HIV-1 co-receptor tropism and disease progression in children and young adults with perinatally acquired HIV-1 infection. The HICCUP Study

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Abstract

Objectives: Retrospective analysis of evolution of HIV tropism and association with disease progression in perinatal HIV–1 infection (PaHIV).

Methodology: Eligible patients with PaHIV were grouped as slow, rapid or long-term non-progressors (LTNP). The V3 region of gp120 was sequenced from stored plasma samples and tropism determined by geno2pheno algorithm (FPR 5.75%). Logistic regression with generalised estimating equations assessed factors associated with R5 virus. Time to tropism change was assessed using standard survival methods.

Results: At baseline (n=48) median age was 12 years (IQR 9.3–14.8), 52% were female, 79% were Black African, 96% were non-B subtypes and 81% (39/48) had R5-using virus. Median follow-up was 7.7 years (308.6 person-years), with a median of five (range 1–14) samples per subject (total 252). Analysing all samples, R5 virus was associated with higher current CD4 cell count (median 520 cells/mm3 vs 202 for X4, P=0.0005), LTNP (35% vs 11%, P=0.05), non-Black ethnicity (74% vs 89%, P=0.05) and female gender (55% vs 28%, P=0.005). Twelve of 38 (31%) with R5 virus at baseline switched to X4/dual-using virus, with an estimated 5-year risk of switch of 24.4% (95% CI 19.7–39.2%) predicted by lower current CD4 cell count (unadjusted HR 0.62/50 cells higher, 95% CI 0.47–0.81, P=0.0006). Eleven of 19 (58%) with X4/dual-using virus subsequently had R5 virus at one or more time points.

Conclusion: Maraviroc was a treatment option for 81% at 12 years, falling to 56% at 18 years, with lower CD4 cell count predictive of co-receptor switching. Paediatric studies of CCR5 antagonists should be expedited to ensure they are an early treatment option before tropism switching occurs.

Keywords: co-receptor tropism, perinatal HIV, CCR5 antagonist, children, adolescents

Introduction

HIV cell entry requires binding of the viral gp120 envelope protein to a CD4 receptor and to a chemokine co-receptor, usually either CCR5 or CXCR4. The majority of infants with perinatally acquired HIV-1 infection are infected with viral strains that use the CCR5 chemokine co-receptor (termed R5 virus) to gain cell entry, although occasional infants have been described with viruses that use the CXCR4 co-receptor (termed X4 virus) [1,2]. Similarly, in horizontally infected adults R5 virus is typically the transmitted strain, with the emergence of X4 virus and/or dual tropic R5/X4 virus frequently associated with higher plasma HIV viral loads, lower CD4 cell counts and faster disease progression [3–5]. Whether the emergence of X4 virus is the cause of, or secondary to, immune destruction and the impact of antiretroviral therapy (ART) remains unclear. Much of the data available in adults is for B clade viral strains; however, the majority of perinatally infected children worldwide harbour non-B clade virus and there is some evidence in adult populations suggesting viral tropism differs between clades. With disease progression X4 virus is reported to occur in approximately 50% of adults with subtype B, with lower rates reported for Clade C (0–30%), the commonest HIV subtype globally, accounting for almost half of all infections [6–12].

Paediatric data on the evolution of viral tropism and disease progression are sparse. Cross-sectional analysis of a South African paediatric cohort with predominantly clade C subtypes found 54% of those failing ART had X4 or dual tropic X4/R5-using virus compared to 10% of ART-naive children; however, the median ages were 7.9 years compared to 0.9 years, respectively [13].
time in a perinatally infected paediatric population and includes both ART-experienced and naive children by retrospective analysis of viral tropism in stored plasma samples over a 10-year period.

Methods

Subjects

Fifty adolescents with perinatally acquired HIV-1 infection were recruited from the ‘Family’ and ‘900’ clinics at St Mary’s Hospital, Imperial College Healthcare NHS Trust, London, UK between May 2012 and May 2013. Subjects were selected on criteria that included: aged 10 years or older; confirmed perinatally acquired HIV-1 infection by positive HIV DNA PCR prior to their 10th birthday; positive maternal HIV status; and to have been in follow-up at the study centre for at least 5 years with stored plasma samples available.

