Role and evolution of viral tropism in patients with advanced HIV disease receiving intensified initial regimen in the ANRS 130 APOLLO trial

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Objectives: The aims of the study were to assess in patients with advanced HIV disease receiving antiretroviral therapy (ART) intensification with enfuvirtide (i) resistance at virological failure (VF), (ii) impact of baseline tropism on immunovirological response, and (iii) HIV-1 DNA tropism evolution during ART.

Methods: The ANRS 130 APOLLO randomized trial evaluated in naive patients the immunovirological impact of standard ART without (control arm) or with enfuvirtide. Tropism was determined on RNA and DNA by V3-loop sequencing interpreted using the Geno2Pheno algorithm.

Results: At baseline the median CD4 cell count was 30 cells/mm³. Among the 170 patients assessable in this virological substudy, HIV-1 RNA tropism was as follows: 60% of viruses were R5 and 40% were R5X4/X4. HIV-1 DNA tropism was as follows: 54% were R5 and 46% were R5X4/X4. At week 24, 39% and 49% of patients experienced VF in the enfuvirtide and control arms, respectively. In the enfuvirtide arm, only resistance-associated mutations to enfuvirtide were detected. In the control arm, two patients displayed drug-resistant viruses at the time of VF. No impact of baseline tropism was observed on immunovirological response, regardless of the study arm. Among the 25 patients experiencing DNA tropism switch between baseline and week 24, 16 (64%) switched from R5 to R5X4/X4. These latter were mostly successfully suppressed patients receiving enfuvirtide and exhibiting poorer immunological response.

Conclusions: Baseline RNA tropism had no impact on the immunovirological response. Drug resistance mutations were only detected for the fusion inhibitor. Finally, the mechanism of replenishment of the viral cellular reservoir with X4 viruses observed needs to be further analysed.

Keywords: HIV DNA, resistance, enfuvirtide, tropism, switch

Introduction

The Agence nationale de recherches sur le SIDA et les hépatites virales (ANRS) 130 APOLLO trial assessed the impact of intensification of standard combined antiretroviral therapy (cART) with enfuvirtide on CD4 cell count response at week 24 in antiretroviral-naive, severely immunosuppressed, HIV-1-infected patients, all with CD4 counts <200 cells/mm³ at baseline. The primary outcome measure, defined by the proportion of patients with a CD4 count >200 cells/mm³ at week 24, showed that intensification did not improve CD4 cell response at week 24 despite enhanced virological success.1 Previous in vitro studies showed that higher concentrations of enfuvirtide were required to inhibit R5 virus compared with...
HIV tropism and immunovirological response

X4 virus. However, in a Phase III clinical trial with a large number of patients, the in vivo virological and immunological responses of enfuvirtide-based treatment were similar, regardless of baseline tropism. In vivo, few data are available on the evolution of HIV-1 RNA and DNA tropism in patients receiving enfuvirtide-containing treatment.

The objectives of this virological substudy of the ANRS 130 APOLLO trial were to analyse the emergence of resistant viruses at the time of virological failure (VF), to determine the impact of baseline viral tropism on virological and immunological responses, and to assess the evolution of viral tropism between baseline and week 24 in the cellular viral reservoir in the context of severely immunosuppressed HIV-1-infected patients.

Patients and methods

Study population

The ANRS 130 APOLLO trial was a multicentre, nationwide, open-label randomized controlled trial of three versus four antiretroviral drugs at the initial phase of cART in severely immunosuppressed HIV-infected patients. From April 2006 to December 2008, a total of 195 patients were randomized: 101 in the enfuvirtide arm (background regimen of tenofovir/emtricitabine and lopinavir/ritonavir or efavirenz) plus enfuvirtide for the first 6 months of cART) and 94 in the control arm (background regimen of tenofovir/emtricitabine and lopinavir or efavirenz). The definition of virological response was defined as plasma HIV-1 RNA values at weeks 24, 36 and 48 all <50 copies/mL. VF was defined as one of the following criteria: (i) age ≥18 years; (ii) written informed consent; (iii) naive HIV-1-infected patients; (iv) either asymptomatic with CD4 <100 cells/mm³ or with past or present history of stage B or C HIV disease with CD4 <200 cells/mm³.

