Kinetics of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance-associated mutations in HIV-1 blood reservoir in NNRTI-experienced people with HIV: the KINNDeR study

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BACKGROUND

In blood mononuclear cells of people living with HIV (PLWH) with an history of virological failure (VF) some viral strains archived as proviral DNA can harbour mutations conferring resistance to antiretroviral (ARV).

OBJECTIVE

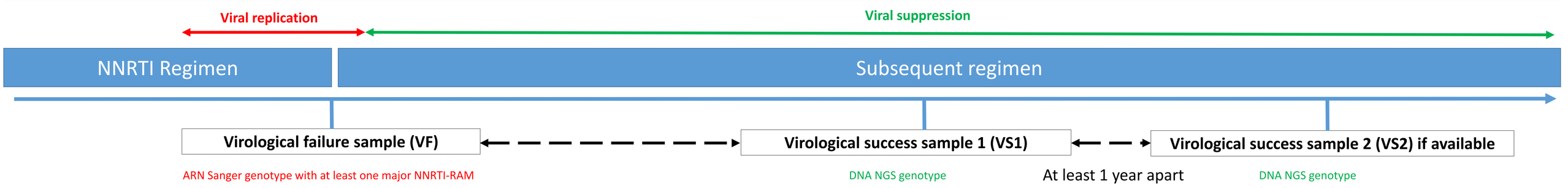
The objective of our study is to describe the temporal evolution of archived resistance-associated mutations (RAM) to non-nucleoside reverse transcriptase inhibitor (NNRTI) using Next Generation Sequencing (NGS).

METHODS

This French single-center retrospective study included patients with :
- Age ≥ 18 years with a history of VF on a NNRTI-containing regimen
- A plasma genotype with one or more major NNRTI-RAM detected by Sanger sequencing (RNA) at time of VF
- At least one blood sample after virological suppression (VS) on subsequent ARV regimens (VS1 and VS2, if available), at least 12 months apart (Fig 1)
- Study was approved by local ethics committee and consent of patients was obtain

NGS sequencing with the ABL® technology of the reverse transcriptase gene and quantification of DNA viral load (Biocentric®) were performed on DNA extracts, a 2% threshold was set for all mutations. Mutational Viral Load (MVL) was calculated by multiplicating the mutation % with the DNA viral load (HIV-1 DNA log_{10} c/10^6cells). Statistics were univariate. The Mann-Whitney test was used for unpaired data and the Wilcoxon test for paired data.

SAMPLES AND STUDY DESIGN



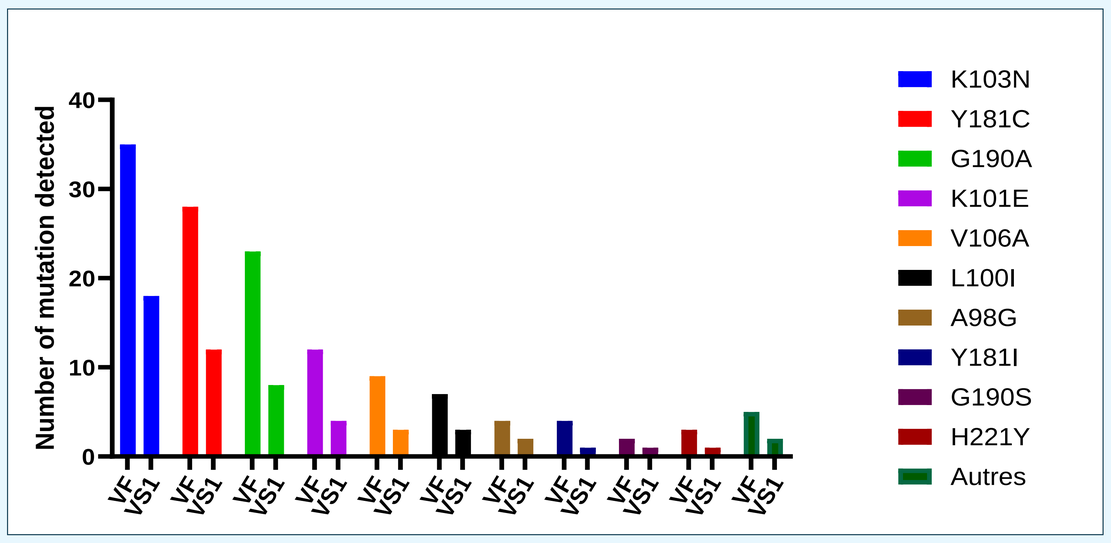
RESULTS

Patients Characteristics at VS1 (n=79)

