Supplementary information

Dose-response Curve Slope Sets Class-Specific Limits on Inhibitory Potential of Anti-HIV Drugs

Lin Shen[1,2], Susan Peterson[1], Ahmad R. Sedaghat[1], Moira A. McMahon[1,2], Marc Callender[1], Haili Zhang[1], Yan Zhou[1], Eleanor Pitt[1], Karen S. Anderson[3], Edward P. Acosta[4], Robert F. Siliciano[1, 5]

[1]Department of Medicine, [2]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 733 N. Broadway, Baltimore, MD 21205, USA.
[3]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, SHM B350B, New Haven, CT 06520, USA.
[4]Division of Clinical Pharmacology, University of Alabama at Birmingham School of Medicine, 1670 University Boulevard, Birmingham, AL 35294, USA.
[5]Howard Hughes Medical Institute, 733 N. Broadway, Baltimore, MD 21205, USA.
Correspondence should be addressed to R.F.S. (rsiliciano@jhmi.edu).

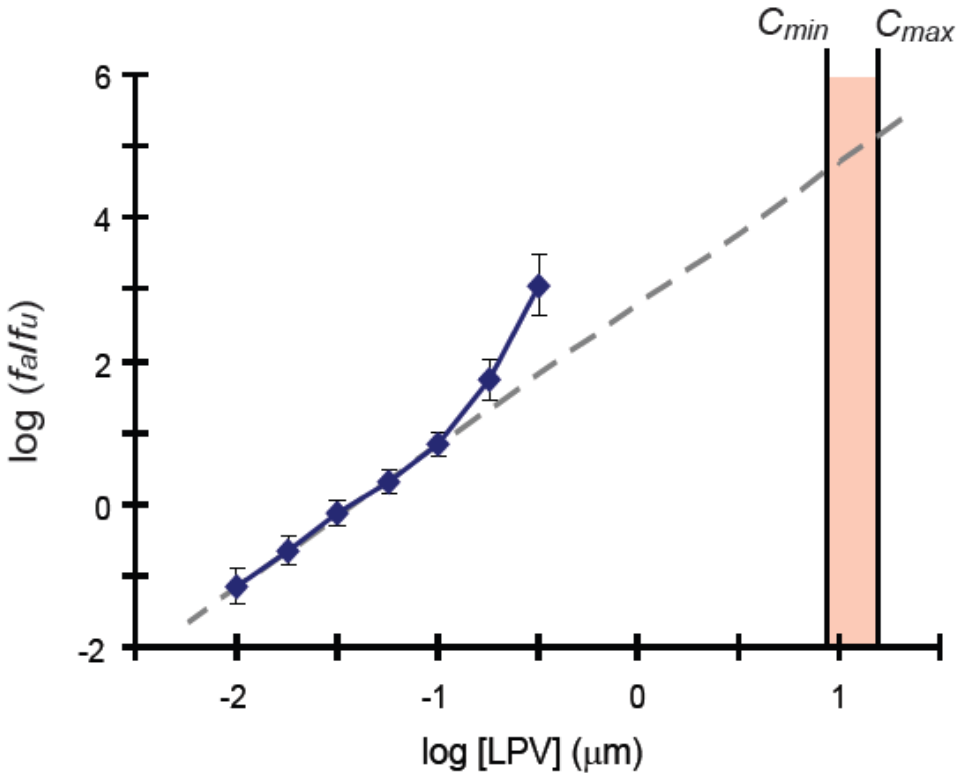

Figure S1 Median effect plot for the protease inhibitor lopinavir (LPV). The dashed line representing the minimal slope was used for calculation of IC_{50}, *m* value, and IIP. Due to the upward inflection of the curve, the actual IIP could be higher. Each data point is the mean ± s.d. from at least three experiments. Where error bars would be hidden by the symbol, they are show at the edge of the symbol.

