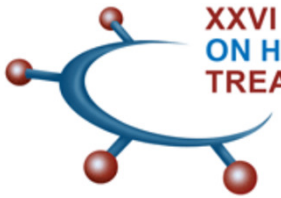
HIV DNA Genotyping by UDS compared with cumulative HIV RNA Genotypes in Pretreated Patients

Mathieu BLOT, Yannis DUFFOURD, Hélène GIRAUDON, Emilie TISSERAND, Arnaud SALMON-ROUSSEAU, Sophie MAHY, Marielle BUISSON, Martin CHEVARIN, Michel DUONG, Pascal CHAVANET, Anne WALDNER,
Lionel PIROTH, **Ali SI-MOHAMMED**

CHU
Centre Hospitalier Universitaire
Dijon Bourgogne



XXVI INTERNATIONAL WORKSHOP
ON HIV DRUG RESISTANCE AND
TREATMENT STRATEGIES
6 - 8 November 2017 Johannesburg, South Africa

Background

- The peripheral blood mononuclear cells (PBMC) include archived DRM that reflect previous treatment regimens failure;

- Highly diversified DRM in proviral DNA lead to their an inconsistent detection by Sanger technology;

- Ultra Deep Sequencing (UDS) is more prone technology to detect minor variants;

- Thus, UDS of proviral DNA may be an interesting alternative to detect HIV-1 RAMs in situations where RNA genotyping by Sanger sequencing is not informative.

Objectives

The **aim** of our study was to evaluate the **pertinence of UDS** to explore **archived DRM** in comparison to cumulative HIV RNA genotyping performed by Sanger approach

Patients and Methods (I)

* 29 HIV-infected patients experiencing several lines of antiretroviral therapy were included;

* For each patient, at least one HIV-1 RNA drug resistance genotyping was available;

* For UDS of HIV-1 DNA, samples used in this study were emanated from routine HIV-1 DNA genotyping resistance testing.

Patients and Methods (II)

- UDS was performed on HIV-1 PR and RT fragments from PCR products amplified according to ANRS recommended procedures;

- PCR was performed in **triplicate** (in first PCR step) for each DNA sample;

- Paired-end indexed libraries for MiSeq, using the Nextera DNA sample preparation kit (Illumina, San Diego, CA)

- The generated reads were then processed in the bioinformatics pipeline developed in-house (Bioinformatics team; CHU Dijon - FHU TRANSLAD - Team GAD

Bioinformatics analysis

Raw reads

QC & trimming
fastqc *trimmomatic*

Alignement
Bwa & custom parameters

Clean & sort
samtools

PRE-PROCESSING

Noise estimation
adequation chi2

Calling vs control
Fischer Exact Test

Comparison to background on position
Independence chi2

Annotation
Provided tsv files

VARIANT CALLING

VIGO

Variant Interpretation

INTERPRETATION

➪ Reads (Qscore > 30) were considered and aligned against the HIV HXB2 K0 3455 reference sequence.

➪ Archived DNA RAMs with a 1% frequency were then compared with those identified with the cumulative RNA GS.

