Published in final edited form as:

Pediatr Infect Dis J. 2016 July ; 35(7): 777–781. doi:10.1097/INF.0000000000001158.

Phenotypic Co-receptor Tropism in Perinatally HIV-Infected Youth Failing Antiretroviral Therapy

Allison L. Agwu, MD., ScM^{1,2,*}, Tzy-Jyun Yao, PhD³, Susan H. Eshleman, MD, PhD⁴, Kunjal Patel, DSc, MPH³, Wei Huang, MD⁵, Sandra K. Burchett, MD⁶, George K. Siberry, MD, MPH⁷, and Russell B. Van Dyke, MD⁸ for the Pediatric HIV/AIDS Cohort Study

¹Division of Pediatric Infectious Diseases, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD

²Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

³Center for Biostatistics in AIDS Research (CBAR), Harvard School of Public Health Boston, MA

⁴Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

⁵Monogram Biosciences, South San Francisco, CA

⁶Boston Children's Hospital and Harvard Medical School, Boston, MA

⁷Maternal and Pediatric Infectious Disease Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD

⁸Section of Infectious Diseases, Department of Pediatrics, Tulane University School of Medicine, New Orleans, LA

Abstract

Background—Perinatally HIV-infected (PHIV) children and youth are often heavily treatment-experienced, with resultant antiretroviral (ARV) resistance and limited treatment options. For those with virologic failure (VF), new agents such as CCR5 (R5) antagonists may be useful; however, reports of R5 antagonist susceptibility in children have mostly relied on genotypic testing, which may not accurately reflect the phenotypic tropism of the viral populations. We characterized phenotypic co-receptor usage among PHIV children and youth with VF on ARV treatment (ART) to identify predictors of CXCR4 (X4) tropism which preclude R5 antagonist use.

Methods—Plasma samples with >1,000 HIV RNA copies/mL were obtained from 73 PHIV-infected ART-treated children and youth (age 9–21 years) enrolled in the multi-center Pediatric HIV/AIDS Cohort Study. Samples were analyzed using the Trofile™ phenotypic assay. Multiple logistic regression was performed to identify factors associated with detectable X4-tropism.

*Corresponding Author: Allison L. Agwu, M.D., ScM, 200, N. Wolfe St. Room 3145, Baltimore, MD 21287 Phone: 410-614-3917 Fax: 410-614-1491, ageorg10@jhmi.edu.

Note: The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the National Institutes of Health or U.S. Department of Health and Human Services.

Results—Tropism results were obtained for 59 (81%) of the 73 children and youth; 32 (54%) had X4-tropism. Persistent viremia (80% of HIV RNA measurements >400 copies/mL) was associated with detectable X4-tropism (adjusted odds ratio (aOR) 6.6, 95% CI 1.4, 31.4), while longer cumulative nucleoside reverse transcriptase inhibitor (NRTI) use was associated with lower risk of X4-tropism (aOR 0.6, 95% CI 0.5, 0.9).

Conclusions—Using a phenotypic assay, >50% of PHIV children and youth with VF had X4-tropism, similar to that in treatment-experienced adults, and higher than the 30% reported for children using genotypic assays. Persistent viremia and shorter NRTI exposure are associated with X4-tropism in children and youth and may help target phenotypic testing to those most likely to benefit from R5 antagonist.

Keywords

CCR5; co-receptor tropism; perinatally HIV-infected; adolescents; youth

INTRODUCTION

HIV antiretroviral drug resistance, whether acquired at the time of infection or as a result of non-adherence to antiretroviral (ARV) treatment (ART), limits treatment options. Many perinatally HIV-infected (PHIV) children and youth were exposed to multiple ART regimens without viral load suppression due to lack of availability of suppressive regimens, non-adherence, or having acquired a resistant isolate with primary infection, leading to drug resistance [1]. In one study in the United States (U.S.), 19% of infected infants had at least one drug resistance mutation on initial genotypic testing [2]. In a European cohort, 12% of PHIV children had developed triple-class ARV drug resistance within five years of ART initiation [2]. Children infected with multi-class ARV resistance require more ARV options for their treatment in order to achieve virologic suppression.

