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Introduction

- In large French cohorts of patients (seroconverters SEROCO, HIV controllers, long-term nonprogressors ALT – Agence Nationale de Recherches sur le SIDA ANRS), HIV reservoir size is usually estimated by **quantification of total HIV DNA in peripheral blood mononuclear cells (PBMC)**.
- The HIV DNA level in PBMC is predictive of disease progression in seroconverters.
- The HIV DNA level permits to distinguish groups of patients: HIV controllers had lower HIV DNA level than ALT, who had themselves lower HIV DNA level than patients who do not control HIV infection.
- **Is the HIV DNA quantification feasible and reproducible in rectal biopsies?**
- **What size has the HIV reservoir in some profound tissues for these different groups of patients, particularly those who had a low level of HIV DNA in PBMC?**
- **Does the peripheral blood compartment reflect well the HIV reservoir in profound tissues?**

Patients

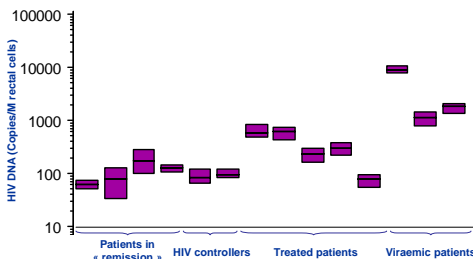
- **4 patients in "remission"** i.e. patients who had initiated HAART during the primary infection, were treated during 25 to 87 months before interruption and presented a sustained viral and immunologic control > 30 months after interruption (HIV-1 RNA < 50 copies/mL (1.7 Log) and a CD4 cell count greater than 500 cells/μL)
- **2 HIV controllers (infected by HIV for 6 and 21 years, < 5000 copies HIV RNA/mL (3.7 Log), > 700 CD4 cells / μL without antiretroviral treatment)**
- **5 patients with infection efficiently controlled on HAART initiated during chronic phase**, HIV-1 RNA < 50 copies/mL (1.7 Log) since over 12 months
- **3 viraemic patients**, median HIV-1 RNA: 31600 copies/mL (4.5 Log), never treated or without antiretroviral treatment since over 18 months
- All patients agreed to participate and gave their consent.

Methods

- **Quantification of total HIV-1 DNA**, which included integrated and unintegrated HIV DNA, by **real-time PCR (LTR region - ANRS)**
 - in PBMC
 - in rectal biopsies (Average of 2 PCR results in 4 rectal biopsies (i.e. 8 results per patient))
- **Determination of the median proportion of CD4 cells in rectal biopsies by immuno-staining**
- **Statistical analysis:** Spearman and Mann-Whitney tests

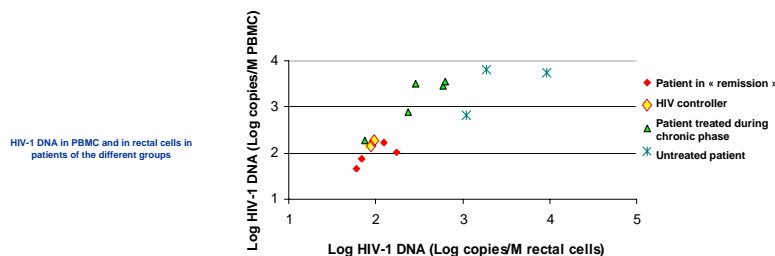
Feasibility and reproducibility study of HIV DNA quantification in rectal biopsies

- A total of 112 HIV DNA PCR has been done.
- **HIV-1 DNA was detectable in all the 56 rectal Biopsies.**
- HIV DNA quantification is **feasible** even in very small rectal biopsies.
- This **box plots representation** underlies that the **HIV DNA quantification in the 4 pieces of rectal biopsies for each patient was very reproducible.**



Introduction

Results



- **Good correlation between HIV-DNA in PBMC and in rectal biopsies** ($r=0.898$, $p=0.001$ and $r=0.799$, $p=0.004$ with HIV-DNA in rectum expressed in Log cp/10⁶ rectal cells or Log cp/10⁶ CD4+ rectal cells respectively).

Virologic and immunologic characteristics of the 4 groups of patients

| Status | CD4+ T cells count cells/mm ³ | median HIV-1 RNA in blood plasma | | median HIV-1 DNA in PBMC | | CD4+ cells count | | median HIV-1 DNA in rectal biopsies | |
|---|---|----------------------------------|------|-----------------------------|-----|-----------------------------|-------------------------------------|-------------------------------------|--|
| | | copies/ mL | Log | copies/10 ⁶ PBMC | Log | cells/ million rectal cells | copies/10 ⁶ rectal cells | Log | |
| patients in "remission" | 930 | < 50 | <1.7 | 80 | 1.9 | 14519 | 100 | 2.0 | |
| HIV controllers | 810 | 2043 | 3.3 | 160 | 2.2 | 15385 | 100 | 2.0 | |
| patients treated during chronic infection | 503 | <50 | <1.7 | 3100 | 3.5 | 32885 | 320 | 2.5 | |
| viraemic patients | 425 | 31600 | 4.5 | 5000 | 3.7 | 7308 | 2000 | 3.3 | |

*HIV-DNA level in PBMC permitted to distinguish two groups of patients: (A) 7 patients with HIV-DNA <2.7 Log cp/10⁶ PBMC (median: 2.2 Log) including 4 patients in "remission", 2 HIV controllers, 1 patient treated during chronic infection and (B) 7 patients with HIV-DNA >2.7 Log cp/10⁶ PBMC (median: 3.5 Log) including 4 patients on HAART from chronic infection and 3 untreated patients. HIV-DNA in rectal biopsies was significantly lower for group A than group B (medians 1.9 vs 2.8 Log cp/10⁶ rectal cells, $p=0.002$).

Conclusions

- HIV DNA can be quantified in rectal biopsies with a good reproducibility.
- Patients in "remission" and HIV controllers showed a very low HIV-DNA level, not only in blood but also in some profound anatomical reservoirs such as rectum.
- HIV-DNA level in blood reflects HIV-DNA level in rectal biopsies for patients at different stages of HIV infection, efficiently treated and untreated.
- Similar studies should be investigated with other profound reservoirs (beum, jejünium); however such investigations are much more complicated and are not performed usually in clinical practice whereas rectal biopsies are realised during proctologic explorations (i.e. HPV).

