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Development of a list of mutations associated with NNRTI resistance for use in clinical research

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Abstract

Background
Current lists of NNRTI resistance-associated mutations (RAMs) are based on experience with the three approved NNRTIs (delavirdine [DLV], nevirapine [NVP] and efavirenz [EFV]). The widely used IAS-USA HIV-1 Drug Resistance Mutation list, Fall 2006 revision, includes 14 reverse transcriptase (RT) mutations associated with resistance to the currently available NNRTIs: L100I, K103N, V106A, V106M, V108I, Y181C, Y181I, Y188C, Y188H, Y188L, G190A, G190S, P225H, and P236L. Given the development of novel NNRTIs with a higher genetic barrier to resistance and the early evidence of activity in the presence of these mutations, a more comprehensive list of NNRTI RAMs was made.

Methods
A broad review of the existing literature on NNRTI resistance *in vitro* and *in vivo* was performed. Additionally, research on the mechanism of HIV-1 resistance to the currently approved NNRTIs, and to NNRTIs formerly and currently in clinical development was conducted. This research included (1) the in-vitro activity of NNRTIs tested against HIV-1 site-directed mutants or recombinant clinical HIV-1 isolates, (2) the emergence of mutations in resistant strains selected *in vitro* starting from wild type or NNRTI-resistant HIV-1, and (3) the analysis of genotype and phenotype of HIV-1 isolates from patients on a failing NNRTI-containing regimen.

Results
The proposed list included 41 RT mutations: A98G, L100I, K101E, K101P, K101Q, K103H, K103N, K103S, K103T, V106A, V106M, V108I, E138G, E138K, E138Q, V179D, V179E, V179F, V179G, V179I, Y181C, Y181I, Y181V, Y188C, Y188H, Y188L, G190A, G190C, G190E, G190Q, G190S, H221Y, P225H, F227C, F227L, M230I, M230L, P236L, K238N, K238T, and Y318F. All the mutations included in this list were observed, either alone or in combination with others, in at least 15 different strains from a panel of 17,390 HIV-1 clinical isolates. Phenotypic data from site-directed mutants helped to establish the degree to which mutations or combinations of mutations contribute to the resistance against the different NNRTIs.

Conclusions
This list provides researchers with a more complete overview of RT mutations playing a role in NNRTI resistance and can be used to guide in-vitro and clinical research on the mechanism of HIV-1 resistance to current and investigational NNRTIs.

Introduction

- Current lists of NNRTI RAMs are based on clinical experience with the three currently approved NNRTIs DLV, EFV and NVP.
- The widely used IAS-USA Drug Resistance Mutation list 2006[1], includes 14 RT mutations associated with resistance to the currently available NNRTIs.
- Given the development of next-generation NNRTIs with a higher barrier to resistance and the early evidence of their activity in the presence of NNRTI mutations from the IAS-USA Drug Resistance Mutation list, the goal of this study was to obtain a more comprehensive list of NNRTI RAMs to guide in vitro and clinical research.

Methods

- An extensive review of the existing literature on HIV-1 resistance to NNRTIs.
- A comprehensive analysis of available lists of NNRTI mutations.
  - IAS-USA Drug Resistance Mutation list 2006[1] (n = 14)
  - resistance notes from the Stanford University HIV Drug Resistance Database updated in August 2006[2] (n = 29)
  - Virco®TYPE mutations[3] 2005 (n = 38)
  - Los Alamos National Laboratory Database[4] (n = 51)
- In-vitro testing of the antiviral activity of NNRTIs against HIV-1 site-directed mutants or recombinant clinical HIV-1 isolates.
- Analysis of the mutations emerging from wild type or mutant HIV-1, following in-vitro selection in the presence of NNRTIs.
- Genotypic and phenotypic analysis of HIV-1 recombinant clinical isolates from patients on a failing NNRTI-containing regimen.

