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The Inhibitory Quotient as a Predictor of Viral Evolution Following Viral Load Rebound During Lopinavir/r Therapy

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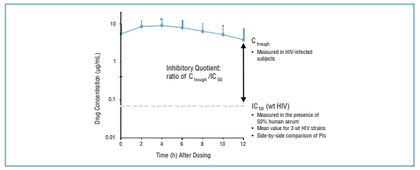
BACKGROUND

The inhibitory quotient (IQ) is a model for understanding and evaluating drug pharmacology that encompasses both pharmacokinetics and drug susceptibility (Figure 1). Originally articulated for the effects of antibiotics,[1] the IQ can be applied to HIV protease inhibitors (PIs) as the ratio of the C_{trough} and the IC_{50}. In order for a pharmacodynamic model such as the IQ to be useful, it should be (a) standardized and (b) predictive of the *in vivo* efficacy of more than one drug.

Figure 1. IQ: A Standardized Model for Understanding PI Pharmacodynamics



In two studies of PI therapy (lopinavir/ritonavir [LPV/r] and indinavir/ritonavir) in PI-experienced patients, the IQ was a significant predictor of virologic response through 48 weeks.[2]

For protease inhibitors, a high IQ value may also provide a high genetic barrier to the evolution of resistance if many mutations in protease are required to generate a reduction in drug susceptibility sufficient to overcome drug levels. At intermediate IQ values where the genetic barrier is compromised, selective pressure for evolution of resistance might be expected to be high if replication occurs. In contrast, if the IQ is low (<1), the drug is unlikely to exert significant selective pressure for the accumulation of additional protease mutations.

To further explore the relevance of IQ in PI pharmacology, the relationship between baseline IQ and viral evolution during viral rebound in PI-experienced patients treated with LPV/r based regimens was examined. Patients included in this analysis were enrolled in two Phase II clinical studies of LPV/r in PI-experienced patients (Table 1).

- Study M97-765 evaluated the activity of LPV/r plus nevirapine (NVP) and NRTIs in 70 single PI-experienced, NNRTI-naïve patients.
- Study M98-957 examined the activity of LPV/r plus efavirenz (EFV) and NRTIs in 57 multiple PI-experienced, NNRTI-naïve patients.

Table 1. LPV/r Phase II Program in PI-Experienced Patients

Study	M97-765	M98-957
Prior ARV	Single PI	Multiple PI
# Patients	70	57
Duration	96 weeks	48 weeks
Other ARV	NVP, NRTIs	EFV, NRTIs
<400 c/ml, on treatment	81%	80%
<400 c/ml, ITT M=F	63%	65%

METHODS

- Genotypes were examined from patients who either failed to suppress plasma HIV RNA to <400 copies/mL or experienced a sustained rebound in plasma HIV RNA to >400 copies/mL while on therapy with LPV/r. Patients qualified for this analysis if they participated in lopinavir pharmacokinetic evaluations in Study M97-765 or M98-957 and had baseline lopinavir phenotypic susceptibility data available for computation of the IQ.
- Baseline and rebound viral isolates from Study M98-957 were submitted to Virco where genotype including the entire protease gene was determined by population sequencing and phenotypic testing was performed by the Antivirogram method.
- Baseline and rebound viral isolates from Study M97-765 were submitted to Virologic where genotype including the entire protease gene was determined by population sequencing and phenotypic testing was performed by the PhenoSense method.
- Steady state C_{trough} was determined for a subset of patients (week 24, n=12) receiving LPV/r with nevirapine in Study M97-765 and from patients receiving LPV/r with efavirenz in Study M98-957 (week 5, n=52).
- Inhibitory quotient values for each patient were calculated by the following equation:

$$IQ = \frac{\text{Observed } C_{trough}}{\text{Fold change in } IC_{50} \text{ X wild-type } IC_{50} \text{ (50\% human serum)}}$$

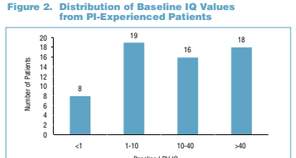
- Serum-adjusted IC_{50} values were taken from Molla et al.[3] and represent the mean IC_{50} for three wild-type laboratory strains (IIB, HXB2, and pNL4-3) in the presence of 50% human serum plus 10% fetal calf serum. The value used for LPV is 0.06 µg/mL. The free fraction of LPV in 50% human serum has been shown to approximate the free fraction in 100% human serum.[4]

RESULTS

Inhibitory Quotients for Single and Multiple PI-Experienced Patients Entering LPV/r Therapy

- The distribution of LPV IQ values for PI-experienced patients entering LPV/r therapy in Studies M97-765 (n=9) and M98-957 (n=52) is shown in Figure 2. A broad range of IQ values was observed, with a range from 0.05 to 279.

