

The Evolution of Drug Resistance Interpretation Algorithms: ANRS, REGA and Extension of Resistance Analysis to HIV-1 Group O and HIV-2

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Key Words

HIV-2 · HIV-1 group O · Drug resistance · Algorithms

Abstract

Antiretroviral drug resistance is mostly linked to a complex interaction of several amino acids with variable importance or a single amino acid. To facilitate the interpretation of observed mutation patterns, hospital university centers have developed several interpretation systems. All the currently available interpretation algorithms evolved, are being continuously updated and have been improved during the last decade. Some discrepancies are still evident that are partially smoothed by link of the individual programs with other systems. After the interpretation of HIV-1 group M subtype B mutations, a refined algorithm for the other group M subtypes was developed followed by the interpretation of HIV-1 group O and HIV-2 mutations. The process of improvement is ongoing, due to the better understanding and interpretation of single and cluster mutations and the availability of new antiretroviral substances. The knowledge gained from the experience of HIV drug resistance testing has been used to establish the interpretation of HBV polymerase mutations and will be extended for the treatment of HCV infected with protease inhibitors.

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Introduction

When AIDS was described in 1981 the disease burden was very high. In 1987 the first antiretroviral drugs were administered [1] and benefit for the patient was generally very short. Due to the development of resistance and thus drug inactivity, the capacity to interfere with HIV replication was already lost in some patients after 6 weeks. Some years later, the phenotypic and genotypic analysis of a patient's HIV by nucleic acid sequencing allowed the identification of several key amino acids responsible for the induction of resistance. The introduction of new substances for antiretroviral therapy (ART) caused further mutations. In 1997, the analysis of the same amino acid mutations in different patients was the beginning of the development of interpretation algorithms in centers in Paris (Agence Nationale de Recherches sur le Sida; ANRS), Leuven (Rega Institute; REGA) and Stanford (Stanford University, HIV database; HIVdb).

Algorithms were initially based on the HIV-1 group M subtype B and subsequently extended to further subtypes. Since 2004/2005 they have included HIV-2 groups A and B and quite recently HIV-1 group O. With close communication between the centers and open access to the interpretation algorithm, the process of learning and improving is still continuing.

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The enzymes of HIV types and groups are similar but also individual. In consequence, some of the amino acid mutations that evolve under the drug pressure in HIV-1 group M subtype B are naturally present in the group O virus and in HIV-2. Structural compensation of the virus to circumvent the ART drug pressure in group M is different from that in group O and HIV-2, e.g. the cleavage sites for the protease (PR) and active site for the reverse transcriptase (RT). The effective functioning of the enzymes PR and RT evolves under selection pressure and is maintained during HIV particle production. In the infected human cell, viral enzyme function is hampered by the ART applied only as long as no resistance evolves. To delay the generation of drug resistance ART, combination therapy is indispensable and the knowledge of which drug combination can perform best for the improvement of the health status of the patient. For the latter a detailed and sophisticated drug resistance interpretation algorithm was developed and remains necessary [2].

ANRS – the Internet address is: <http://www.hivfrenchresistance.org/>. First interpretation programmes were instituted around 2002 and are continuously being improved.

REGA – the Internet address is: <http://jose.med.kuleuven.be/lab/>. For direct determination of the HIV-1 group M subtype see: <http://hivdb.stanford.edu/>. HIV-1 group M subtype interpretation is based on the evaluation of more than 4,000 nucleic acid sequences from mostly African patients [3]. The REGA algorithm can be found under: <http://jose.med.kuleuven.be/lab/index.php?id=30>. Here, it is indicated that drug resistance interpretation for HIV-2 was available with version 7 in 2007 and that the latest version was refined in June 2009.

Stanford HIVdb – the Internet address is: <http://hivdb.stanford.edu/> (see Tang et al. [4]).

Additional data bases for the interpretation of mutated amino acids are: geno2pheno (<http://www.geno2pheno.org/>), see also Thielen and Lengauer [5] and Lengauer [6], Virco (<http://vircolab.com/>) in Pattery et al. [7] and HIV-GRADE (<http://www.hiv-grade.de/cms/grade/>) in Obermeier et al. [8].

The algorithms are primarily based on the evaluation of subtype B resistance. The resistance interpretation offered also takes into account the naturally occurring amino acid mutations in strains that were not subtype B and amino acids that facilitate the generation of resistant strains [2, 9]. Naturally occurring amino acids linked with ART drug resistance were especially found in group M subtype C and G strains in the PR genome in positions 20, 36 and 82 (table 1) [10].