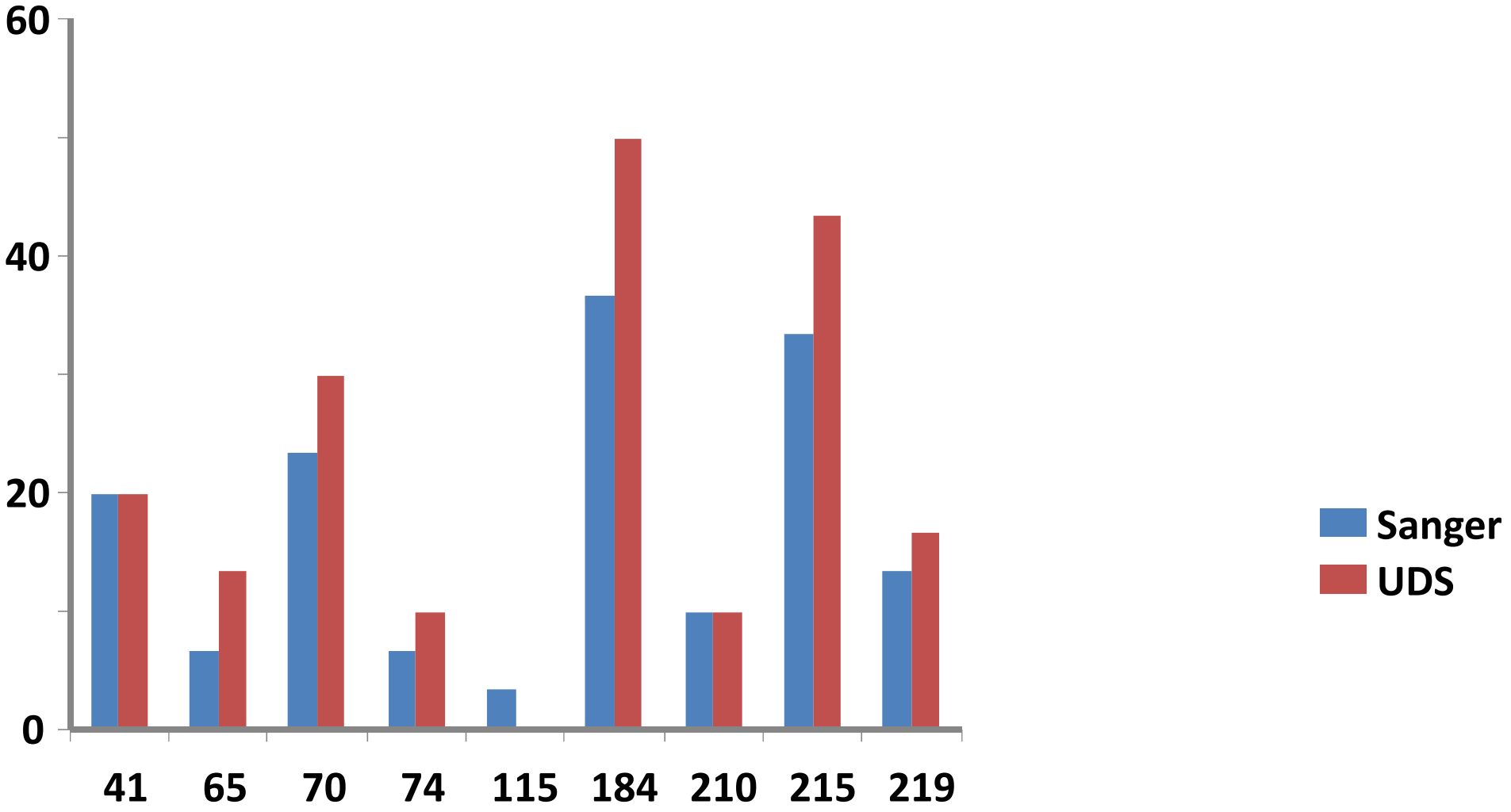
Design

	Previous RNA Sanger genotypes (Years, months) *according to time of UDS DNA*				**Cumulative Sanger RNA Genotype**	**UDS DNA Genotype**	
RAMs profile	14,9	13,2	12,9	3,9		Depth	Frequency
RT-41L	1	0	0	0	**1**	26954	16,2
RT-67N	1	0	1	0	**1**	36458	67,6
RT-69N	0	0	1	0	**1**	38384	5,1
RT-70R	1	0	0	0	**1**	43046	64,1
RT-101R	0	1	1	1	**1**	67555	37,3
RT-181C	1	0	0	0	**1**	65673	7,0
RT-184I	0	0	0	0	**0**	69210	9,5
RT-184V	0	0	0	1	**1**	69210	14,7
RT-190A	1	1	1	0	**1**	59888	11,4
RT-215F	1	0	0	0	**1**	56712	35,5
RT-215I	0	1	1	0	**1**	**0**	**0**
RT-219Q	1	0	1	0	**1**	48539	59,2
RT-230I	0	0	0	0	**0**	41653	8,7
RT-230T	1	0	0	0	**1**	**0**	**0**

Results (I)