The CCR5 antagonists are available for use in adults and are under evaluation in children [3]. They have the potential to be included as components of ART that may result in virologic suppression for individuals with ARV drug resistance with limited treatment options. CCR5 (R5) is a co-receptor that HIV uses to infect CD4⁺ T-lymphocyte (CD4) cells; most patients harbor viruses that preferentially use the R5 co-receptor during acute and early infection. However, the proportion of viruses that use R5 for cell entry tends to decrease over time, with emergence of viruses that use an alternative co-receptor, CXCR4 (X4) [3]. The presence of X4 or dual-mixed (DM), X4 and R5-tropic HIV, precludes the response to therapy with a R5 antagonist [4]. The few pediatric studies in the literature have reported X4 or DM prevalence rates of 19–33% in cohorts of children on and off antiretroviral therapy [5–7]. However, these studies have primarily used genotypic assays to assess HIV tropism, which may be less accurate and less clinically relevant than phenotypic tropism assays [8]. Genotypic tropism assays are based on sequencing of the V3-coding region of HIV-1 *env*, which is the principal determinant of co-receptor usage [9]. Those assays use algorithms and bioinformatics programs to predict co-receptor usage. Genotypic assays have specificities ~90%, but are relatively insensitive (sensitivity ~50%–70%) for detecting X4- or DM-tropic HIV; while phenotypic assays have sensitivities and specificities above 95% [8, 10]. Studies have reported variability in concordance of tropism results by

genotypic and phenotypic assays [5, 11, 12]. Thus, studies, preferably using phenotypic assays, are needed to evaluate the R5 antagonist activity in PHIV-infected children. In this study, we used a phenotypic assay to characterize co-receptor usage and factors associated with X4-using tropism in PHIV children, adolescents, and young adults who have virologic failure, the patient population where evaluation of their potential utility would be most critical.

METHODS

We analyzed data from PHIV children and youth enrolled in the Adolescent Master Protocol (AMP) of the Pediatric HIV/AIDS Cohort Study (PHACS) [13]. From 2007 to 2009, AMP enrolled 451 PHIV children and youth age 7 – 16 years into an ongoing prospective cohort study at 15 sites in the United States including Puerto Rico. The study protocol was reviewed and approved by the institutional review board (IRB) of each participating site, and written informed consent was obtained from each child's parent/legal guardian or from older participants as allowed by the local IRB. Written assent was obtained as appropriate. Participants were followed prospectively with study visits every six months up to August 2010, then annually. All eligible participants had complete documentation of health and ART exposure history [13]. Our study sample was limited to AMP participants who were on ART and had evidence of virologic failure on ART with a viral load (VL) > 1000 copies/mL (limit for successful assay performance) at their last blood collection before July 1, 2012, and had at least 1 mL plasma available from that sample (volume needed to perform the assay). If participants were Maraviroc or another R5 antagonist experienced (currently n=2 or previously n=0), the most recent sample with a viral load >1000 copies/mL prior to drug initiation was selected for analysis. We used the enhanced sensitivity Trofile™ assay (ESTA) (Monogram Biosciences) [10, 14]. The ESTA, capable of detecting R5-, X4-, or DM variants present at 0.3% of the virus quasispecies, was the phenotypic assay used in the development, evaluation, and licensure of the R5 antagonists [10, 14]. Briefly, full length envelope (*env*) sequences were amplified by RT-PCR and cloned into an *env* expression vector as *env* libraries. A HIV-1 genomic vector containing a luciferase report gene was then used to co-transfect human embryonic kidney 293 cell cultures with patient *env* expression vectors. Co-receptor tropism of pseudoviruses was evaluated by infecting U87 cells expressing CD4 and either CCR5 or CXCR4 co-receptors. Viruses were classified as R5, X4 or DM tropic based on the production of luciferase activity in U87 CD4 CCR5 and U87 CD4 CXCR4 cells, and the specific inhibition of luciferase activity by CCR5 or CXCR4 inhibitors [14].

Demographics, Clinical characteristics and ARV use

Socio-demographic information included were age at time of plasma drawn for tropism testing, birth year, race, ethnicity and gender. Lifetime clinical characteristics, including CD4 cell count, HIV-1 RNA concentration, Center for Disease Control and Prevention (CDC) clinical classification, and lifetime ARV use were obtained by medical chart abstraction or from prior studies.

Per the PHACS AMP protocol, all CD4 and viral load (VL) measurements were recorded from historical measures prior to entry into PHACS, at baseline, and every 6 months thereafter. The measurements were done in clinical laboratory improvement amendments (CLIA) certified clinical laboratories at the respective sites. All available CD4 counts were assessed and characterized as follows, current CD4 (known CD4 closest to date of specimen used for tropism testing), and nadir CD4 count (lowest CD4 count identified from all historical records through the time of specimen drawn for tropism testing). We also assessed the relationship between recent advanced immunosuppression and X4-tropism by examining if those who had nadir CD4 < 200 cells/mm³ within one year of specimen drawn for tropism assessment were more likely to have X4-tropism. Viral load measurements were characterized as current (VL measurement on the same date as the specimen used for tropism testing), or past viral loads which were used to assess cumulative viremia. Cumulative viremia was measured by calculating the proportion of results with VL > 400 copies/mL, among all available RNA PCR test results up to specimen collection for tropism testing.