	Median	IQR
Age (years)	58	55-62
Zenith plasma HIV-1 RNA (log_{10} c/ml)	5.4	4.7-5.8
CD4 nadir (/mm³)	127	35-225
Duration of replication on NNRTI (days)	310	120-1088
Time between HIV diagnosis and VS1, (years)	26	21-30
Time between VF and VS1 (years)	16	11-18
Time between VS1 and VS2 (years)	3	2-5
Duration of viral suppression before VS1 (years)	10	5-13
HIV-1 DNA at VS1 (log_{10} c/10^6 cells) (n=79)*	2.8	2.5-3
HIV-1 DNA at VS2 (log_{10} c/10^6 cells) (n=62)*	2.7	2.4-2.9
Zenith viral load at VF**	4.2	3.6-4.9

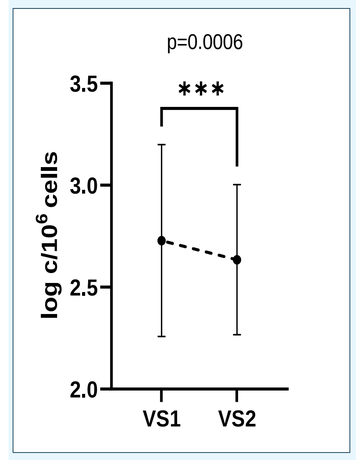
* p=0.0006 DNA viral load decreased significantly between VS1 and VS2
**NNRTI at VF : Nevirapine (n=51) Efavirenz (n=27) and Etravirine (n=1)

NNRTI RAM detected at VF (RNA Sanger) and at VS1 (DNA NGS)



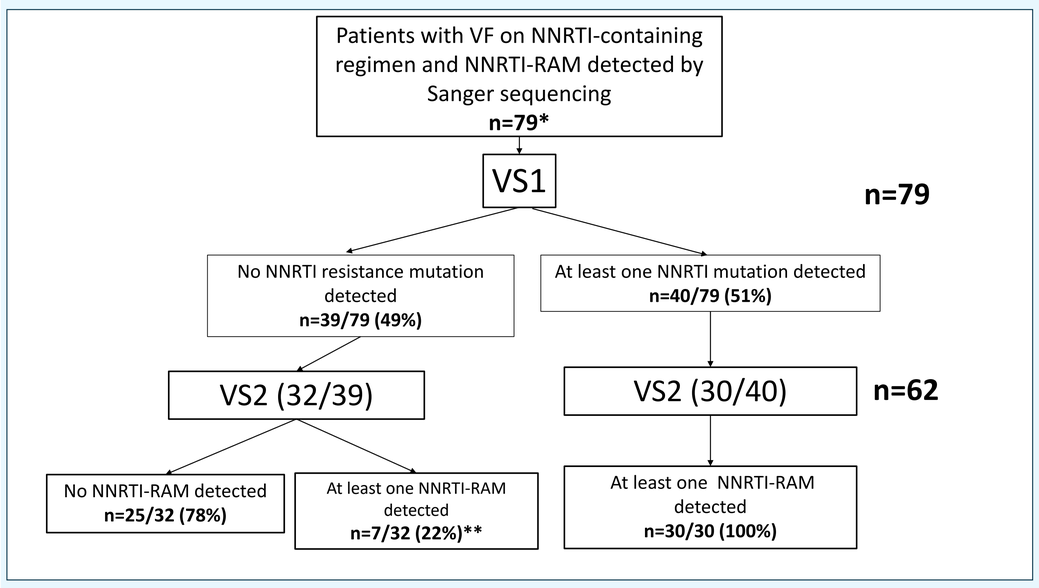
The frequency of detection of the mutations between Sanger sequencing at VF and NGS sequencing at VS1 decreased at the same rate for every mutation

Evolution of DNA viral load



DNA viral load decreased significantly between VS1 and VS2 (p=0.0006)

Flow chart



*84 patients meet the inclusion criteria, NGS DNA sequencing was successful in 79 patients (94%). The 5 patients without NGS result were excluded.

** 9 NNRTI-RAM with a median percentage in NGS of 9% [7.5-18]

At VS1, patients with persistent NNRTI-RAM had a higher zenith plasma HIV-1 RNA viral load at VF (p=0.014), a higher current HIV-1 DNA viral load (p=0.075) and a longer duration of replication on NNRTI (p=0.091).