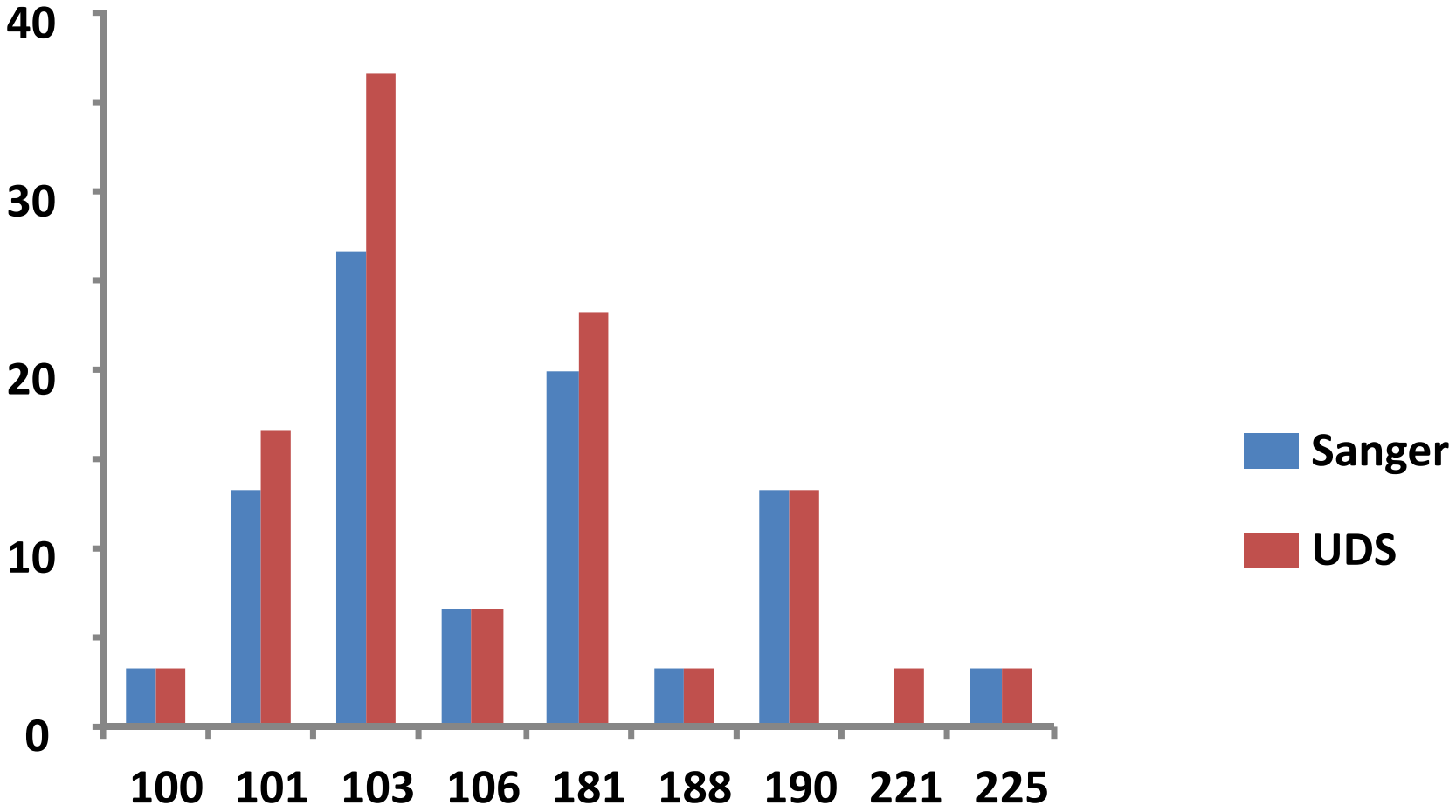
	HIV-1 infected patients n= 29
Demographic characteristics	
Age, median (IQR)	51.2 (45.8-57.5)
Female, number (%)	14 (49)
Immunovirological features	
CD4 count, median (IQR) (cells/mm3)	517 (209-800)
RNA viral load, median (IQR) (log copies/mL)	2.18
Genotyping by Sanger, number/patient, median (IQR)	2 (1-4)
Genotypic resistance analyses	
Number of RAMs detected by Cumulative RNA-GbS	290
Number of RAMs detected by DNA-UDS	398