Baseline data collection

Baseline data collected were retrieved from patient records and included: age, ethnic group, weight and height, presenting history, nadir CD4 cell count and viral load, CDC disease status, ART history, HIV-1 protease and reverse transcriptase resistance–associated mutations, hepatitis B/C status, CD4 cell count and viral load in response to therapy. Subjects were assigned to a clinical group dependent on the history and age of ART exposure:

♦ Group 1: ART initiated after 10th birthday
♦ Group 2: ART initiated prior to 5th birthday
♦ Group 3: ART naive at study recruitment

Three clinical groups were selected using age at starting ART as a surrogate marker of disease progression in an era when commencement of ART was driven by CD4 cell count thresholds/clinical disease rather than current universal treatment of all diagnosed infants. Group 2 included children with rapidly progressive disease, presenting and requiring ART in the pre–school years; Group 1 included those with slower disease progression who had survived until at least 10 years of age without ART; and Group 3 those who had yet to require ART on current UK guidance of treatment at CD4 count thresholds of 350 cells/mm$^3$.

Sampling

Stored samples, previously submitted for routine clinical monitoring plasma viral load assay, were identified that had a detectable viral load >500 copies/mL prior to ART or at time of virological failure. Samples were analysed as follows:

♦ Groups 1 and 2: yearly prior to treatment initiation and at subsequent time points of virological failure
♦ Group 3: at 2-yearly intervals

Additionally all participants had a ‘current’ tropism assay performed at recruitment/latest clinic appointment, using HIV-1 proviral DNA for those suppressed or with low level viraemia (<500 copies/mL) and using plasma RNA if the viral load was greater than 500 copies/mL.

Genotypic analysis of HIV-1 tropism

HIV-1 tropism was assessed according to guidance from BHIVA [19]. Viral RNA was extracted from plasma, reverse-transcribed and the V3 region of the HIV-1 envelope gene PCR amplified and sequenced using in-house primer sequences (unpublished). Each RNA extract was amplified and sequenced in triplicate. Sequences were edited with Sequencher v4.7 (Gene Codes, Ann Arbor, MI) with a cut-off of 20% for minority peaks. Derived sequences were submitted to the ‘geno2pheno’ algorithm (www.geno2pheno.org) to give a prediction of viral tropism. Sequences were analysed by the clonal analysis algorithm and assigned as R5 or X4 virus using a cut-off false-positive rate of 5.75%, without reference to additional clinical data or the presence of a resistance-associated mutation at codons 11 or 25. For subjects who had low (<500 RNA copies/mL) or undetectable viral loads (<50 copies/mL) at recruitment, sequencing was performed on HIV-1 proviral DNA extracted from peripheral blood mononuclear cells and assigned R5 or X4 tropism based on the same FPR cut-off of 5.75%.

Statistical analyses

Baseline was defined as the date of the first sample measured on each child. Baseline characteristics of the study population – age, sex, ethnicity, nadir CD4 cell count and viral load, and ART history – were summarised by clinical group, recording absolute numbers, percentages, median and interquartile range (IQR). Amongst those with more than one sample measured, the number and percentage that experienced a change in tropism on at least one occasion was calculated, stratified by clinical group and tropism at the time of the first sample. To further investigate changes in tropism over time, the subset of individuals with initial R5 virus and at least one subsequent sample was considered. Individuals were followed until the date of a change from R5 to X4/dual tropic virus or the last available sample, whichever occurred first. Kaplan–Meier methods were used to investigate the time to tropism switch, and Cox proportional-hazards models were used to investigate factors associated with this. Only univariable results were calculated, due to a small number of events.

Finally, to ensure that information on all samples taken was summarised, logistic regression with generalised estimating equations was used to assess factors associated with the presence of R5 virus. Statistical significance was assumed for $P$ values below 0.05 and analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC).

Ethical approval

The study was approved by the National Research Ethics Service (NHS Trust Ref 11/LD/1314) and sponsored by Imperial College Healthcare Trust (JROSM0223). Consent was obtained from adolescents aged 16 years and older, with adolescent assent and parental/carer consent obtained for those aged 12–15 years. Each participant received a unique study number, with data pseudo-anonymised under the study number.