Current ARV exposure was defined as ARV exposure at the time of specimen collection for tropism testing. Lifetime exposure to all ARVs was collected with start and stop dates for each ARV prescribed to calculate the duration of exposure to each ARV class just prior to collection of the specimen for tropism testing. An ART regimen change was defined as a change in one or more drug in the regimen [13]. Combination antiretroviral therapy (cART) was considered as any regimen containing at least 3 drugs from at least 2 different drug classes or a triple nucleoside reverse-transcriptase inhibitor (NRTI) regimen including zidovudine, lamivudine, and abacavir. Individual drug classes including NRTI, non-nucleoside reverse-transcriptase inhibitor (NNRTI), protease inhibitor (PI), fusion inhibitor (FI) and integrase inhibitor (II) were also considered. Exposure to a specific ARV class was counted if the exposure lasted at least 3 days. Cumulative lifetime ARV exposure was calculated by summing over all ART intervals in the record up to the date of the specimen drawn for tropism testing. For each drug class, ever exposure (Yes/No) and lifetime duration were studied. The age of first ARV exposure and first cART initiation were also evaluated. The number of distinct regimens ever received was counted.

Data on antiretroviral genotypic resistance

All available genotypic resistance testing results up to the date of specimen drawn for phenotypic tropism testing were analyzed for resistance mutations using the Stanford HIV Drug Resistance Interpretation Program (Version 6.0) [15]. The numbers of scored and/or commented distinct mutations by drug class, specifically NRTI, PI and NNRTI, for each participant were extracted. For participants who had multiple records, the total numbers of distinct mutations was used.

Statistical methods

The proportion of participants with detectable X4-tropism, including X4 only or X4/R5 dual viruses, was calculated along with a Clopper-Pearson exact 95% confidence interval (CI). Demographics, clinical characteristics (e.g., current and lifetime viremia, current CD4, lifetime CD4 nadir, and CD4 nadir within one year of the date of the specimen drawn for

tropism testing), ARV exposure measures and antiretroviral genotypic resistance measures were summarized overall and compared between those with and without detectable X4-tropism using Fisher's exact tests or Wilcoxon rank sum tests as appropriate to evaluate the crude associations. Potential predictors of detectable X4-tropism were investigated using both univariable and multivariable logistic regression models. In addition to demographics, potential predictors included clinical characteristics, and ARV exposures if the p-values of the crude associations were < 0.10 . The multivariable model was reduced to a final model based on stepwise selection, which at each step evaluated all covariates not yet in the model and included the most significant covariates with $p < 0.10$ and for the covariates already in the model, only kept those with $p < 0.10$, until no more inclusion or exclusion were allowed. Age at tropism testing was kept in the final model regardless of its significance level. Because of small sample size, sensitivity analyses were conducted using exact logistic regression in each univariate analysis. Analyses were conducted using SAS statistical software (Version 9.4). All p-values were two-sided.

Results

Of the 451 participants, 116 had VL >1000 copies/mL, 88/116 were on ART and therefore met our eligibility criteria; and of these, 73/88 had a repository specimen available for co-receptor usage analysis. Co-receptor analyses were successful in 81% (59/73) of the samples tested, with the remaining 14 (19%) having insufficient sample volume for analysis. We examined differences between those with virologic failure who had tropism testing performed (N=59) vs. those who did not due to either lack of specimen or unsuccessful test (N=29). There were no demographic differences between the two groups, though those who had successful testing had higher median VL (4.16 vs. 3.66 log₁₀ HIV RNA copies/mL; $p < .001$) and started ARV treatment at a younger age 0.68 years vs. 2.25 years ($p = .05$). Table 1 summarizes socio-demographic, clinical, and ARV use characteristics for the cohort. Overall, 32/59 (54.2%, exact 95% CI: 40.8% - 67.3%) had detectable X4-tropism (X4/DM), with two subjects having only X4-tropism (2/59, 3.4%, exact 95% CI 0.4–11.7%) and 57/59 having dual tropism.

Lower current CD4 count was significantly associated with higher prevalence of detectable X4-tropism. The median CD4 count for participants with X4-tropism was significantly lower than for those without (278 cells/mm³ vs. 517 cells/mm³, $p < 0.01$), with a higher prevalence of X4 tropism in those with CD4 counts < 500 cells/mm³ compared with ≥ 500 cells/mm³ (68% and 22%, respectively, $p < 0.01$). Participants with a nadir CD4 < 200 cells/mm³ within one year prior to the date of specimen collected for tropism testing had a higher prevalence of detectable X4-tropism, with marginal statistical significance (79% vs. 47%, $p = 0.06$). The prevalence of X4-tropism did not differ by CDC class. There was also no association with antiretroviral genotypic resistance.