Table 1. Protease amino acid resistance mutations

Position	M:A	M:B	M:C	M:G	O:A	O:B	O:C	HIV-2A	HIV-2B
10	I	L			I	I	V	P	P
20		K	R	I				P	P
24		L							
30		D						(N)	
36	I	M	I	I	I	I	I	I	I
46		M	I					I	I
47		G							
48		G							
50		I							
54		I							
62		I	V	V	T	T	T	V	V
71		A			V	V	V	V	V
77		V	I	I				I	I
84		I				L	L		
90		L							

Numbers show the position of selected amino acids that occur in the majority of strains of HIV-1 group M subtype A, B, C and G, group O clades A, B and C [17], and HIV-2 groups A and B and which are responsible for PR inhibitor resistance or impaired activity.

Interpretation of Amino Acid Mutations in HIV-1 Group M

As already mentioned, all available ART drugs were developed in countries where HIV-1 M subtype B shows the highest prevalence and these drugs were primarily evaluated against subtype B strains. Drug resistance prediction models were trained on results obtained with HIV strains grown in culture with a reduced replication rate, and taking sequence data of isolates of patients according to treatment response and failure. A further way of monitoring the efficacy of an algorithm is by a follow-up of patients whose therapeutic regimen was changed because of resistance development and for whom the beneficial drug action in the new combination could be evaluated. Identification of the key amino acids leading to treatment failure is one item; another is weighing the importance of a single mutation or the combination of several mutations in the different parts of the HIV genome. In 2003, even within 26 subtype B strains, divergent results were obtained by 9 available algorithms; the Virco and geno2pheno interpretation programs were included in this particular study [11]. Because the system for interpretation is complex, it cannot be avoided that discordant results are obtained with different interpretation algorithms.

Table 2. RT and NRTI amino acid resistance mutations

Amino acid position	M:A	M:B	M:C	M:G	O:A	O:B	O:C	HIV-2A	HIV-2B
41		M						K	
65	(R)	K							
67		D							
69		T							
74		L							
75		V							
77		F							
115		Y							
151		Q							
184		M							
210		L			Y	Y	Y	G	N
215		T							
219		K	(Q)						

Selected amino acid positions for resistance induction of NRTI are given compared to HIV-1 group M subtype B, for subtype A, C and G, for group O clades A, B and C [17], and for HIV-2A and HIV-2B.

The study of van de Vijver et al. [12] showed a divergence of 25–70% in the results (i.e. resistant and possibly resistant) of a virtual phenotypic assay and a genotypic interpretation, in strains from 15 European countries, e.g. for dideoxyinosine (ddI) using the genotypic ANRS and phenotypic virco TYPE algorithm. Despite the reported discrepancies, a considerable convergence of the 3 interpretation systems ANRS, HIVdb and REGA in >3,700 patients from Europe was found in the prediction of virological response for up to 48 weeks [13] and in >4,600 patients from the United States applying the ViroSeq interpretation system in addition to these other 3 [14]. Results of all studies request further refinement of drug-specific genotypic susceptibility scores to improve the virological response predictions.

Drug resistance prevalence is still dependent on the frequency of the drug administered, and thus of the enforced selection pressure. Single point mutations against nucleoside reverse transcriptase inhibitors have been found in 74%, against nonnucleoside reverse transcriptase inhibitors in 49% and against PR inhibitors in 36%. Dual-drug resistance was present in 46% and triple-drug resistance in 20% of all strains [12]. Among approximately 500 patients from France and Switzerland, complete resistance against 1, 2 or 3 classes of ART drugs was found in 37, 15 and 4%, respectively, using the ANRS algorithm and in 27, 23 and 24%, respectively, using the HIVdb al-

Table 3. RT and NNRTI amino acid resistance mutations

Amino acid position	M:A	M:B	M:C	M:G	O:A	O:B	O:C	HIV-2A	HIV-2B
98		A		G/S					
100		L							
101		K			Q	Q	Q		
103		K			Q	Q	Q	R	R
106		V							
108		V							
179		V			E	E	E		
181		Y			C	C		I	I
188		Y						L	L
190		G						A	A

Numbers show the position of selected amino acids that occur in the majority of strains of HIV-1 group M subtype A, B, C and G, group O clades A, B and C [17], and HIV-2 groups A and B and which are responsible for NNRTI resistance or impaired activity.

gorithm [15]. This discrepancy illustrates how hard it may be to reach an unambiguous conclusion, despite the efforts made over a decade and the data sets of >10,000 patients.