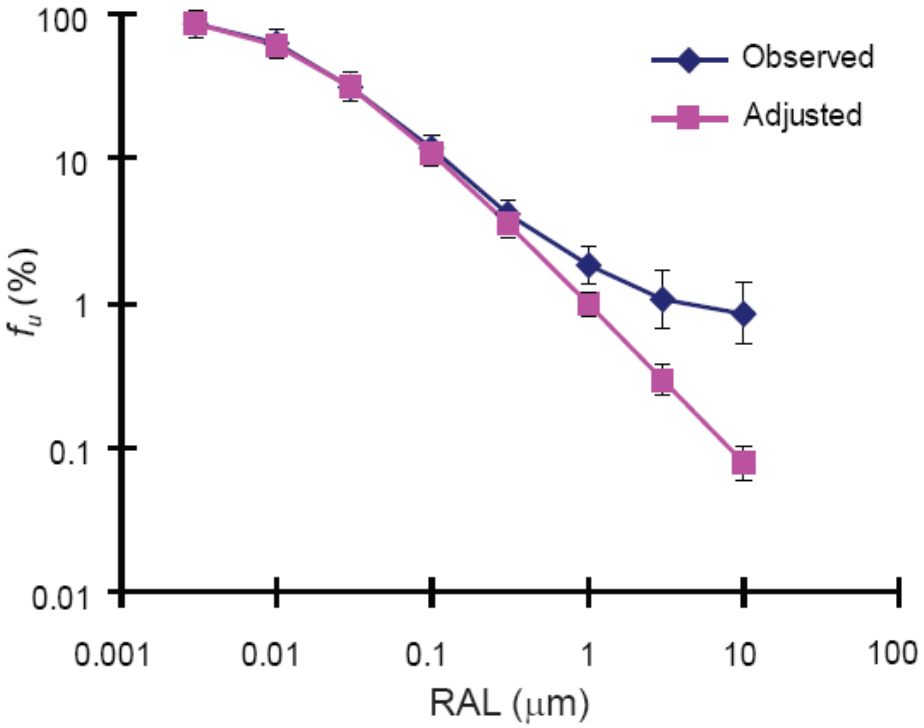

Figure S2 Analysis of dose-response curves for raltegravir (RAL). Log-log dose-response curves for RAL are shown. The original dose-response curve (blue) based on observed percentage of GFP^+ cells and the adjusted dose-response curve (purple) corrected for the contribution of cells with unintegrated virus are both shown. The correction was carried out according to **Equation 6**. Each data point is the mean ± s.d. from at least three experiments. Where error bars would be hidden by the symbol, they are show at the edge of the symbol. IC_{50}, *m* and IIP values for RAL are based on the adjusted dose-response curve.

Supplementary Table 1 Summary of Dose-response Curve Parameters of Anti-HIV-1 Drugs

Drug	IC_{50} (nm)	*m* value	r^2	Inhibitory Quotient[a]			Instantaneous Inhibitory Potential[a,b]		
				C_{min}/ IC_{50}	C_{ave}/ IC_{50}	C_{max}/ IC_{50}	C_{min}	C_{ave}	C_{max}
NRTI									
3TC	29.8 ± 12.7	1.15 ± 0.12	0.987 ± 0.007	62.5	101.4	513.1	2.12	2.36	3.17
ABC	34.4 ± 15.9	0.95 ± 0.15	0.970 ± 0.005	1.4	50.9	304.6	0.40	1.67	2.40
AZT	182.3 ± 50.3	0.85 ± 0.12	0.988 ± 0.008	0.0002	2.4	24.4	0.0005	0.50	1.23
d4T	552.4 ± 199.3	1.13 ± 0.14	0.986 ± 0.008	0.017	0.85	4.2	0.0059	0.28	0.80
ddI	179.4 ± 36.3	1.07 ± 0.17	0.966 ± 0.012	0.0067	2.4	219.4	0.0033	0.55	2.50
FTC	7.4 ± 3.0	1.18 ± 0.10	0.988 ± 0.006	49.0	226.9	980.3	2.03	2.81	3.56
TDF[c]	168.4 ± 37.3	0.97 ± 0.13	0.972 ± 0.013	2.4	2.7	6.3	0.53	0.57	0.86
NNRTI									
EFV	5.4 ± 0.9	1.69 ± 0.08	0.978 ± 0.009	1042.5	1427.8	2401.4	5.11	5.34	5.72
NVP	81.4 ± 25.4	1.55 ± 0.15	0.981 ± 0.010	133.7	195.6	309.0	3.30	3.56	3.87
DLV	171.3 ± 76.4	1.56 ± 0.18	0.993 ± 0.005	62.3	101.2	158.4	2.82	3.15	3.45
TMC125	4.3 ± 0.7	1.81 ± 0.23	0.990 ± 0.006	160.0	203.4	376.0	4.00	4.19	4.68
TMC278	3.9 ± 0.7	1.92 ± 0.20	0.991 ± 0.006	ND	ND	ND	ND	ND	ND
Protease inhibitor[d]									
ATV	13.6 ± 1.6	2.69 ± 0.28	0.986 ± 0.010	66.1	199.8	460.6	4.91	6.20	7.17
APV[e]	144.2 ± 18.2	2.09 ± 0.14	0.990 ± 0.009	19.9	39.7	99.3	2.73	3.35	4.18
DRV	23.6 ± 5.1	3.61 ± 0.32	0.993 ± 0.005	215.3	410.6	626.4	8.46	9.47	10.14
IDV	90.9 ± 10.0	4.53 ± 0.36	0.982 ± 0.009	20.1	60.0	137.6	5.91	8.06	9.69
LPV	35.8 ± 7.2	2.05 ± 0.12	0.992 ± 0.005	244.6	343.2	435.8	4.91	5.21	5.43
NFV	166.8 ± 44.8	1.81 ± 0.23	0.987 ± 0.010	13.7	21.5	30.6	2.07	2.43	2.70
SQV	45.3 ± 4.4	3.68 ± 0.23	0.995 ± 0.006	19.8	120.0	293.2	4.78	7.66	9.09
TPV	242.0 ± 43.6	2.51 ± 0.36	0.993 ± 0.005	147.1	244.5	320.6	5.45	6.01	6.30