Patients were included with the following eligibility criteria: (i) age ≥18 years; (ii) written informed consent; (iii) naive HIV-1-infected patients; (iv) either asymptomatic with CD4 <100 cells/mm³ or with past or present history of stage B or C HIV disease with CD4 <200 cells/mm³.

Plasma and whole blood specimens were collected for virological analysis at baseline and at week 24 of cART.

Written informed consent was obtained from all patients. The protocol was reviewed and approved by an ethics committee (Comité de Protection des Personnes) and competent health authorities (Agence Française de Sécurité Sanitaire des Produits de Santé). The trial was conducted in accordance with the Declaration of Helsinki.

Definition of virological response

In the virological substudy of the ANRS 130 APOLLO trial, virological success was defined as plasma HIV-1 RNA values at weeks 24, 36 and 48 all <50 copies/mL. VF was defined as one of the following criteria: (i) all HIV-1 RNA values during the follow-up were >50 copies/mL, defined as no virological response; (ii) the HIV-1 RNA level became <50 copies/mL but only after week 24, defined as slow kinetics of viral load decrease; (iii) two consecutive HIV-1 RNA values >50 copies/mL following one HIV-1 RNA value <50 copies/mL, defined as viral rebound. One isolated HIV-1 RNA value >50 copies/mL was defined as a viral blip. Immunological response was defined as reaching a CD4 cell count >200 cells/mm³ at week 24.

Genotypic resistance tests

Bulk sequencing of the protease (PR) and reverse transcriptase (RT) regions was performed at baseline in all patients in the study and at week 24 in patients experiencing VF. Bulk sequencing of the gp41 region was performed at baseline and at week 24 in patients included in the enfuvirtide arm and experiencing VF.

Genotypic prediction of co-receptor use

The gp120 sequence analysis comprising the complete V3 loop sequence was performed, from plasma collected at baseline in all patients and at week 24 in patients experiencing VF, according to the sequencing procedures and primer sequences described at http://www.hivfrenchresistance.org. Viral tropism was also assessed in whole blood specimens in all study patients both at baseline and at week 24. HIV-1 DNA was extracted from whole blood with the Qiagen Whole Blood Extraction Kit (Qiagen, Hilden, Germany). PCR and nested PCR were then performed according to the ANRS procedures.

Virological substudy population

Among the 195 patients included in the ANRS 130 APOLLO trial, the virological substudy was performed in 177, 159, and 162 samples for RT, PR, and V3 loop regions, respectively, in HIV-1 RNA, and in 156 samples for V3 loop region in HIV-1 DNA. The HIV-1 subtype was analysed at baseline in 170 patients.

Patient characteristics

The baseline median HIV-1 RNA load and CD4 cell count of patients included in the ANRS 130 APOLLO study were 5.4 log₁₀ copies/mL (IQR = 5.0–5.8) and 30 cells/mm³ (IQR = 12–72), respectively.

Sixty-six percent (n = 113) of patients assessed in the virological substudy were infected with HIV-1 subtype B. A large genetic diversity was observed among the 57 patients infected with...
HIV-1 ‘non-B’ subtypes, mostly represented by the CRF02_AG (n=29; 51%).

**Genotypic resistance tests at baseline**

Baseline PR, RT and gp41 sequencing was successful in 99%, 94% and 98% of samples, respectively. Baseline genotypic resistance analysis showed that at least one DRM was observed in 19 samples, leading to a prevalence of transmitted drug resistance of 11.4% (95% CI 6.5%–16.3%). DRMs to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and enfuvirtide were observed in 11 (6.6%), 4 (2.4%), 5 (3.2%), and 0 (0.0%) samples, respectively. Genotypic resistance data at baseline are shown in Table 1. No significant difference in the prevalence of DRMs was observed between HIV-1 subtype B and ‘non-B’ subtypes (5% versus 14%, P=0.12). A similar prevalence of DRMs was observed in both study arms (10% versus 14%, P=0.48).

**Profiles of virological failure in the enfuvirtide and control arms**

Thirty-nine percent (38/97) and 49% (45/92) of patients experienced VF in the enfuvirtide and control arms, respectively. The HIV-1 RNA level at the time of VF was low (median=106 copies/mL; IQR=72–252). The rate of virological response was similar between patients infected with HIV-1 B subtype and those infected with ‘non-B’ subtypes (54% versus 59%, P=0.62). In patients experiencing VF, baseline DRMs were more frequently found in the control arm than in the enfuvirtide arm (20% versus 5%, P<0.005). Eleven of the 19 patients with baseline DRMs experienced VF (58%).

