EPIDEMIOLOGICAL AND MOLECULAR EVOLUTION OF THE HIV-1 CRF94 : BIRTH OF CRF132

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BACKGROUND

In 2018, we reported the emergence of the new HIV-1 recombinant CRF94_02.B.F2 involved in a large transmission cluster of 49 French MSM mostly infected in 2016-2017. This CRF94 strain raised concerns about enhanced virulence. This study reports the molecular and epidemiological evolution of this CRF94 until June 2022.

METHODS

- In 2021-2022, the sequence databases of the laboratories of the French ANRS MIE network were screened for patients diagnosed with an HIV-1 CRF94 virus or a similar *pol* gene recombination pattern.
- Subtypes of the collected strains were confirmed by phylogenetic analysis of their sequences coding for the protease/reverse transcriptase (1070bps) and the integrase gene (696bps), with the 2018 compendium dataset of the Los Alamos National Laboratory (LANL). Phylogenetic trees were constructed using IQ-Tree with a GTR-G nucleotide substitution model and ultra fast boostrap (Fig. 1).
- The DeepChek® assay Whole Genome kit and the DeepChek® analysis sofware were used to obtain five complete genomes from 5 strains classified as "close" but distinct from the CRF94 cluster. Recombination breakpoints of these five sequences were estimated using SimPlot and RDP5.
- The statistical analyses of biological parameters were performed with Mann-Whitney and LogRank tests.
- A Kaplan Meier survival analysis was used to compare the viremia decay after the treatment initiation.

Figure 1. Phylogenetic trees for the 98 Prot-RT sequences and the HIV-1 sequence compendium 2018. Stage of HIV infection : (▲< 1 year; ▼ AIDS; ● undetermined). Year of diagnosis : (**blue (2013-14)**; **pink (2015)**; **yellow (2016-17)**; **red (2018-19)** and **green (2020-22)**

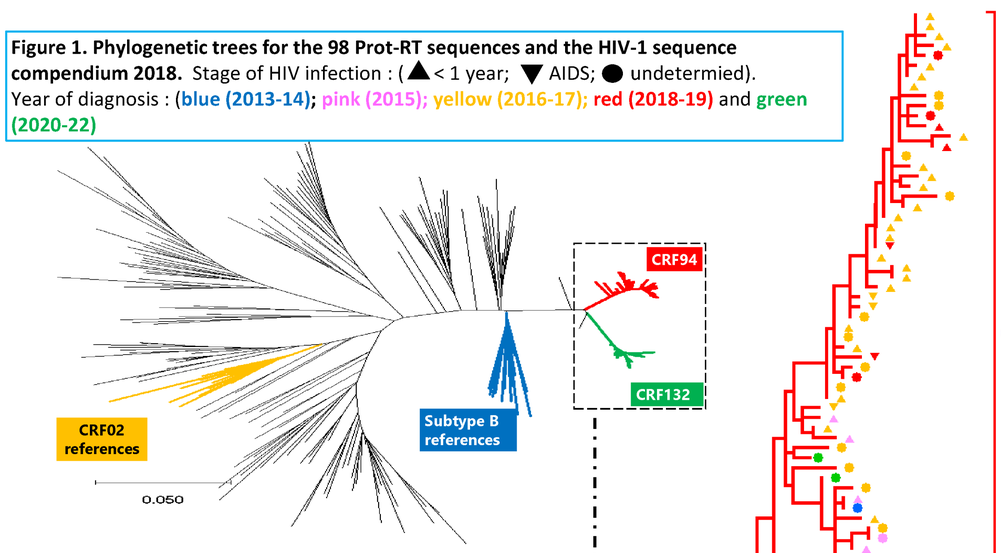
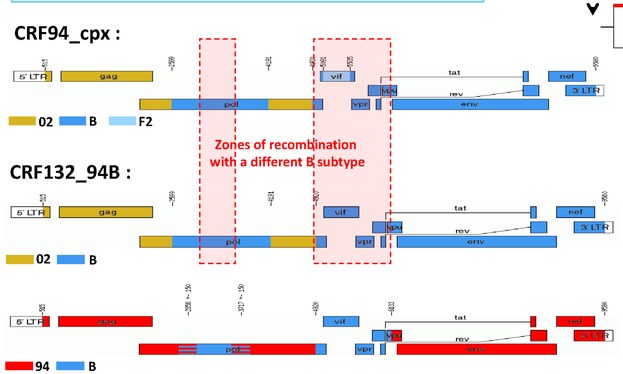


Figure 2. Identified breakpoint positions according to HXB2 subtype B reference.



* New detection of ***CRF94_cpx*** infection virtually ***stopped after 2019.***

* Meanwhile, the related ***CRF132_94B*** was ***discovered*** in a new cluster of MSM.