Changes in DNA mutational viral load (MVL) at VS2 (samples with NNRTI-RAMs detected at VS1) (n=30)

In situations where at least one major NNRTI-mutation was detected on long-term suppression (VS1 and VS2), the median DNA mutational viral load decreased over time from 193 to 43 copies/10^6 cells (p<0.001) (all mutations combined)

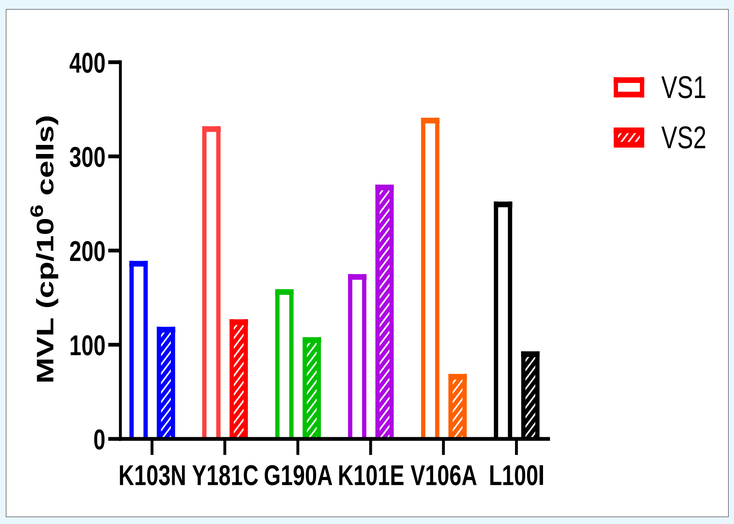


Fig 1 : Change in DNA mutational viral load between VS1 and VS2 for the 6 most frequent mutations detected at VS1

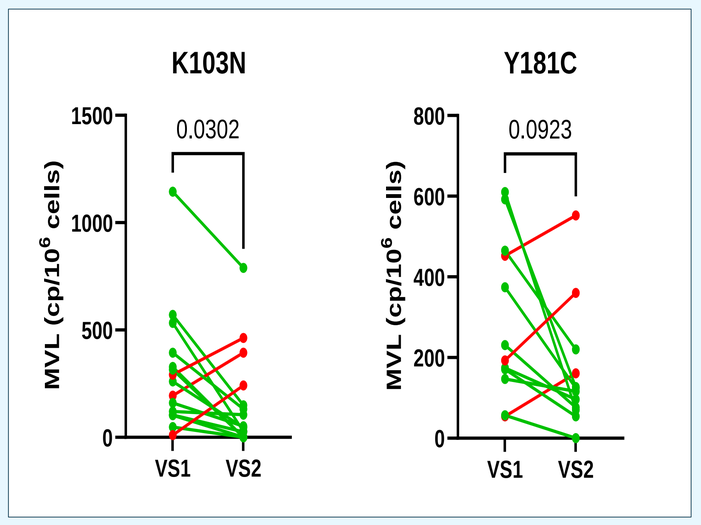


Fig 2 : Change in K103N and Y181C mutational DNA viral load between VS1 and VS2 (NGS)

CONCLUSION

- ❖ NNRTI RAM were not detected in HIV reservoir (circulating cells) in half of the patients following virological failure on NNRTI regimen, with ARN genotype harboring NNRTI RAM, after a median duration of VS of 11 to 18 years. However, when DNA NGS sequencing was repeated 3 years apart, 22% of patients with no mutations at first time point were found to have mutations at second time point.
- ❖ The persistence of NNRTI RAM at VS1 was associated with a higher DNA viral load, a higher zenith RNA viral load and a longer duration of replication on NNRTI regimen.
- ❖ The kinetics of all major NNRTI-RAM appear similar. However, for persistent NNRTI-RAM a significant decreased in mutational viral load was observed and those mutations could be on the way of being cleared from the HIV reservoir.
- ❖ Clearance of archived NNRTI resistance mutations could be achieved in long-term virologically controlled patients as shown with M184V (1,2). Further studies are needed to assess whether DNA mutational viral load evaluated with NGS can guide prescription of a long-acting NNRTI or a new- generation NNRTI in virologically suppressed patients with history of failure on NNRTI regimen.

Reference : 1 : Palich et al. (2022). Kinetics of Archived M184V Mutation in Treatment-Experienced Virally Suppressed HIV-Infected Patients. J Infect Dis
2 : Nouchi et al. (2018). Dynamics of drug resistance-associated mutations in HIV-1 DNA reverse transcriptase sequence during effective ART. J Antimicrob Chemother