SUPPLEMENTARY INFORMATION

Cooperative Effects of Drug-Resistance Mutations in the Flap Region of HIV-1 Protease

Jennifer E. Foulkes-Murzycki[1], Christina Rosi[1], Nese Kurt Yilmaz[1], Robert W. Shafer[2], Celia A. Schiffer[1*]

[1]Department of Biochemistry and Molecular Pharmacology,
University of Massachusetts Medical School,
364 Plantation Street, Worcester, MA 01605

[2]Division of Infectious Diseases, Department of Medicine, Stanford University,
Stanford, CA 94305

*** Corresponding Author**
Email: Celia.Schiffer@umassmed.edu

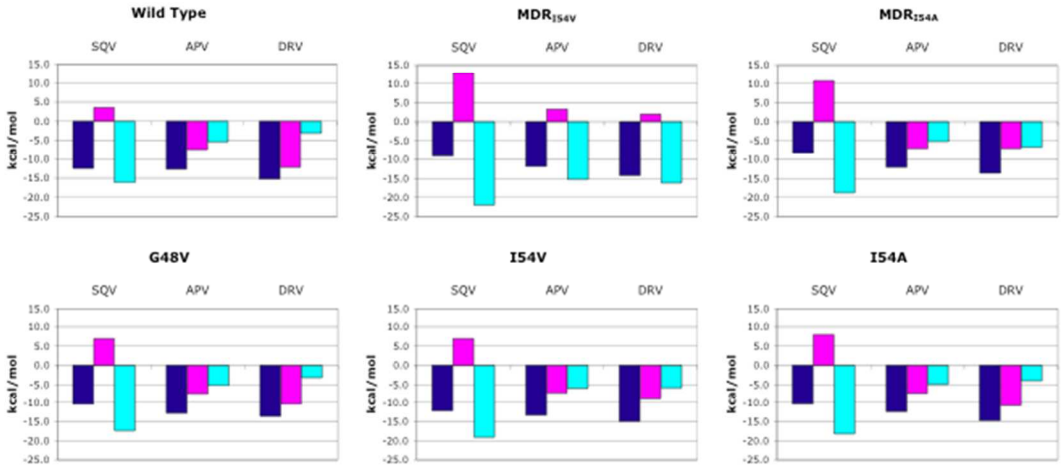

Figure S1. Thermodynamic parameters for binding of each protease variant to APV, DRV, and SQV (data from Table 1 of main manuscript). The dark blue, magenta and cyan bars are the free energy (ΔG), enthalpy (ΔH), and entropy (TΔS) of binding, respectively.

Patient Sequence Analysis for I54A Mutation

Methods. HIV-1 protease sequences were obtained from patients who had plasma samples submitted for genotypic resistance testing at Stanford University Hospital. The genotypic resistance testing of these samples was performed using a previously described sequencing protocol (*1*). Briefly, RNA was extracted from 0.2–0.5 mL of plasma using a guanidine-thiocyanate lysis reagent. Reverse-strand cDNA was generated from viral RNA, and first-round PCR was performed using Superscript One-Step RT-PCR (Life Technologies). Nested PCR was used to amplify a 1.3-kb product encompassing the protease gene and the first 300 residues of the RT gene. Direct PCR cycle-sequencing was performed using AmpliTaq DNA FS polymerase and rhodamine terminators (Applied Biosystems Inc.).

Results. We performed a detailed analysis of the patient sequences in the Stanford HIV drug resistance database (*2*) containing the I54A mutation. Antiretroviral treatment histories, plasma HIV-1 RNA levels, and CD4-lymphocyte counts were available on 22 patients in whom I54A occurred (Table S1). Mutations at positions 10, 62, 63, 71, and 82 were particularly common among isolates with I54A. L10I/V/F occurred in 21 patients, I62V in 16 patients, L63P/A/T in 20 patients, A71V/I/T in 19 patients, and V82A/T/F/I in 20 patients. Other co-occurring drug-resistance mutations included M46I/L in 8, G48V in 6, and L90M in 8 patients. Hence, the spectrum of mutations co-occurring with I54A was not significantly different from that of mutations occurring with I54V. Interestingly, in 9 of 13 patients with a preceding sequence, I54V was found to precede I54A. Among patient sequences containing either I54V or I54A, L10I occurred in 87% of sequences, G48V in 33%, and V82A in 70%, ensuring the clinical relevance of both Flap+ protease variants examined in this study.