A slow kinetics of viral load decrease was observed in 37% of VFs and was more frequently observed in the control arm than in the enfuvirtide arm (P<0.01). Viral blips were more frequently observed in the enfuvirtide arm than in the control arm (47% versus 22%, P=0.02); however, all viral blips observed in the enfuvirtide arm occurred after week 24, thus after enfuvirtide withdrawal.

**HIV-1 RNA and HIV-1 DNA tropism at baseline**

At baseline, plasma HIV-1 RNA tropism determination was successful in 134 of the 162 assessable samples (83%). Overall, 81 viruses (60%) were classified as R5-tropic, and 53 (40%) as R5X4/X4-tropic. The proportion of patients harbouring R5X4/X4 viruses at baseline was 39% in both the enfuvirtide and control arms. Baseline HIV-1 DNA tropism analysis was successful in 121 of the 156 assessable samples (78%). Overall, HIV-1 DNA tropism was as follows: 65 viruses (54%) were classified as R5-tropic and 56 (46%) as R5X4/X4-tropic. The distribution of R5 and R5X4/X4 viruses in RNA and DNA according to the CD4 cell count is depicted in Figure 1. The proportion of R5X4/X4 viruses was similar in RNA and in DNA (P=0.21).

Viral tropism results were available in 110 paired plasma and whole blood samples. At baseline, HIV-1 RNA and DNA tropism were concordant in 77% of cases (n=85). Among the discordances, 14 (56%) were attributable to the prediction of R5 in plasma RNA and R5X4/X4 in proviral DNA, and 11 (44%) were

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Control arm</th>
<th>Enfuvirtide arm</th>
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<tbody>
<tr>
<td></td>
<td>NRTI</td>
<td>PI</td>
</tr>
<tr>
<td>Baseline</td>
<td>T215C/D/E/S (n=4)</td>
<td>Y181C (n=1)</td>
</tr>
<tr>
<td></td>
<td>M41L (n=2)</td>
<td>none</td>
</tr>
<tr>
<td>Virological failure</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

Table 1. Genotypic resistance data of the ANRS 130 APOLLO trial at baseline and at the time of virological failure.

NA, not applicable; FI, fusion inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VF, virological failure.

Drug resistance mutations were identified using the ANRS algorithm (version 21, October 2011, http://www.hivfrenchresistance.org).

7 The T215Y mutation was already present at baseline for this patient.
impact of baseline HIV-1 RNA tropism on immunovirological response

Overall, virological success was observed in 52% \((n=42)\) and 64% \((n=34)\) of patients with baseline R5 and R5X4/X4 viruses, respectively \((P=0.12)\), with no differences according to the study arm. Immunological response was observed in 42% \((n=34)\) and 32% \((n=17)\) of patients with baseline R5 and R5X4/X4 viruses, respectively \((P=0.19)\). No difference was observed between the study arms, with 30% and 38% of immunological responders harbouring baseline R5X4/X4 HIV-1 RNA tropism in the enfuvirtide and control arms, respectively \((P=0.38)\).

genotypic resistance tests at the time of VF

Genotypic resistance testing was performed in (i) plasma samples with HIV-1 RNA \(>50\) copies/mL at week 24 \((n=45)\), and (ii) plasma samples with viral rebound \((n=10)\). Among the 55 samples fulfilling the criteria for genotypic resistance testing, a plasma sample was available for 47 of them. Sequencing succeeded in 6 of 16 (38%), 24 of 47 (51%) and 29 of 47 (62%) samples for the gp41, RT and PR regions, respectively.

In the enfuvirtide arm, no resistance-associated mutations to NRTIs, NNRTIs or PIs were detected at the time of VF. Resistance-associated mutations to enfuviritide were detected in two samples among the six tested, showing the G36D mutation in both cases. In the control arm, two patients displayed drug-resistant viruses at the time of VF, including one who exhibited virus with DRMs at baseline; both were receiving lopinavir as a third agent. Genotypic resistance data at VF are presented in Table 1.

