CONCISE COMMUNICATION

Viral rebound in semen after antiretroviral treatment interruption in an HIV therapeutic vaccine double-blind trial

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Objectives: This study aimed to determine the timing and level of HIV rebound in blood and seminal plasma and to characterize the HIV rebounding populations after antiretroviral treatment interruption (ATI) in HIV-1-infected participants enrolled in a therapeutic vaccine trial.

Design: A 12-week (W) ATI period was proposed at W36 to patients enrolled in the VRI02/ANRS149-LIGHT trial. Paired blood and semen samples were collected before (W32 or W36) and during ATI (W38, W40, W42, W44, and W48).

Methods: HIV-RNA and HIV-DNA were quantified sequentially from blood and semen samples. Ultradeep sequencing (UDS; Roche/454) of partial env HIV-DNA/RNA (C2V3) was performed in both compartments.

Results: HIV-RNA rebounded in blood plasma and seminal plasma of all ten participants after ATI [median peak of 5.12 $\log_{10}$ cp/ml (range: 4.61–6.35) and 4.26 $\log_{10}$ cp/ml (3.20–4.67), respectively]. HIV-RNA rebound was detected in blood plasma as soon as W38 in 8/10 patients, and in seminal plasma between W38 and W40 in 8/10 patients. Phylogenetic approaches showed intermingled HIV-RNA populations from plasma and semen during ATI, suggesting a lack of viral compartmentalization between blood and semen.

Conclusion: Our data demonstrate rapid and high HIV rebound in semen after ATI, raising concerns about high risk of HIV sexual transmission during HIV cure trials.

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Introduction

Currently, many therapeutic and vaccine trials are being offered to HIV-infected participants to explore different strategies for HIV remission. These trials usually involve a discontinuation of combined antiretroviral therapy (cART). Recent findings show that short-term antiretroviral treatment interruptions (ATI) do not necessarily lead to expansion of the persistent HIV reservoir nor irreparable damages to the immune system in the peripheral blood [1]. However, another question remains unaddressed: what is the impact of ATI on HIV replication in genital tract? Using cART now dramatically reduces the risk of sexual transmission [2]. The risk of transmission to sexual partners during ATI is an important concern, and HIV cure strategies would not be acceptable if they did not prevent sexual transmission as well.

Previous studies on viral rebound in genital compartment during ATI are limited by the small number of participants, and by their cross-sectional design [3–5]. Some authors have evidenced a compartmentalization between blood and semen, which could allow for independent viral replication in genital male tract [6–8].

Participants enrolled in a HIV therapeutic vaccine trial (VRI02/ANRS149-LIGHT) agreed to provide regular and repeated paired blood and semen samples during a programmed ATI. Our main goal was to compare the timing and level of HIV blood plasma viral load (bpVL) and semen plasma viral load (spVL) during ATI. The secondary goals were to compare the level of HIV-DNA in peripheral blood mononuclear cells (PBMCs) before and after ATI, to quantify HIV-DNA in nonsperm cells (NSC) in semen, and to characterize HIV rebounding populations in both compartments.

Material and methods

Study population

Our study was designed among male participants enrolled in the VRI02/ANRS149-LIGHT therapeutic vaccine randomized double-blind trial (ClinicalTrials.gov identifier: NCT01492985). Briefly, this trial assessed the effectiveness of a combination of two therapeutic vaccines in HIV-positive participants on cART for at least 18 months. Participants were randomized to receive the active vaccine or placebo between week 0 (W0) and W24. The primary outcome was the level of bpVL at W48, after the 12-week ATI (between W36 and W48). cART could be resumed at W48 or before if HIV-RNA rebound occurred depending on $CD4^+$ cell count changes. The ethics committee of Ile-de-France IX approved the substudy protocol, and the 10 participants provided their written informed consent.

Semen samples collection

Participants provided semen samples at W36 (at the time of ATI), W38, W40, W42, W44, and W48 (during ATI). These samples were collected in sterile containers by self-masturbation after a 48-h abstinence period. Seminal plasma and cellular fraction were separated by centrifugation, and frozen at −80°C.

