# NGS in routine HIV-1 resistance diagnostic - frequency of additional resistance relevant mutations in 2% and 1% population proportions correlated to viral load and additional patient follow-ups

Martin Obermeier, Robert Ehret, Marcel Schütze

**MIB Medizinisches Infektiologiezentrum Berlin,**
*Contact: Obermeier@mvz-mib.de*

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## BACKGROUND

The technologies of next generation sequencing (NGS) 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. Clinical data is rare. We here report the frequency of additional resistance relevant mutations in population proportions of greater than 2% and greater than 1% in routine laboratory testing of HIV-1 Protease and Reverse Transcriptase resistance testing and correlate them to viral load. Therapy implications for patients with relevant minor viral populations were monitored.

## 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 mutations using 10%, 2% and 1% minority cut-offs. Besides the overall increase in mutations, 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 1000 and 2000 c/mL(= mutational load) were calculated, therapy data and follow up for those patients was monitored as far as available.

## RESULTS

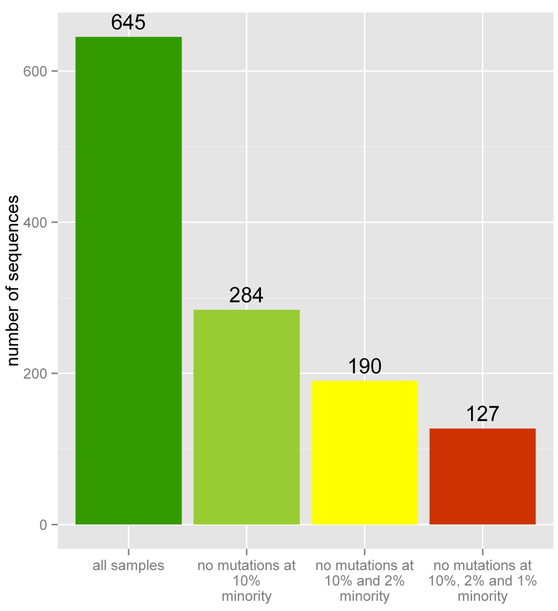


**Fig. 1: No resistance-relevant mutations with different cut-offs**

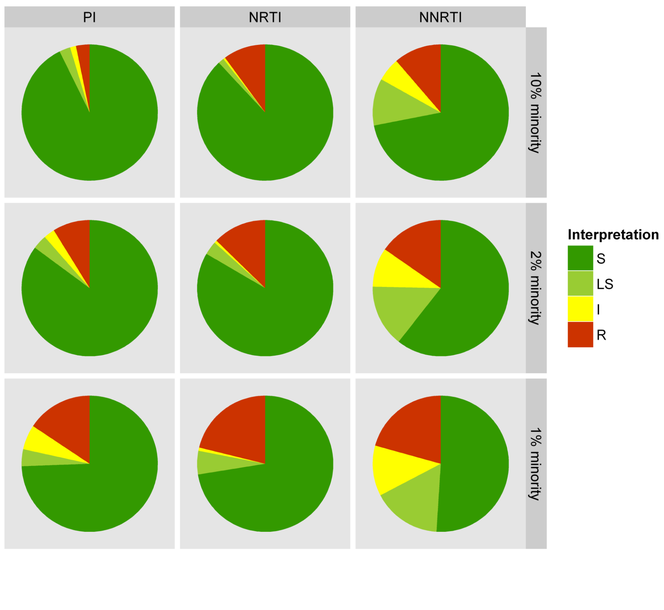


**Fig. 2: Resistance interpretation with different cut-offs sorted by drug-classes**

**Table 1: Samples with additional mutations >2% causing at least for one drug one step up in resistance interpretation and having mutational loads above 1.000 or 2.000 copies per mL sorted to drug-classes with therapy data and follow-up**