Participants with detectable X4-tropism had significantly higher current VLs compared to participants with R5-tropism only (median 4.6 vs. 3.8 log₁₀ copies/mL, $p < 0.01$). Indeed, the prevalence of X4-tropism was 35%, 65%, and 86% for subjects with a current VL of 1,000–10,000 copies/mL, 10,000–100,000 copies/mL, and $>100,000$ copies/mL ($p = 0.02$), respectively. The proportion of lifetime VL values > 400 copies/mL was significantly higher

for participants with detectable X4-tropism than for those without (88.5% vs. 75.4%, $p = 0.03$).

Eighty three percent (49/59) were receiving combination ART (cART) at the date of the specimen drawn for tropism testing and all were receiving ART. There were no significant associations between prevalence of X4-tropism and current cART regimens. In assessing crude associations, participants with detectable X4-tropism were older when they first started ART (median 1.6 years vs. 0.4 year, $p = 0.02$), and had shorter lifetime exposure duration of cART (10.3 years vs. 12.1 years, $p = 0.04$). Those with detectable X4 tropism tended to have shorter lifetime exposure to specific ARV classes vs. R5-only tropism, specifically NRTI (11.9 years vs. 14.5 years, $p < 0.01$), PI (8.8 years vs. 11.0 years, $p = 0.01$) and NNRTI (1.6 years vs. 3.0 years, $p = 0.09$). The number of distinct regimens ever received up to the date of the specimen drawn for tropism testing was similar between the two tropism groups (median 7 vs. 6).

In multivariable analyses (Table 2), having 80% of lifetime recorded VLs > 400 copies/mL was associated with higher odds of X4-tropism (aOR=6.56, 95% CI: 1.37, 31.44, $p = 0.02$) and a longer duration of NRTI exposure was associated with a lower odds of detectable X4-tropism (38% lower per additional year of exposure, aOR=0.62, 95% CI: 0.45, 0.87, $p < 0.01$) after adjusting for age on the date that the specimen was drawn for tropism testing. A higher current VL and CD4+ count < 500 cells/mm³ were associated with increased odds of detectable X4-tropism with marginal statistical significance (VL: aOR = 2.52 per log₁₀ copies/mL, 95% CI: 0.90, 7.10, $p = 0.08$; CD4+: aOR=4.28, 95% CI: 0.80, 22.96, $p = 0.09$).

DISCUSSION

This is the largest study to date assessing phenotypic HIV tropism in PHIV children and youth. We observed a high (54%) prevalence of detectable X4-tropism among 59 PHIV children and youth (median age 16 years) with virologic failure. The proportion of lifetime VL measures > 400 copies/mL was significantly independently associated with X4-tropism; VL and CD4 count at the time of testing were also associated with X4-tropism with marginal statistical significance. Given the relatively high proportion of X4-tropism seen in our study, earlier use of R5 antagonists could be considered to achieve VL suppression.

Studies of HIV tropism in PHIV children and youth have mostly used V3 genotypic assays and have reported X4 prevalence rates of 19–33% [6, 7]. However, there are several limitations with this approach, including: no phenotypic confirmation, inability to detect minority viral populations, and determinants of X4 tropism residing outside of the V3 region [9]. In the only other study of the ESTA™ assay in PHIV children, 36 cART-experienced PHIV Spanish children (median age: 14.6 years, range: 10.9–17.0) had an overall prevalence of X4-tropism of 33%, substantially lower than our finding [5]. In general, we demonstrated higher prevalence rates of X4/DM virus using the ESTA™ assay than those that have used genotypic testing in PHIV children and youth [6, 7]. Interestingly, in a longitudinal British cohort study of PHIV children that used a genotypic tropism assay, 11% of children had tropism results switch from X4/DM to R5 at subsequent time points, raising concerns about the reliability of the genotypic test [7]. Although the DHHS guidelines recommend

genotypic testing as an alternative, it may under-report X4-tropism and thereby falsely predict the activity of CCR5 antagonists for those failing cART [16].

Our findings of associations between X4/DM tropism and prolonged lifetime viremia > 400 copies/mL and higher VL at time of tropism testing are consistent with other studies in adults [17, 18]. They are also consistent with the evolution of viral tropism with longer periods of uncontrolled viral replication which usually occurs in the setting of either delayed ART or virologic failure of ART due to use of suboptimal regimens or non-adherence. Our findings that shorter duration of NRTI exposure, were associated with X4-tropism also likely relates to later ART initiation, correlating with the increased time for unchecked viral replication and evolution, which occurs even without the selective pressure of ART.

