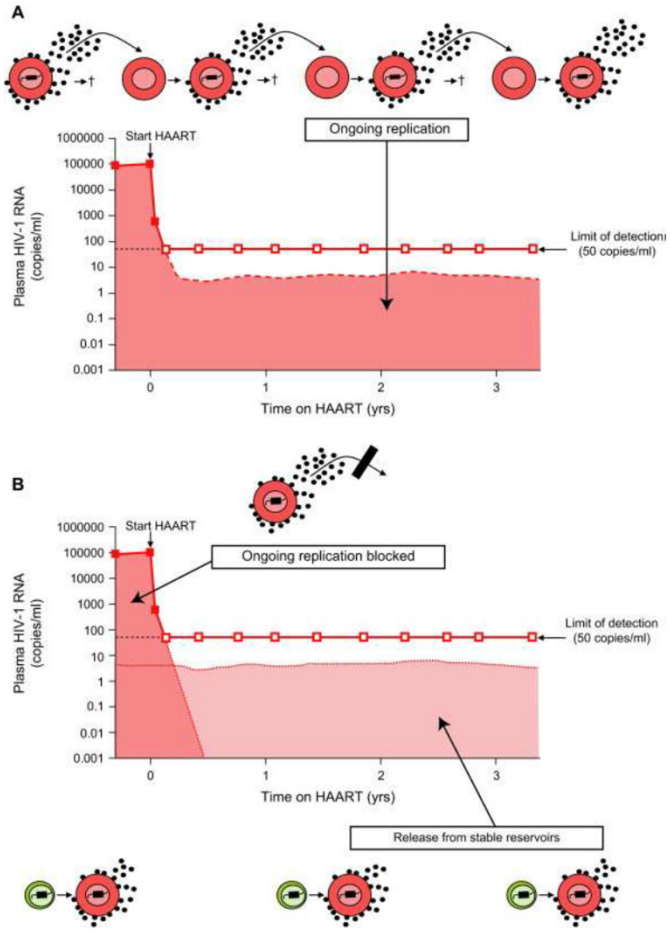
Nature of low level viremia



- Ongoing replication despite cART
- Virus production due to activation of latently infected cells
- Release of viral RNA from defective cells

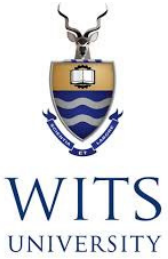
Shen, Siliciano, J Allergy Clin Immunol 2008

APOBEC mediated hypermutations in HIV

- APOBEC enzymes are responsible for G to A hypermutations in HIV genomes
- Help protect humans and mammals from viral infections
- Hypermutations inhibit the viral replication and virion formation of HIV due to frequent stop codons

