Four Amino Acid Changes in HIV-2 Protease Confer Class-Wide PI Susceptibility

CROI 2015 Abstract # 588

Dana N. Raugi[1], Robert A. Smith[1], Matthew Coyne[1], Julia Olson[1], Kara Parker[1], Selly Ba[2], Papa Salif Sow[2], Moussa Seydi[2], and Geoffrey S. Gottlieb[1,3];
for the University of Washington-Dakar HIV-2 Study Group

Departments of [1]Medicine/Allergy & Infectious Diseases and [3]Global Health, University of Washington, Seattle, Washington, USA
[2]Service des Maladies Infectieuses Ibrahima Diop Mar, Centre Hospitalier National Universitaire de Fann, Universite Cheikh Anta Diop de Dakar, Dakar, Senegal

Contact: Dana N. Raugi, Medicine/AID, Box 358061, University of Washington, Seattle, WA 98109, raugid@uw.edu, tel: 206-543-8729

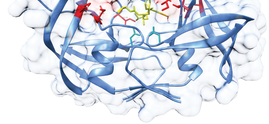
BACKGROUND

- HIV-2 is endemic in West Africa and has achieved limited spread elsewhere. It affects 1-2 million people worldwide.
- Treatment of HIV-2 is complicated by intrinsic resistance to non-nucleoside reverse transcriptase inhibitors and the fusion inhibitor enfuvirtide, as well as a low genetic barrier to nucleoside/nucleotide reverse transcriptase inhibitor resistance.
- Protease inhibitor (PI)-based regimens are the mainstay of antiretroviral therapy for HIV-2, but the virus exhibits some degree of resistance to the majority of protease inhibitors, except saquinavir, darunavir, and lopinavir.
- The mechanisms for this resistance are largely unknown.
- HIV-1 and HIV-2 proteases share just 40-50% sequence identity, but structural studies indicate that the active sites differ by only four amino acids, at residues 32, 47, 76, and 82.

A

```
                A         B         C         B         A
                10        20        30        40        50        60        70        80        90        99
HIV-1   PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI GGFIKVRQYD QILIEICGHK AIGTVLVGPT QVNIIGRNLL TQIGCTLNF
        ** ** ** ** * ** * ******** *       *       **  *** **** *   **    *   * * * * ** *** * * *  **
HIV-2   PQFSLWKRPV VTAYIEGQPV EVLLDTGADD SIVAGIELGN NYSPKIVGGI GGFINTKEYK NVEIEVLNKK VRATIMTGDT PINIFGRNIL TALGMSLNL
```

B



Sequence and Structure of HIV-2 Protease

A. Amino acid alignment of HIV-1_{NL4-3} and HIV-2_{ROD9} proteases. (Adapted from Menendez-Arias and Tozser, Trends Pharmacol. Sci. 29:42). Identical residues are marked with red asterisks. Grey boxes A, B, and C indicate the dimerization domain, active site/carboxy-terminal triad, and flap region, respectively. Yellow boxes indicate the residues mutated in this study: I32V, V47I, M76L, and I82V in HIV-2_{ROD9}.

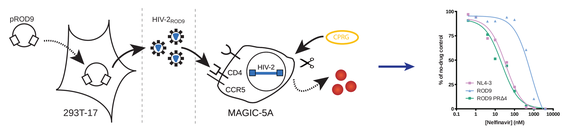
B. Location of the engineered amino acid replacements in the crystal structure of wild-type HIV-2 protease complexed with amprenavir (PDB # 3S45). Active site aspartate residues are shown in green sticks, amprenavir is shown in yellow, and mutated amino acids are shown in red.

STUDY AIM

To examine the contributions of HIV-2 protease amino acids I32, V47, M76, and I82 to the virus's intrinsic PI resistance.