* ***CRF94 is more virulent*** than CRF132, probably related to the origin of ***F2 subtype*** of the ***vif gene***.

Table 1 . Epidemiological data at the time of diagnosis (percentage or median value (IQR))

	CRF94 (n=63)	CRF132 (n=32)	
Male	98%	100%	
MSM	95%	97%	
Median age (years)	**34 (28-43)**	30 (25-33)	**P=0,0183**
Year of diagnosis	97% in 2013-2019	90% in 2020-2022	
Infection date <1 year AIDS stage	57 % 10 %	77 % 0%	P=0,1425

Table 2 . Biological data at the time of diagnosis (median value (IQR))

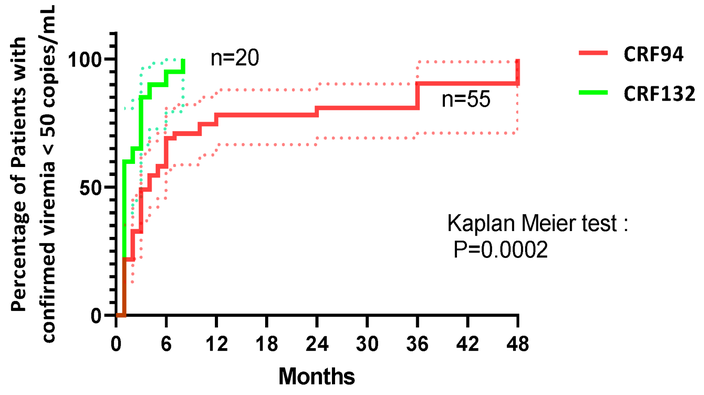
	CRF94	CRF132	
Viral Load (log_{10} copies/mL)			
All HIV infection stages	**5,42 (4,88-5,98)** n=61	4,42 (3,78-5,33) n=31	**P=0,0006**
Acute infections excluded	**5,22 (4,81-5,74)** n=37	4,49 (3,92-5,15) n=16	**P=0,0100**
CD4 count (per mm^3)			
All HIV infection stages	358 (199-550) n=61	**508 (414-686)** n=31	**P=0,0017**
Acute infections excluded	258 (159-408) n=37	**482 (377-716)** n=16	**P=0,0015**

RESULTS

- 49 new HIV-1 sequences were collected and added to the 49 identified in the previous study, before 2018.
- Phylogenetic analyzes of these 98 strains showed that 63 clustered within the CRF94 branch, and 32 were included in a new distinct cluster. The last 3 strains were not included in any of those two large clusters (Fig. 1)
- The analysis of 5 complete genomes, (GenBank accession numbers: ON901787-88-89-90-91) selected from the new cluster, revealed a new recombinant form : the CRF132_94B. It presents the same CRF94 pattern with two new subtype B inclusions in the *pol* gene and in accessory genes. As a result, the *vif* gene changed from F2 to B subtype.
- Except 3 sequences, the cluster CRF132 appeared after 2019 while only 2 CRF94 was detected.
- The comparison of epidemiological and biological parameters between the CRF94 and 132 clusters are resumed in tables 1 and 2. The patients infected with CRF94 had significantly higher viral load (delta of 1 log copies/mL) and lower CD4 count at the time of diagnosis. In addition, 95% of patients in the CRF132 cluster were aware of PrEP.
- Finally, on treatment , patients infected with the CRF94 achieved a confirmed viremia <50 copies/mL significantly later than those infected with the CRF132 (Fig.3) (p=0.0002).

Figure 3 : Time to achieve two consecutive viral loads <50 copies/mL after traitment initiation.

Percentages of patients receiving integrase inhibitor were not significantly different between CRF94 and CRF132 groups: 69% and 75%, respectively.



CONCLUSIONS

- New infection with CRF94 strain were no longer detected after 2019. It's possibly due to: i) the planned targeted prevention actions carried out in the cluster area, ii) the expansion of PrEP, or iii) to the COVID epidemic with a drastic drop in the tourist activity of the company around which the cluster had developed.
- However, during the same period, a new related recombinant CRF132_94B was discovered in another area of the Paris region.
- The biological parameters suggest a lower virulence of CRF132, possibly due to the change of the *vif* gene which has changed from the F2 (CRF94) to the B subtype (CRF132).
- This molecular change of the *vif* gene between CRF94 and CRF132 reinforces previous observations reporting a greater virulence of the HIV-1 subtype F, (Pernas.B *et al.* AIDS, 2014; Cid-Silva. P *et al.* AIDS, 2018), with especially a higher efficacy of Vif against APOBEC3 (Binka. *et al.* M, J.Virol, 2012).
- Contrary to the CRF94, we could not identify a particular population or transmission place, that could allow specific prevention measures for CRF132.

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