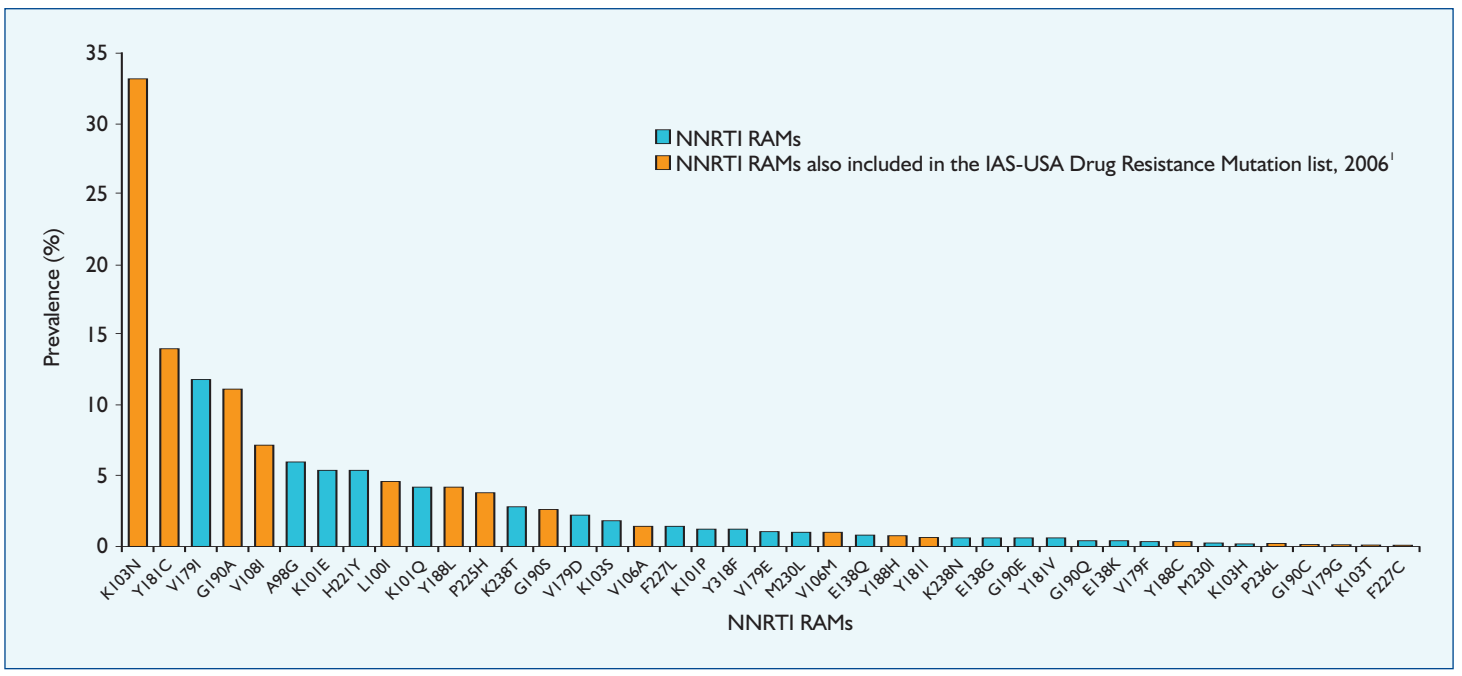
Results

- The list of NNRTI RAMs resulting from this extensive analysis includes 41 HIV-1 RT mutations.
- For each mutation the following results are presented in **Table 1**
  - the prevalence of the mutation in a panel of 19,689 HIV-1 recombinant clinical isolates collected at Virco between 2004 and 2006, with at least one NRTI or one primary PI mutation from the IAS-USA Drug Resistance Mutation list 2005[5]. This prevalence is also illustrated in **Figure 1**
  - the number of nucleotide substitutions needed to obtain the indicated amino acid
    - o the wild type codon as in the HIV-1/HXB2 reference strain
    - o the mutated codon as the most prevalent codon in clinical samples (mainly clade B)
  - the fold change (FC) in EC_{50} value for EFV, NVP and TMC125 for the corresponding site-directed mutant
  - the reference to one or more peer reviewed publications, which demonstrate NNRTI-associated resistance in vitro and/or in clinical samples.

Table 1. List of NNRTI RAMs.

