Medizinisches Infektiologiezentrum Berlin

Next generation sequencing in routine HIV-1 resistance diagnostic – frequency of additional resistance relevant mutations in 2% and 1% population proportions

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Robert Ehret

Berlin

Concept

- **background**
- **methods**
  NGS sequencing and interpretation
- **results**
  account of resistance relevant mutations
  differences in resistance levels
  appearing major mutations in lower population proportions
- our course of action in reporting results

background

next generation sequencing (NGS) has made the way into routine diagnostics in HIV-1 resistance testing

it is still a subject of debate which percentage is reported to the physicians and what is the relevance of mutation detected in lower frequencies

I want to report now the frequency of resistance relevant mutations in population proportions of greater than 10%, 2% and 1% of our routine laboratory testing of HIV-1 Protease and Reverse Transcriptase

methods

we use an in-house PCR protocol for amplification of the HIV-1 POL region

the library preparation and sequencing is performed in Kaiserslautern by Martin Däumer and Alexander Thielen, Seq IT GmbH & Co.KG, Labor Dr. Thiele

they use the Illumina MiSeq and report only sequences of HIV-1 POL with >100 reads with seven cut-offs to us, with more than 1%, 2%, 5%, 10%, 15%, 20% and 30% proportion of the population

we can check for each position the rate of reads, aminoacids, templates

interpretation is performed with the HIV-GRADE online tool (http://www.hiv-grade.de)

HIV-Grade algorithm

HIV-GRADE version 12/2015

Sequence Analysis | Mutation List Analysis

Sequence: O-176_POL.fasta_1 O-176-10

Sequence Date	20-May-2016
Algorithms	GRADE ANRS HIVDB Rega geno2pheno

Length of included Sequences:

- **Sequence includes PR: codons: 1 - 99**
- **Sequence includes RT: codons: 1 - 342**

Subtype and % similarity to closest reference isolate (adopted from Stanford hivDB):

1. PR: B (97.6%)
2. RT: B (94.0%)

Gene	Differences from Consensus B	Drug Resistance Mutations
PR	E35D, M36I, N37D, L63P	E35D
RT	V35I, R83K, D123E, I135T, E138A, K166R, R172K, D177E, M184V, Y188L, G196E, R211K, T215Y, V245M, E248D, A288S, V292I, E297K, I326V, Q334D	E138A, M184V, Y188L, T215Y

NNRTI	GRADE 12/2015			ANRS 25_09/2015			HIVDB 7.0.1			Rega 9.1.0			geno2pheno 3.4			Final Rating
	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	
EFV	Y188L	Resistance	R	Y188L	Resistance	R	Y188L	High-level resistance *(Score:60)*	R	Y188L	Resistant GSS 0 *(Score:2)*	R	9.7 : R	2.5 : R (>2.1)	R	
ETR	E138A, Y188L	Intermediate	I	E138A	Possible resistance	I	E138A, Y188L	Low-level resistance *(Score:25)*	I	Y188L, E138A	Susceptible GSS 1 *(Score:0.75)*	S				
NVP	Y188L	Resistance	R	Y188L	Resistance	R	Y188L	High-level resistance *(Score:60)*	R	Y188L	Resistant GSS 0 *(Score:2)*	R	24. : R	3.2 : R (>2.4)	R	
RPV	E138A, Y188L	Resistance *(Score:4)*	R	Y188L, E138A	Resistance	R	E138A, Y188L	High-level resistance *(Score:75)*	R	E138A, Y188L	Resistant GSS 0 *(Score:4)*	R				

Scored mutations for Drugclass NNRTI : E138A, Y188L

http://www.hiv-grade.de

HIV-Grade
Four interpretation-level:

