Impact of hypermutation on the results of deep sequencing analysis of proviral DNA for baseline HIV-1 drug resistance.

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Introduction

- Current baseline HIV resistance testing: RNA Sanger sequencing fails to detect minor variants (<25% of the viral population).
- Resistant mutations may fade away from the circulation due to the overgrowth by more fit back reverted variants.
- Minority resistant variants have been found in plasma of untreated individuals using deep sequencing or allele specific PCR.
- Research questions:
  - added value of deep sequencing for HIV baseline resistance testing?
  - testing of proviral DNA: a more complete picture of the resistance potential?

Methods

- **Patient selection**: 20 HIV-1 positive patients:
  - 16 with ≥ 1 drug resistant mutation in baseline Sanger sequencing
  - 9 RT215 revertants / (N)NRTI
  - 4 control patients (WT virus)
  - VL >10.000 c/ml
  - plasma and buffy coat sample

Sample ID	Clade	Baseline Sanger TDRM	
		PR	RT
12040	B		103N
12083	B		41L
11063	B		41L
08094	B		215E
08087	B		215E
10027	B		215E
08074	B		215D
09046	B		215E
13039	B		215E
10075	B		215E
09087	B		67N, 215C, 215S
10030	B		67N, 69D, 215C
12054	02AG	20I	103N, 108I, 184V, 225H
11072	F2		
10096	B		
10029	B		
12122	B		

Methods

- **Experimental setup**:

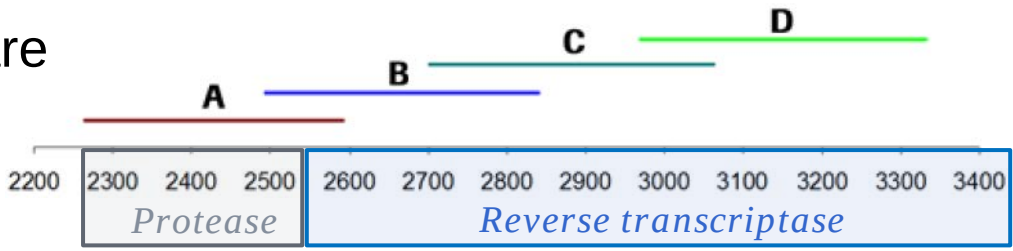
HIV plasma RNA | HIV cellular DNA

Baseline Sanger Sequencing

454 deep sequencing
Protease and *Reverse Transcriptase*

454 SEQUENCING

Roche AVA software



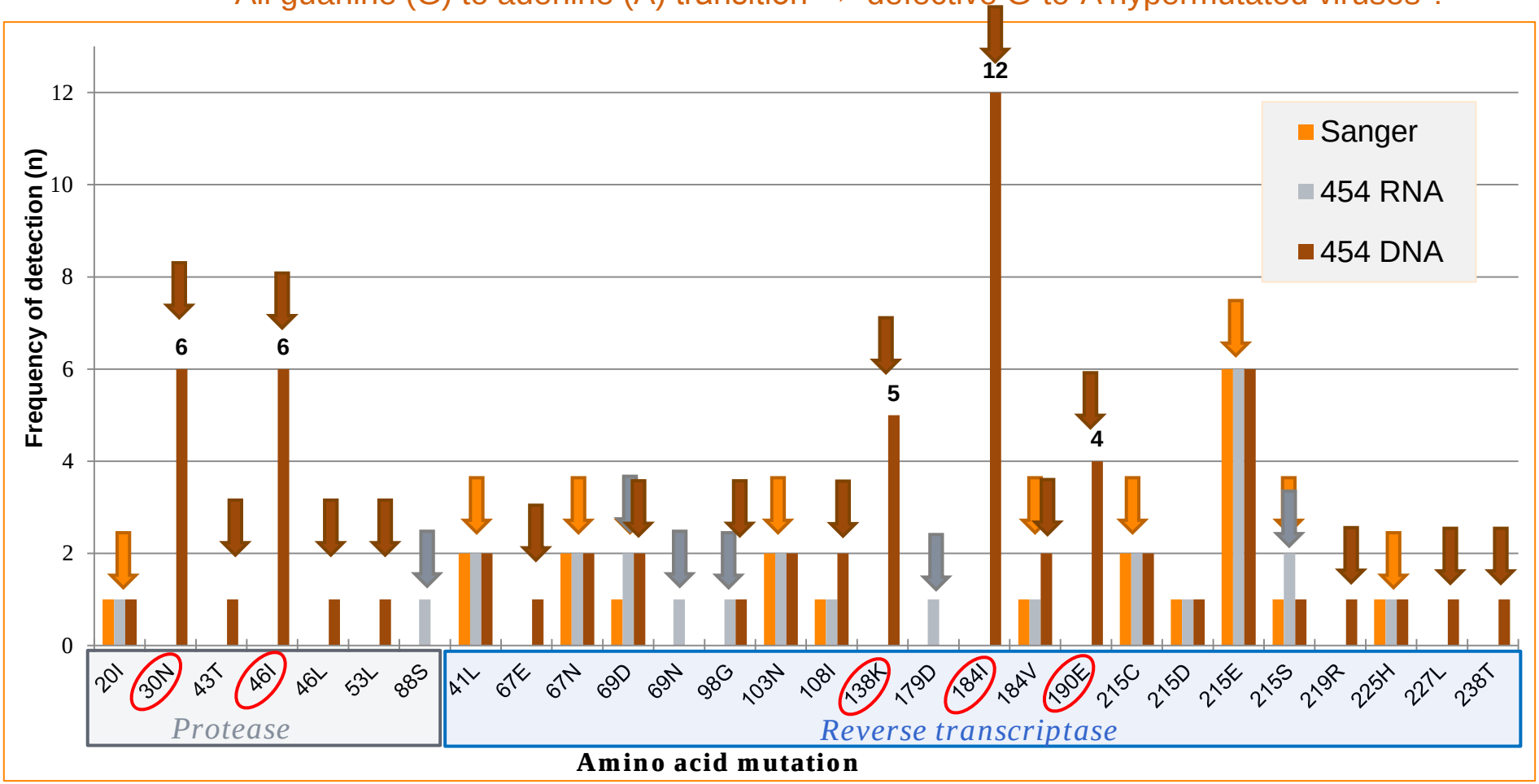
Cut-off minorities: 1% (min.5 reads; forward & reverse)

