretroviral therapy in children and adults has brought many young clinicians and researchers to work with you. In the past, you have provided me with crucial advice when choosing the directions for the future work. I was privileged to have co-authored a manuscript with you that is included in this thesis. Your support and guidance have meant a great deal to me and I look forward to have other opportunities to collaborate with you in the future.

Dear **Prof. dr. Charles Boucher**, **Prof. dr. Teun van Gelder**, and **Prof. dr. Catherijne Knibbe**, I would like to extend my sincere gratitude for the commitment you have made to support my work and to review the thesis. You represent for me the example of the truly European academic excellence – both through your personal achievements and through the academic mentorship you have extended to me by accepting your role in this committee. Your participation in this thesis served this work in the best possible way.

**Prof. dr. E. Capparelli**, dear Edmund, at the time of finalizing this thesis we have spent a wonderful time working on a couple of projects with you and your research group in San Diego. There could not be a better moment for me to recognize the impact of your mentorship on my career and research development. Your vision of the pediatric HIV pharmacology research is hard to surpass by any level of expertise. You have mentored me through my previous research award and you continue to support my current projects. Your responses to all my queries always come in the most efficient and productive manner.

**Prof. dr. M. Neely**, dear Michael, I am very grateful to you for the unique collaboration we have developed over recent years. It has become a truly fascinating experience to work with you and to learn about population pharmacokinetic approach from someone who is so knowledgeable and so passionate about it. We have co-authored several of the original studies included in this thesis and this collaborative work has truly become a highlight of my research in recent years. As both of us continue to develop our projects, I look forward to many years of successful work together in the future.

Dear leadership of **Children's National Medical Center (CNMC) and Children's Research Institute**: Prof. Peter Holbrook, Prof. David Wessel, Prof. Nalini Singh, Prof. Larry D'Angelo, Prof. Naomi Luban, Prof. Mark Batshaw, Prof. Mendel Tuchman, Prof. Jill Joseph, Prof. Max Coppes, Prof. Eric Hoffman, Prof. Patricio Ray and Prof. Stephen Teach, I am profoundly grateful for the opportunity to work and conduct the research at our Hospital and Research Institute. I am proud to be associated with the Faculty at **the George Washington University**. Your support and excellence in clinical care and research at CNMC has made this thesis possible.

**Prof. dr. S. Soldin**, dear Steve, you were the first to welcome me in the field of pediatric HIV pharmacology research at CNMC. Your remarkable achievements in the bioanalytical science have allowed our laboratory to develop and validate unique methods of determining the concentrations of antiretroviral drugs in body fluids. Because of your establishment of this expertise, many projects on the pharmacology, pharmacogenetics and pharmacodynamics of antiretroviral therapy became possible in our Program. I have always found in you the best ally for new projects and an eager collaborator and mentor.

Dear **Keetra Williams**, as a clinic and research study coordinator for many projects you have become my strongest supporter, my right hand and most trusted designee. Your commitment to the good of our patients is exemplary. Your availability to be reachable for our patients' needs during day and night hours, your deep respect to patients and warm and caring personality have earned you the reputation of a trusted and reliable provider among our children and their families. You are particularly liked by our adolescent patients who appreciate your honest and bold way of talking to them, your sincerity and fearless nature. Your role in this thesis is well deserved and admired by me and your colleagues.

Dear **SIS staff**, your team work is aimed to provide the best care to our patients and families. It is my privilege to lead the Program that unites so many excellent and dedicated individuals untied by this goal. Throughout our multiple meetings and daily interactions, I never stop being amazed at your insightful approach and drive to improve the care we provide. Without the trusted and comfortable medical home the clinical research is impossible. You do provide this home to our children and their families.

Dear **GCRC staff**, your availability and support have made the research in the therapy of pediatric HIV possible in our institution. You were always there for me and our patients. You listened to our concerns and requests and responded to every single one of them. Because of the experience that you provided many of our children and families have acquired a completely new appreciation and motivation for clinical research. In fact, many of them are eager to return to see you and open for the clinical research advocacy.

