“FALSE ART FAILURE” FROM IDENTICAL HYPERMUTATED HIV NUCLEIC ACID IN PLASMA

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Background and preliminary data

HIV-1 viral load (VL) monitoring is premised on an elevated VL indicating HIV-1 replication due to drug resistance or poor adherence.

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 performed on 3 separate samples over a 3-month period, all yielded identical hypermutated sequences.

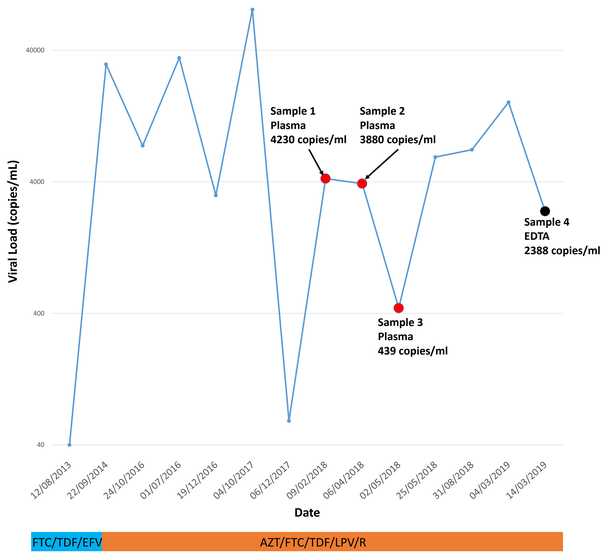
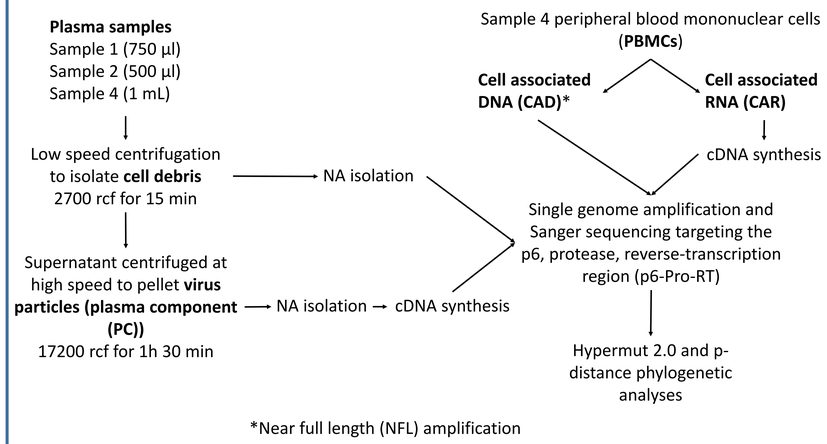


Figure 1: Log scale viral load data plot, with ART regimens. Plasma samples 1, 2 and 3 are indicated by red dots. EDTA sample 4 taken for further investigation is indicated by black dot.

Methods



An HIV-1 positive adult female patient on long term cART presented with virological failure. Investigation revealed this case of false cART failure is caused by a large cell population continuously releasing identical defective HIV DNA and RNA, detectable in plasma for over 13 months.

Aim

To investigate the apparent virological failure by determining the source of HIV genomic material: distinguishing cell debris and virus particles.

Results

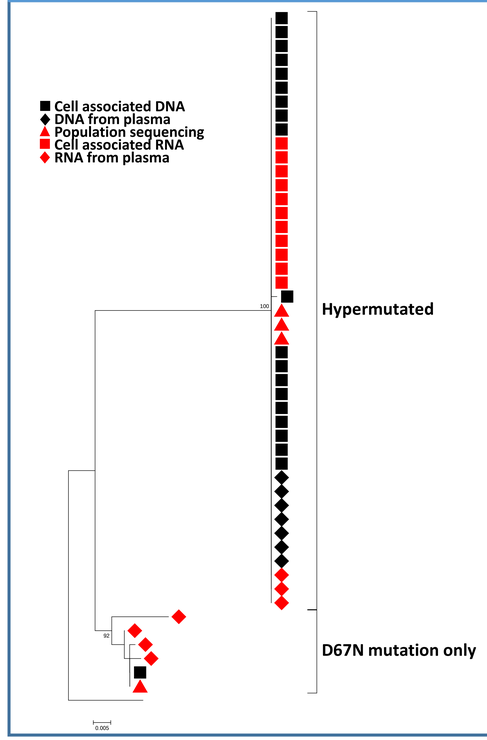


Figure 2: P-distance phylogenetic tree of all nucleotide sequences generated for the p6-Pro-RT region. Plasma population sequences from drug resistance testing is included in phylogenetic tree as reference.

Results - Continued

The APOBEC hypermutated strain is highly transcribed into RNA, based on the endpoint dilutions of single genome sequencing, cell associated RNA (1:2187) was found to be about 81 times more concentrated than cell associated DNA (1:27).

Partial gag sequences of NFL products indicate a stop codon in p24, resulting in truncated p24 capsid protein.

Table 1: Summary of PCR and sequencing results indicating detected mutations

Sample	Component	Sequence results	Mutations detected
Sample 1	Plasma	Population drug resistance seq	APOBEC
	Cell debris	p6-Pro-RT SGS	APOBEC
	Virus pellet	cDNA p6-Pro-RT SGS	APOBEC
Sample 2	Plasma	Population drug resistance seq	APOBEC
	Cell debris	p6-Pro-RT SGS	APOBEC
	Virus pellet	cDNA p6-Pro-RT negative	PCR negative
Sample 4 plasma	Plasma	Population drug resistance seq	D67N
	Cell debris	p6-Pro-RT SGS	APOBEC
	Virus pellet	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

Discussion and Conclusions

19/20 HIV-1 DNA and 11/11 HIV-1 cellular RNA 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.

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