Figure 2. Distribution of Baseline IQ Values from PI-Experienced Patients



Impact of LPV Inhibitory Quotient on Viral Evolution in Protease During Viral Rebound

- Three distinct patterns of protease genotypic evolution were seen, depending on the baseline LPV IQ values. Genetic evolution during viral load rebound on LPV/r therapy was observed in 7/9 patients with an intermediate range LPV IQ and in 0/3 and 0/4 patients with low and high LPV IQs, respectively (low vs. intermediate IQ, p=0.045; intermediate vs. high IQ, p=0.021; Table 2).

Table 2. LPV Inhibitory Quotients for PI-Experienced Patients Experiencing Viral Load Rebound or LPV/r Therapy

IQ Range	Pt #	LPV C_{trough}* (µg/mL)	Baseline LPV IC_{50} (fold increase over wt)	LPV IQ	Protease Evolution Following Rebound
Low	A1	0.99	63 fold	0.26	No
	A2	1.36	64 fold	0.35	No
	A3	3.66[a]	96 fold	0.64	No
Intermediate	B1	5.36	67 fold	1.3	Yes
	B2	4.33	44 fold	1.6	No
	B3	2.72	17 fold	2.7	Yes
	B4	10.10	57 fold	3.0	Yes
	B5	4.28	20 fold	3.6	Yes
	B6	7.29	34 fold	3.6	No
	B7	4.84	26 fold	3.1	Yes
	B8	4.82	12 fold	6.7	Yes
	B9	1.72	2.8 fold	10	Yes
High	C1	1.03	0.7 fold	25	No
	C2	2.76	0.7 fold	66	No
	C3	4.49	0.7 fold	107	No
	C4	6.38	0.7 fold	152	No

* LPV concentrations determined while patients were receiving LPV/r with NVP or EFV which results in greater PK variability and ~33% reduction in LPV trough concentrations compared to LPV/r dosing in the absence of an NNRTI.
[a] Calculated C_{trough} based on mean values from subjects receiving LPV/r with efavirenz.

- Viral rebound isolates from 14 of the 16 patients evaluated demonstrated at least a 10-fold reduction in phenotypic susceptibility to the NNRTI received, relative to wild-type virus.
  - NNRTI phenotypic susceptibility at rebound was reduced <10-fold in the other two patients evaluated (5.4-fold in patient C3 and 1.3-fold in patient C1), both of whom had a high LPV IQ.
  - The lack of significant evolution in either protease or reverse transcriptase during viral rebound in patients C3 and C1 strongly suggests that periods of reduced adherence or drug holiday may be the cause of the viral rebound. This hypothesis is supported by the observation that both patients experienced viral resuppression to <400 copies/mL after initial rebound with continued therapy.
- LPV phenotypic susceptibility and protease genotype at baseline and virologic rebound are presented for patients with low, intermediate, and high LPV IQ values in Table 3.

Table 3. Phenotypic Susceptibility to Lopinavir and Protease Genotype for Viral Isolates at Baseline and Following Rebound

