# Skewed distribution of HIV-2 reservoir with limited input of central memory T-cells

**Assia Samri**[1*], Charlotte Charpentier[2*], Mélanie Bertine[2], Mariama Diallo[1], Sophie Even[1], Sophie Matheron[3], Rodolphe Thiébaut[4], Brigitte Autran[1,5], Françoise Brun-Vezinet[6] and the ANRS CO5 IMMUNOVIR-2 Study Group

*equal contributors
1. UPMC Univ Paris 06, UMRS CR7 INSERM U1135 CIMI - F-75013, Paris, France
2. IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France
3. IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, Service de Maladies Infectieuses et Tropicales, Hôpital Bichat, AP-HP, Paris, France
4. INSERM U1219, INRIA SISTM, Univ. Bordeaux, Bordeaux, France
5. AP-HP, Hôpital Pitié-Salpêtrière, Département d'Immunologie, F-75013, Paris, France
6. Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France

## BACKGROUND AND OBJECTIVES

HIV-2 is a Lentivirus responsible for a less pathogenic infection than the infection caused by HIV-1, and characterized by a slow clinical progression, a prolonged maintenance of CD4 lymphocytes, a high proportion of untreated patients with undetectable plasma viral load. While the latent reservoir of integrated but inducible HIV-1 provirus is known to predominantly take place in resting memory lymphocytes CD4 (TCM and TTM) and in few macrophages, no data exist on the distribution of HIV-2 in the CD4 + cells.

The objectives were:

-To analyze: i) the latent and inducible blood reservoir of HIV-2, ii) the immune activation in the different sub-populations of peripheral CD4+ cells from untreated patients of the HIV-2 ANRS CO5 cohort.

-To study in the same patients the correlations between the reservoir results and the immunological characteristics of immune activation, and with other host parameters such as lymphocyte aging, anti-HIV2 CD8 T cell response and HLA which are already determined in parallel by an ANRS research consortium.

-To compare the results of the HIV-2 reservoirs to those already obtained in the ALT (Asymptomatic Long Term) ANRS CO15 cohort infected by HIV-1.

## SUBJECTS and METHODS

**Patients**: The patients' characteristics are reported in Table 1.
The study is performed on at least 14 samples from patients infected with HIV-2 of the cohort ANRS CO5, including 12 LTNP (Long Term Non Progressors) and 2 progressors.

**Methods**:
The latent reservoir is studied on sorted CD4+ subsets from CD8 depleted cells as follows: The cells have been thawed and depleted from CD8 cells. Sorting CD4 + total cells is performed on the FACSAria 5 lasers placed in a BSL3, to separate the following subsets:
Naïve T cells (TN): CD45RA+CCR7+CD27+; Central memory T cells (TCM): CD45RA-CCR7+CD27+; Transitional memory T cells (TTM): CD45RA-CCR7-CD27+; Effector memory T cells (TEM): CD45RA-CCR7-CD27-; Resting CD4 T cells: CD25-CD69-HLADR-; Activated CD4 T cells: CD25+CD69+HLADR+; Monocytes: CD3-CD4- or low CD14+ . A detailed cell sorting scheme is shown.

Dried pellets of each sorted fraction are stored for HIV-2 DNA quantification. To do this, DNA was extracted from sorted CD4+ T cells subsets using QIAamp DNA Mini kit (n = 57) or QIAamp DNA Micro kit (n = 41) when subset counted less than 500 000 cells.
HIV-2 DNA viral load was quantified using a real-time PCR assay with a 95% limit of detection (LOD) of 3 c/PCR and a limit of quantification (LOQ) of 6 c/PCR.

The inducible reservoir was evaluated by culturing 5 million of CD8- T- cell from available samples for eleven subjects with anti-CD3+CD28+IL-2+IL-7 or IL-15 up to 30 days.

**Statistics:**
A two-tailed Wilcoxon matched-pairs signed rank test was used to compare cell subsets, and Mann-Whitney test to compare the different groups. A p value lower than 0.05 was considered as a significant. All values given in the text are medians and [IQR 25–75%].