NNRTI RAM	Prevalence in clinical samples (%)	Number of nucleotide substitutions (codon change)	FC in site-directed mutants EFV	NVP	TMC125	Reference(s)
A98G	5.90	1 (GCA→GGA)	2.2	8.1	2.5	Byrnes VW, et al. AAC 1993;37:1576–9; Bacheler LT, et al. AAC 2000;44:2475–84
L100I	4.47	1 (TTA→ATA)	13.1	NA	1.8	Mellors JW, et al. Mol Pharmacol 1993;43:11–6; Johnson VA, et al. Top HIV Med 2006;14:125–30
K101E	5.46	1 (AAA→GAA)	2.9	4.3	1.7	Byrnes VW, et al. AAC 1993;37:1576–9; Bacheler LT, et al. AAC 2000;44:2475–84
K101P	1.18	2 (AAA→CCA)	97.4	>733.4	6.2	Rhee S-Y, et al. AAC 2004;48:3122–6; Parkin NT, et al. AAC 2006;50:351–4
K101Q	4.09	1 (AAA→CAA)	3.8	NA	3.4	Kleim J-P, et al. J Infect Dis 1999;179:709–13; Bacheler LT, et al. AAC 2000;44:2475–84
K103H	0.15	2 (AAA→CAC)	15.6	17.8	1.6	Harrigan PR, et al. AIDS 2005;19:549–54
K103N	33.07	1 (AAA→AAC)	26.7	56.2	0.7	Nunberg JH, et al. J Virol 1991;65:4887–92; Johnson VA, et al. Top HIV Med 2006;14:125–30
K103S	1.76	2 (AAA→AGC)	4.9	36.2	0.9	Kleim J-P, et al. J Inf Dis 1999;179:709–13; Harrigan PR, et al. AIDS 2005;19:549–54
K103T	0.08	1 (AAA→ACA)	1.4	>37.0	1.3	Harrigan PR, et al. AIDS 2005;19:549–54
V106A	1.35	1 (GTA→GCA)	1.8	86.1	0.5	Larder BA. AAC 1992;36:2664–9; Johnson VA, et al. Top HIV Med 2006;14:125–30
V106M	0.89	2 (GTA→ATG)	2.3	6.9	0.8	Loemba H, et al. AAC 2002;46:2087–94; Johnson VA, et al. Top HIV Med 2006;14:125–30
V108I	7.20	1 (GTA→ATA)	1.2	2.7	0.5	Byrnes VW, et al. AAC 1993;37:1576–9; Johnson VA, et al. Top HIV Med 2006;14:125–30
E138G	0.49	1 (GAG→GGG)	2.3	NA	3.8	Pelemans H, et al. Virology 2001;280:97–106
E138K	0.37	1 (GAG→AAG)	1.8	1.7	2.4	Balzarini J, et al. PNAS USA 1994;91:6599–603
E138Q	0.80	1 (GAG→CAG)	7.1	NA	5.1	Pelemans H, et al. Virology 2001;280:97–106; McCreedy B, et al. Antivir Ther 1999;4(Suppl. 1). Abstract 13
V179D	2.14	1 (GTT→GAT)	6.2	5.7	2.6	Byrnes VW, et al. AAC 1993;37:1576–9; Palmer P, et al. Antivir Ther 2003;8(Suppl. 1):S411–2. Abstract 811
V179E	1.01	2 (GTT→GAG)	4.0	2.6	1.1	Byrnes VW, et al. AAC 1993;37:1576–9; Vingerhoets J, et al. 13th CROI 2006 (Abstract 154)
V179F	0.26	1 (GTT→TTT)	>0.4	1.6	0.1	Vingerhoets J, et al. J Virol 2005;79:12773–82; Vingerhoets J, et al. 13th CROI 2006 (Abstract 154)
V179G	0.08	1 (GTT→GGT)	0.6	NA	0.6	Miller MD, et al. XII IHDRW 2003 (Abstract 135)
V179I	11.73	1 (GTT→ATT)	0.9	1.3	0.8	Turner D, et al. AAC 2004;48:2993–8
Y181C	14.03	1 (TAT→TGT)	2.2	207.6	3.9	Nunberg JH, et al. J Virol 1991;65:4887–92; Johnson VA, et al. Top HIV Med 2006;14:125–30
Y181I	0.59	2 (TAT→ATT)	1.6	>55.5	12.5	Shih C-K, et al. PNAS USA 1991;88:9878–82; Johnson VA, et al. Top HIV Med 2006;14:125–30
Y181V	0.46	2 (TAT→GTT)	2.8	2155.9	17.4	Shih C-K, et al. PNAS USA 1991;88:9878–82; Vingerhoets J, et al. 13th CROI 2006 (Abstract 154)