Results

Of 50 participants recruited, two were excluded from analysis as all samples ($n=3$ per patient) from these individuals failed to amplify the V3 region from plasma RNA or proviral DNA and therefore tropism could not be determined.

Baseline data

Of the remaining 48 patients recruited, at baseline, defined as date of earliest retrievable stored sample, the median age was 12 years (IQR 9.3–14.8 years), 52% were female, 79% Black African ethnicity with 46 (96%) patients having non-B clade HIV viral subtypes; most frequently: clade C in 13 (27%), clade A in 10 (21%) and clade A recombinants (A/E, A/D, AE/D, A/G) in 8 (17%). The 48 patients were assigned clinical groups on the basis of their antiretroviral treatment history. Group 1 ($n=20$): ART initiated after their 10th birthday; Group 2 ($n=16$) ART pre 5th birthday; and Group 3 ($n=11$) ART naïve – long-term non progressors (LTNP). One patient had insufficient clinical data to
assign to a study group and was excluded from comparator analysis between groups. At baseline, 81% (39/48) had R5 and 19% (9/48) had dual R5/X4 or X4 viruses. The baseline characteristics and viral tropism by clinical group at study entry are described in Table 1. Of the samples, 222/252 (88%) underwent RNA genotyping.

Follow-up: association with R5 virus
Median duration of follow-up was 7.7 years with a total of 308.6 person-years recorded. Of the 37 who had ever received ART, 15 (42%) were dual, and 22 (58%) ≥ triple class exposed at latest follow-up. HIV-1-associated resistance mutations at any time point were documented in 19/37 (51%) of ART-exposed patients: 7/37 (19%) single-, 9/37 (24%) dual- and 3/37 (8%) triple-class mutations.

A median of five (range 1–14) samples per subject, totalling 252 samples, were successfully sequenced and viral tropism determined. Analysing all 252 samples, the presence of R5 virus was associated with: a higher current CD4 cell count, median 520 cells/mm$^3$ for subjects with R5 virus vs 202 cells/mm$^3$ for X4 virus ($P=0.0005$), being a long term non-progressor (Group 3) versus ever requiring ART (35% vs 11% $P=0.05$), non-Black African ethnicity (74% vs 89%; $P=0.05$) and female gender (55% vs 28%; $P=0.005$) (Table 2). No difference was recorded between groups starting ART early and late.

Tropism switch over time
Three children had only one evaluable sample and were excluded from analysis of tropism switching over the duration of the study period. During a median of 7.7 years of follow-up 19/45 (42.2%) had at least one change in their tropism (Table 3). Lower current CD4 cell count predicted co-receptor switch [unadjusted hazard ratio (HR)=0.62 per 50 cells higher; 95% confidence interval (CI) 0.47–0.81; $P=0.0006$] but not viral load ($P=0.81$), age ($P=0.85$) or clinical group ($P=0.56$) (Table 4).

Of the 39 patients with R5 virus at baseline, 38 (97%) had at least one follow-up sample successfully sequenced. A switch from R5 to dual or X4 occurred in 12/38 (30.8%) during the median 7.7 years of follow-up (Table 3). The estimated 5-year risk of tropism switch from R5 to dual tropic/X4 virus was 24.4% (95% CI 9.7–39.2%), with estimated percentages of a change in tropism at 1 year of 2.8% (95% CI 0.0–8.2%) and 2 years of 5.7% (95% CI 0.0–13.4%) (Figure 1).

Tropism reversion
Of 19 patients who ever had X4/dual tropic virus and at least one

