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Impact of cART & Systemic Inflammation on Semen HIV-1 Reservoir in Primary Infection - ANRS 147 Optiprim

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Background
The risk of sexual transmission is conditioned by the presence of HIV-1 infected cells and viral particles in genital secretions. Infected cells in semen represent an increased risk of HIV-1 cell to cell transmission Primary HIV Infection (PHI) is brief but very efficient in terms of viral transmission. We studied the impact of early-cART in PHI and the influence of the systemic activation on HIV-semen and blood reservoir dynamic.

Methods
Patients from the ANRS-147-OPTIPRIM randomized trial received two years of early-cART. Blood and seminal samples were collected at inclusion and month 24. Total cell-associated-HIV-DNA ((Biocentric, Bandol, France) were quantified in blood) and in semen cells and HIV-RNA in blood (Roche or Abbott) and seminal plasma (roche). Interferon-γ-inducible interferon 10 (IP-10) and interleukin-6 (IL6) were quantified by ELISA in blood plasma. Spearman correlation tests were performed.

Results
Twenty-one patients participated to this substudy (median age: 36 years, time from estimated date of infection: 33 days), 20 were symptomatic and 8 presented acute infection (WB ≤1 Ab).At inclusion, median HIV-RNA was significantly higher in blood (5.39 log_{10}cp/mL) than in semen (4.22) (p< 0.0001).The median of blood and seminal HIV-DNA was 3.59 and 0.31 log_{10}cp/10⁶PBMC respectively. Semen HIV-RNA was correlated with CD4 count (r=-0.54, p=0.018) and CD8 count (r=-0.54 p=0.018). Furthermore, IP-10 was positively correlated with blood HIV-RNA (r=0.46 p=0.046), blood and semen HIV-DNA (r=0.53 p=0.018; r=0.51 p=0.026), IL-6 (r=0.68 p=0.003) and negatively with CD4/CD8 ratio (r=-0.59 p=0.006) (Figure1). Among 8 patients with acute infection, semen-HIV-RNA was correlated with blood-HIV-RNA (r=0.81, p=0.015), CD4 count (r=-0.98, p<0.0001), CD4/CD8 ratio (r=-0.85, p=0.0075).Two years effective cART induced an important decrease of blood and semen HIV-RNA levels <threshold of detection, IP-10 level (p=0.004) but did not impact IL-6 level. Semen HIV-DNA level similarly decreased in all but one patient who maintained high IP-10 and IL-6 levels and who reported the use of recreational drugs at that time point (that might explain this positive result as previously reported).

Conclusions
This is the first evidence of HIV-reservoir cells in semen of patients with PHI, showing that levels are linked with the immunosuppression severity and plasma IP-10 level.. Early treatment allows purging viral particles but also infected cells that reduce the important risk of transmission at PHI.

Background

Primary-HIV-Infection (PHI) is a high-risk period for viral transmission. Few data are available on the efficacy of cART initiated at the time of PHI on HIV genital shedding, and none regarding HIV reservoir in the genital tract (*C.D.Pilcher, AIDS 2007*).

Results from the **ANRS-147 OPTIPRIM trial** showed that the efficacy on HIV blood reservoirs of a two year-early pentatherapy containing raltegravir plus maraviroc did not differ from standard cART (***Chéret et al, Lancet ID 2015***).

Objectives

The objective of this substudy nested in the Optiprim ANRS-147 trial was to assess HIV shedding in semen. Blood and semen HIV-RNA and HIV-DNA were quantified to assess the impact of early treatment in patients with PHI.

Methods

- **Patients presenting with PHI** (inclusion criteria: HIV-1 western-blot (WB) ≤4 antibodies (Ab) and positive HIV-RNA, and CD4<500/µL in case of asymptomatic PHI) were enrolled in the **ANRS-147 OPTIPRIM** study and received two years of an early cART.
  21 patients agreed to participate in this HIV reservoir substudy

- **At inclusion and month 24:**
- Total HIV-DNA, in blood and semen cells were quantified using an ultrasensitive real-time-PCR (Biocentric, Bandol, France).
- HIV-RNA in blood and seminal plasma were quantified using an ultrasensitive real-time-PCR, (Roche or Abbott, Roche respectively)
-IP-10, IL-6, sCD14, sCD163 were quantified in duplicate, with specific ELISA assays (Human IL-6 Platinum ELISA, eBioscience; Human quantikine CXCL10 ELISA,; Human CD14 DuoSet ELISA and Human CD163 DuoSet ELISA, R&D Systems, Minneapolis, Minnesota).

- **Analysis was conducted overall and after differentiating acute (≤ 1Ab on WB) from recent PHI.**
- Wilcoxon signed rank test was used to estimate differences between HIV-RNA levels in semen and blood and Spearman test to estimate correlations with quantitative baseline characteristics.