HIV-1 RNA quantification in blood and semen samples

Blood samples were collected at W32 (before ATI), and at the same time-points described above. HIV-1-RNA in blood plasma was quantified using COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (Roche, Meylan, France), with a detection threshold of 20 copies/ml.

HIV-1-RNA in seminal plasma was quantified using the same method, after one-third dilution to limit the samples' viscosity and the presence of PCR inhibitors [9]. The limit of detection was 60 copies/ml.

HIV-1 DNA quantification in blood and semen samples

Total DNA in whole blood was extracted and purified from W32 and W44 samples, using a QiaSymphony DSP DNA assay (Qiagen, Courtaboeuf, France). Total DNA in semen was extracted and purified from all samples during ATI, using a QiaAmp DNA Micro Kit (Qiagen). Total DNA was quantified with a Qubit Fluorometer (DNA High Sensibility Kit; Invitrogen, Illkirch-Graffenstaden, France).

HIV-1-DNA was quantified from all extracts, using real-time PCR (Generic HIV-DNA Cell; Biocentric, Bandol, France) [10]. For blood, results were reported as HIV-1-DNA cp/10^6 PBMC. For semen, results were reported as HIV-1-DNA cp/10^6 NSC. Lower limit of quantification was 1.3 $\log_{10}$ cp/10^6 PBMC or NSC.

454 pyrosequencing and phylogenetic trees reconstruction

After DNA and RNA extraction, amplicons were prepared for 454 pyrosequencing from partial envelope C2V3 sequence. Subsequent sequencing and phylogenetic analysis

were performed if at least two paired blood and seminal plasma samples successfully amplified were available during rebound.Pyrosequencing on 454 GS Junior (Roche) was performed according to the manufacturer's recommendations (see Appendix, http://links.lww.com/QAD/B385).

Maximum likelihood trees were reconstructed from cleaned and realigned resulting collections of C2V3 haplotypes (see Appendix, http://links.lww.com/QAD/B385).

Statistical analysis

Student's *t* test for paired data was used to compare levels of HIV-DNA before and after ATI. The log-transformation of the data allowed assuming normal distribution. Pearson correlation test was used to determine the correlation between HIV-RNA values in blood plasma and semen plasma during the ATI. No comparison between the 'vaccine group' and the 'placebo group' was processed.

Results

Population characteristics at baseline

Ten participants were enrolled in the 'semen substudy' of the VRI02/ANRS149-LIGHT vaccine trial between April 2015 and December 2015. Median [interquartile range (IQR)] age at baseline was 42 years (32–48), CD4 nadir 412/mm^3 (334–518), pretherapeutic bpVL 4.96 $\log_{10}$ copies/ml (4.43–5.16), time with pVL less than 50 copies/ml before ATI 44 months (36–54), and CD4 count at W36 (at the time of ATI) 768/mm^3 (674–862). cART at the time of the beginning of ATI (W36) included tenofovir/emtricitabine in all the participants, plus darunavir/ritonavir, atazanavir/ritonavir or dolutegravir in seven, two, and one participants, respectively. Four participants received the experimental vaccines (P2, P4, P5, P7), six were in the 'placebo group' (P1, P3, P6, P8, P9, P10).

HIV-RNA rebound levels and kinetics in blood plasma and semen plasma

All the bpVL were less than 20 copies/ml at W36. bpVL rebound occurred as soon as W38 for eight of 10 participants, and at W42 and W44 for the two remaining participants (P6 and P9, respectively) (Table 1, Supplementary Figure 1, http://links.lww.com/QAD/B385). Maximum median bpVL during ATI was 5.12 $\log_{10}$ copies/ml (range: 4.61–6.35), and was comparable to pretherapeutic bpVL.