METHODS

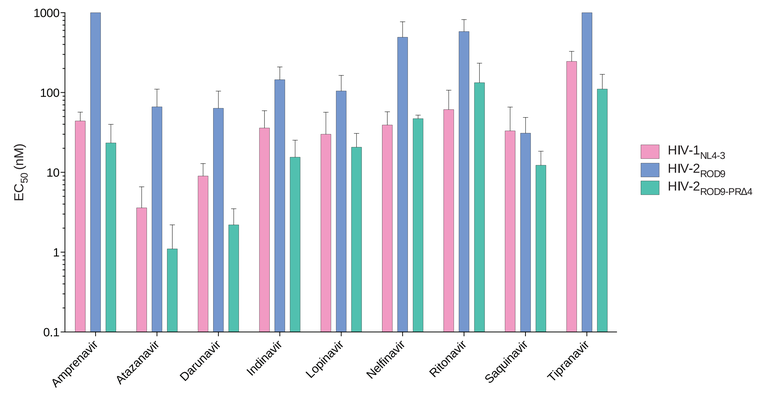
- Site-directed mutagenesis to construct a full-length HIV-2_{ROD9} molecular clone in which codons 32, 47, 76, and 82 were substituted to encode the amino acids found in wild-type HIV-1 (PRΔ4: I32V, V47I, M76L, I82V), as well as clones containing each individual substitution.
- Plasmid DNA (WT HIV-1_{NL4-3}, WT HIV-2_{ROD9}, and mutant HIV-2_{ROD9}) was transfected into 293T/17 cells using chloroquine-mediated calcium phosphate coprecipitation, and virus grown for 48 hours in the presence of protease inhibitors. Virus growth was assayed on MAGIC-5A indicator cells by β-galactosidase cleavage of chlorophenol red-β-D-galactopyranoside (CPRG).



- 50% effective concentrations (EC_{50}) values for each virus were obtained from dose response data against a no-drug control using sigmoidal regression in GraphPad Prism v 6.0f.
- Modeling studies used published crystallographic structures of HIV-1 and HIV-2 complexed with amprenavir. The PRΔ4 protease was constructed by substituting the amino acids in the HIV-2 structure and energy minimizing using YASARA. Structure manipulations and comparisons were performed in Chimera.

RESULTS

HIV-2 PRΔ4 and HIV-1 are similarly susceptible to all FDA-approved PI



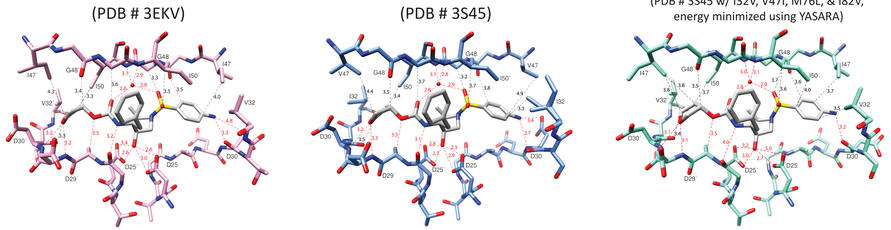
Bars indicate mean EC_{50}s of at least three independent dose-response experiments. Error bars represent standard deviations.

No single amino acid change confers more than 12-fold increase in PI susceptibility

Drug	HIV-1 NL4-3	HIV-2 ROD9	I32V	V47I	M76L	I82V	PRΔ4
Amprenavir	44 ± 13	>1000	550 ± 310 (1.8)	470 ± 220 (2.1)	660 ± 280 (1.5)	>1000 (1.0)	**23 ± 17 (>43)**
Atazanavir	3.6 ± 3.0	66 ± 44	51 ± 18 (1.3)	27 ± 3.4 (2.4)	**5.3 ± 2.1 (12)**	32 ± 5.9 (2.1)	**1.1 ± 1.1 (60)**
Darunavir	9.0 ± 3.9	58 ± 32	**12 ± 0.7 (4.8)**	**17 ± 3.3 (3.4)**	28 ± 7.8 (2.1)	29 ± 2.4 (2.0)	**2.2 ± 1.3 (26)**
Indinavir	36 ± 23	150 ± 64	**46 ± 21 (3.3)**	**30 ± 25 (5.0)**	**15 ± 6.8 (10)**	140 ± 140 (1.1)	**16 ± 9.8 (9.4)**
Lopinavir	30 ± 27	105 ±59	**38 ± 22 (2.8)**	**26 ± 8.7 (4.0)**	120 ± 64 (1.0)	120 ± 98 (1.0)	**21 ± 10 (5.0)**
Nelfinavir	39 ± 18	490 ± 280	370 ± 90 (1.3)	**190 ± 86 (2.6)**	**99 ± 69 (4.9)**	250 ± 130 (2.0)	**47 ± 4.9 (10)**
Ritonavir	61 ± 46	580 ± 240	290 ± 94 (1.6)	510 ± 440 (1.3)	240 ± 190 (2.4)	620 ± 150 (1.0)	**160 ± 100 (3.6)**
Saquinavir	33 ± 33	31 ± 18	19 ± 12 (1.6)	35 ± 7.7 (1.0)	25 ± 12 (1.2)	**93 ± 71 (0.3)**	12 ± 6.1 (2.6)
Tipranavir	250 ± 82	>1000	>1000 (1.0)	**260 ± 69 (>3.8)**	**500 ± 270 (2.0)**	>1000 (1.0)	**120 ± 65 (>8.3)**

Data shown are mean EC_{50} ± standard deviation (fold change compared to wild-type HIV-2_{ROD9}) for at least three independent dose-response experiments.