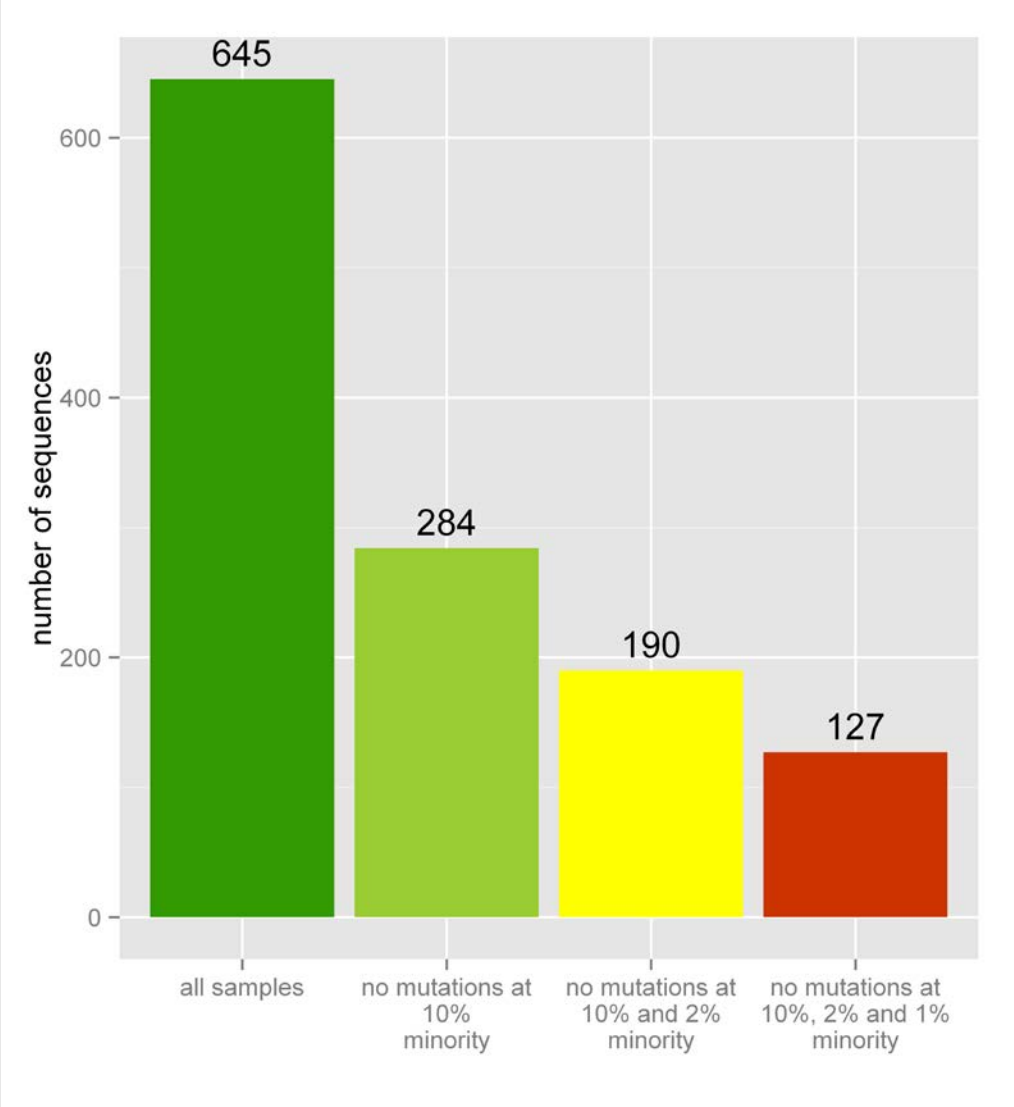
Susceptible

Limited susceptibility

Intermediate resistance

Resistance

results: mutations



samples without resistance relevant mutations observed in the different cut-off groups
44% without or
56% with relevant mutations at 10%