All the spVL were less than 60 copies/ml at W36, except for P7 (2.43 $\log_{10}$ copies/ml at W36, concordant level on two different assays), but less than 60 copies/ml at W38 in this patient. spVL rebound occurred as soon as W38 for 4/10 participants (P1, P4, P5, and P10), and as of W40 for four of 10 participants (P2, P7, P8, and P9). Semen sample was missing at W40 and W42 for P3 and P6,

Table 1. Virological assessment before and during antiretroviral therapy interruption.

Participants	First time-point of HIV-1-RNA rebound in blood plasma	First time-point of HIV-1-RNA rebound in seminal plasma	HIV-1-RNA maximum level in blood plasma ($\log_{10}$ copies/ml) (visit)	HIV-1-RNA maximum level in seminal plasma ($\log_{10}$ copies/ml) (visit)	HIV-1-DNA level in PBMC ($\log_{10}$ cp/10^6 PBMC) (visit)	HIV-1-DNA level in NSC ($\log_{10}$ cp/10^6 NSC) when quantification was possible (visit)
1	W38	W38	5.12 (W44)	3.20 (W44)	1.61 (W32) 3.04 (W44)	–
2[a]	W38	W40	5.12 (W40)	3.81 (W42)	3.02 (W32) 3.30 (W44)	2.02 (W48)
3	W38	W42[b]	5.12 (W40)	4.56 (W42)	3.06 (W32) NA (W44)	–
4[a]	W38	W38	5.30 (W42)	3.40 (W42)	2.76 (W32) NA (W44)	–
5[a]	W38	W38	5.26 (W38)	4.33 (W48)	3.11 (W32) 3.67 (W44)	1.98 (W44)
6	W40	W44[b]	5.07 (W42)	4.27 (W44)	3.16 (W32) 3.57 (W44)	–
7[a]	W38	W40	6.35 (W40)	4.49 (W40)	2.93 (W32) NA (W44)	1.64 (W44) 2.34 (W48)
8	W38	W40	4.96 (W40)	3.65 (W42)	2.41 (W32) 3.09 (W44)	<1.3[c] (W40)
9	W42	W44	4.61 (W44)	4.26 (W44)	<1.3 (W32) 2.50 (W44)	<1.3[c] (W44)
10	W38	W38	4.67 (W48)	4.67 (W48)	3.26 (W32) 3.37 (W44)	<1.3[c] (W44)

NSC, nonsperm cells; NA, not available sample.
[a]Participants assigned to the vaccine group.
[b]Missing HIV-1-RNA value at the previous time-point.
[c]Not quantifiable but detectable signal.

respectively. Maximum median spVL during ATI was 4.26 $\log_{10}$ copies/ml (range: 3.20–4.67). There was a strong correlation between HIV-RNA values from paired blood samples and semen samples during ATI ($r = 0.77$, $P < 0.001$).

HIV-DNA levels in PBMC and nonsperm cells

HIV-DNA was detected and quantified from all, except one, blood samples (Table 1). The median HIV-DNA level was 2.97 $\log_{10}$ cp/10^6 PBMC (IQR: 2.50–3.10) at W32 ($n = 9$) and 3.30 $\log_{10}$ cp/10^6 PBMC (IQR: 3.01–3.47) at W44 ($n = 7$). For participants with available blood samples at W32 and W44, the difference of HIV-DNA level before and after ATI was +0.58 $\log_{10}$ cp/10^6 PBMC (CI95% [+0.09;+1.06], $P = 0.028$).

During ATI, HIV-DNA was detected in NSC of six of 10 participants (Table 1), from seven of 57 semen samples. The median HIV-DNA level was 1.64 $\log_{10}$ cp/10^6 NSC (IQR: 1.02–1.98). No NSC samples were available before ATI.

Phylogenetic analysis of sequences

Phylogenetic analyses were processed in the five individuals, for whom the amplification of at least two paired blood and seminal plasma samples during HIV-RNA rebound was successful (Fig. 1).