Interpretation of Amino Acid Mutations in HIV-1 Group O

This virus has mainly spread in West/Central Africa, especially Cameroon, Equatorial Guinea and Gabon and is found in patients originating from this region or having close contact with people from this region. Some 100 patients infected with HIV-1 group O are known in Portugal and France, as well as a few in Germany, Spain and other countries. Group O viruses may be divided into 3 major clades, A, B and C, indicating nearly the same degree of heterogeneity as the group M virus [15, 17].

As may be seen in tables 1–3, there are some naturally occurring amino acid mutations compared to the group M virus that are associated with resistance and which limit the choice of drugs for specific therapy in group O-infected patients. The group O virus is resistant to NNRTI, while most NRTI and PR inhibitors are active. Resistance mutations restricting integrase inhibitors and the fusion inhibitor T20 are rarely found [17]. To amplify

the genomic regions of the PR, RT and integrase as well as gp41 and the V3 loop, specifically designed primer sets differing from those for group M are needed.

Interpretation of Amino Acid Mutations in HIV-2 Groups A and B

HIV-2 is distributed mainly in West Africa, Angola, Mozambique and on the western coast of India. Infected people can also be found in European countries. The viral load is usually not as high as in HIV-1 infection and the decline of CD4 cells is more moderate [18]. The ANRS and REGA resistance algorithms for interpretation have been available since about 2004 [19].

As shown in table 3, drug susceptibility testing revealed that most of the NRTI were active, while all NNRTI were inactive. Within the repertoire of PR inhibitors, amprenavir and atazanavir failed to be sufficiently active. Inhibition of HIV-2 by enfur tide (T20) was not achieved [20, 21]. Integrase inhibitors are active against HIV-2 [17]. Basics for treatment are described by Camacho [22].

Since the number of HIV-2 patients treated with anti-retroviral drugs is low compared to HIV-1 patients, broad experience on the action of ART in patients is not available, but the development of resistance may well be as fast as in HIV-1 patients [23, 24]. Viral replication in lymphocytes may be high despite the absence of viremia [25].

As was mentioned for the group O, virus-specific primers have to be used to amplify HIV-2 genes and occasionally it is hard to amplify the 5' region of the RT of HIV-2 group B. Participation in international evaluation trials is also recommended to maintain a high standard of quality control [26].

Compensatory Mutations in Gag

Compensatory mutations which lead to restored activity of the nelfinavir-resistant HIV-1 PR were detected in Gag as P453L [27]. A further structure involved in restoring reduced sensitivity to PR inhibitors seems to be the N-terminus of Gag [28]. Polymorphic mutations at positions 128, 437 and 449 of HIV-1 Gag seem to be involved in a virological response in treatment-naive patients receiving two PR inhibitors [29]. Studies of whether similar polymorphic mutations are selected in HIV-2 resistance are currently not available.

Compensatory mutations are also found in the envelope proteins: vicriviroc binds to the V3 loop of gp120. Resistance to vicriviroc binding is not only dependent on amino acid mutations within the V3 loop but may be found in compensatory sites in gp41 [30].

Drug Resistance and Amino Acid Mutations in the Polymerase Gene of HBV

The HBV polymerase acts as RT to complete the double-stranded circular DNA after liver cell entry. The HBV polymerase can be inhibited by some NRTI as lamivudine, emtricitabine and tenofovir. Other substances such as adefovir, entecavir and telbivudine are available. Drug resistance is partially linked to the YMDD motif of the HBV polymerase as observed in the HIV RT [31, 32].

HBV drug resistance interpretation systems are available at HIVdb Stanford, HIV-GRADE and geno2pheno. Due to the overlapping reading frames of HBV polymerase and HB surface antigen, HIV-GRADE and geno2pheno provide not only information on resistance to antiviral drugs but also on immune and detection escape variants of HBV.

Drug Resistance and Amino Acid Mutations in the NS3 PR Gene of HCV

HCV infection is treated with the combination of pegylated interferon- α and ribavirin. Treatment of HCV genotype 1 infection can be essentially improved by addition of the HCV NS3 PR inhibitors boceprevir (Victrelis[®], Merck) and telaprevir (Incivu[®], Jansen). Drug resistance develops quickly under monotherapy [33, 34]. As has been the experience for HIV and HBV, genotypic assays for drug susceptibility evaluation in HCV-infected patients who are treated with PR inhibitors are indispensable. In the analysis of mutations, a geno2pheno tool is also provided for the genotypic determination of drug resistance of the so-called direct-acting antiviral agents, such as PR (NS3), polymerase (NS5B) and NS5A inhibitors.

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