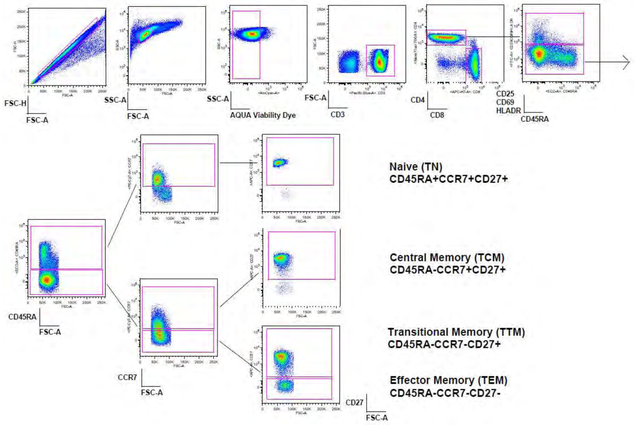
## BASELINE HIV-2 PATIENTS CHARACTERISTICS

| Patients | Gender | Age (years) | CD4 T cell counts (cells/mm³) | Time since diagnosis (years) | Plasma Viral Load (copies/ml) | Ultra Sensitive plasma Viral Load (copies/ml) | HIV-2 DNA load (copies/10⁶ PBMC) | HIV-2 DNA load (log 10 copies/ml /10⁶ PBMC) | HLA A | HLA B | HLA C | Country of Birth |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Long-Term Non-Progressors** | | | | | | | | | | | | |
| 012-073 | M | 52 | 891 | 11.3 | <40 | <1 | 131 | 2.12 | 02/03 | 49/57 | 07/18 | Ivory Coast |
| 036-019 | M | 53 | 1228 | 17.6 | <40 | <1 | 170 | 2.23 | 23/23 | 07/14 | 07/08 | Guinea Bissau |
| 013-049 | F | 52 | 604 | 25.6 | <40 | 19 | 139 | 2.14 | 02/23 | 15/52 | 02/16 | Guinea Conakry |
| 082-005 | M | 57 | 1090 | 23 | <40 | ND | 107 | 2.03 | 23/34 | 53/53 | 04/04 | Ivory Coast |
| 012-101 | F | 34 | 1212 | 12.5 | <40 | 5.6 | 88 | 1.94 | 03/26 | 58/58 | 03/07 | Gambia |
| 012-084 | F | 50 | 1776 | 8.8 | <40 | <1 | 53 | 1.73 | 68/68 | 07/52 | - | Ghana |
| 036-018 | F | 39 | 1036 | 12.7 | <40 | <1 | 53 | 1.73 | 03/03 | 35/53 | 04/04 | Ivory Coast |
| 013-035 | F | 48 | 859 | 9.2 | 101 | 75 | 40 | 1.60 | 34/34 | 15/53 | 02/04 | Guinea Conakry |
| 028-016 | F | 49 | 895 | 11.3 | <40 | <1 | 28 | 1.45 | 02/68 | 15/51 | 14/16 | Ivory Coast |
| 012-009 | F | 54 | 1118 | 27.3 | <40 | 2.4 | 10 | 0.99 | 03/74 | 14/15 | 07/08 | Guinea Conakry |
| 013-037 | M | 59 | 1300 | 8.8 | <40 | <1 | <6 | - | 01/29 | 44/57 | 06/16 | Colombia |
| 012-006 | M | 44 | 707 | 20.5 | <40 | 1.3 | 187 | 2.27 | 03/23 | 35/53 | 04/04 | Ivory Coast |
| **Progressors** | | | | | | | | | | | | |
| 012-045 | F | 43 | 858 | 22.7 | <40 | <1 | 10 | 1.00 | 1/33 | 15/35 | 04/14 | France |
| 012-088 | M | 70 | 502 | 7.4 | 117 | 58 | 127 | 2.10 | 02/02 | 27/53 | 02/04 | Senegal |
| Median | | 51 | 966 | 12.6 | | 4* | 88* | 1.94* | | | | |
| IQR | | | 820-1216 | 9.7-22 | | 2.1-9 | 40-131* | 1.53-2.13 * | | | | |

ND: not done
* in patients with detectable HIV-2 DNA **or RNA**

**Patients Characteristics:** HIV-2 controllers were part of the ANRS CO5 VIH-2 cohort and included in the ANRS IMMUNOVIR 2/RESERVOIRS study, which focused on the study of patients with nonprogressive infection. All patients in the current study (Table) had characteristic features of HIV controllers (i.e., asymptomatic treatment naive individuals).
Patients were infected since in median 12.6 [IQR: 9.7-22] years, the median CD4 counts was 966 [IQR: 820-1216] cells/mm$^3$.
Plasma viral load (pVL) was <40 c/mL in 12 patients, among them 4 had a positive ultra-sensitive pVL median 4 c/mL [IQR=2.1-9]. Median total HIV-2 DNA in PBMC was above the LOQ in 13 patients with a median of 1.94 $\log_{10}$ c/10$^6$ PBMC [IQR=1.53-2.13].