In each individual, sequences were highly homogenous soon after ATI, with genetic diversity increasing over time. Tree topologies showed an absence of segregation of blood plasma and seminal viruses, which is consistent with the lack of compartmentalization at any given time-point with intermingled sequences from both compartments overtime, and suggest possible replenishment from PBMC. Of note, in participant 4, premature resumption of cART yielded a dramatic population restriction at the time-point following cART resumption.

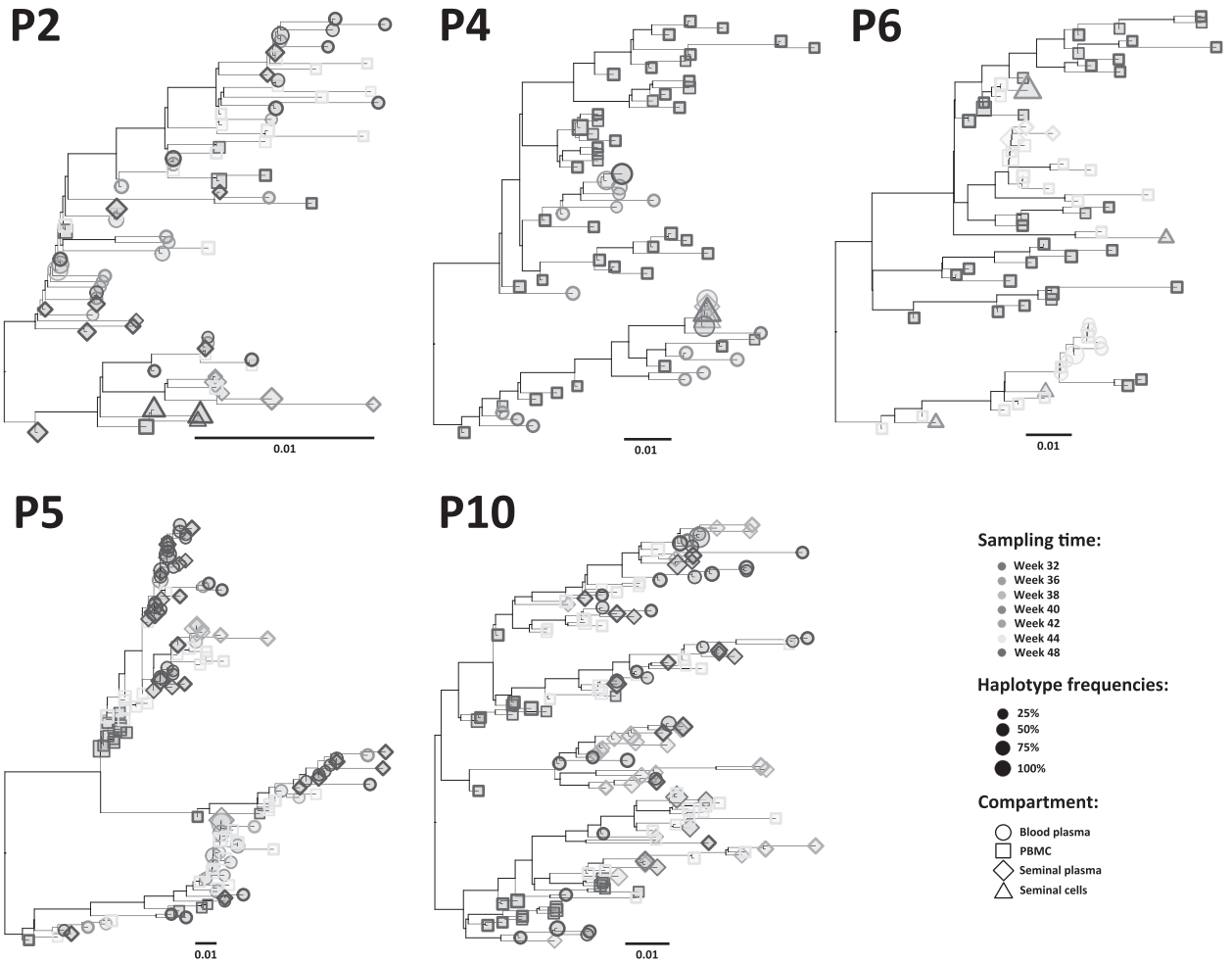

Fig. 1. Phylogenetic trees from HIV-DNA and HIV-RNA ultradeep sequenced from PBMC, seminal cells, blood plasma and seminal plasma (patients 2, 4, 5, 6 and 10). Legend: The collections of C2V3 haplotypes obtained from each samples were realigned using ClustalW and piped to FastTree 2 for approximate maximum likelihood trees reconstruction, and subjected to codon-based phylogenetic analyses using a Tamura Nei substitution model with a gamma-distributed substitution rate.

Discussion

We report here the first longitudinal study assessing HIV-RNA rebound in blood plasma and seminal plasma during a structured ATI, allowing sequential collection of paired samples. Early HIV-RNA rebound was observed in all participants in both compartments, before the fourth week of ATI in eight 10 of them, reaching high levels of spVL. This finding supports evidence of a very high risk of sexual transmission during self-driven cART breaks [11] or during ATI stemming from clinical trials [12]. Thus, prevention strategies for HIV-negative partners of HIV-infected participants undergoing ATI need reinforcement (counselling, condoms, PrEP) [13]. One HIV transmission occurred from a VRI02/ANRS149-LIGHT vaccine trial participant to his partner, despite conselling provided during the study, unfortunately confirming the increased risk of HIV acquisition through sexual intercourse during ATI.

The level of pre-ATI PBMC HIV-DNA measured in our participants was consistent with previously published data on long-term virologically controlled subjects [14]. Here we described a significant increase in PBMC HIV-DNA after ATI, in keeping with another recent study [15]. Clarridge *et al.* [1] showed that the increase in markers of HIV reservoir is transient and returns to baseline 6–12 months after the resumption of cART.

HIV-DNA was detected in NSC of six of 10 patients, at least at one time-point during ATI, corresponding to 12% of all the tested samples. This finding is consistent with the assessments carried out by Gantner *et al.* [16] on a larger sample size of patients with similar virological suppression time.