Drug	IC_{50} (nm)	*m* value	r^2	Inhibitory Quotient[a]			Instantaneous Inhibitory Potential[a,b]		
				C_{min}/ IC_{50}	C_{ave}/ IC_{50}	C_{max}/ IC_{50}	C_{min}	C_{ave}	C_{max}
Fusion inhibitor									
T20	35.9 ± 6.8	1.65 ± 0.14	0.992 ± 0.002	20.5	25.1	30.9	2.17	2.31	2.46
T1249	11.2 ± 4.6	1.97 ± 0.17	0.966 ± 0.007	ND	ND	ND	ND	ND	ND
Integrase inhibitor									
L870812	228.2 ± 91.1	0.99 ± 0.08	0.995 ± 0.003	ND	ND	ND	ND	ND	ND
RAL	14.9 ± 1.8	1.10 ± 0.05[f]	0.987 ± 0.007	13.6	89.8	268.2	1.27	2.15	2.67
GS9137	28.1 ± 12.3	0.95 ± 0.04	0.989 ± 0.005	10.7	29.2	59.1	1.04	1.43	1.71
L240	15.2 ± 4.0	1.24 ± 0.12[f]	0.992 ± 0.006	ND	ND	ND	ND	ND	ND
L525	86.1 ± 24.0	1.00 ± 0.06	0.989 ± 0.004	ND	ND	ND	ND	ND	ND

Values are means ± s.d. where available.

[a]The C_{min}, C_{ave} and C_{max} values used for calculation were obtained from supplementary reference (1) for the following drugs: AZT, d4T, 3TC, ddI, NVP, DLV, ATV, NFV, IDV, and SQV. The C_{min}, C_{ave} and C_{max} values used for calculation were obtained from FDA package insert for the following drugs: ABC, FTC, TDF, EFV, TMC125, APV, LPV, TPV, and T20. The C_{min}, C_{ave} and C_{max} values for DRV, RAL and GS9137 were obtained from supplementary references (2)–(4) respectively. The IQ and IIP values for TMC278, T1249, L870812, L240, and L525 are not determined (ND) because their C_{min}, C_{ave} and C_{max} values are not available.

[b]An IIP value > 3.5 indicates calculation from extrapolation.

[c]For TDF, IC_{50} and *m* values in the Table are for tenofovir. IIP values were calculated based on the C_{min}, C_{ave} and C_{max} plasma levels of tenofovir in subjects taking the standard dose of the prodrug TDF.