HIV Pseudo viremia from large defective cell population

JC Botha, K Steegen, L Hans, A Karstaedt, S Carmona, D Reddy, GU van Zyl

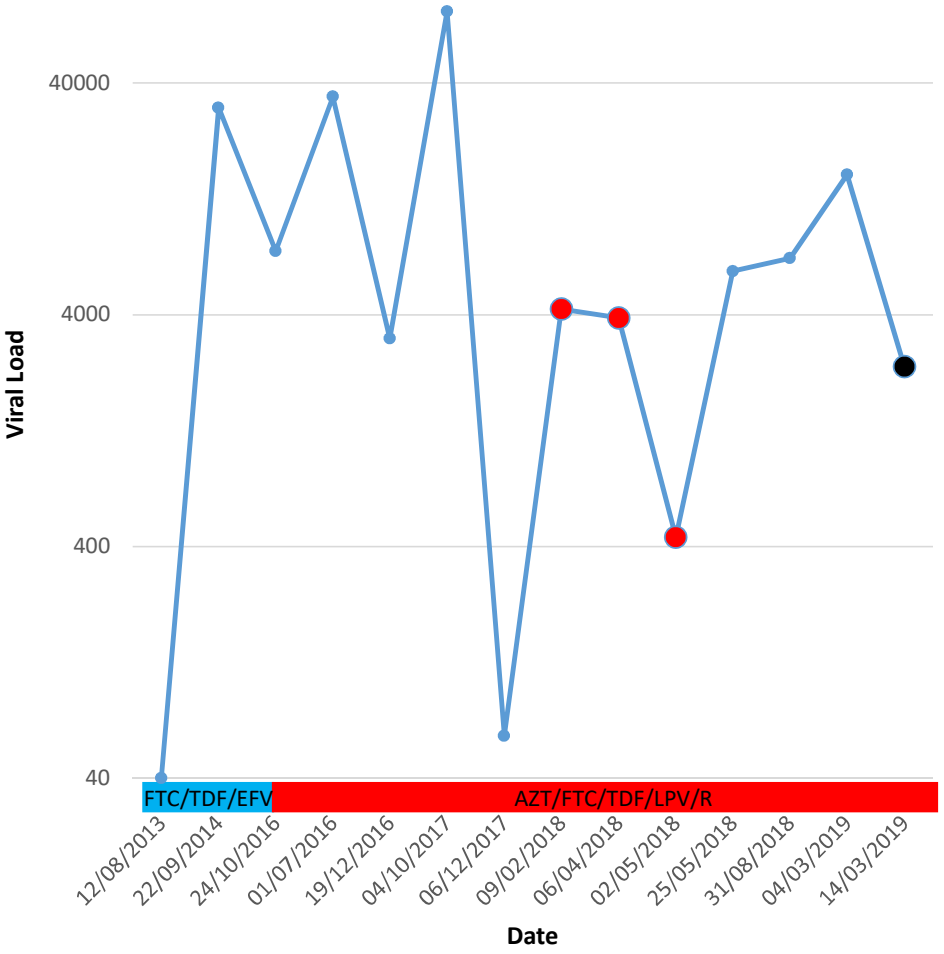


Case presentation and hypotheses

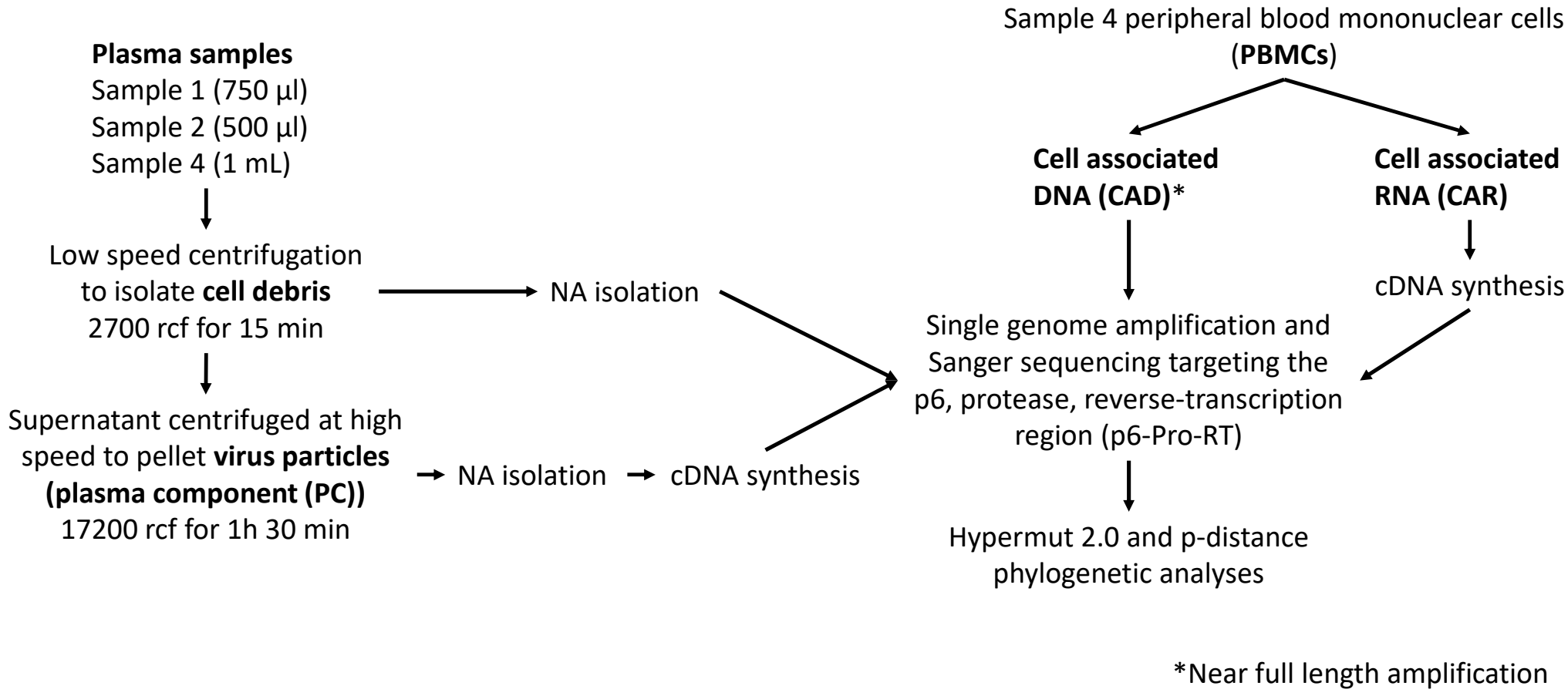
- A 46 year old HIV-1 positive adult female patient on long term cART presented with virological failure
- Drug resistance testing by Sanger population sequencing was performed on 3 separate samples, in a Gauteng laboratory, over a 3-month period, yielding identical hypermutated sequences
- Possible explanations:
  - A hypermutated CD4 cell clone, perhaps infected with a helper provirus, that through complementation can produce free plasma HIV with defective hypermutated genomes?
  - HIV infected cell lysis with leakage into ‘plasma’; but how is the identical hypermutated sequence maintained in plasma over a 3-month period?
- The three plasma samples and an EDTA sample (taken 13 months after the first plasma sample) were sent to our lab for further investigation