HIV-1 DNA tropism at week 24

At week 24, HIV-1 DNA tropism was assessed in 155 samples and V3 loop sequencing succeeded in 138 (89%) samples. Overall, in whole blood samples 63 viruses (46%) were classified as R5-tropic and 75 (54%) as R5X4/X4-tropic. Similar proportions of R5X4/X4 viruses were observed at baseline and week 24 \((P=0.19)\), regardless of the study arm.

Paired HIV-1 DNA tropism results at baseline and at week 24 were available in 96 patients. HIV-1 DNA tropism remained unchanged in 74% of cases \((n=71)\), including 33 R5 viruses and 38 R5X4/X4 viruses. The proportion of patients evidencing HIV-1 DNA tropism switch between baseline and week 24 was not different between the enfuvirtide and control arms \((44\%\text{ versus }56\%, P=0.28)\).

Patients experiencing a tropism switch had a lower baseline CD4 cell count (median=14 cells/mm\(^3\)) than patients with stable tropism in the cellular viral reservoir (median=47 cells/mm\(^3\), \(P=0.0073)\). The proportion of patients evidencing tropism switch tended to be higher in patients with virological success than in those experiencing VF \((33\%\text{ versus }16\%, \(P=0.051)\). Among the 25 patients experiencing HIV-1 DNA tropism switch, 9 (36%) switched from R5X4/X4 at baseline to R5 at week 24, while 16 (64%) switched from R5 to R5X4/X4 (Table 3). The proportion of patients with R5 to R5X4/X4 switch was twice as high in the enfuvirtide arm than in control arm, but with no statistical significance \((21\%\text{ versus }12\%, P=0.24)\).

We assessed the impact of an R5 to R5X4/X4 DNA tropism switch on immunological response. A higher proportion of immunological responders was found in patients with stable R5 DNA tropism between baseline and week 24 \((n=21, 64\%)\) than in patients with an R5 to R5X4/X4 DNA switch \((n=4, 25\%)\) \((P=0.013)\). If we focused on patients with virological success in the enfuvirtide arm, 78% \((n=7)\) and 0% were immunological responders in patients with stable R5 DNA tropism and in those with R5X4/X4 DNA tropism switch, respectively \((P=0.003)\).

Table 2. Tropism determination of paired plasma HIV-1 RNA and whole blood HIV-1 DNA samples at baseline

<table>
<thead>
<tr>
<th>Plasma HIV-1 RNA</th>
<th>Whole blood HIV-1 DNA</th>
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<tbody>
<tr>
<td></td>
<td>R5</td>
</tr>
<tr>
<td>R5</td>
<td>53</td>
</tr>
<tr>
<td>R5X4/X4</td>
<td>32</td>
</tr>
</tbody>
</table>

attributable to the prediction of R5X4/X4 in plasma RNA and R5 in proviral DNA (Table 2).
Table 3. Tropism determination of paired whole blood HIV-1 DNA samples at baseline and at week 24 of antiretroviral therapy

<table>
<thead>
<tr>
<th>HIV-1 DNA tropism</th>
<th>baseline</th>
<th>week 24</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>R5</td>
<td>33 (34)</td>
<td></td>
</tr>
<tr>
<td>R5X4/X4</td>
<td>R5X4/X4</td>
<td>38 (40)</td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td>R5X4/X4</td>
<td>16 (17)</td>
<td></td>
</tr>
<tr>
<td>R5X4/X4</td>
<td>R5</td>
<td>9 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In this study, performed in severely immunosuppressed patients with a median CD4 count of 30 cells/mm³ receiving initial CART, we showed a low rate of selection of resistance mutations in case of VF. The baseline HIV-1 RNA tropism had no impact on the rate of immunovirological response. A change in HIV-1 DNA tropism between baseline and week 24 was observed in 26% of patients. In most cases DNA tropism switch was from R5 at baseline to R5X4/X4 at week 24 in patients successfully receiving an enfuvirtide-containing regimen.

Despite a more pronounced immunodeficiency in patients included in the ANRS 130 APOLLO trial, they displayed similar viral characteristics to chronically infected French patients recently described regarding: (i) the distribution of HIV-1 subtypes, with 34% of ‘non-B’ subtypes versus 42%, respectively; (ii) half of new HIV-1 ‘non-B’ subtypes due to the CRF02_AG recombinant (44% versus 42%, respectively); and (iii) the prevalence of transmitted drug resistance (11.4% versus 10.6%, respectively).