Pt #	LPV IQ	Baseline LPV IC_{50} (fold increase over wt)	Rebound LPV IC_{50} (study day of rebound)	Baseline Protease Genotype[a]	Rebound Protease Genotype[b]
A1	0.26	63 fold	66 fold (Day 93)	L10I, R41K, M46L, G48V, I54T, L63P, A71L, I72I/T, V77I, V82A, I93L	H69H/Y
A2	0.35	64 fold	51 fold (Day 114)	L10V/F, M46I, I54V, L63P, H69K, A71V, V82A, L90M, I93L	No change
A3	0.64	96 fold	108 fold (Day 153)	L10F/I, K20R, L24I, L33I, E35D, M36I, N37D, R41K, M46L, G48S, F53L, I54V, K55R, R57K, L63P, I64V, V82A	L10I
B1	1.3	67 fold	85 fold (Day 508)	L10I, M46I, I54V, Q58E, D60D/E, I62I/V, L63L/P, I64V, I72I/M, V77I, V82F, L90M, I93L	I64I/V, H69H/R, G73C[c]
B2	1.6	44 fold	43 fold (Day 248)	L10I, T12D, K14R, I15V, K20R, L24I, E35D, M36I, R41K, M46L/V, F53F/L, I54V, L63P, H69K, K70R, A71V, V82A, N88N/S	No change
B3	2.7	17 fold	30 fold (Day 434)	L10I, K14R, L24I, M46I, I54V, L63P, H69Q, A71V, V77I, V82A, I93M	L33L/F, I72I/V
B4	3.0	57 fold	177 fold (Day 493)	L10I/V, I13V, K14K/R, K20K/R, L33F, E35D, M36I, R41K, F53L, I54V, D60E, Q61Q, I62V, L63P, A71V, G73G/S, V82V/A, I84I/V, L90M	T4T/P, E34E/V, M46M/I, I47I/V, K55K/R
B5	3.6	20 fold	43 fold (Day 336)	L10I, G48V, I54V, L63P, A71V, I72M, V77I, V82A, L90M, I93L	M46M/L
B6	3.6	34 fold	60 fold (Day 83)	L10I, K20R, E35D, M36I, R41R/K, K43T, F53L, I54V, I62V, L63P, A71V, V82A, L90M	N37N/T
B7	3.1	26 fold	59 fold (Day 867)	V3I, L10F, E35D, M36V, R41K, I54V, D60E, Q61E, I62V, L63P, I64V, V82A	(V3I), L24I, N37S, M46I, F53F/L, R57R/K
B8	6.7	12 fold	51 fold (Day 506)	L10I, I19R, N37N/Y, R41K, I54V, D60E, L63P, A71V, V77I, V82T, L90M, I93L	L10F, K20I/M/V, E34K, M36I, I62V, H69H/Q, V82S
B9	10	2.8 fold	99 fold (Day 112)	L10L/I, I15V, G16G/E, K20R, E35D, M36I, R41K, I54I/V, R57K, Q61N, L63L/H, I64I/M, K70K/R, L89M, L90L/M	I54A/V, Q61D, A71V, I72R, V82A, L89I, L90M, Q92K
C1	25	0.7 fold	0.5 fold (Day 342)	D30N, R41K, M46M/I, I62V, L63P, V75V/I, V77I, I93L	(D30N), V77V/I
C2	66	0.7 fold	0.3 fold (Day 848)	V3I, D30N, E35D, M36M/I, S37N, K45R/K, L63P, I64L	(V3I), (D30N), (S37N)
C3	107	0.7 fold	0.7 fold (Day 168)	V3I, L10I, D30N, L63T, I64V, E65D, A71V, V77V/I, N88D	N88G/D
C4	152	0.7 fold	1.1 fold (Day 195)	V3I, I13V, S37N, R57K, L63P	No change

[a] Protease mutations highlighted in red are those associated with PI resistance according to the Data Analysis Plan of the HIV Collaborative Working Group[5] as well as those found to be associated with reduced *in vitro* susceptibility to LPV.[6] These include L10F/I/R/V, K20M/R, L24I, D30N, V32I, L33F, M36I, M46I/L, I47V, G48V, I50V, F53L, I54L/T/V, L63P, A71I/L/T/V, G73S/A, V77I, V82A/F/T, I84V, N88D and L90M.
[b] Only protease mutations which differ from the baseline sequence are included. Mutations in parentheses were present at baseline but not in the rebound isolate.
[c] G73C has been found to be associated with other resistance mutations in a search of the Stanford database.

DISCUSSION

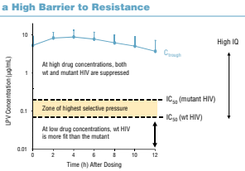
The Inhibitory Quotient as a Model for Viral Evolution During Viral Rebound on LPV/r Therapy

- The **inhibitory quotient (IQ)** provides a quantitative measure of the relationship between drug trough concentrations in the plasma (the point in the dosing cycle where the patient is most vulnerable to viral replication) and viral drug susceptibility. The IQ may also be a semi-quantitative estimate of drug potency *in vivo*.
- The **zone of highest selective pressure** for viral evolution in protease is present at LPV concentrations wherein the first mutant has a significant replication advantage over the wild-type virus. This advantage is expected to be the greatest between the IC_{50} values for the wild-type and first mutant. At higher drug concentrations, replication of both wild-type and first mutant are significantly inhibited and overall replication is low. At lower drug concentrations, the wild-type virus has some fitness advantage and overall replication is high.

No Viral Evolution Observed with High LPV IQ

- In instances where a **high IQ** is achieved, plasma trough levels of LPV are sufficiently high so as to remain well above the zone of highest selective pressure for resistant mutants, even in situations where LPV/r dosing intervals may extend past 12 hours (Figure 3). Therefore, significant viral replication is not expected to occur during adherent periods. Detectable viral load rebound in such patients is most likely due to periodic non-adherence.
  - Of the 4 patients with IQ >20 who experienced viral load rebound on LPV/r therapy in this analysis, two patients had viral load resuppression to <400 copies/mL on continued therapy (Patient C3 and C1), and the other two patients had periods of nonadherence reported by the investigator (Patients C2 and C4).
  - In cases where markedly reduced adherence is present, LPV plasma concentrations decline through the zone of highest selective pressure. At these concentrations of LPV, the corresponding concentrations of RTV are no longer sufficient to adequately inhibit the cytochrome P450 (CYP) 3A mediated metabolism of LPV. Consequently, LPV concentrations are expected to decline rapidly through the zone of highest selective pressure because of the high inherent (uninhibited) metabolism rate of LPV. Under these circumstances, there may be only small periods of time during which the selection of mutants is favored.

