Next-generation-sequencing in routine HIV-1 resistance diagnostics – frequency of additional resistance relevant mutations in 2% and 1% population proportions correlated to viral load and additional patient follow-ups

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BACKGROUND

NGS-technologies have made their way into routine-diagnostics in HIV-1 resistance testing. The report of mutations of at least 10% of the viral population is chosen by many laboratories due to its equivalency to Sanger-sequencing minority detection. The relevance of mutations detected by NGS technologies in low frequencies is still a subject of debate. Clinical data is rare. We here report the frequency of additional mutations in population-proportions of greater than 2% and 1% in routine laboratory testing and correlate them to viral load. Therapy implications for patients with relevant minor viral populations were monitored.

METHODS

645 HIV-1 resistance tests (reverse transcriptase/protease) performed between 10/2014 and 04/2016 with an in house PCR followed by NGS (Illumina MiSeq, sequences reported with >100 reads only) were analyzed. Sequences were interpreted by HIV-GRADE (http://www.hiv-grade.de) for resistance mutations using 10%, 2% and 1% minority cut-offs. A specific focus laid on differences in reported resistance associated mutations and resistance levels (e.g. additional drug class or further drugs same class). The proportion of subpopulations harbouring additional mutations with greater than 2000 c/mL (= mutational load) were calculated, therapy data and follow up for those patients was monitored as far as available.

RESULTS

Tables A: drug specific resistance relevant mutations detected with different cut-offs

Darunavir

Cut-off/ mutations	10%	2%	1%
11I	7	17	27
32I	0	2	3
33F	5	5	5
47V	0	3	18
50V	0	5	11
54L	1	2	2
73S	8	11	16
76V	1	1	2
84V	2	3	9
89V	1	3	4
sum	25	52	97

Tenofovir

Cut-off/ mutations	10%	2%	1%
41L	15	17	20
65R	10	11	49
67N	16	19	23
70E	1	4	9
70R	8	12	19
115F	0	2	3
210W	10	12	13
215FY	9	9	10
219EQ	13	19	28
sum	82	105	174

Atazanavir

Cut-off/ mutations	10%	2%	1%
10FI	101	109	115
24I	1	3	3
32I	0	2	3
33F	5	5	5
46I	8	28	42
48V	0	0	3
50L	0	0	0
53L	5	9	18
54AMV	4	7	9
73ACST	8	11	17
82AFT	6	8	18
84AV	2	3	9
88S	2	8	12
90M	8	10	11
sum	150	203	265

Lamivudin

Cut-off/ mutations	10%	2%	1%
65R	10	11	49
184V	40	45	52
184I	17	25	29
151M	2	2	2
sum	69	83	132

Rilpivirin

Cut-off/ mutations	10%	2%	1%
90I	31	41	53
101EP	7	13	18
138KRAG QS	34	51	68
181ICV	15	18	21
188L	4	4	4
189I	21	38	51
230IL	12	22	26
sum	124	187	241

Efavirenz

Cut-off/ mutations	10%	2%	1%
101P	1	1	1
103HNST	42	49	53
106M	4	5	7
188L	4	4	4
190ACEQS	15	29	37
230L	2	3	3
sum	68	91	105

Table B: Samples with additional mutations >2% causing one step up in resistance interpretation for at least one drug and having mutational loads above 1.000 or 2.000 copies per mL sorted to drug-classes with therapy data and follow-up