[d]Except where indicated in **Fig 4b,c** and for NFV, the IIP values for the protease inhibitors are calculated based on plasma levels with ritonavir boosting.

[e]For APV, IC_{50} and *m* values in the Table were measured using APV. IIP values were calculated based on the C_{min}, C_{ave} and C_{max} plasma levels of APV in subjects taking the standard dose of the prodrug fosamprenavir.

[f]The *m* values reported for RAL and L240 were adjusted by **Equation 6**.

Supplementary notes

The decay of IIP as a function of time

Assuming that subsequent doses are missed, drug concentration decreases according to first order elimination, then

$$C_t = C_{\max} e^{-kt} \tag{7}$$

where k is the elimination rate constant (k = ln(2) / $t_{1/2}$), t is the time after C_{max} is reached, and $t_{1/2}$ is the half life of the drug.

Combining **Equations 3–5** and **Equation 7**, the decay rate of IIP can be expressed as follows:

$$\frac{d}{dt}(IIP) = \frac{1}{\ln(10)} \bullet \frac{-mk\left(\frac{C_{\max}}{IC_{50}}\right)^m e^{-mkt}}{1+\left(\frac{C_{\max}}{IC_{50}}\right)^m e^{-mkt}} \tag{8}$$

In this system, m ranges from about 1 to 5, and in general C_{max} >> IC_{50}

Therefore, if $t < 1/mk$, **Equation 8** can be simplified as follows:

$$\frac{d}{dt}(IIP) \approx \frac{-mk}{\ln(10)} \approx -0.3 \bullet \frac{m}{t_{1/2}} \tag{9}$$

Therefore, the initial decay rate of IIP is faster if m is large and $t_{1/2}$ is small.

$t_{1/2}$ values were obtained from FDA package insert or from references (1)–(4).

Source of clinical trial data

The data from clinical trials used in **Fig. 4c** are as followed: ACTG 5095 Gulick, 2004[5]; 2NN van Leth, 2004[6]; DuPont006 Staszewski, 1999[7]; ACTG 384 Robbins, 2003[8]; BMS034 Squires, 2004[9]; ACTG 5142 Riddler, 2008[10]; ARTEMIS Ortiz,

2008[11]; TITAN Madruga, 2007[12]; KLEAN Eron, 2006[13]; CASTLE Molina, 2008[14]; M98-863 Walmsley, 2002[15]; NEAT Rodriguez-French, 2004[16].

IIP is only one of the factors that contribute to clinical outcome

The promising new integrase inhibitor RAL[17], when used together with the NRTIs tenofovir disoproxil fumarate (TDF) and lamivudine, causes a faster initial decline in viral load than a comparable EFV-based regimen[18]. A potential explanation for this rapid drop in viral load despite a relatively low IIP is that the drop in viral load may reflect the fact that RAL acts later in the virus life cycle, leaving a population of infected cells that already have integrated proviruses and that have a faster average decay rate[19]. Note that for this analysis, protease inhibitors may be considered to act at the beginning of the life cycle since virions that have completed maturation when the protease inhibitors are started can still infect cells and those cells can go on to produce immature virus particles before the block imposed by the drug is encountered again. Therefore, for the current five clinically available drug classes, protease inhibitors act as the earliest stage of the virus life cycle, whereas integrase inhibitors act at the latest stage of the virus life cycle. Thus the rapid drop in viral load does not necessarily indicate greater antiviral activity. Despite a relatively low IIP, RAL may prove to be an excellent antiretroviral drug because of its favorable side effect profile, activity against viruses resistant to protease inhibitors or reverse transcriptase inhibitors[18,20], and potentially for other reasons related to drug distribution and synergy.

For the NRTIs, it is also necessary to consider the levels and decay rates of the

active, intracellular triphosphate forms of these drugs, which are very different from those of the prodrugs[1]. The low values of IIP_{Cmin} for NRTIs are due to low C_{min} values for the prodrugs, reflecting their rapid clearance from the plasma. Despite the relatively low IIP_{Cmin} of NRTIs, these drugs are clinically useful in part because the active triphosphate forms of some NRTIs have very long intracellular half lives (e.g. for TDF, the active intracellular form has a half life >60 h)[1].

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