A comprehensive evaluation of how HIV populations colonize and migrate between blood and the male genital tract is critical to assess the risk of sexual transmission of HIV, and to design possible eradication strategies. Here, we did not find evidence suggesting local viral production in semen after cART discontinuation. Indeed, intermingled HIV populations from blood and semen argue against the idea of autonomous HIV replication in the male genital tract over time. These findings suggest that, if a therapeutic vaccine was to be effective on the control of HIV-RNA replication in blood plasma after cART discontinuation, it should also allow for similar control in semen.

Our study has several limitations. First, the sample size is small but our study has the advantage of being longitudinal. Unfortunately, the study was not designed to show differences in HIV-RNA rebound in vaccine and nonvaccine recipients. Second, HIV-DNA was detectable in few NSC samples, which might have underestimated the potential role of NSC HIV-DNA in the origin of spVL. Third, the interpretation of phylogenetic trees may have been hampered by the lack of HIV sequences at several time points. However, the frequency and variety of sampling in these participants remain unique in ATI studies and provide invaluable information for exploring the dynamics of HIV in blood and the genital tract following ART interruption. Finally, the 454/Roche sequencing platform leads to several technical limitations (see Appendix, http://links.lww.com/QAD/B385), limiting the interpretation of phylogenetic data.

Overall, the rapid and intense HIV-RNA rebound observed very early both in blood and semen after ATI emphasizes the need for targeted prevention strategies to reduce the risk of sexual transmission during all the trials involving treatment interruptions. Intermingled rebounding HIV sequences between blood and semen suggest that future therapeutic vaccines or HIV cure strategies should be a useful tool to reduce cART prolonged exposure while allowing for a sustained reduction in the risk of sexual transmission.

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Previous presentation of results: This work has been presented at the 9th IAS conference on HIV science, Paris, France, in July 2017.

Conflicts of interest

Authors declare to have no conflict of interests regarding this study.

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