increasing to 70.5% at 2% cut-off

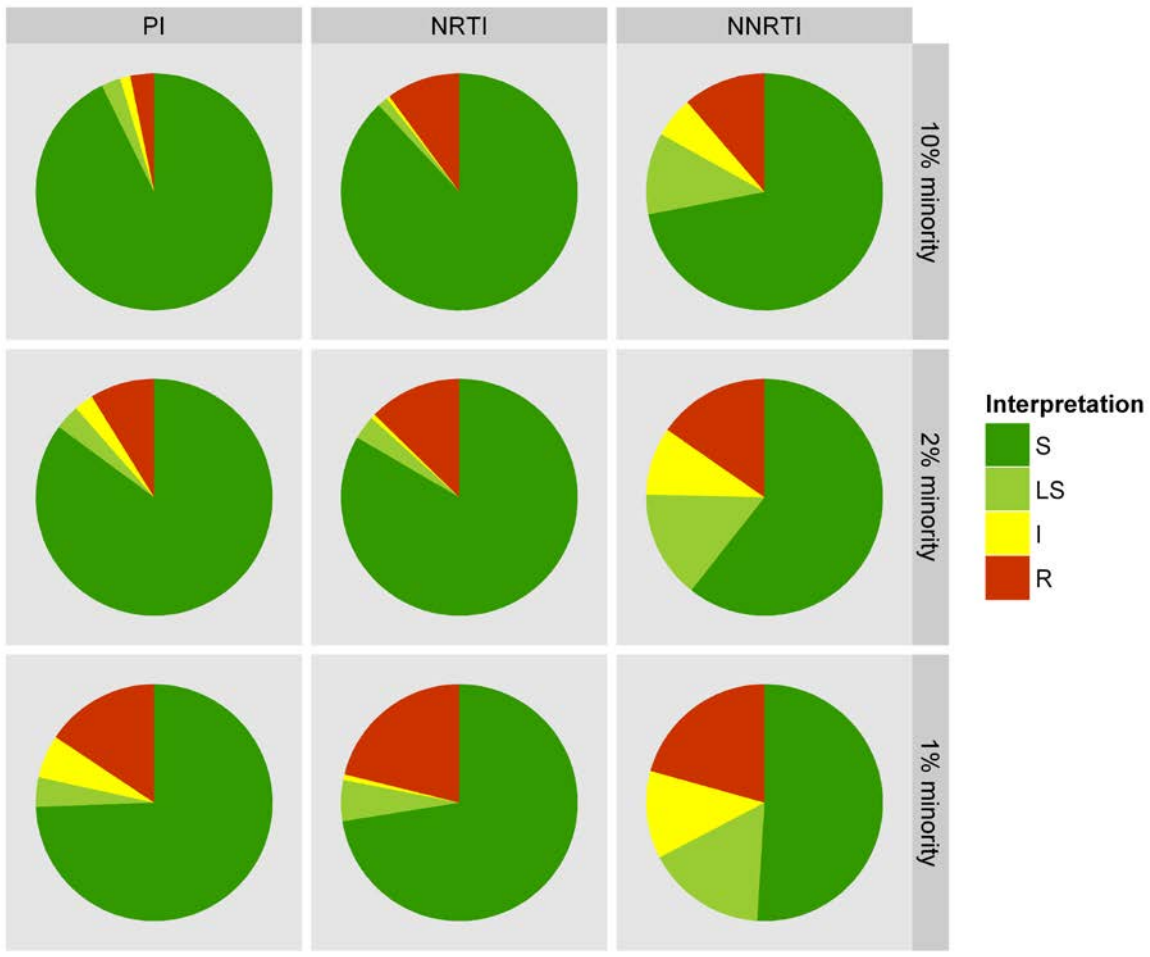
and to 80.3% at 1% cut-off

most often PI mutations (subtype B $_{n=483}$):
10FIV, 20MIRT, 71V, 46IL, 73S, 82AFT

most often NRTI mutations:
184IV, 118I, 65R, 70ENR, 219ENQ

most often NNRTI mutations:
103NRST, 138AGK, 189I, 90I, 106AIM, 190EAGSV, 230ILV

results: interpretation



sequences interpreted for all three drug classes in the different cut-off groups

as the mutation-rate the level of resistance is increasing with lowering the cut-off

that´s true for all drug classes, with the highest proportions for NNRTIs

results: PI

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

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

exemplarily two broadly used protease inhibitors and their detected specific mutations

some mutation are more or less stable with different cut-offs

others differ a lot

APOBEC ?

results: NRTI

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

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

exemplarily two broadly used NRTIs and their detected specific mutations

some mutation are more or less stable with different cut-offs

others differ a lot

APOBEC ?

results: NNRTI

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

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

exemplarily two broadly used NNRTIs and their detected specific mutations

same game, same results

some mutation are more or less stable with different cut-offs

others differ at least with a factor of 2 – 2.5

APOBEC ?

appraisal and output

so far, we take the 10% cut-off sequence an fill out our report by sorting the detected mutations to the belonging drugs and give a estimation if the drug is useful, less or not useful anymore

if there appear minorities above 2% we put them in brackets and write a text with something like:

The relevance of these minorities is not well known and not clinically validated. If there are enough therapeutical possibilities you should consider these mutations and optionally do without this drug.

and you?

what are your experiences ?

do you find something totally different ?

what percentage of minorities do you react on ?

and what do you tell your physicians or patients?

Many thanks to:

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Thank you for your attention !

Robert Ehret

Berlin