Patient viral load data

- Viral load data represented in log scale graph
- Residual plasma from samples 1-3 (red dots):
  - Sample 1: 09-02-2018 (1.5 ml), VL: 4230 copies/ml
  - Sample 2: 06-04-2018 (0.5 ml), VL: 3880 copies/ml
  - Sample 3: 02-05-2018 (0.5 ml), VL: 439 copies/ml
- EDTA sample 4: 14-03-2019 VL: 2388 copies/ml (black dot)
- ART regimen (indicated by bar in Figure):
  - 2013 – 10/2014 (blue): FTC/TDF/EFV
  - 10/2014 – 14/03/2019 (red): AZT/FTC/TDF/LPV/R



Materials and methods



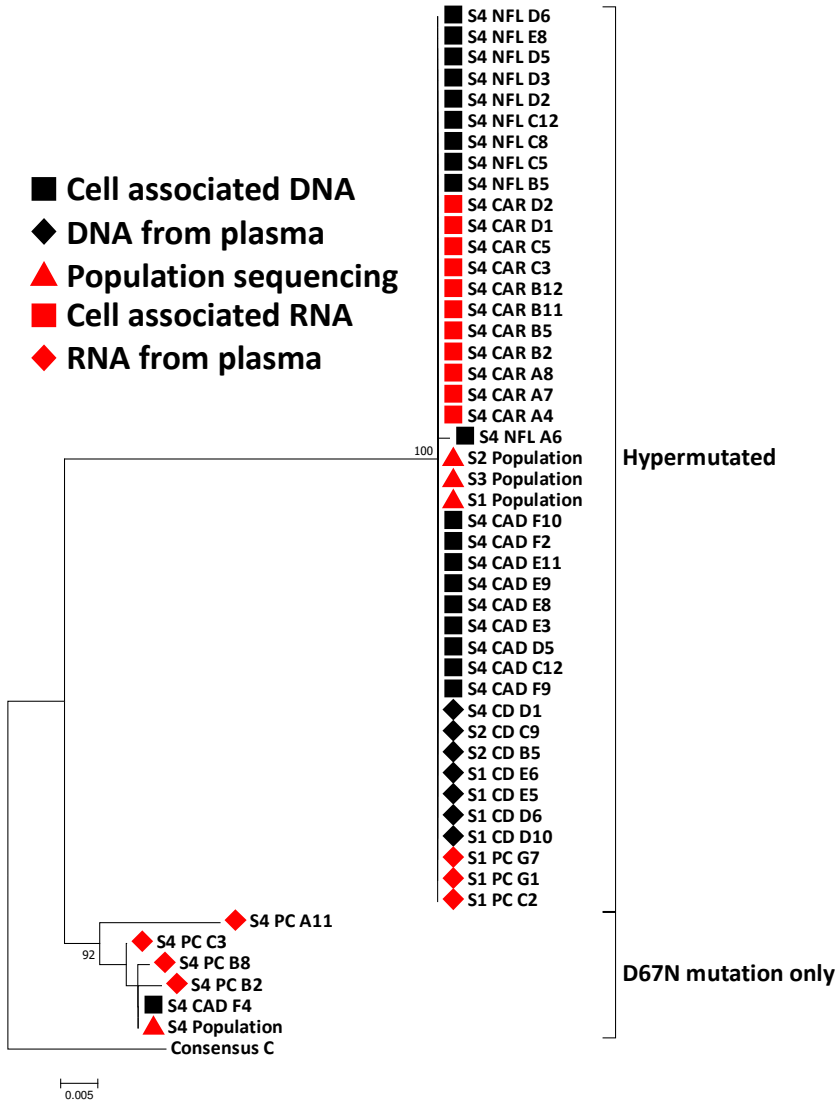
*Near full length amplification

Breakdown of sequencing results

Sample	Component	Sequence results	Mutations detected
Sample 1	Plasma	Population drug resistance seq	APOBEC
	Cell debris	p6-Pro-RT SGS	APOBEC
	Plasma component	cDNA p6-Pro-RT SGS	APOBEC
Sample 2	Plasma	Population drug resistance seq	APOBEC
	Cell debris	p6-Pro-RT SGS	APOBEC
	Plasma component	cDNA p6-Pro-RT negative	PCR negative
Sample 4 plasma	Plasma	Population drug resistance seq	D67N
	Cell debris	p6-Pro-RT SGS	APOBEC
	Plasma component	cDNA p6-Pro-RT SGS	D67N
Sample 4	PBMC	CAD p6-Pro-RT SGS	1/10 – D67N 9/10 – APOBEC
		CAD NFL (p6-Pro-RT region)	APOBEC
		CAR p6-Pro-RT SGS	APOBEC

Results

- APOBEC strain detected in all components, except sample 2 & 4 PC.
- Population sequences from drug resistance testing included for comparison
- p6-Pro-RT CAD-SGS endpoint dilution (1:27)
- p6-Pro-RT CAR-SGS endpoint dilution (1:2187)
- Partial gag sequences of NFL products indicate a stop codon in p24, resulting in truncated p24 capsid protein



Conclusions

- 19/20 CAD and 11/11 CAR sequences from PBMCs are identical to APOBEC hypermutated sequences from cell debris and plasma component
- This suggests a major APOBEC mutated population consisting of identical p6-Pro-RT sequences (possible CD4+ T-cell clone)
- The APOBEC strain is highly transcribed, as detected by CAR-SGS (endpoint dilution 1:2187)
- Production of viral proteins and packaging of viral genomes are highly unlikely given the hypermutated genome with at least one stop codon in gag-p24
- The cause of “virological failure” appears to be a large population of cells with identical hypermutated proviruses undergoing cytolysis, releasing cellular nucleic acid including HIV DNA and mRNA into plasma
- Release of cellular nucleic acids into plasma may be an underappreciated cause of false virological failure and likely influence patient care

Thank you

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