Fifty-eight percent of patients with baseline primary resistance experienced VF, although all received three active drugs. As previously described, the presence of transmitted drug resistance is associated with a higher risk of VF, suggesting the role of minority resistant variants. In addition, higher baseline viral loads are known to be associated with a profile of slow kinetics of HIV-1 RNA decrease and an increased risk of VF. In the present study, 75% of patients displayed baseline HIV-1 RNA > 5 log₁₀ copies/mL. A previous study showed the correlation between the delay in obtaining undetectable viral load and the risk for the selection of drug-resistant viruses. In our series, HIV-1 RNA lever at VF was low (median=106 copies/mL) and was associated with a low rate of selection of resistance, except for enfuvirtide, as expected with the use of a low genetic barrier drug. One patient exhibited a multiresistant virus at the time of VF, suggesting that VF could be due to the re-emergence of archived transmitted resistance mutations as reported in one patient in the CASTLE study.

In our study 81% of patients received a PI, lopinavir, as a third agent, which could explain the low rate of selection of resistance observed in the case of VF, as reported in the ACTG 5142 trial. All viral blips, most common in the enfuvirtide arm, occurred after enfuvirtide withdrawal, arguing for an initial regimen with four antiretroviral drugs in this profile of severely immunosuppressed patients with high plasma viral load, and suggesting that a longer period of enfuvirtide intake might have been virologically more potent.

In the present study, 40% of baseline plasma viruses were R5X4/X4, a level similar to that previously described in such immunosuppressed patients. The concordance between HIV-1 DNA and HIV-1 RNA tropism was 77%. In other studies the rate of concordance reached 82% to 90%. The higher level of concordance possibly explained by the higher median CD4 cell count, around 300 cells/mm³, compared with a median of 30 cells/mm³ in our trial. A previous report, assessing patients receiving their initial antiretroviral combination, showed the absence of impact of viral tropism on virological response as we observed in the APOLLO clinical trial. Our results are similar to those of the Phase III studies of enfuvirtide (TORO 1 and TORO 2 trials) showing no correlation between baseline tropism and immunovirological response to an enfuvirtide-based regimen.

Conversely, an impact of viral tropism on virological response was observed in the ARTEN study, which compared nevirapine and atazanavir, with a weaker virological response in patients with baseline X4 viruses than in patients with R5 viruses at week 24 (61% versus 83%, P=0.001), and in the group of HIV-1 subtype-B-infected patients at week 48.

Saracino et al. reported a similar percentage (28%) of HIV-1 DNA tropism evolution at month 12 of antiretroviral therapy. In this latter study, as observed in our trial, changes were mainly a switch from R5 to X4 tropism (in about 60% of cases). In the study of Saracino et al., the occurrence of the switch was not related to enfuvirtide use or virological response, but was correlated with the CD4 cell count nadir. In our study, the median baseline CD4 cell count was significantly lower in patients experiencing a tropism switch compared with patients with stable tropism in the cellular viral reservoir. This switch away from R5 tropism, more pronounced in the enfuvirtide arm, could partly explain the lack of improved CD4 cell count recovery despite virological suppression.

In conclusion, the intensification of initial CART with enfuvirtide in patients with advanced HIV disease did not improve immunological reconstitution in spite of a higher rate of virological response. The phenomenon of R5 to R5X4/X4 DNA tropism switch in the cellular reservoir between baseline and week 24, in particular in the enfuvirtide arm, may have negatively impacted the immune reconstitution expected with the intensified regimen. The occurrence of viral blips after enfuvirtide withdrawal in addition to viral cellular reservoir replenishment with X4 viruses could have prevented demonstration of the benefit of this four-drug regimen as initial cART. In conclusion, intensification with other classes of antiretroviral drugs, without impact on viral tropism switch, might display more beneficial immunovirological outcomes.

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Members of the APOLLO study group

Saint Jean Hospital, Paris: H. Aumaître, M. Medus, N. Benmakhlouf, C. Brochier, L. Cotte, V. Joly, F. R., N. C. V. and P. Y. recruited patients in the trial and contributed to the analysis and interpretation of the data. B. V. and L. L. performed the virological analysis. C. C., D. D., V. J., and C. F. contributed to the analysis and interpretation of the data. C. C., D. D., V. J., and C. F. contributed to the writing of the manuscript. All authors contributed to the critical reviewing of the manuscript.

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