### Table 1. Baseline characteristics of children at time of study entry

|                | Group 1: Late ART | Group 2: Early ART | Group 3: LTNP |
|----------------|-------------------|--------------------|--------------|
| Total*         | 20                | 16                 | 11           |
| CD4 (cells/mm$^3$) | 460 (330–760)     | 810 (520–1000)     | 670 (510–810) |
| CD4 group      | ≤200              | 1 (5%)             | 0 (0%)       |
|                | 201–350           | 5 (25%)            | 2/15 (13.3%) |
|                | 351–500           | 5 (25%)            | 1/15 (6.7%)  |
|                | 501+              | 9 (45%)            | 12/15 (80.0%)|
| VL (copies/mL) | Median (IQR)      |                   |              |
|                | (1691–44431)      | (157–10,359)       | (2067–7514)  |
| VL group (n)   | <500              | 4 (20%)            | 5/15 (33.3%) |
|                | 500–10,000        | 5 (25%)            | 6/15 (40.0%) |
|                | 10,001–50,000     | 6 (30%)            | 3/15 (20.0%) |
|                | 50,001+           | 5 (25%)            | 1/15 (6.7%)  |
| Clade          | C                 | 7/17 (41.2%)       | 3/13 (23.1%) |
|                | Non-C             | 10/17 (58.8%)      | 10/13 (76.9%)|
| Ethnicity      | Black African     | 17 (85%)           | 11 (68.8%)   |
|                | Other             | 3 (15%)            | 5 (31.3%)    |
| Gender         | Female            | 9 (45%)            | 9 (56.3%)    |
|                | Male              | 11 (55%)           | 7 (43.8%)    |
| Age            | Median (IQR)      | 12 (9–15)          | 11 (8–13)    |
| Tropism        | R5                | 15 (75%)           | 12 (75.0%)   |
|                | X4                | 2 (10%)            | 2 (12.5%)    |
|                | Dual/mixed        | 3 (15%)            | 2 (12.5%)    |

* One child (with R5 virus at study entry) had insufficient clinical data to assign to a study group

LTNP: long-term non-progressors, IQR: interquartile range

Figure 1. Kaplan–Meier estimate of probability of tropism switch from R5 to X4/dual tropic virus over study follow-up (n=38)
In this cohort of adolescents with perinatally acquired HIV-1 infection, more than 80% had R5 virus at 12 years of age, indicating that CCR5 antagonists may be a treatment option as a component of ART. The estimated 5-year risk of tropism switch from R5 to X4/dual tropic virus was 24% with, as in adult studies, lower CD4 cell count predictive of co-receptor switching [4]. As the cohort entered adult care and approach their third decade living with HIV, the proportion with R5 virus at all time points sequenced had fallen to just over 50%. This is comparable to a cross-sectional genotypic co-receptor tropism analysis of 55 Spanish adolescents, median age 18.2 years, 90% clade B, of whom 40% harboured X4/dual tropic strains [18]. As such, almost half of perinatally infected adolescents currently enter adult care with reduced treatment options for HIV. In this small cohort no difference was seen in tropism switching between those who started ART early in life, at less than 5 years of age, and those who started later, at over 10 years of age, and may reflect the small numbers and heterogeneity of CD4 cell count thresholds at diagnosis and subsequently starting ART within the cohort. Additionally, due to the small sample size (36 samples with CXCR4 virus on 18 children), we were unfortunately unable to perform multivariate analysis. Therefore the observed univariate differences in tropism switching between genders and ethnicity may be due to confounding factors.

Table 2. Factors associated with having a CCR5-using virus, considering all samples over follow-up