Results (II) ~ NRTI

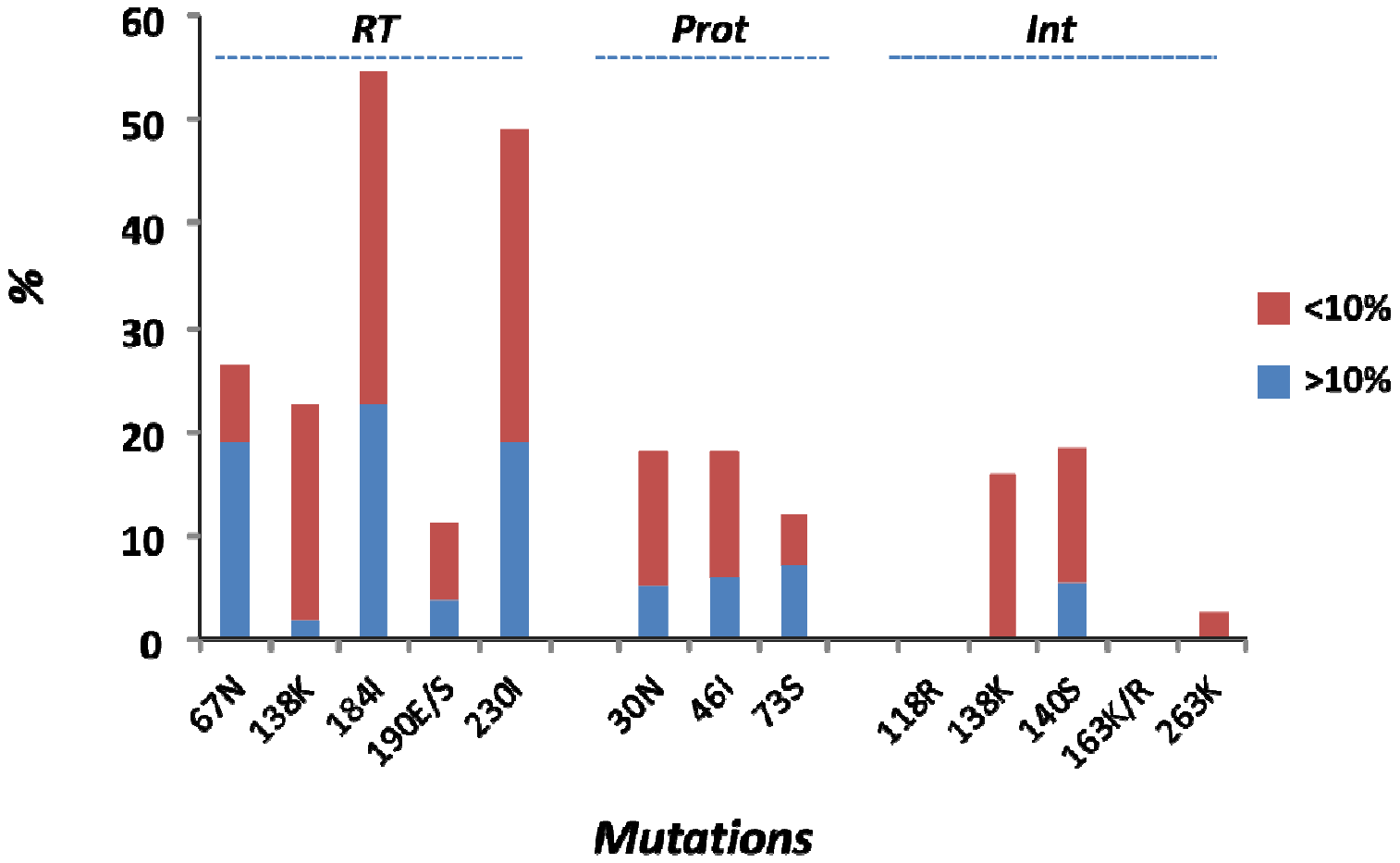


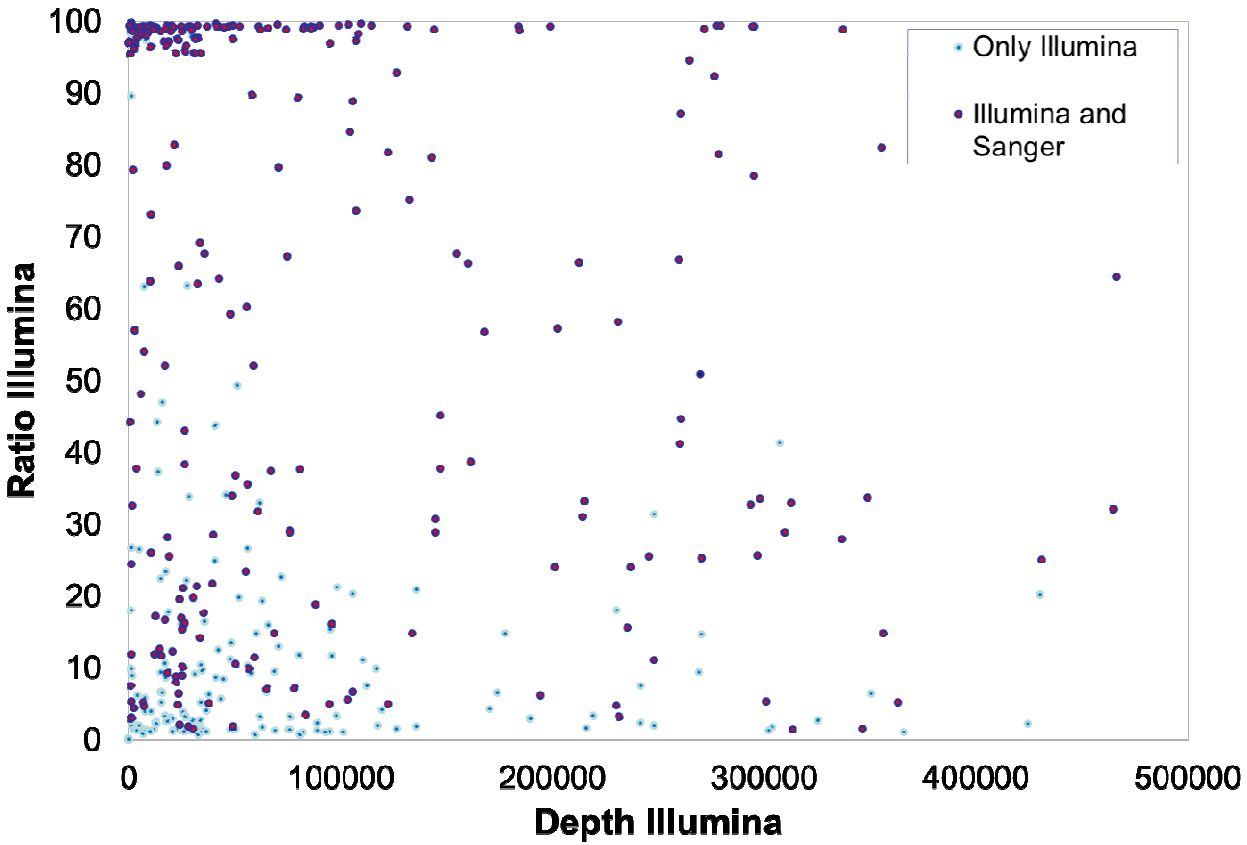
	RAMs profile	Time of RNA genotyping	UDS DNA	
		6,4	Depth	Frequency
RT	RT-70R	0	62252	32,9
	RT-115F	1	**0**	**0**
	RT-184V	1	88499	18,8
Protease	**PROT-30N**	0	23960	4,7
	PROT-36I	1	32705	21,3
	PROT-46I	0	34182	2,7
	PROT-62V	1	24771	99,3
	PROT-64M	0	28133	63,1