Dear Pediatric Research Pharmacology Unit (PPRU) team, **Elaine Williams** and **Ruby Daniels**, your expertise and superior organization have helped to conduct the clinical studies presented in this thesis. The calm and efficient pace with which you work, the knowledge of the principles of clinical research, high level of responsiveness to every minor request and attention to every detail have always given me peace of mind. I knew that the things were done when you were responsible for them. Looking forward to working with both of you on the new projects in the future.

Dear paranimphen **Ron Van Schaik** and **Monique van Dijk**, your help and support have made this day very special for me. Ron, you have shared my work in the past years and helped tremendously with the pharmacogenetic component of our studies and the data analysis. Monique, your friendship and warmth have always supported John and me. Both of you have made remarkable achievements in your fields and I am proud to have you as my paranimphen. Thank you for all the work you have done, all the advice you have given and all the arrangements you have made to make this defense day so beautiful.

Dear **Margo Terlouw**, you embrace the symbol of the Dutch woman for me. Your high precision in work, extreme punctuality and thoroughness, attention to every detail, great sense of responsibility and straightforward honest communication have become a pleasure to be surrounded by during the preparation of this thesis. I had a full trust in every piece of editing and design you did and find this book to be the most charming PhD among others. Your love and pride in your country are obvious and very inspiring.

Dear **Galina** and **Natalia Drozdova**, дорогие Галина и Наталья, in the last 20 years, I have only had a chance to see you on a few occasions, yet there are relationships that stay as close to the heart as they can be. Because of Galina's mentorship and recommendation, I was selected to become a postgraduate student in the US, the path that started my clinical and research career abroad and brought me to this work. Your warmth and love have extended not only to me, but also to my family. I always look forward to sit at the table in your cozy Moscow home and am grateful to you for sharing your lives with us.

Dear father **Yuri A. Rakhmanin**, дорогой папа, I am proudly presenting you with the reflection of my work. I learned from you an indomitable spirit in work, perseverance in every single endeavor and sense of the great responsibility for colleagues and mentees. Throughout my childhood and youth I have witnessed you mentoring young people, many of whom hold now high academic appointments. You fought tough battles and you were able to win them. You always walked to the top of the mountain, you reached it and you looked up at the higher one to climb. I am happy to see you at the glory of your success at this stage of your life.

Dear mother **Natella L. Rakhmanina**, дорогая мама, I do not have enough words to thank you for everything you have done for me. Your love for me and your family is one of the rare kind of self sacrifice that only a few people can achieve and can understand. Your bright and inquisitory mind, noble and honest heart and deep loving soul shine the light on me throughout my life. You protected every minute of my childhood in the difficult years of our life in a couple of rooms of the old house shared with 5 other families. You inspired me and created the best possible learning environment for me during my school and university years. You followed me and supported me throughout the most difficult years of accreditation of my diploma in the US. You embraced my husband and followed me to the Netherlands and then back to the US. You were there for us when our son was born to whom you give the same love you have given me. You are indeed the best mother and the best grandmother in the world. What is even more – you have always been and remain мой дружок.

Dear husband **John van den Anker**, lieverd, the love of my life, from the day we saw each other for first time you have been my strength and protection, my caring and tender friend, my devoted loving husband. Your stubborn Dutch character, your incredible determination and self discipline have brought you a great success in



your career. You taught me and continue teaching me many secrets of professional and academic success. You are proud for every successful step I make and you are standing by me in every failure and difficulty to support me. You will make me laugh, you will dry my tears. You will talk to me and I will always listen, even if I try to deny it in my own stubbornness. You have shared your love for Deborah, Nico and Thea with me, and through you I love them too. Your love for me and our son have made me the happiest woman in the world and I only hope that my love brings you the same happiness as you have given me.

Dear son **John van den Anker**, родной Ванечка, here comes the time to say the most important word to the most important man in my life. You have given me the joy and happiness every mother wishes to have in life. You have made me many times proud of you even though you are still a young man. When one stops to look at the scope of work one has done or evaluates the new step in their life, the important things become very straightforward. This thesis is the stop before moving on and I use this time to tell you a few simple things that I believe matter in life the most. Be good and truthful, live your life with dignity and strong sense of who you are. Work hard, respect and help others. Read good books. Be wise, have good friends and a good wife. *Мамма.*