|                           | CCR5       | CXCR4       | Dual / mixed | P       |
|---------------------------|------------|-------------|--------------|---------|
| Total samples             | 252        | 203         | 36           | 13      |
| Method used               | DNA        | 21 (10.3%)  | 6 (16.7%)    | 3 (23.1%)| 0.12    |
|                           | RNA        | 182 (89.7%) | 30 (83.3%)   | 10 (76.9%)|         |
| CD4 (cells/mm³) Median (IQR) | 520 (346–730) | 202 (68–327) | 300 (145–775) | 0.0005  |
| CD4 group                | ≤200       | 21/191 (11.0%) | 21/35 (60%)  | 4/12 (33.3%)|         |
|                           | 201–350    | 30/191 (15.7%) | 9/35 (25.7%)  | 3/12 (25.0%)|         |
|                           | 351–500    | 39/191 (20.4%) | 4/35 (11.4%)  | 1/12 (8.3%)|         |
|                           | 501+       | 101/191 (52.9%) | 1/35 (2.9%)  | 4/12 (33.3%)|         |
| VL (copies/mL) Median (IQR) | 5,874 | 22,024 | 12,938 | 0.3403 |
|                           | <500       | 44/192 (22.9%) | 9 (25%)    | 5/12 (41.7)  |         |
|                           | 500–1,000  | 68/192 (35.4%) | 10 (27.8%)  | 2/12 (16.7%)|         |
|                           | 1,001–5,000| 65/192 (33.9%) | 12 (33.3%)  | 4/12 (33.3%)|         |
|                           | 5,001+     | 15/192 (0.08%) | 5 (13.9%)  | 1/12 (8.3%)|         |
| Clade                     | C          | 58/174 (33.3%) | 14/34 (41.2%)| 4 (30.8%)| 0.35    |
|                           | Non-C      | 116/174 (66.7%) | 20/34 (58.8%)| 9 (69.2%)|         |
| Group                     | 1 Late ART | 74/201 (36.8%) | 21 (58.3%)  | 9 (69.2%)| 0.12    |
|                           | 2 Early ART| 76/201 (37.8%) | 11 (30.6%)  | 4 (30.8%)| 0.06    |
|                           | 3 LTNP     | 51/201 (25.3%) | 4 (11.1%)   | 0 (0%)|         |
| Ethnicity                 | Black African | 150 (73.9%) | 32 (88.9%)  | 9 (69.2%)| 0.05    |
|                           | Other      | 53 (26.1%) | 4 (11.1%) | 4 (30.8%)|         |
| Sex                       | Female     | 112 (55%) | 10 (27.8%) | 7 (53.8%)| 0.005   |
|                           | Male       | 91 (44.8%) | 26 (72.2%) | 6 (46.2%)|         |
| Age (years)               | Median (IQR) | 16 (13–18) | 17 (15–19) | 17 (5–11)| 0.0339  |

* Comparing CCR5 vs CXCR4: calculated from logistic regression with GEEs to account for repeated samples taken on children
IQR: interquartile range; GEE: generalised estimating equation

Table 3. Proportion of participants who experienced tropism switch, by tropism at first sample and clinical group

|                           | Total* n | Tropism changed at least once over follow-up |
|---------------------------|----------|---------------------------------------------|
| Tropism at first sample   |          |                                             |
| R5                        | 38       | 12 (30.8%)                                  |
| X4                        | 2        | 2 (100.0%)                                  |
| Dual                      | 5        | 5 (100.0%)                                  |
| Group                     |          |                                             |
| 1 Late ART                | 19       | 10 (52.6%)                                  |
| 2 Early ART               | 14       | 7 (50.0%)                                   |
| 3 LTNP                    | 11       | 2 (18.2%)                                   |
| Tropism at first sample and group |          |                                             |
| 1 Late ART                | 14       | 5 (35.7%)                                   |
| R5                        | 2        | 2 (100.0%)                                  |
| X4                        | 3        | 3 (100.0%)                                  |
| 2 Early ART               | 12       | 5 (41.7%)                                   |
| R5                        | 0        | —                                           |
| X4                        | 2        | 2 (100.0%)                                  |
| 3 LTNP                    | 11       | 2 (18.2%)                                   |

* 45 children with ≥2 samples recorded were eligible for inclusion (one child with initial R5 and two with X4 virus were excluded), of whom 44 had sufficient clinical data to be assigned to a clinical group (one further child with initial X4 virus excluded)

In this cohort of adolescents with perinatally acquired HIV-1 infection, more than 80% had R5 virus at 12 years of age, indicating that CCR5 antagonists may be a treatment option as a component of ART. The estimated 5-year risk of tropism switch from R5 to X4/dual tropic virus was 24% with, as in adult studies, lower CD4 cell count predictive of co-receptor switching [4]. As the cohort entered adult care and approach their third decade living with HIV, the proportion with R5 virus at all time points sequenced had fallen to just over 50%. This is comparable to a cross-sectional genotypic co-receptor tropism analysis of 55 Spanish adolescents, median age 18.2 years, 90% clade B, of whom 40% harboured X4/dual tropic strains [18]. As such, almost half of perinatally infected adolescents currently enter adult care with reduced treatment options for HIV. In this small cohort no difference was seen in tropism switching between those who started ART early in life, at less than 5 years of age, and those who started later; at over 10 years of age, and may reflect the small numbers and heterogeneity of CD4 cell count thresholds at diagnosis and subsequently starting ART within the cohort.