Resistance mutations: Stanford* + SDRM list°

* http://hivdb.stanford.edu/
° *Bennett, et al. 2009.*

Results

- Samples successfully deep sequenced (both RNA & DNA): **17/20** (85%)
- All 21 mutations detected by population sequencing were also observed after deep sequencing, both in RNA and DNA
- Additional mutations detected in RNA **(n=6)** and DNA **(n=42)**
- **Five** codon positions account for 83% of mutations only present in DNA
  - All guanine (G) to adenine (A) transition → defective G-to-A hypermutated viruses ?



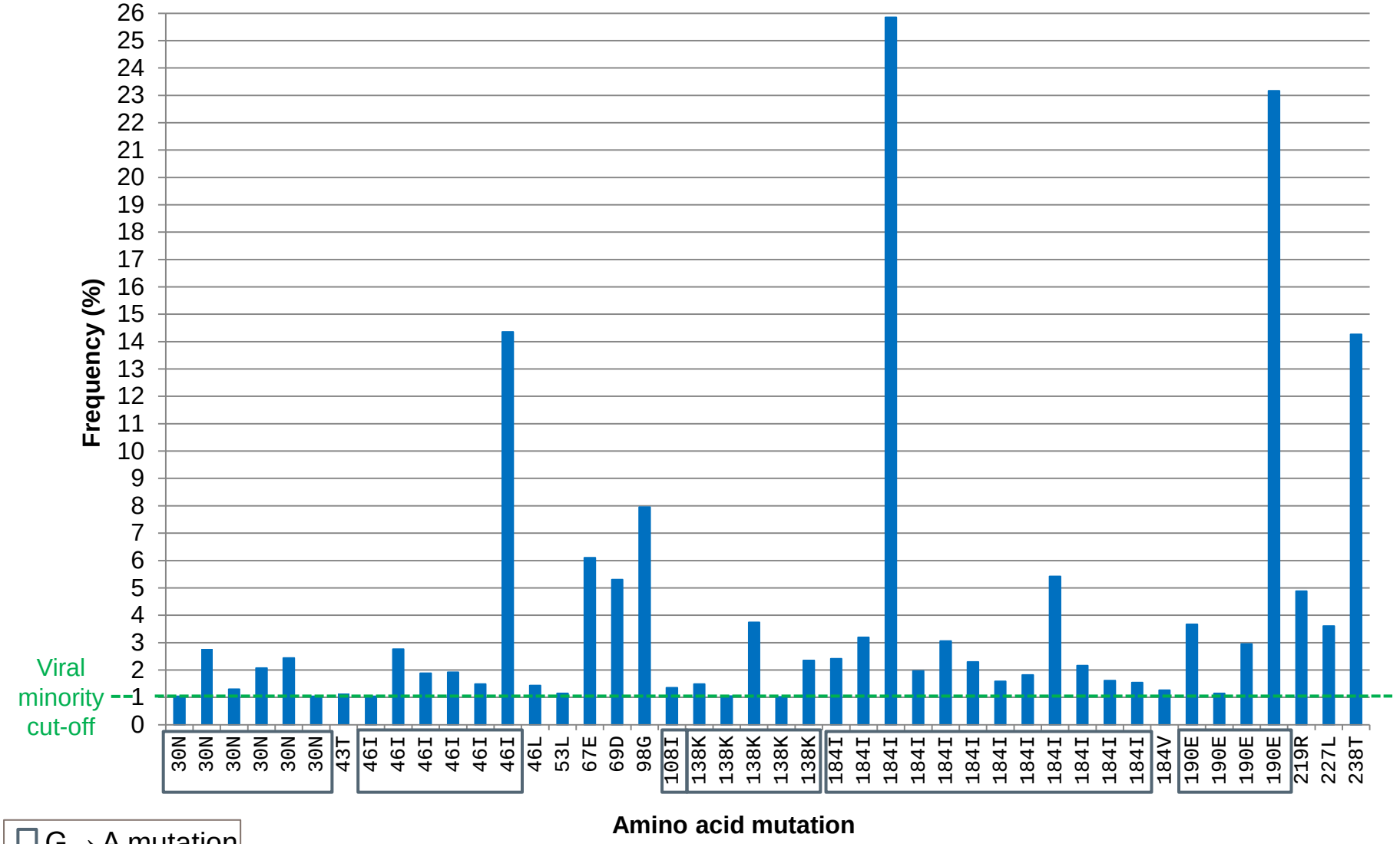
Results

- Screening of all DNA / RNA deep sequencing reads with minority resistance mutations for APOBEC-induced hypermutation (Hypermut 2.0 software[x]).

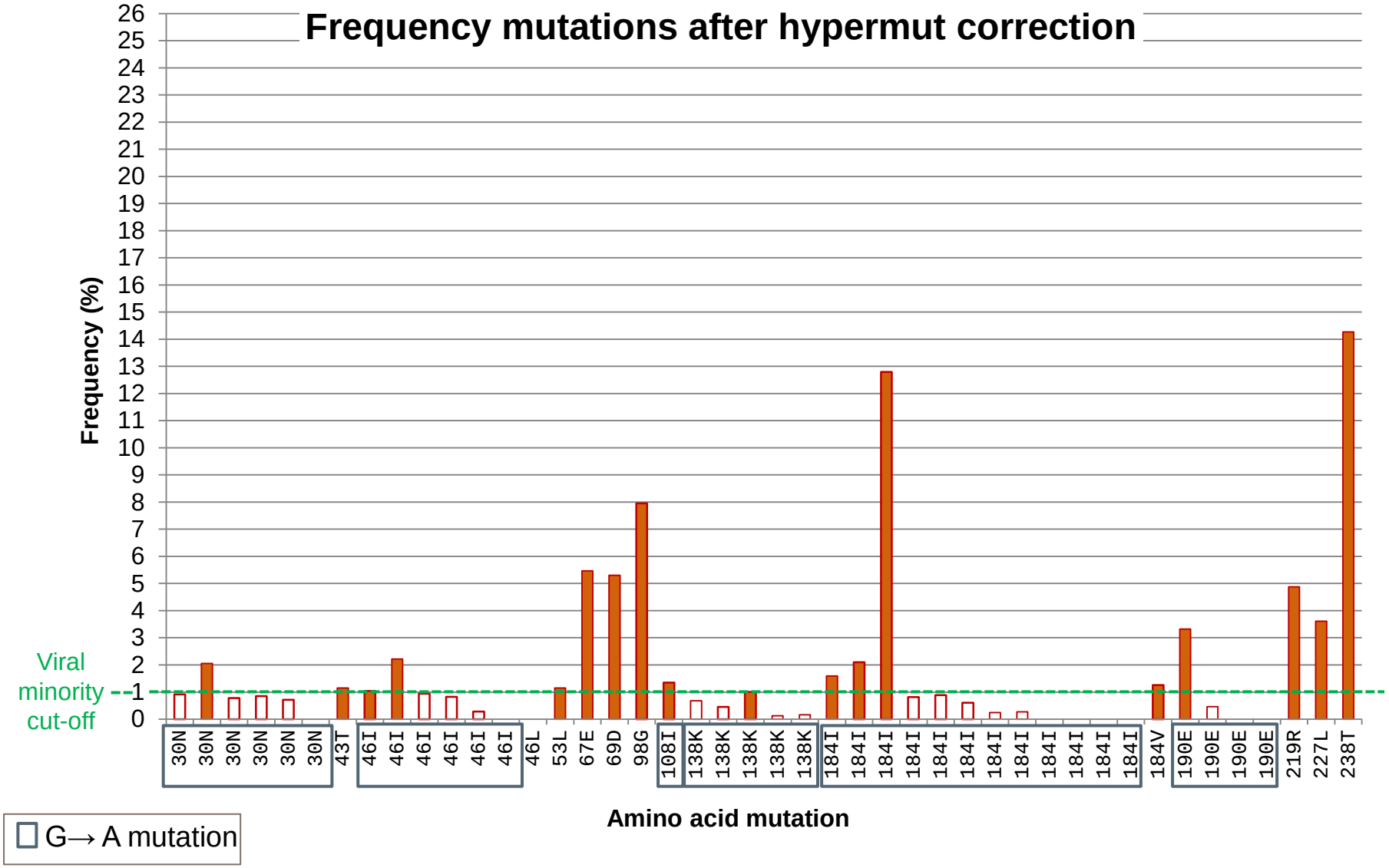
	454 RNA	454 DNA
Hypermutated reads	0/2593	1919/3744
Removal of hypermutated reads ⇩		
Remaining minority mutations	*n = 6* *PR*: 88S *RT*: 98G 69D, 69N 215S 179D	n = 18 *(43%)* *PR*: 30N, 43T, 46I (2x), 53L *RT*: 67E, 69D, 98G, 108I, 138K, 184I (3x), 184V, 190E, 219R, 227L and 238T

