Differentiating APOBEC- and drug-resistant populations by clonal analysis of near full-length proviral HIV-1 genome in highly treatment experienced people living with HIV

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(1) Objective

Objective: HIV-1 genotypic resistance testing from proviral DNA can be beneficial in switch settings where therapy history is incomplete and/or viral RNA is undetectable. However, interpretation of a proviral resistance profile can be challenging, since APOBEC-induced mutations may also appear at drug-resistance-associated positions (APOBEC-missense-mutations) and the majority of proviral variants is defective (>90-95%). APOBEC proteins ("apolipoprotein B mRNA editing enzyme, catalytic polypeptide") play an essential role in defense against intracellular virus infections by deaminating cytidine bases to uracil, leading to G to A hypermutations and thus inducing nonsense (e.g. stop codons) and missense mutations.
Here, we describe a near-full-length amplification and long-read sequencing approach to discriminate populations with APOBEC-missense-mutations from replication-competent populations by covariation analysis in highly treatment experienced PLWH.

(2) Methods

17 heavily treatment experienced and - except two - virologically suppressed (median: 10 years) PLWH were included in this study (Tab. 1). A single round, near full-length PCR was done from HIV-1 proviral DNA followed by short-read- (Illumina MiSeq) and long-read- (Oxford nanopore technology [ONT]) sequencing approach (Fig.1).

patient-ID	Age	gender	first diagnosed	ART duration yrs	viral load (cop/ml)	CD4 (/µl)	current ART
1	64	m	1993	29	<40	1146	ETR, DRV/r
6	48	w	1997	25	<40	266	3TC, DOR, MVC, FTV
12	46	m	2001	13	<40	644	DRV/r, RAL
16	41	m	2013	9	<40	622	DRV/r, DTG
17	46	m	1997	25	<40	421	DOR, DTG
19	54	m	1995	25	<40	1657	3TC, DOR, DTG
22	55	m	1996	25	<40	1325	ABC, 3TC, DTG
27	65	m	1994	28	<40	468	LPV/r, DTG
31	65	m	1990	26	57	1163	DOR, DTG
37	60	m	1989	26	<40	580	TAF, FTC, DRV/c
38	61	w	1987	30	<40	2030	LPV/r, DTG
42	57	m	2000	22	<40	583	AZT, 3TC, DTG
47	51	m	2012	10	<40	1108	3TC, DRV/r, MVC
50	62	m	2004	18	<40	991	TDF, FTC, ETR, DRV/r
53	35	m	2010	12	<40	598	TAF, FTC, DRV/c
56	57	m	2004	18	86	332	TAF, FTC, BIC
58	68	m	1998	24	<40	988	DRV/r, RAL

Table 1. Patient characteristics

Analysis was performed using SeqIT's drug resistance testing pipeline for MiSeq data and an in-house developed analysis pipeline for ONT-data and the combined/hybrid analysis.
Covariation analysis was used to extract linkage information of mutations on ONT reads. Mutations were considered for analysis when confirmed by the MiSeq approach. Potential APOBEC-missense-mutations in Protease, Reverse Transcriptase and Integrase were determined using Stanford hivdb. Replication incompetence of individual variants was defined as presence of premature stop codons found anywhere in the genome.
The replication competence of variants with potential APOBEC-missense-mutations and Non-APOBEC-Resistance-Mutations was compared with the Mann-Whitney U-Test.

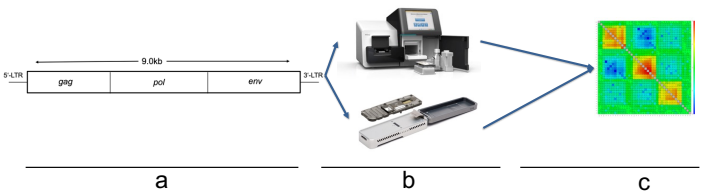

Figure 1. Near-full-length genome amplification (a) and sequencing strategy (b) of proviral HIV-1 near-full-length genome followed by covariation analysis (c).

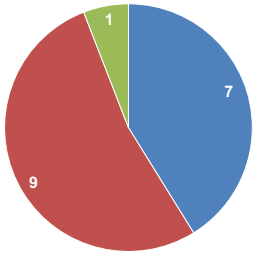

Figure 2. PLWH with replication-competent only (blue), mixed (red) or only defective viral variants (green).

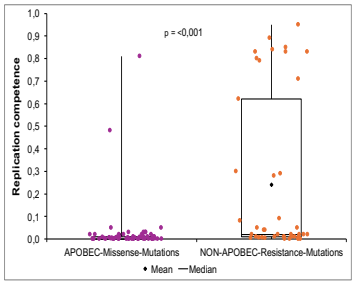

Figure 3. Comparison of relative replication competence of all variants carrying APOBEC-Missense- and/or NON-APOBEC-Mutations at resistance associated positions.

(3) Results

No APOBEC-induced premature stop codons were observed in 7/17 samples, indicating a fully replication-competent population. 1 sample showed a population with stop codons in >99% of the genomes, indicating almost complete replication incompetence. Mixed populations with and without premature stop codons were detected in 9/17 samples (Fig.2). These showed a total of 89 mutations at resistance associated positions with frequencies between 1% and 99%. In 8/9 samples drug resistant populations were replication-competent with frequencies of replicative viruses between 0,2% and 95%. By using mutation linkage analysis within individual viruses, mutations of ambiguous origin such as M184I or M230I in the Reverse Transcriptase could clearly be classified as resistance- or APOBEC associated. There is a significant difference (p<0,001) between the potential APOBEC-missense-mutations and the other resistance mutations (Fig.3). Of particular interest is patient 19, (Fig.4), since the overall resistance pattern may have an impact on the susceptibility to Emtricitabine, Doravirin and Dolutegravir. The entire viral population was found to be replication-incompetent indicating that this might be an explanation for the ART being sufficient.

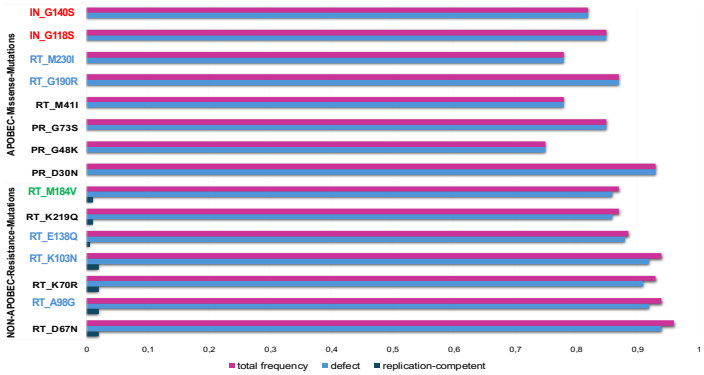

Figure 4. Patient 19: APOBEC induced missense mutations (upper part) at resistance associated positions are completely covarying with replication-incompetent variants. Mutations potentially critical to the current ART DTG (red), 3TC (green) and DOR (blue) are highlighted

(4) Conclusions

By using near-full-length amplification of the HIV-1 genome in combination with long-read sequencing, we were able to discriminate APOBEC- from potential replication competent populations in the proviral DNA. All 17 PLWH were virologically suppressed despite critical resistance profiles in some cases, indicating that APOBEC related stop codons may have an impact on viral replication competence of drug-resistant variants. The characterization of a proviral population in terms of replication competence might be helpful in ART switch settings with limited options.