Additionally, due to the small sample size (36 samples with CXCR4 virus on 18 children), we were unfortunately unable to perform multivariate analysis. Therefore the observed univariate differences in tropism switching between genders and ethnicity may be due to confounding factors.

In routine clinical practice a genotypic co-receptor tropism sequence, performed within 3 months prior to starting/switching ART, determines whether a CCR5 antagonist is a potentially active agent within an ART regimen [19]. Surprisingly, more than a half
of those ever sequenced shown to harbour X4 virus reverted to R5 virus at a subsequent time point. The relatively frequent ‘switching’ between R5 and X4 virus, and vice versa, demonstrated in this cohort, highlights a need for vigilant clinical monitoring of virological response in children and adolescents receiving maraviroc. On current evidence, detection of X4 virus at any time should be considered long-lasting and preclude the use of CCR5 antagonists. Clinical outcome data for the use of maraviroc in paediatric patients following tropism determination by genotypic testing methods are limited. However, recent preliminary data suggest that CCR5 antagonists may have a therapeutic role in patients with a mixture of R5 and X4 viruses and warrant further investigation, particularly in those with multidrug resistance and limited treatment options [20,21].

Almost half of the adolescents were triple-class ART experienced and, of those adolescents who had ever received ART, one-third had accumulated mutations associated with resistance to two or more drug classes, further limiting future ART treatment options. Globally, 30% of children with PaHIV are triple-class experienced, with up to 80% of multidrug-resistant paediatric patients harbouring X4/dual tropic viruses [14]. Paediatric studies of CCR5 antagonists should be expedited, particularly as early use of such antagonists may not be an effective component of ART. Further investigation into the optimal sequencing of antiretroviral classes is required for this unique population who currently face a lifetime on antiretroviral therapy.

Consideration should, however, be given to existing data from adult studies. In a post hoc analysis of the MERIT study in patients with R5 virus confirmed by enhanced sensitivity Trolife assay, maraviroc was non-inferior to efavirenz in treatment-naive adults infected with R5 virus out to 5 years of follow-up [22]. More recently a treatment-naive nucleoside-sparing strategy of boosted darunavir with maraviroc was stopped due to increased virological failure compared to the standard of care of boosted darunavir with tenofovir/emtricitabine [23].

Initial paediatric data suggest that maraviroc is safe, well tolerated and effective with both an oral solution and paediatric tablets in development [24,25]. A4001031, a Phase IV study in treatment-experienced children age 2 to <18 years harbouring R5 virus is ongoing and due to report in 2019. Given the central nervous system penetration of maraviroc, the potential role in younger children, and warrant further investigation, particularly in those with multidrug resistance and limited treatment options [20,21].

In conclusion half of the children born with HIV will enter adult life with X4/dual tropic virus, for whom the existing CCR5 antagonist may not be an effective component of ART. Further investigation into the optimal sequencing of antiretroviral classes is required for this unique population who currently face a lifetime on antiretroviral therapy.

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Table 4. Factors associated with a switch in viral tropism

| Hazard ratio | 95% CI | P value |
|-------------|-------|---------|
| Current CD4 cell count | | |
| Per 50 cells higher | | | |
| 0.62 | 0.47–0.81 | 0.0006 |
| Group | | |
| 1 Late ART | | | |
| 2.45 | 0.46–12.99 | 0.56 |
| 2 Early ART | | | |
| 1.54 | 0.28–8.48 | |
| 3 LTNP | | | |
| 1.00 (reference) | | | |
| Current viral load | | |
| Per 1 log10 copies/mL higher | | | |
| 0.92 | 0.46–1.84 | 0.81 |
| Age | | |
| Per year older | | | |
| 1.02 | 0.85–1.21 | 0.85 |

Results from Cox proportional hazards regression model (univariate due to limited number of events)

CI: confidence interval

higher rates of tropism switching from R5 to X4 virus. Conversely, in the current era of universal ART for all children diagnosed in the first 5 years of life under World Health Organization (WHO) guidelines and at higher CD4 cell count thresholds for older children, current paediatric populations may have lower rates of tropism switching, providing viral suppression and CD4 cell counts are maintained, which can be a challenge, particularly through adolescence. As failure on maraviroc is rarely associated with resistance, it has the potential to preserve future treatment options and provides a well-tolerated option for a population with limited treatment options.
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