Results (III) ~ NNRTI

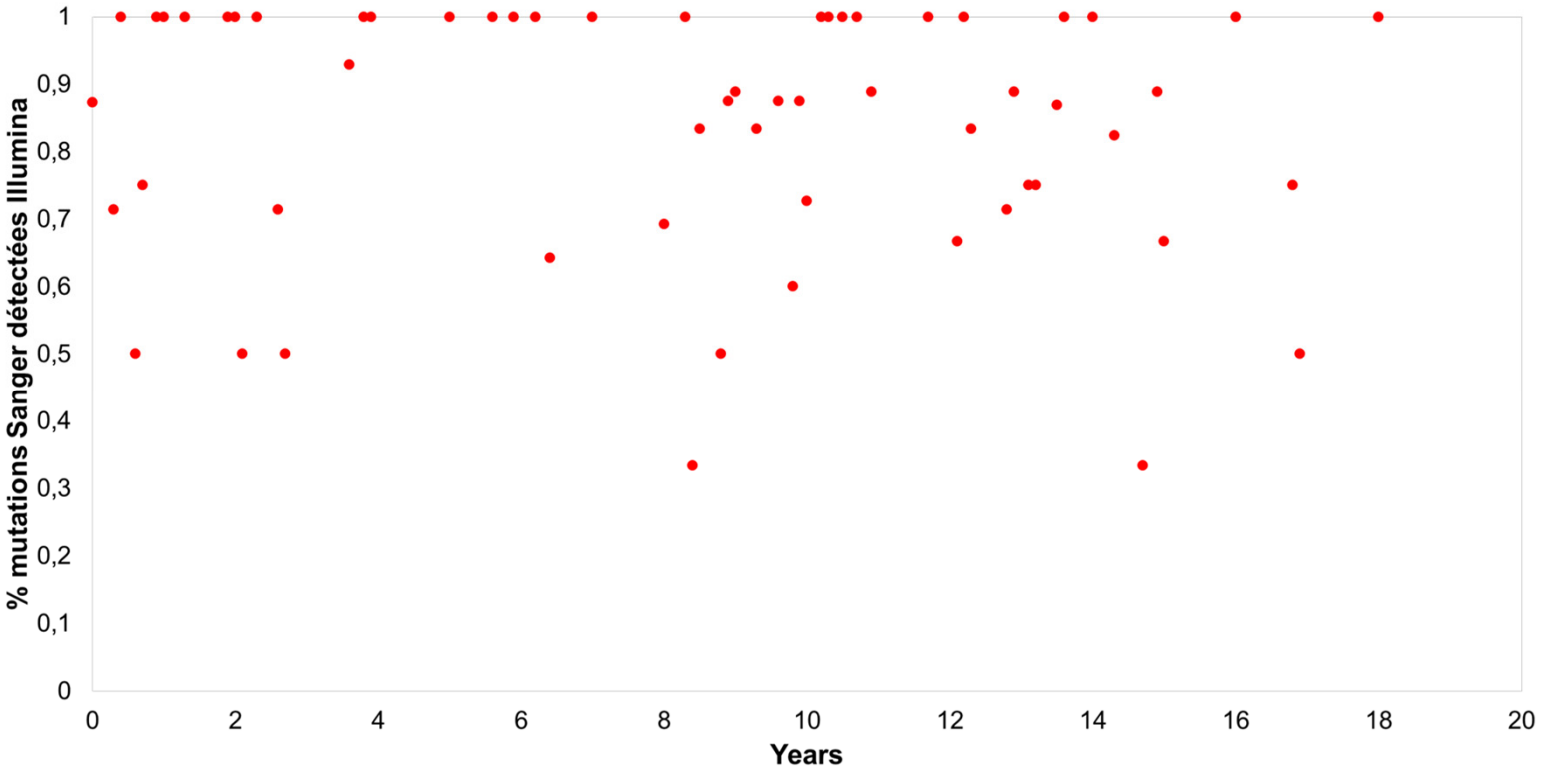


Prevalence of APOBEC HIV-1 Drug Resistance Mutations

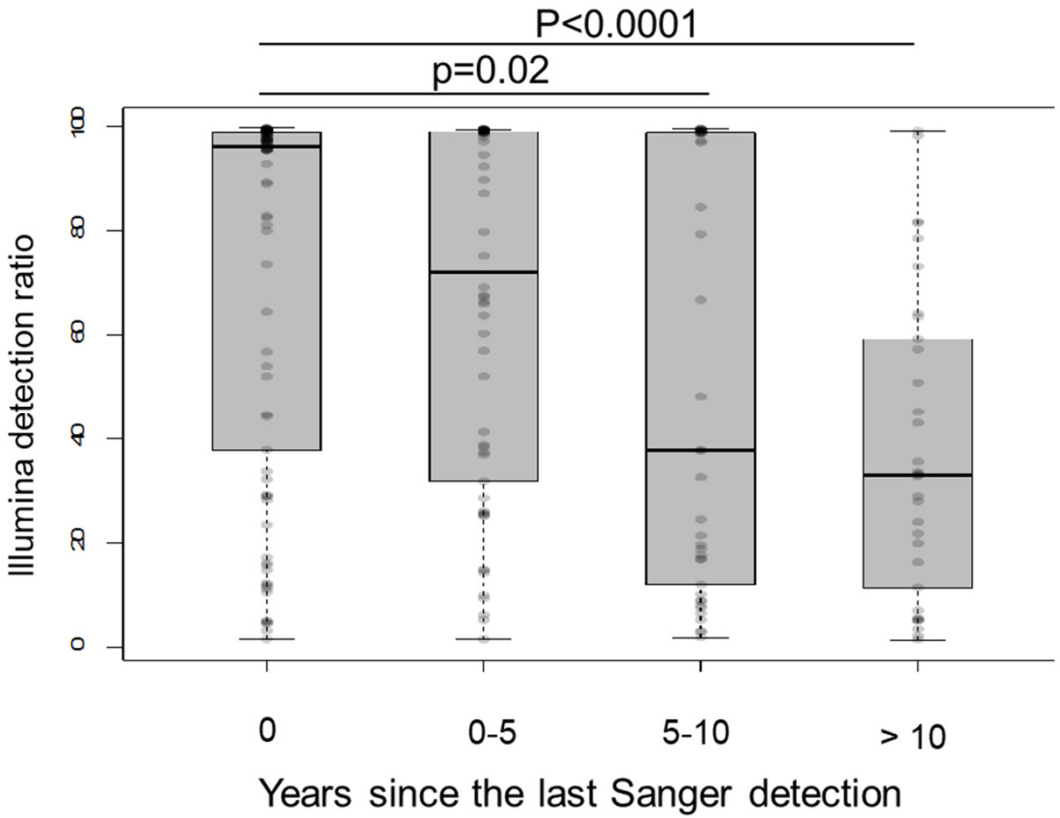




⇨ **Sensitivity of DNA UDS not affected by deepth (number of reads)**



⇨ Sensitivity of DNA UDS ~ # ~ by the age of RAMs detected by Sanger



☛ **Frequency of RAMs in UDS-DNA AFFECTED by the age of Sanger RNA**

Conclusion

- Our strategy based on a **large amount of DNA included in PCR** assays was highly sensitive and may allow us to deal with the fluctuation of archived RAMs in the DNA compartment;

- RAMs detected in DNA by UDS seems to be not influenced by the age of their detection in RNA;

- This approach has to be validated by a prospective study before being used routinely

- Unresolved questions…

Infectious diseases department

- Pr Pascal CHAVANET
- Pr Lionel PIROTH
- Dr Marielle BUISSON
- Dr Michel DUONG
- Dr Sophie MAHY
- Dr Arnaud SALMON-ROUSSEAU
- Dr Anne WALDNER
- Mrs Sandrine GOHIER
- Dr Mathieu BLOT

Virological laboratory

- Mrs Hélène GIRODON
- Valérie LAURENT
- Mrs Michèle VILLAUME
- Dr Ali SI-MOHAMMED

GAD team – bioinformatic analyses

- Mr Yannis DUFFOURD
- Mrs Emilie TISSERAND
- Mr Martin CHEVARIN

DRCI

- Mrs Florence MARTEL
- Mr Antoine LEZE
- Mrs Maud CARPENTIER
- Mrs Lysiane JONVAL
- Mrs Emeline MOREAU

Epidemiology and hospital hygiene departement

- Dr Ludwig Serge AHO

DIJON teaching hospital, FRANCE