We report an association between X4/DM tropism and lower current CD4 count, a finding that has been seen in adult studies [17–20]. In a Brazilian study of PHIV children, associations between genotypic X4-tropism, lower CD4 nadir, and disease severity (CD4 < 200 cells/mm³ or clinical event) were also reported [21]. In contrast to other studies, we did not find an association between X4-tropism and greater number of mutations associated with NRTI, PI or NNRTI resistance [22]. It is possible that our sample was enriched with youth with ART resistance and we were, therefore, underpowered to detect differences.

Our study is strengthened by use of the phenotypic Trofile™ assay. We were restricted to patients that had available samples in the repository, which led to limited sample size and statistical power to detect clinically significant associations. Due to limitations with sample size and our data structure, we utilized odds ratio instead of prevalence ratio as the measure of association in our analyses to avoid numerical issues. Although odds ratios give higher magnitudes than their corresponding prevalence ratios, the chance of misidentification of a significant association is comparable. While the study may not be generalizable to all PHIV children and youth in treatment, the demographic and clinical characteristics of the PHACS AMP cohort favorably compares to those of other U.S. cohorts in the literature[23, 24] and therefore the study findings may have broader implications.

This study provides insight into patient and disease characteristics that might help direct resources for phenotypic tropism testing and potential R5 antagonist use. Studies are needed to evaluate whether more optimal timing of the use of R5 antagonists before development of X4-tropism would be in initial or first switch regimens rather than as currently used as components of salvage regimens when the likelihood of X4-tropism is increased.

Acknowledgments

Sponsorship: Dr. Agwu was supported by the National Institutes of Allergy and Infectious Diseases (1K23 AI084549). PHACS was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development with co-funding from the National Institute on Drug Abuse; the National Institute of Allergy and Infectious Diseases; the Office of AIDS Research; the National Institute of Mental Health; the National Institute of Neurological Disorders and Stroke; the National Institute on Deafness and Other Communication Disorders; the National Heart, Lung, and Blood Institute; the National Institute of Dental and Craniofacial Research; and the National Institute on Alcohol Abuse and Alcoholism, through cooperative agreements with the Harvard University School of Public Health (HD052102, 3 U01HD052102-05S1, 3 U01 HD052102-06S3; principal investigator, George Seage; project director: Julie Alperen) and the Tulane University School of Medicine (HD052104, 3U01 HD052104-06S1; principal investigator, Russell Van Dyke; co-principal investigator, Kenneth Rich; project

director, Patrick Davis). The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the sponsoring agencies.

We thank the children and families for their participation in PHACS, and the individuals and institutions involved in the conduct of PHACS. Dr. Agwu was supported by the National Institutes of Allergy and Infectious Diseases (1K23 AI084549). The study was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development with co-funding from the National Institute on Drug Abuse, the National Institute of Allergy and Infectious Diseases, the Office of AIDS Research, the National Institute of Mental Health, the National Institute of Neurological Disorders and Stroke, the National Institute on Deafness and Other Communication Disorders, the National Heart Lung and Blood Institute, the National Institute of Dental and Craniofacial Research, and the National Institute on Alcohol Abuse and Alcoholism, through cooperative agreements with the Harvard University T.H. Chan School of Public Health (HD052102) (Principal Investigator: George Seage; Project Director: Julie Alperen) and the Tulane University School of Medicine (HD052104) (Principal Investigator: Russell Van Dyke; Co-Principal Investigator: Kenneth Rich; Project Director: Patrick Davis). Data management services were provided by Frontier Science and Technology Research Foundation (PI: Suzanne Siminski), and regulatory services and logistical support were provided by Westat, Inc (PI: Julie Davidson).

The following institutions, clinical site investigators and staff participated in conducting PHACS AMP in 2013, in alphabetical order: **Ann & Robert H. Lurie Children's Hospital of Chicago:** Ram Yogeve, Margaret Ann Sanders, Kathleen Malee, Scott Hunter; **Baylor College of Medicine:** William Shearer, Mary Paul, Norma Cooper, Lynnette Harris; **Boston Children's Hospital:** Sandra Burchett, Nancy Karthas, Betsy Kammerer; **Bronx Lebanon Hospital Center:** Murlu Purswani, Mahboobullah Baig, Anna Cintron; **Children's Diagnostic & Treatment Center:** Ana Puga, Sandra Navarro, Patricia Garvie, James Blood; **Jacobi Medical Center:** Andrew Wiznia, Marlene Burey, Molly Nozyce; **Rutgers - New Jersey Medical School:** Arry Dieudonne, Linda Bettica, Susan Adubato; **St. Christopher's Hospital for Children:** Janet Chen, Maria Garcia Bulkley, Latreaca Ivey, Mitzie Grant; **St. Jude Children's Research Hospital:** Katherine Knapp, Kim Allison, Megan Wilkins; **San Juan Hospital/Department of Pediatrics:** Midnela Acevedo-Flores, Heida Rios, Vivian Olivera; **Tulane University Health Sciences Center:** Margarita Silio, Medea Jones, Patricia Sirois; **University of California, San Diego:** Stephen Spector, Kim Norris, Sharon Nichols; **University of Colorado Denver Health Sciences Center:** Elizabeth McFarland, Alisa Katai, Jennifer Dunn, Suzanne Paul; **University of Miami:** Gwendolyn Scott, Patricia Bryan, Elizabeth Willen.