Comparison of protein-amprenavir interactions in HIV-1, HIV-2, and HIV-2 PRΔ4 proteases reveals possible clues to binding differences



Oxygen, nitrogen, and sulfur atoms are depicted in Corey-Pauling-Koltun coloring, water molecules coordinated by amprenavir and the main-chain nitrogen atoms of I50 and I50' are shown as red spheres. Dotted black lines indicate potential hydrophobic and C-H···O interactions and dotted red lines indicate hydrogen bonds. Atomic distances are shown in angstroms.

STRUCTURAL COMPARISON

- HIV-1 and HIV-2 protease structures show similar orientation of the inhibitor within the substrate binding cleft.
  - Interactions between amprenavir and the amino acids in the P1 and P1' sites, as well as the hydrogen bonding interactions between the catalytic aspartates at D25 and D25' and the residues within the flap region, are conserved between HIV-1 and HIV-2.
  - Subtle differences occur in the P2 and P2' sites, including the loss of a potential C-H···π interaction between position 47 and the aniline ring of amprenavir, increased distance between the main chain carbonyl of D30' and the tetrahydrofuran (THF) group, and lengthening of the hydrogen bond between the D30 main chain carbonyl and the aniline amino group of amprenavir.
- HIV-2 PRΔ4 shares common features with HIV-1
  - In the energy-minimized predictions for HIV-2 PRΔ4, P1 and P1' interactions appear quite similar to wild-type HIV-2.
  - The side chain of V47', main chain carbonyl oxygen of D30', and main chain nitrogen atoms on D29' and D30' are in closer proximity to the THF moiety.
  - The substitution of the longer isoleucine side chain at position 47 brings the side chain closer to the aniline ring, potentially restoring the C-H···π interaction noted in HIV-1.
  - The main chain carbonyl group of D30 is rotated approximately 40 degrees relative to wild-type HIV-2, resulting in a confirmation similar to HIV-1 and shorter hydrogen bonding distance between the carbonyl oxygen and the aniline NH_2 of amprenavir.

CONCLUSIONS

- Substituting four active site amino acid residues in HIV-2 protease to the corresponding amino acids from HIV-1 results in a replication-competent virus which exhibits a pattern of PI susceptibility comparable to that of HIV-1.
- The four amino acid changes appear to work synergistically to confer a greater level of PI susceptibility than the sum of each individual change.
- HIV-2 resistance to amprenavir appears to result from a diminution or loss of stabilizing interactions between protease and the THF and aniline groups of the inhibitor. Our data suggest the mutations in PRΔ4 help stabilize amprenavir in the P2 and P2' pockets of HIV-2 protease, possibly accounting for the increased susceptibility of HIV-2 PRΔ4 to amprenavir in culture.
- These findings enhace our overall understanding of the genetic basis of PI susceptibility, and demonstrate that active site residues in protease are the primary determinants of intrinsic HIV-2 PI resistance.

ACKNOWLEDGEMENTS

These studies were supported by grants to GSG from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID; 2R01-AI060466), the UW Center For AIDS Research (CFAR, an NIH-funded program; P30 AI027757), and the UW Royalty Research Fund (A92723).

We thank the NIH AIDS Research and Reference Reagent Program for providing wild-type HIV-1 and HIV-2 strains.

Additional UW-Dakar HIV-2 Study Group members include: Macoumba Toure, Ndeye Mery Dia Badiane, Louise Fortes, Cheikh T. Ndour, Jacques Ndour, Fatou Niasse, Fatima Sall, Fatou Traore, Khadim Faye, Sophie Chablis, Marie Pierre Sy, Bintou Diaw, Mbaye Ndoye, Amadou Bale Diop, Marianne Fadam Diome (Clinique des Maladies Infectieuses Ibrahima Diop Mar, Centre Hospitalier Universitaire de Fann, Universite' Cheikh Anta Diop de Dakar, Dakar, Senegal); Alassane Niang, ElHadji Ibrahima Sall, Ousseynou Cisse, Jean Philippe Diatta, Raphael Bakhoum, Juliette Gomis, (Région Médicale de Ziguinchor, Ziguinchor, Casamance, Senegal), Stephen Hawes, Noelle Benzekri, Donna Kenney, Joshua Stern, Qinghua Feng, Kim Wong, John Lin, Steve Cherne, Nancy Kiviat, Brad Church, Alexandra Hernandez, Alexandra Montano, Kate Parker, Lindsey Blankenship, Mariah Oakes, Sally Leong, Vincent Wu (University of Washington, Seattle, Washington).