Supplementary References

1. Shafer, R. W., Hertogs, K., Zolopa, A. R., Warford, A., Bloor, S., Betts, B. J., Merigan, T. C., Harrigan, R., and Larder, B. A. (2001) High degree of interlaboratory reproducibility of human immunodeficiency virus type 1 protease and reverse transcriptase sequencing of plasma samples from heavily treated patients, *J. Clin. Microbiol. 39*, 1522-1529.
2. Shafer, R. W. (2006) Rationale and uses of a public HIV drug-resistance database, *J. Infect. Dis. 194 Suppl 1*, S51-58.

Table S1. Sequence information for patients with I54A mutation.

Patient Number	Prior Amino Acid at 54[a]	Protease Mutations Present in First Isolate with I54A[b]
795	I54V	L10I, I15V, M36I, **G48V**, **I54A**, I62V, I64V, **V82A**
1144	I54V	L10V, T12KT, L19I, **M46L**, **G48V**, **F53Y**, **I54A**, **Q58E**, Q61E, I62V, L63P, A71V, I72V, T74S, V77I, **V82TA**, **L90M**, I93L
1415	Unknown	L10I, **E34Q**, **K43T, I54A**, L63P, A71V, **V82A**, Q92K
1595	I54V	L10I, I13V, L19V, K20R, E35D, M36I, N37D**, I54A**, I62V, L63P, I66F, A71I, **V82T**
3871	Unknown	L10I, I13V, K14R, I15V, K20M, M36V, N37T, **I54A**, I62V, I64V, A71V, **V82F**, L89I
4304	Unknown	L10I, K20R, E34EK, E35D, M36I, N37D, **I54AV,** I62V, L63P, A71V, V77VI, **V82A**, L**90M**, I93L
4426	Unknown	L10I, L19T, N37S, **M46LM**, **F53LF**, **I54AV**, L63PL, A71V, **V82A**
4463	I54V	L10I, K20R, E35D, M36I, **G48V, I50V,** I**54A**, I62V, L63AP, A71V, **V82T**, Q92K
5015	I54V	L10I, I13V, K20I, E35D, M36I, N37D, **M46I, I54A**, R57K, L63P, I64V**, I66F**, A71V, I72LI, **V82T, L90ML**
6406	Unknown	L10I, **K43T, G48V, I54A**, I62V, L63P, I64V, V77I, **V82A, L90M**
6492	I54V	L10I, I15V, **M46I, G48V, I54A, Q58E**, I62V, L63A, T74K, **V82A**, L89M
6802	Unknown	L10I, L19T, **G48V**, **I54A**, I62V, L63P, A71V, V77I, **V82A**, **L90M**, I93L
6867	Unknown	L10I, I13V, **L33F, I54A, Q58E**, I62V, L63P, I66F, A71V, **V82A, L90M**
7950	I54V	**L10F, V11IV**, I13V, K20RK, **D30N**, **L33I**, E35D, M36I, N37C, **I54A**, I62V, L63P, A71T, **N88D**, I93L
14302	WT	L10I**, I54A**, I62V, L63P, A71V, **V82A**
14395	I54V	L10I, I13V, L19VL, K20I, L33I, E35D, M36I, N37DN, **M46I**, **I54A**, L63P, C67L, H69K, A71IV, I72MI, **V82A, L89V, L90M**
14612	Unknown	L10I, L19I, K20R, E35D, M36I, N37D, **I54A**, I62V, L63P, C67F, H69R, A71I, I72M, **G73S**, V82I, **L90M**
22055	Unknown	L10I, N37D, **M46I, I54A**, D60E, I62V, L63P, I64V, A71V, I72R, **V82A**
22166	WT	T12S, L19I, K43R, **M46I, I54A**, **Q58E**, L63P, A71V, V77I, **V82A**, I93L
27937	WT	L10I, N37DN**, M46I, I54A**, D60E, Q61G, I62V, L63T, I66L, A71V, I72R, V77I, **V82A**, I93L
35586	I54V	L10V, T12P, K14R, K20R, E34D, E35D, M36I, R41K, K45R, **I54A**, I62V, L63T, A71I, L89M, I93L
42138	WT	L10I, E34EK, M46MI, I54A, R57K, L63P, A71V, I72IR, V82A

[a] Prior I54 genotypes are considered to be unknown if no previous genotype was available and the patient had received multiple protease inhibitors.
[b] Nonpolymorphic drug-resistance mutations are indicated in **boldface**.