Reference List

1. Agwu AL, Fairlie L. Antiretroviral treatment, management challenges and outcomes in perinatally HIV-infected adolescents. *J Int AIDS Soc.* 2013; 16:18579. [PubMed: 23782477]
2. Castro H, Judd A, Gibb DM, Butler K, Lodwick RK, van SA, et al. Risk of triple-class virological failure in children with HIV: a retrospective cohort study 1. *Lancet.* 2011; 377:1580–1587. [PubMed: 21511330]
3. Wilkin TJ, Gulick RM. CCR5 antagonism in HIV infection: current concepts and future opportunities 2. *Annu Rev Med.* 2012; 63:81–93. [PubMed: 22034870]
4. Beck IA, Jang M, McKernan-Mullin J, Bull M, Wagner T, Huang S, et al. Monitoring of HIV type 1 DNA load and drug resistance in peripheral blood mononuclear cells during suppressive antiretroviral therapy does not predict virologic failure. *AIDS Res Hum Retroviruses.* 2012; 28:780–788. [PubMed: 22081867]
5. Briz V, Garcia D, Mendez-Lagares G, Ruiz-Mateos E, de Mulder M, Moreno-Perez D, et al. High prevalence of X4/DM-tropic variants in children and adolescents infected with HIV-1 by vertical transmission. *Pediatr Infect Dis J.* 2012; 31:1048–1052. [PubMed: 22828644]
6. Di Biagio A, Parisini A, Bruzzone B, Prinapori R, Lauriola M, Paolucci S, et al. Genotypic determination of HIV tropism in a cohort of patients perinatally infected with HIV-1 and exposed to antiretroviral therapy. *HIV Clin Trials.* 2014; 15:45–50. [PubMed: 24518213]
7. Foster, C.; Kaye, S.; Smith, C.; Mackie, N. Longitudinal Evolution of Tropism in Perinatal HIV-1 Infection: The HICCUP Study. Conference on Retroviruses and Opportunistic Infections; Boston, MA, USA. 2014.
8. Lin NH, Kuritzkes DR. Tropism testing in the clinical management of HIV-1 infection. *Curr Opin HIV AIDS.* 2009; 4:481–487. [PubMed: 20048714]
9. Huang W, Toma J, Fransen S, Stawiski E, Reeves JD, Whitcomb JM, et al. Coreceptor tropism can be influenced by amino acid substitutions in the gp41 transmembrane subunit of human immunodeficiency virus type 1 envelope protein. *J Virol.* 2008; 82:5584–5593. [PubMed: 18353956]