Y188C	0.24	1 (TAT→TGT)	2.1	36.5	0.2	Richman DD. AAC 1993;37:1207–13; Johnson VA, et al. Top HIV Med 2006;14:125–30
Y188H	0.66	1 (TAT→CAT)	7.6	5.5	0.3	Sardana VV, et al. J Biol Chem 1992; 267:17526–30; Johnson VA, et al. Top HIV Med 2006;14:125–30
Y188L	4.06	2 (TAT→CTT)	31.9	173.4	0.9	Shih C-K, et al. PNAS USA 1991;88:9878–82; Johnson VA, et al. Top HIV Med 2006;14:125–30
G190A	11.10	1 (GGA→GCA)	6.8	105.0	0.8	Bacolla A, et al. J Biol Chem 1993;268:16571–7; Johnson VA, et al. Top HIV Med 2006;14:125–30
G190C	0.09	2 (GGA→TGC)	NA	NA	NA	Huang W, et al. J Virol 2003;77:1512–23
G190E	0.48	1 (GGA→GAA)	NA	NA	NA	Kleim J-P, et al. AAC 1993;37:1659–64; Bacheler LT, et al. AAC 2000;44:2475–84
G190Q	0.38	2 (GGA→CAA)	NA	NA	NA	Kleim J-P, et al. Virology 1994;200:696–701; Huang W, et al. J Virol 2003;77:1512–23
G190S	2.48	2 (GGA→AGC)	94.4	177.1	0.2	Kleim J-P, et al. Virology 1994;200:696–701; Johnson VA, et al. Top HIV Med 2006;14:125–30
H221Y	5.45	1 (CAT→TAT)	NA	NA	NA	Gonzales MJ, et al. AIDS 2003;17:791–9; Saracino A, et al. J Med Virol 2006;78:9–17; Perno CF, et al. AIDS Rev 2006;8:179–90
P225H	3.70	1 (CCT→CAT)	2.2	2.8	1.0	Kleim J-P, et al. J Infect Dis 1999;179:709–13; Bacheler LT, et al. AAC 2000;44:2475–84; Johnson VA, et al. Top HIV Med 2006;14:125–30
F227C	0.02	1 (TTC→TGC)	NA	NA	NA	Andries K, et al. AAC 2004;48:4680–6
F227L	1.28	1 (TTC→CTC)	0.7	2.9	0.4	Balzarini J, et al. AIDS Res Hum Retroviruses 1998;14:255–60; Parkin NT, et al. AIDS 2000;14:2877–87
M230I	0.18	1 (ATG→ATA)	4.7	13.1	2.4	Kleim J-P, et al. J Infect Dis 1999;179:709–13
M230L	0.91	1 (ATG→CTG)	5.7	13.9	3.4	Rhee S-Y, et al. AAC 2004;48:3122–6; Huang W, et al. Antivir Ther 2000;5(Suppl. 3):24. Abstract 30
P236L	0.12	1 (CCT→CTT)	2.4	4.6	1.3	Dueweke T, et al. PNAS USA 1993;90:4713–7; Johnson VA, et al. Top HIV Med 2006;14:125–30
K238N	0.55	1 (AAA→AAC)	2.7	NA	1.7	The Stanford University HIV Drug Resistance Database (http://hivdb6.stanford.edu/). NNRTI resistance notes updated in August 2006
K238T	2.76	1 (AAA→ACA)	3.4	NA	2.4	Rhee S-Y, et al. AAC 2004;48:3122–6; Demeter LM, et al. J AIDS Hum Retrovirol 1998;19:135–44
Y318F	1.11	1 (TAT→TTT)	0.6	1.5	1.4	Pelemans H, et al. J Biol Chem 1998;273:34234–9; Harrigan PR, et al. J Virol 2002;76:6836–40

Figure 1. Prevalence of the NNRTI RAMs in the panel of 19,689 HIV-1 recombinant clinical isolates.



Conclusions

- This list of 41 NNRTI RAMs provides a comprehensive overview of mutations playing a role in the mechanism of HIV-1 resistance to NNRTIs.
- The list can be used to guide further in vitro and clinical research on the mechanism of HIV-1 resistance to current and investigational NNRTIs.

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