ID	subtype	VL [c./mL]	PI-RAM	additional PI-RAM	PI more at 2%	NRTI-RAM	additional NRTI-RAM	NRTI more at 2%	NNRTI-RAM	additional NNRTI-RAM	NNRTI more at 2%	VL in minority	date RA	therapy RA	follow-up
rcent population		[100]	[10]	[02]		[10]	[02]		[10]	[02]		[02]			
10426285	B	3200000			0			0		V90I	1	64000	10.06.2015	TDF/FTC, RAL	VL 320.000 RA 12/16 106I, 184V
10425535	D	590000		M46IRS, G48CDEWY, I50GSV, F53FL, I54LPT, A71V	6			0			0	11800	08.06.2015	no therapy	TDF/FTC, DRV/r 12/15, nd
10480657	B	480000		E35G	1			0		K103N	1	9600	04.01.2016	no therapy	TDF/FTC, DTG success
10379553	B	400000			0			0		Y318F	1	8000	03.12.2014	pause	3TC/ABC/DTG success
10502043	CRF01_AE	398000	H69K	H69K	0			0		V106I, V189I	2	7960	31.03.2016	no therapy	TDF/FTC, RAL success (with blips)
10393031	B	390000			0	M184MV	K70R, M184V	1	E138AE	E138A	0	7800	02.02.2015	TDF/FTC/RPV/c	no change no INI-RAM (compliance?)
10387025	B	280000		K20R	1			0		K103N	1	5600	12.01.2015	no therapy	TAF/FTC/EVG/c success
10379550	B	260000			0			0		Y318FL	1	5200	03.12.2014	no therapy	TDF/FTC, RAL success
10494842	B	112000			0			0		V106I	1	2240	26.02.2016	no therapy	TDF/FTC/EVG/c success
10409027	B	96000			0			0		V189I	1	1920	07.04.2015	no therapy	nd
10414003	B	85000			0			0		V189I	1	1700	23.04.2015	no therapy	TDF/FTC, DTG success
10491937	B	85000			0			0		E138G	1	1700	15.02.2016	no therapy	TDF/FTC, DRV/r success
10430389	A	76000	H69K	V11I, K20EIV, D30EGKNRS, V32EIK, H69K	5			0			0	1520	29.06.2015	no therapy	no therapy
10438499	B	74000			0		M41L	1			0	1480	28.07.2015	pause	TDF/FTC, DTG success (with breaks)
10481467	B	66000			0			0		E138K	1	1320	06.01.2016	no therapy	3TC/ABC/DTG success
10388791	B	57683			0			0		E138K	1	1154	16.01.2015	no therapy	TDF/FTC/RPV success

RESULTS

In the evaluation period, we performed 645 NGS resistance tests. 483 (74,9%) of sequences were identified as subtype B. No drug resistance associated mutations were reported by the HIV-GRADE tool for 284 (44%) sequences with a cut-off of 10%. A loss of the wildtype status regarding the resistance levels compared to a 10% cut-off was observed for 101 samples at a cut-off of 2% and for 211 samples in the 1% cut-off group. The increase of resistance when lowering the cut-off could be shown for all drug classes with the highest proportions in the NNRTI drug-class 190 (29,5%) and 127 (19,7%) with cut-offs of 2% and 1% respectively.

With a cut-off of 10% in 148 samples (105 of them with a non-B subtype) only PI relevant mutations could be detected. We found samples with mutations only relevant for NRTIs in 21 samples and only for NNRTI in 100 samples. Additional mutations could be detected in 94 of the samples using a 2% minority cut-off. This increased to 157 samples more when utilizing a cut-off of 1%. This corresponds to an additional mutational load of >2000 c/mL in 76 cases with a 2% minority cut-off and additional 134 mutations at 1% cut-off.
Changes based on specific mutations for some broadly used drugs are shown in the tables. For Tenofovir for example the specific selected mutations (65R, 70E, 115F) show substantially higher incremental factors than the probably by other drugs selected TAMs.

CONCLUSIONS

A relative high proportion (56%) of investigated sequences showed resistance mutations at a minority cut-off of 10% increasing substantially lowering the cut-off range to 2 or 1% in number of mutation and also regarding resistance-levels. Relevance of mutations in these low-percentages is often discussed. The concept of “mutational load” tries to correlate the viral load with the proportion of mutation in the whole viral population. Despite the low percentage these viral quasi-species can be detected in relevant absolute quantities which increases the probability of detecting viable resistant virus. There is a clear need for clinical evaluation of the relevance of mutations in the low-percentage range for resistance interpretation due to its broader use in clinical routine.