10. Reeves J, Coakley E, Petropoulos C, Whitcomb J. An enhanced sensitivity Trofile HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5: a review of analytical and clinical studies. *J Viral Entry*. 2009; 3:94–102.
11. Verhofstede C, Brudney D, Reynaerts J, Vaira D, Fransen K, De Bel A, et al. Concordance between HIV-1 genotypic coreceptor tropism predictions based on plasma RNA and proviral DNA. *HIV Med*. 2011; 12:544–552. [PubMed: 21518222]
12. Vandekerckhove LP, Wensing AM, Kaiser R, Brun-Vezinet F, Clotet B, De Luca A, et al. European guidelines on the clinical management of HIV-1 tropism testing. *Lancet Infect Dis*. 2011; 11:394–407. [PubMed: 21429803]
13. Van Dyke RB, Patel K, Siberry GK, Burchett SK, Spector SA, Chernoff MC, et al. Antiretroviral treatment of US children with perinatally acquired HIV infection: temporal changes in therapy between 1991 and 2009 and predictors of immunologic and virologic outcomes. *J Acquir Immune Defic Syndr*. 2011; 57:165–173. [PubMed: 21407086]
14. Whitcomb JM, Huang W, Fransen S, Limoli K, Toma J, Wrin T, et al. Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. *Antimicrob Agents Chemother*. 2007; 51:566–575. [PubMed: 17116663]
15. Shafer RW. Rationale and uses of a public HIV drug-resistance database. *J Infect Dis*. 2006; 194(Suppl 1):S51–58. [PubMed: 16921473]
16. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. [Accessed (11/15/2015)] Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at <http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf>
17. Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. Change in coreceptor use correlates with disease progression in HIV-1--infected individuals. *J Exp Med*. 1997; 185:621–628. [PubMed: 9034141]
18. Hunt PW, Harrigan PR, Huang W, Bates M, Williamson DW, McCune JM, et al. Prevalence of CXCR4 tropism among antiretroviral-treated HIV-1-infected patients with detectable viremia. *J Infect Dis*. 2006; 194:926–930. [PubMed: 16960780]
19. Koot M, Keet IP, Vos AH, de Goede RE, Roos MT, Coutinho RA, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. *Ann Intern Med*. 1993; 118:681–688. [PubMed: 8096374]
20. Wilkin TJ, Su Z, Kuritzkes DR, Hughes M, Flexner C, Gross R, et al. HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. *Clin Infect Dis*. 2007; 44:591–595. [PubMed: 17243065]
21. Almeida FJ, Zapparoli MS, Moreira DH, de Cavalcanti JS, Rodrigues R, Berezin EN, et al. Association of X4 tropism with disease progression in antiretroviral-treated children and adolescents living with HIV/AIDS in Sao Paulo, Brazil. *Braz J Infect Dis*. 2014; 18:300–307. [PubMed: 24275366]
22. Wagner TA, Frenkel LM. Potential limitation of CCR5 antagonists: drug resistance more often linked to CXCR4-utilizing than to CCR5-utilizing HIV-1. *AIDS*. 2008; 22:2393–2395. [PubMed: 18981780]
23. Agwu ALFJ, Rutstein R, Korthuis PT, Gebo KA. Changes in Advanced Immunosuppression and Detectable HIV Viremia Among Perinatally HIV-Infected Youth in the Multisite United States HIV Research Network. *Journal of the Pediatric Infectious Diseases Society*. 2013
24. Setse RW, Siberry GK, Gravitt PE, Moss WJ, Agwu AL, Wheeling JT, et al. Correlates of sexual activity and sexually transmitted infections among human immunodeficiency virus-infected youth in the LEGACY cohort, United States, 2006 2. *Pediatr Infect Dis J*. 2011; 30:967–973. [PubMed: 22001904]

Table 1

Demographics, Current and Historic CD4+ Measures, CDC Classification, and Viral Load by Phenotypic Tropism

	Tropism CCR5 only (N=27)	CXCR4 or Dual (N=32)	Total (N=59)	P-Value [*]
Age (years)				
Min, Max	8.7, 19.7	9.9, 20.7	8.7, 20.7	0.49
Median (Q1, Q3)	16.8 (13.8, 18.5)	16.2 (12.7, 18.2)	16.3 (13.0, 18.5)	
Race/Ethnicity[#]				
Black/Non-Hispanic 17 (63%)		25 (78%)	42 (71%)	0.63
Non-Black/Hispanic 6 (22%)		6 (19%)	12 (20%)	
Non-Black/Non-Hispanic 2 (7%)		1 (3%)	3 (5%)	
Female 19 (70%)		20 (63%)	39 (66%)	0.59
Current CD4 count $\downarrow$ (cells/mm³)[*]				
Min, Max	0, 1,185	14, 991	0, 1,185	0.001
Median (Q1, Q3)	517 (343, 660)	278 (81, 443)	391 (128, 530)	
Current CD4 count $\downarrow$ < 500 cells/mm³[*]				
	13 (48%)	28 (88%)	41 (69%)	0.002
Nadir CD4 count (cells/mm³) (lifetime)				
Min, Max	0, 629	7, 465	0, 629	0.02
Median (Q1, Q3)	292 (119, 423)	144.00 (29, 283)	217 (40, 362)	
Nadir CD4 count < 200 cells/mm³ and occurred within 1 year				
	3 (11%)	11 (34%)	14 (24%)	0.06
CDC HIV clinical classification				
Not symptomatic 7 (26%)		1 (3%)	8 (14%)	0.08
Mildly symptomatic 6 (22%)		12 (38%)	18 (31%)	
Moderately symptomatic 6 (22%)		8 (25%)	14 (24%)	
Severely symptomatic 8 (30%)		11 (34%)	19 (32%)	
Current VL $\downarrow$ (copies/mL)				
1,000–10,000 17 (63%)		9 (28%)	26 (44%)	0.02
10,000–100,000 9 (33%)		17 (53%)	26 (44%)	
>100,000 1 (4%)		6 (19%)	7 (12%)	
% of lifetime VL values > 400 copies/mL				
Min, Max	18.8, 100.0	8.8, 100.0	8.8, 100.0	0.03
Median (Q1, Q3)	75.4 (48.5, 91.7)	88.5 (77.0, 95.4)	81.3 (71.4, 92.7)	
% of VL values > 400 copies/mL 80%				
	9 (33%)	22 (69%)	31 (53%)	0.01
Age (years) first started ARV				
Min, Max	0.2, 10.6	0.2, 9.6	0.2, 10.6	0.02
Median (Q1, Q3)	0.4 (0.2, 1.0)	1.6 (0.5, 2.9)	0.7 (0.3, 2.4)	
Age (years) first started cART				
Min, Max	0.2, 13.6	0.2, 15.6	0.2, 15.6	0.41