## Methods: cell-sorting scheme

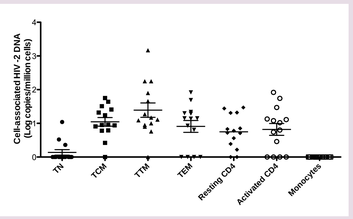



## CONCLUSIONS

**Overall, these HIV-2-infected patients had low circulating HIV-2 reservoirs that were quantifiable in only 5 of the 14 patients tested, mainly distributed in TTM. HIV-2 DNA was undetectable among monocytes suggesting that these cells do not constitute a reservoir for the HIV-2. Among these 5 patients, HIV-2 was reactivable *in vitro* in 3 of the 4 assessable patients. These results confirm the hypothesis of a limited reservoir in TCM, thus supporting the concept of the relative protection of central-memory T cells as an attribute of low pathogenicity models of HIV/SIV infection.**

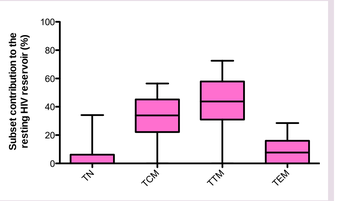
## RESULTS

### Total HIV-2-DNA measurement in peripheral blood cell subsets



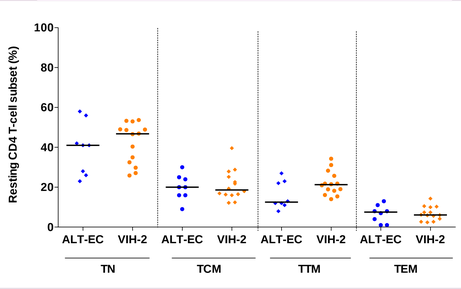

After sorting, HIV-2 DNA was undetectable among monocytes, and above the LOQ only in TTM from 4 patients (median=2.25 [IQR: 1.99-2.94] $\log_{10}$ c/10$^6$ cells) and in TCM from 1 patient (1.75 $\log_{10}$ c/10$^6$ cells). HIV-2 DNA levels were above the LOD in 3, 12, 9 and 10 patients in TN, TCM, TTM and TEM, respectively.

### Resting CD4+ T-cell subsets contribution to the HIV-2 reservoir



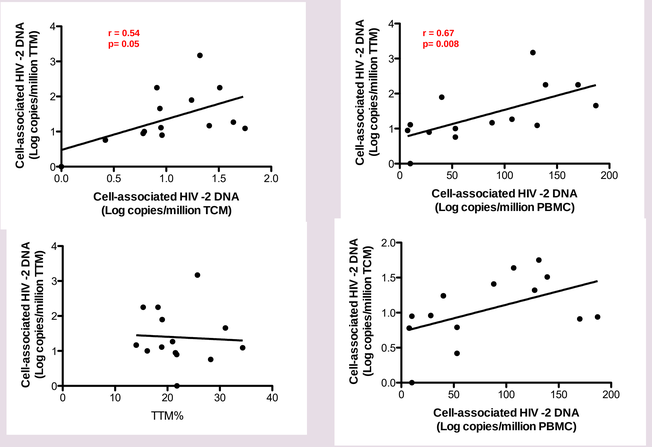

Each subset contribution to the pool of infected resting CD4 T cells was then calculated by taking into account the infection level and frequency in blood of each subset.
The median contribution of TN, TCM, TTM and TEM to the HIV-2 reservoirs was 0%, 33%, 46% and 8%, respectively.

### The repartition of resting CD4 T-cell subsets: phenotypes Comparison with the ALT study



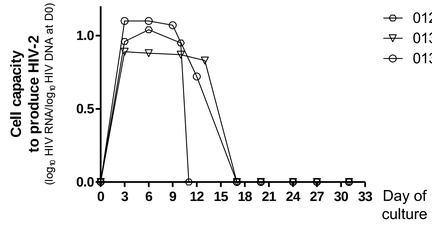

Comparing the resting CD4 subsets frequency of the fourteen HIV-2 controllers individuals to the one of eight HIV-1 controllers individuals showed a similar CD4 subsets repartition among these HIV-infected individuals.

### HIV-2 reservoirs in TTM and TCM subsets: correlation studies




We determined the relationship between: TTM and TCM infection levels; TTM and total PBMC infection levels; TTM infection levels and % of TTM in blood ; TCM and total PBMC infection levels.
The HIV-2 DNA levels in TTM were positively correlated to those in PBMC (p=0.008; r=0.67) and to those in TCM (p=0.05; r=0.54)

### Inducibility of HIV in CD8- T cell




After a strong TCR-stimulating signal (anti-CD3/anti-CD28 co- stimulation) and IL-2 + IL-7 or IL-15 stimulation at D0, HIV-2 RNA was detected in only 3 of the 11 tested samples. These three patients had the highest HIV-2 DNA values that were quantified in TTM (n=2) or TCM (n=1).