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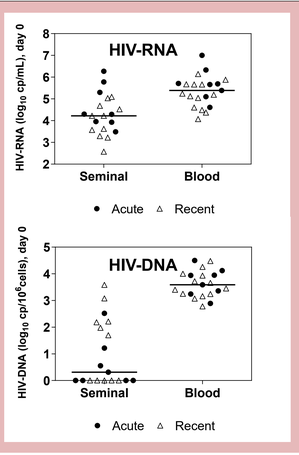
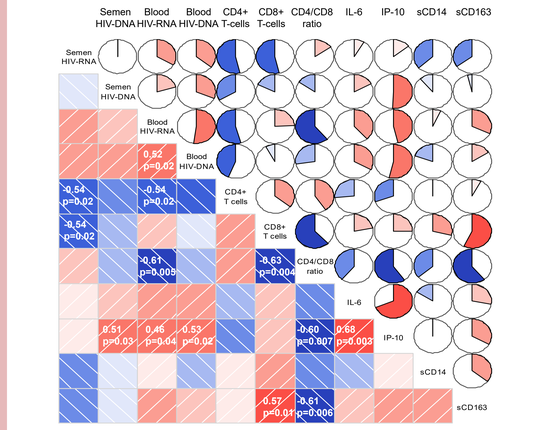
At enrollment

PATIENTS CHARACTERISTICS	Semen study N=21
Men	21 (100%)
MSM	19 (90%)
Age, years	36 (20-59)
Place of birth:	
Europe	18 (85%)
Sub-Saharan Africa	1 (5%)
Other	2 (10%)
Symptomatic primary infection	20 (95%)
Acute primary infection	8 (38%)
Time between estimated time of infection and enrolment (days)	33 (19- 49)
Seminal plasma HIV-RNA, log cp/mL n=19	4.22 (2.57- 6.27)
Seminal cell-associated HIV-DNA, log cp/10⁶ cells n=19	0.31 (0.00-3.58)
Seminal HIV-DNA	
Detectable	10 (53%)
Undetectable	9 (47%)
Blood plasma HIV-RNA, log cp/mL	5.39 (4.07- 7.00)
Blood cell-associated HIV-DNA, log cp/ 10⁶ PBMC	3.59 (2.78- 4.50)
CD4 count, cells per µL	465 (163 -1116)
CD8 count, cells per µL	1088 (438-5148)
CD4 to CD8 ratio	0.42 (0.14- 1.18)
HIV-1 subtype B (vs. non-B)	13 (62%)
R5 HIV-1 tropism (vs. X4)	21 (100%)
IL-6, pg /mL n=16	1.11 (0.10- 4.30)
IP-10, pg/mL n=19	184.93 (93.88 - 1910.87)
sCD14, pg/mL n=19	2.10 (1.35 - 9.02)
sCD163, pg/mL n=19	0.45 (0.25 - 1.60)

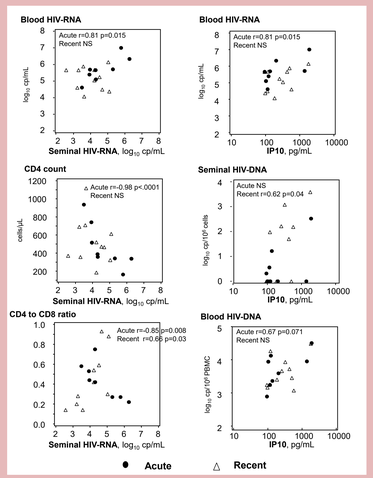
Demographic and baseline characteristics were similar in ANRS-147 total and Substudy populations
- Data are number (%) or median (min-max).
- MSM= men who have sex with men. PBMC = peripheral blood mononuclear cells.
- Acute HIV infection was defined by the presence of one band or fewer on HIV-1 western blot plus detectable plasma HIV-RNA.
- Seminal plasma HIV-RNA and HIV-DNA and biomarkers data were missing for two participants.



HIV-RNA and HIV-DNA levels at inclusion were significantly higher in blood than in semen (median Δ=1.21 and 2.69 respectively for paired data, p-value <.0001).

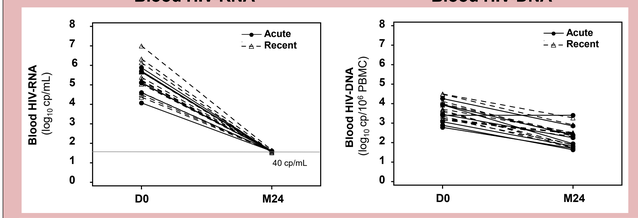


Heatmaps and pie-charts indicate associations between the variables. Red color displays a positive correlation and blue a negative correlation. Their intensity and size of pie, represent the strength of the association. Correlations are noted when their p-value < 0.05.

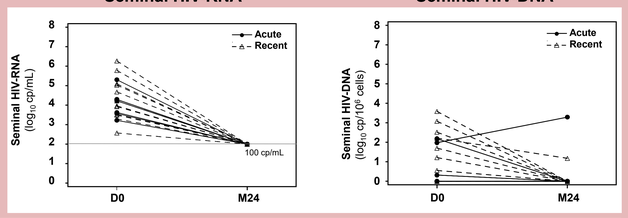


Correlations among patients:
- **Acute infection** (n=8) or
- **Recent infection** (n=11)

Impact of two years of early-cART



Blood and seminal HIV-RNA/DNA evolution between D0 and M24
All patients had indetectable level of blood and seminal HIV-RNA at M24.
Interestingly, the only one patient with seminal HIV-DNA increase during treatment reported use of recreational drugs (cocaïn) at M23, which might explain this positive HIV-DNA quantification at M24.



Conclusions

- This is the **first study quantifying HIV-reservoir cells in semen** of patients with acute or recent infection.
- The seminal reservoir is progressively established from acute to recent PHI, as well as blood IP-10 level underlying the major role of this cytokine during PHI.
- HIV reservoirs in semen are linked with the immunosuppression severity.
- Treating as early as possible in PHI **allows not only purging viral particles but also infected cells to limit cell to cell transmission and reduce drastically the risk of HIV transmission.**