	Tropism CCR5 only (N=27)	CXCR4 or Dual (N=32)	Total (N=59)	P-Value[*]
Median (Q1, Q3)	2.8 (0.6, 5.9)	3.8 (1.8, 5.8)	3.2 (1.2, 5.9)	
Cumulative duration (years) on cART				
Min, Max	0.1, 16.2	2.3, 15.1	0.1, 16.2	0.04
Median (Q1, Q3)	12.1 (10.8, 13.7)	10.3 (7.9, 12.5)	11.5 (8.9, 13.2)	
Number of NRTI, PI, and NNRTI class mutations				
0	1 (4%)	1 (3%)	2 (4%)	0.88
1	5 (20%)	5 (17%)	10 (18%)	
2	10 (40%)	10 (33%)	20 (36%)	
3	9 (36%)	14 (47%)	23 (42%)	
Missing	2	2	4	

^{*} P-value by Wilcoxon rank sum test for age, current and nadir CD4 and % viral loads over 400, and by Fisher's exact test for all the rest. Characteristics with p< 0.05 are bolded.

[#] Race was missing in 2 of 27 participants with CCR5 tropism; # Two Hispanic participants were missing information on race. Both did not have detectable CXCR4. There were no Black Hispanic participants in this study, although there were some in the AMP cohorts.

[⊥] Current = known value at the time of specimen drawn for tropism testing; for VL (current VL was measured on the same date of the specimen used for tropism testing, while current CD4 is the CD4 closest to the date of the specimen used for tropism testing, but may not be on the same date.

Abbreviations: CDC: Center for Disease Control and Prevention; min=minimum; max: maximum; Q1: first quartile; Q3: third quartile; VL: viral load; ARV: antiretroviral; cART: combination antiretroviral therapy NRTI: nucleoside/nucleotide reverse transcriptase inhibitor, PI: protease inhibitor, NNRTI: non- nucleoside/nucleotide reverse transcriptase inhibitor

Table 2

Univariable and Multivariable Logistic Regression Models for Detectable CXCR4 Tropism by Demographics, Disease status and ART experience (N = 59)

Variable	Univariable			Multivariable		
	Odds Ratio	95% Confidence Interval	P- value	Adjusted Odds Ratio	95% Confidence Interval	P- value
Age(years)	0.94	(0.80,1.12)	0.50	1.21	(0.87, 1.68)	0.26
Black Race	1.68	(0.51,5.51)	0.39			
Hispanic or Latino	0.55	(0.16,1.84)	0.33			
Female	0.70	(0.24,2.09)	0.53			
Current [‡] log viral load [#] (copies/mL)	3.10	(1.34,7.13)	0.008	2.52	(0.90, 7.10)	0.08
% of viral loads > 400 copies/mL	4.40	(1.47,13.15)	0.008	6.56	(1.37, 31.44)	0.02
CDC Class C	1.24	(0.41,3.74)	0.70			
Current [‡] CD4 count* < 500 cells/mm ³	7.54	(2.07,27.42)	0.002	4.28	(0.80, 22.96)	0.09
Nadir CD4 (cells/mm ³) (lifetime)	1.00	(0.99,1.00)	0.02			
Nadir CD4 < 200 cells/mm ³ and occurred within 1 year	4.19	(1.03,17.07)	0.05			
Age (years) first started ARV	1.20	(0.91,1.58)	0.20			
Cumulative duration on cART (years)	0.89	(0.77,1.04)	0.14			
Cumulative duration on NRTI (years)	0.81	(0.68,0.97)	0.02	0.62	(0.45, 0.87)	0.005
Cumulative duration on PI (years)	0.86	(0.74,1.00)	0.05			
Cumulative duration on NNRTI (years)	0.89	(0.75,1.05)	0.17			

[‡]Current = known value at the time of specimen drawn for tropism testing; for VL (current VL was measured on the same date of the specimen used for tropism testing, while current CD4 is the CD4 closest to the date of the specimen used for tropism testing, but may not be on the same date.

Abbreviations: ART: antiretroviral therapy; cART: combination antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor