Deciphering the HIV-2 Proviral Reservoir in Antiretroviral-naive Individuals

Poster #562

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BACKGROUND

- HIV-2 infection is considered as an attenuated viral infection
- PBMC HIV-2 reservoir has been shown to be smaller than that of HIV-1 with a higher proportion of APOBEC3-induced defective proviruses

- **The aim of this study was to characterise the composition of the HIV-2 cellular reservoir among ARV-naive PWH2 by identifying the different forms of defective proviruses**

METHODS

- HIV-2 ARV-naive participants (PWH2) were recruited from the ANRS national HIV-2 cohort during 2020
- Sequencing of protease, RT and integrase was performed using GridION (Oxford Nanopore Technologies, detection threshold: 5%)
- Bioinformatic analyses were performed using an in-house script (APOBEC3-induced mutations and hypermutations, stop codons)
- We considered all hypermutated pol sequences and those containing a stop codon as defective
- We set up **a specific HIV-2 Intact Proviral DNA Assay (IPDA)**, for both HIV-2 A and B groups, targeting the *Psi* and *env* to quantify 5'- and 3'-defective (due to large internal deletions), env APOBEC3-hypermutated and intact proviral DNA (figure 1,2)

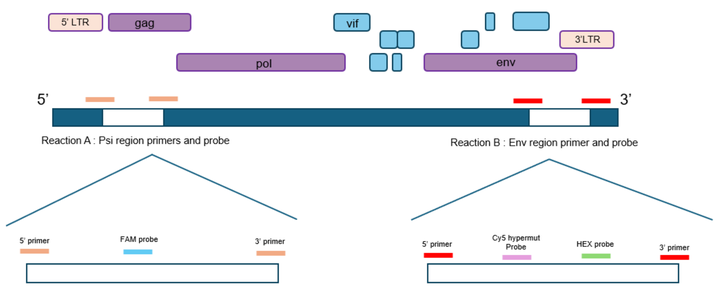


Figure 1: Intact proviral DNA assay set up

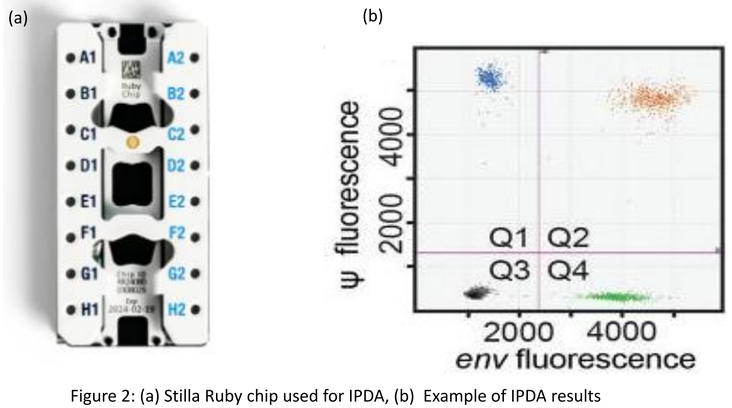


Figure 2: (a) Stilla Ruby chip used for IPDA, (b) Example of IPDA results

- In this cross-sectional study of 57 ARV-naive PWH2, all but 2 with VL <40 c/mL, a specific HIV-2 IPDA was set up to describe the composition of the reservoir

- We showed that a very small proportion of PWH2 harbors intact proviruses and that most of the proviruses constituting the reservoir are hypermutated

RESULTS

Patients

57 PWH2 from the ANRS I MIE national HIV-2 cohort during 2020
Median age : 56 years
Median Nadir CD4 cells : 560/mm^3
61% women

Virological status

55 patients with spontaneously VL <40 c/mL
2 patients with VL <200 c/mL

Nanopore sequencing was successful for 32 samples for protease and RT regions and 20 samples for integrase region
HIV-2 DNA was detectable by IPDA for 30 samples

Nanopore sequencing

- In median **APOBEC3 mutations** were found in **18%** of the APOBEC3 mutation sites in **RT** [IQR = 17%-21%] vs **11%** [IQR = 6%-16%] in **protease** (p=0.0001) (figure3)

- 5 **(16%)** patients had APOBEC3-induced defective proviruses either hypermutated or harboring stop codons in **protease** and/or **RT**

- **1** (5%) patient had APOBEC3-induced defective proviruses on **integrase** gene due to a stop codon

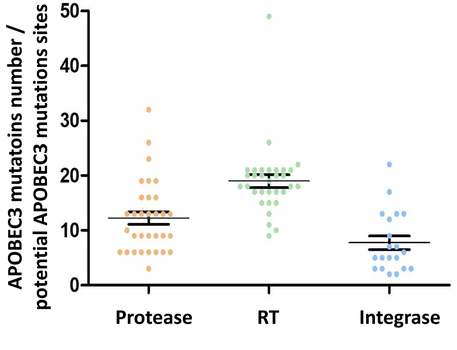


Figure 3: Ratio of APOBEC3 mutations number on potential APOBEC3 mutations sites in sequenced regions

IPDA

- HIV-2 total DNA was <LOQ for 27 samples (48%)

- **Median HIV-2 total DNA**: 303 c/10^6 PBMC (IQR = 189-764)

- 19 PWH2 **(63%)** harbored ***env*-hypermutated** proviruses (figure 5)

- 15 PWH2 **(50%)** harbored proviruses **defective** at either the 5' or 3' end

- 5 (8%) PWH2 harbored proviruses both env-hypermutated and 5'/3'-defective

- **Intact** proviruses were detected in only **one** participant (3.3%)

- **Composition of the reservoir in PBMC**: (figure 4) average of:
  12% 3'-defective proviruses,
  19% 5'-defective proviruses
  67% *env*-hypermutated proviruses

CONCLUSIONS

- In this cross-sectional analysis, we were able to describe the composition of a part of the HIV-2 viral reservoir
- This study describes for the first time the proportion of defective and intact HIV-2 proviruses in ARV-naïve PWH2. A small proportion of ARV-naive individuals harbors intact proviruses, while most proviruses constituting the reservoir are hypermutated or carry large internal deletions
- These findings are consistent with the attenuated profile of HIV-2 infection

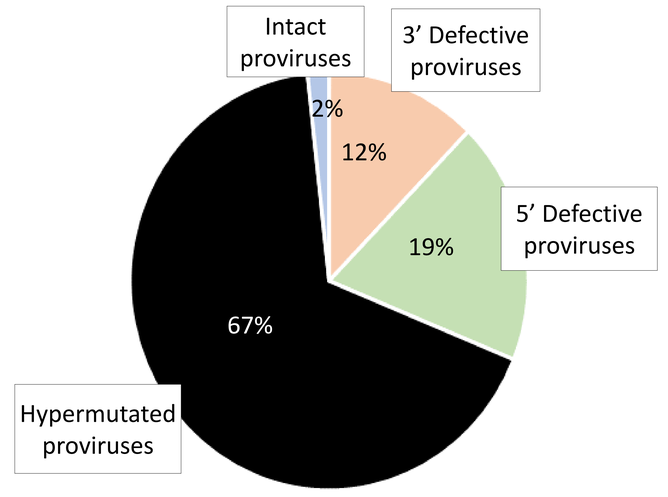


Figure 4: HIV-2 reservoir composition

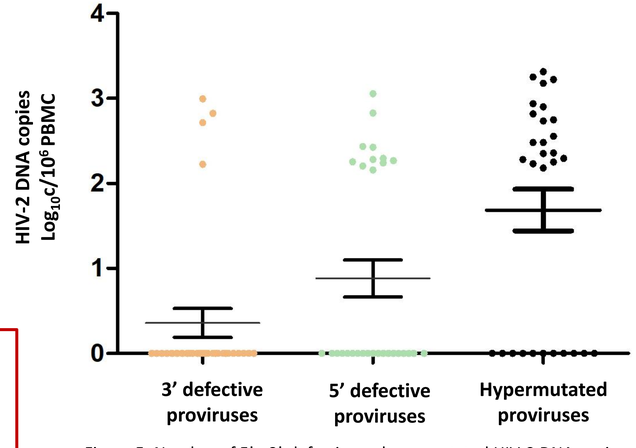


Figure 5: Number of 5'-, 3'-defective or hypermutated HIV-2 DNA copies (c/10^6 PBMC)

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