[x] *Rose, PP and Korber, BT., 2000.* www.hiv.lanl.gov

Results:
Minorities of drug resistance mutations in DNA



Results: additional mutations only in 454DNA



Results

Sample ID	Clade	Baseline Sanger TDRM		Additional TDRM Deep Seq RNA		Additional TDRM Deep seq DNA	
		PR	RT	PR	RT	PR	RT
12040	B		103N				
12083	B		41L				
11063	B		41L				108I
08094	B		215E				
08087	B		215E				
10027	B		215E				
08074	B		215D				
09046	B		215E			30N 46I	190E
13039	B		215E				184I
10075	B		215E				138K 184I
09087	B		67N 215C 215S		69D	46I	67E 69D 184V 184I
10030	B		67N 69D 215C		69N 215S	43T 53L	
12054	02AG	20I	103N 108I 184V 225H	88S	98G		98G 219R 227L 238T
11072	F2						
10096	B						
10029	B				179D		
12122	B						

Results

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12083	B		41L				
11063	B		41L				*108I*
08094	B		215E				
08087	B		215E				
10027	B		215E				
08074	B		215D				
09046	B		215E			*30N 46I*	*190E*
13039	B		215E				*184I*
10075	B		215E				*138K 184I*
09087	B		67N 215C 215S		**69D**	*46I*	**67E 69D** *184V 184I*
10030	B		67N 69D 215C		**69N 215S**	**43T 53L**	
12054	02AG	20I	103N 108I 184V 225H	**88S**	**98G**		**98G 219R 227L 238T**
11072	F2						
10096	B						
10029	B				**179D**		
12122	B						

Conclusions

- Interpretation of the results of HIV-1 proviral DNA deep sequencing is hampered by the presence of hypermutated defective viruses.
- G-to-A hypermutation may results in the false identification of D30N and M46I in protease and E138K, M184I and G190E in RT.
- Cleanup of hypermutated reads is needed. Running the hypermut software sufficient?
- No minority resistance detected in RNA of patients with singleton mutations or 215 revertants pointing to onward transmission as the cause of their presence.

Acknowledgements

AIDS Reference Laboratory Ghent:

Delfien Staelens, Els Demecheleer, Marlies Schauvliege, Sylvie Dinakis, Laura Hebberecht, Leen Vancoillie, Virginie Mortier and Chris Verhofstede.

Patients of Ghent University hospital