Figure 3. High LPV IQ Provides a High Barrier to Resistance



- The time to reach the zone of highest selective pressure and the duration of replication within the zone after dosing with LPV/r can be estimated based on modeling of LPV pharmacokinetics and viral replication dynamics.
  - The LPV phenotypic susceptibility of the first mutant isolate is assumed to be reduced 3-fold or less, relative to wild-type virus based on *in vitro* data from molecular clones/passaged viruses with single primary mutations with or without additional secondary mutations (Table 4).

Table 4. *In Vitro* Activity of ABT-378 Against Viruses with 1 Primary Mutation, with or without 1-2 Secondary Mutations

Virus Genotype	Source	Fold IC_{50} (LPV)	Reference
V82A	Molecular clone	1.9-2.7*	3
V82F	Molecular clone	1.5*	3
V82T	Molecular clone	1.5-1.8*	3
V82S[◊]	Molecular clone	1.5-3.1*	3
G48V	Molecular clone	0.5-1.6*	3
I84V	Molecular clone	1.2-1.7*	3
L90M	Molecular clone	1.0-1.8*	3
V82A,I54V	Molecular clone	0.8-1.3*	3
V82F,I54V	Molecular clone	1.2-2.0*	3
V82T,I54V	Molecular clone	0.8-2.2*	3
I84V,L10F	Passaged virus	4	7
I84V,L10F,M46I	Molecular clone	6	7
I50V,M46I	Molecular clone	4	Data on file

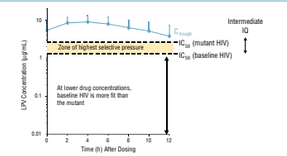
* Values represent determinations both in the presence and absence of 50% human serum.
◊ V82S is a double base pair mutation from the wild-type sequence.

- At steady-state, following a dose of LPV/r 400/100 mg, the median estimated time for plasma levels to decay to the upper range of IC_{50} values for single mutants (upper boundary of zone of highest selective pressure) is 20.5 hours. This time would approximate missing a scheduled LPV/r BID dose by 8 hours.
- The estimated median time for LPV concentrations to decay through the zone of highest selective pressure is approximately 3.5 hours (range 2.5-5 hours). Since viral maturation through proteolytic processing by active (uninhibited) wild-type HIV protease is not instantaneous (estimated half-life up to 1.5 hours for production of mature, infectious particles)[7] and the kinetics of HIV protease containing primary mutations can be substantially impaired compared to wild-type protease,[8] the selective production of infectious virus containing primary resistance mutations may be minimal during this period.

Viral Evolution Observed with Intermediate LPV IQ

- The probability for viral evolution in protease during rebound is greatest in situations when an **intermediate IQ** is present. While LPV trough levels are still expected to remain above the zone of highest selective pressure, variability in absorption, metabolism, or adherence may allow LPV concentrations to drop into the zone, where they may remain for a longer period of time since RTV concentrations are still inhibiting the CYP 3A mediated metabolism of LPV (Figure 4).

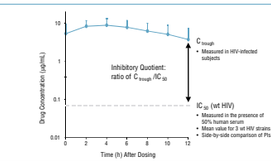
Figure 4. Selective Pressure for Viral Evolution in Protease is Present with Intermediate LPV IQ



No Viral Evolution Observed with Low LPV IQ

- When a **low IQ** (value of one or less) is present, LPV susceptibility of the baseline isolate is sufficiently reduced such that LPV plasma concentrations are unable to completely suppress viral replication. Therefore, no selective pressure is present for viral evolution since additional mutations could further compromise fitness without providing a growth advantage over the baseline isolate (Figure 5).

Figure 5. The Barrier to Resistance is Essentially Overcome with Low LPV IQ



CONCLUSIONS

- Viral evolution during viral load rebound on LPV/r therapy appears to be related to the LPV IQ.
- At low IQ, selective pressure appears to be insufficient to drive evolution.
- At intermediate IQ, evolution occurs since selective pressure is high and the genetic barrier is low.
- At high IQ, the high pharmacologic barrier prohibits the selection of mutants, suggesting that virologic rebound may be a consequence of periodic non-adherence in these patients. Modeling predicts that LPV concentrations would first drop into the zone of highest selective pressure 8 hours after missing a dose on the LPV/r 400/100 mg BID regimen and would remain there for a period of time that may only allow for a minimal production of infectious mutant virus.

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