| ID | sub-type | VL [c./mL] | PI-RAM | additional PI-RAM | PI more | NRTI-RAM | additional NRTI-RAM | NRTI more | NNRTI-RAM | additional NNRTI-RAM | NNRTI more | VL in minority | date RA | therapy RA | follow-up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| >% population | | [100] | [10] | [02] | [02] | [10] | [02] | [02] | [10] | [02] | [02] | [02] | | | |
| 10426285 | B | 3200000 | | | 0 | | | 0 | | V90I | 1 | 64000 | 10.06.15 | no therapy | TDF/FTC, RAL VL 320.000 RA 12/16 106I, 184V |
| 10425535 | D | 590000 | | G48CDEWY, I50GSV, F53FL, I54LPT, A71V | 6 | | | 0 | | | 0 | 11800 | 08.06.15 | no therapy | TDF/FTC, DRV/c 12/15, success |
| 10480657 | B | 480000 | | E35G | 1 | | | 0 | | K103N | 1 | 9600 | 04.01.16 | no therapy | TDF/FTC, DTG success |
| 10379553 | B | 400000 | | | 0 | | | 0 | | Y318F | 1 | 8000 | 03.12.14 | pause | 3TC/ABC/DTG success |
| 10502043 | RF01_A | 398000 | H69K | H69K | 0 | | | 0 | | V106I, V189I | 2 | 7960 | 31.03.16 | no therapy | TDF/FTC, RAL success (with blips) |
| 10393031 | B | 390000 | | | 0 | M184MV | K70R, M184V | 1 | E138AE | E138A | 0 | 7800 | 02.02.15 | TDF/FTC/RPV | no change no INI-RAM (compliance?) |
| 10387025 | B | 280000 | | K20R | 1 | | | 0 | | K103N | 1 | 5600 | 12.01.15 | no therapy | TAF/FTC/EVG/c success |
| 10379550 | B | 260000 | | | 0 | | | 0 | | Y318FL | 1 | 5200 | 03.12.14 | no therapy | TDF/FTC, RAL success |
| 10494842 | B | 112000 | | | 0 | | | 0 | | V106I | 1 | 2240 | 26.02.16 | no therapy | TDF/FTC/EVG/c success |
| | | | | | | | | | | | | | | | |
| 10409027 | B | 96000 | | | 0 | | | 0 | | V189I | 1 | 1920 | 07.04.15 | no therapy | nd after RA |
| 10414003 | B | 85000 | | | 0 | | | 0 | | V189I | 1 | 1700 | 23.04.15 | no therapy | TDF/FTC, DTG success |
| 10491937 | B | 85000 | | | 0 | | | 0 | | E138G | 1 | 1700 | 15.02.16 | no therapy | TDF/FTC, DRV/r success |
| 10430389 | A | 76000 | H69K | V11I, K20EIV, D30EGKNRS, V32EIK, H69K | 5 | | | 0 | | | 0 | 1520 | 29.06.15 | no therapy | no therapy |
| 10438499 | B | 74000 | | | 0 | | M41L | 1 | | | 0 | 1480 | 28.07.15 | pause | TDF/FTC, DTG success (with breaks |
| 10481467 | B | 66000 | | | 0 | | | 0 | | E138K | 1 | 1320 | 06.01.16 | no therapy | 3TC/ABC/DTG success |
| 10388791 | B | 57683 | | | 0 | | | 0 | | E138K | 1 | 1154 | 16.01.15 | no therapy | TDF/FTC/RPV success |

## RESULTS

In the evaluation period, we performed 645 NGS resistance tests. 483 (74,9%) of the sequences were identified as subtype B. No drug resistance associated mutations were reported by HIV-GRADE 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, 21 samples with only NRTI and 100 samples with only NNRTI mutations. Regarding the resistance level the same drift could be observed, a loss of wild type status (shown in darker green in Fig. 2) lowering the cut-off to 2% and 1%. 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 (Fig. 2).

At a minority cut-off of 2% we detected mutations in 94 more samples as compared to a cut-off of 10%, 39 of them or 41% with a mutational load above 2.000 c/mL and 70 > 1.000 c/mL (74%). The number with additional mutations utilizing a cut-off of 1% increased to 157 samples. Tab 1 summarizes 16 patients that showed additional mutations at 2% cut-off, 9 with a mutational load of > 2.000 c/mL and 7 with > 1.000 c/mL. The mutations resulted in a higher resistance classification by HIV-GRADE for at least one drug. Mutations, viral loads, therapies and follow-up data are listed. We could not document an influence of minority populations on therapy choice or outcome.

## CONCLUSIONS

A relative high proportion (56%) of investigated sequences showed resistance mutations at a minority cut-off of 10%. This high percentage of resistance increases substantially lowering the cut-off range to 2 or 1% not only by number of mutations but 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 quasispecies (above 2%) can be detected in relevant absolute quantities (41% >2.000 c/mL and 74% >1.000 c/mL) which increases the probability that these mutations represent viable resistant virus. Our data pool, especially in the number of therapy follow up is to small to recognize an influence on therapy outcome. Another limitation of this observation is the missing of data for Integrase resistance because many (firstline-) therapies include Integrase inhibitors meanwhile (s. Tab 1). Developments in this direction should be included in the future.

There is still 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.

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