Frequency of additional resistance relevant mutations in 2% and 1% population proportions in next generation sequencing in routine HIV-1 resistance diagnostics

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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 mutation detected in lower frequencies is still a subject of debate. We here report the frequency of additional mutations in population-proportions of greater than 2% and 1% in routine laboratory testing.

METHODS

All HIV-1 resistance tests of the reverse transcriptase Inhibitors (RTI) and protease Inhibitors (PI) performed with an in house PCR followed by NGS (Illumina MiSeq, sequences reported with >100 reads only in seven steps with more than 1%, 2%, 5%, 10%, 15%, 20% and 30% proportion of the population) between 10/2014 and 04/2016 were analyzed. Sequences were interpreted with the HIV-GRADE online tool (http://www.hiv-grade.de) for resistance relevant mutations using a 10%, 2% and 1% minority cut-off. Besides the subtype and the overall increase in mutations, a specific focus were differences in reported resistance associated mutations. We analyzed potential increase in resistance levels (e.g. additional drug class or further drugs in the same class).

RESULTS

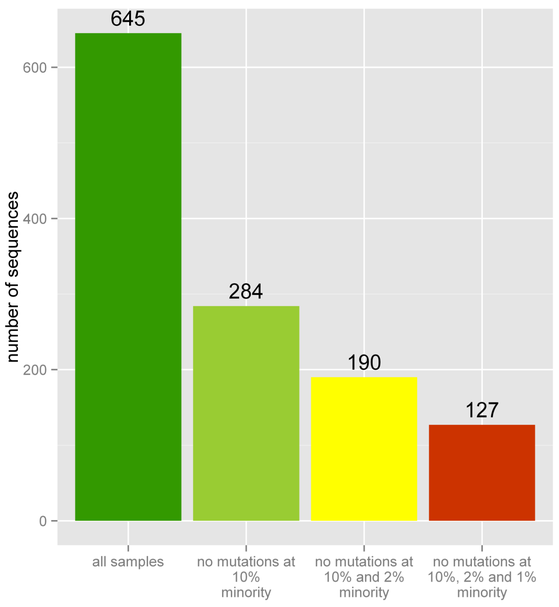

Fig. 1: No resistance-relevant mutations with different cut-offs

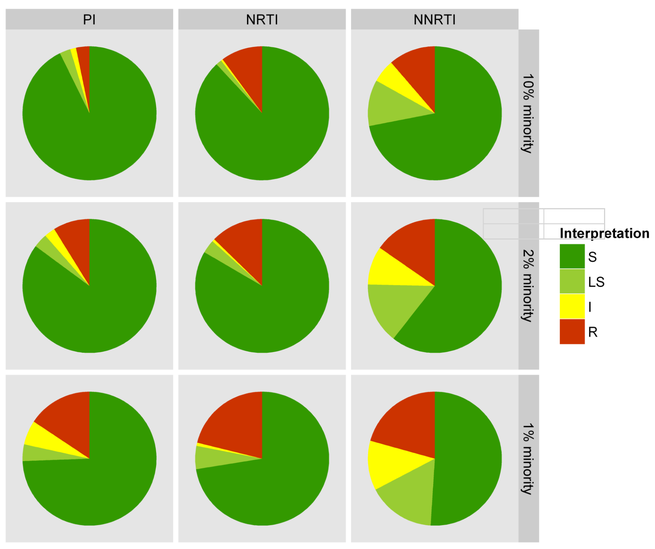

Fig. 2: Resistance interpretation with different cut-offs sorted by drug-classes

Tables to 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

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%, 190 (29,5%) and 127 (19,7%) with cut-offs of 2% and 1% respectively (s. Fig 1). 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. At a minority cut-off of 2% we detected mutations in 94 more samples as compared to a cut-off of 10%. This increased to 157 samples more when utilizing a cut-off of 1%. A loss of the wildtype status regarding the resistance levels compared to a 10% cut-off (412 samples 63,9%) 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 (s. Fig. 2).
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 relatively high portion (56%) of investigated sequences showed resistance relevant mutations at a minority cut-off of 10%. Even removing the non-B subtype sequences, containing only secondary mutations or subtype specific mutations, still left a proportion of 50% sequences with resistance-associated mutations. This high percentage of resistance increases substantially lowering the cut-off range to 2 or 1%. That´s true not only for the numbers of mutations but also regarding resistance-levels. There is a clear need for clinical evaluation of the relevance of mutations in the low percentage range in NGS for resistance interpretation due to its broader use in clinical routine.