

**ICAAC 2006**  
**Monogram Biosciences**  
**Abstract Book**  
**September 27<sup>th</sup>-30<sup>th</sup>**



biosciences  
monogram

The Mark of  
Individualized Medicine

**The Number of HIV Primary NRTI Mutations Correlates Directly with Other antiretroviral (ART) Associated Mutations and Indirectly with Replicative Capacity (RC) and Reduced Drug Susceptibility (RS)**

L. Ross<sup>1</sup>, N. Parkin<sup>2</sup>, and R. Lanier<sup>1</sup>

<sup>1</sup>GlaxoSmithKline, RTP, NC, and <sup>2</sup>Monogram BioSciences, S San Francisco, CA

**Background:** Selection for specific resistance associated mutations (RAM) in subjects following ART failure may result from many factors. Changes in RC, RS (as Fold-change in IC50 [FC]), and RAM prevalence (type and class) were examined as a function of the number of major NRTI mutations (NAMs) present in clinical samples.

**Methods:** Samples submitted for resistance testing from 2003-onward (n=35,222) were evaluated. RS was defined by drug specific cut-offs (Dec 2005). Major and minor RAMs were as defined by the IAS-USA (Oct 2005); samples with mixtures at major NAM sites were excluded. FTC, TPV and ddC were not evaluated.

**Results:**

# of NAMs	N	RC	Mean #						% samples with at least one drug FC > cutoff		
			minor NRTI RAMs	NNRTI RAMs	Major PI RAMs	NRTIs w/ FC >cutoff (Max 6)	NNRTIs w/ FC > cutoff (Max 3)	PIs w/ FC > cutoff (Max 7)	NRTI	NNRTI	PI
0	14363	97.8	0.2	0.3	0.1	0	0.8	0.3	3.4%	33.6%	12.6%
1*	4092	68.9	0.3	0.8	0.5	1.7	1.5	1.2	93.9%	53.8%	33.0%
2	1559	61.5	0.8	1.0	1.0	2.7	1.8	2.4	94.2%	66.6%	48.9%
3-4	4189	54.3	1.4	1.0	1.9	4.1	1.8	4.8	99.4%	65.9%	73.3%
5-6	2516	46.6	1.9	1.3	2.5	5.0	2.0	5.4	100%	75.1%	84.4%
7-8	133	43.9	1.5	1.5	2.5	5.4	2.1	5.3	100%	75.2%	82.0%

\*78% M184IV; 7% K65R

**Conclusions:** Increases in NAMs correlated with increases in RAMs and RS for all drug classes, although PI RAMs and RS increased at a slower rate than NNRTI RAMs and RS. RC also declined, with a substantial drop after detection of one NAM, possibly due to reduced fitness of the 65R or 184I/V. The relatively high percent of patients without NAMs but with NNRTI associated mutations may result from earlier selection of these mutations following virologic failure, and/or persistence following transmission of NNRTI RAMs.

## Impact of Protease (PR) Mutations L33F/I, V82A, I84V, and L90M on Ritonavir (RTV)-Boosted Protease Inhibitor (PI) Susceptibility

Peter J. Piliero<sup>1</sup>, Neil Parkin<sup>2</sup>, Douglas Mayers<sup>1</sup>

<sup>1</sup>Boehringer Ingelheim, Ridgefield CT; <sup>2</sup>Monogram Biosciences, South San Francisco, CA

**Introduction:** Tipranavir (TPV) is a novel PI active against many PI resistant HIV-1 isolates. PR mutations L33F/I, V82A, I84V, and L90M are known to confer resistance to PIs. We sought to evaluate the impact of these mutations on resistance to currently available RTV-boosted PIs.

**Methods:** All routine patient testing samples received by Monogram Biosciences for drug resistance phenotype and genotype testing since January 2000 and containing at least one drug-selected PR mutation or L33I/F were examined to evaluate the impact of L33F/I, V82A, I84V, and L90M on the proportion of samples resistant to different RTV-boosted PIs using recently updated clinical cut-offs. The number of samples per group varied depending on the time of drug approval. Resistance was defined by an IC<sub>50</sub> fold-change > 8 (TPV), 55 (lopinavir, LPV), 11.5 (amprenavir, APV), 20 (atazanavir, ATV), and 12 (saquinavir, SQV).

**Results:** In the presence of 1 or more of the mutations examined, the percentage of isolates resistant to each PI was lowest for TPV (see table).

# Mutations	TPV (n=2082)	LPV (n=9142)	APV (n=9142)	ATV (n=8325)	SQV (n=9142)
1	5.9%	14.4%	16.2%	21%	30.9%
2	17.4%	40.4%	50.4%	57.8%	75.6%
3	38.1%	69.6%	85.1%	78.9%	88.2%
4	69.4%	82.3%	96.0%	91.7%	97.6%

**Conclusions:** Isolates containing single or combinations of mutations L33F/I, V82A, I84V, and L90M are more commonly resistant to LPV, ATV, APV, and SQV than TPV. This analysis is consistent with the genotypic guidance for tipranavir found in the APTIVUS package insert. Quantitative differences exist related to the specific combination of these mutations present; therefore phenotypic testing may provide additional guidance.

## **Estimation of Clinical Cutoffs for Susceptibility to HIV-1 Protease Inhibitors (PIs) Based on Pharmacokinetic (PK), Protein Binding, and *in vitro* Wild-type Virus Susceptibility Data.**

**N. Parkin**<sup>1</sup>, E. Coakley<sup>1</sup>, K. Limoli<sup>1</sup>, L. Trinh<sup>1</sup>, C. Chappey<sup>1</sup>, and E. Acosta<sup>2</sup>  
<sup>1</sup>Monogram Biosciences, South San Francisco, CA and <sup>2</sup>Univ. of Alabama, Birmingham AL.

### **Background**

The ability of PIs to suppress virus replication is dependent in part on the susceptibility of the virus and the concentration of free drug in plasma. The level of PI resistance, reported as fold-change in IC<sub>50</sub> (FC), associated with a decrease in efficacy (the lower clinical cutoff, LCCO) has been derived experimentally for some PI regimens, but is unknown for PIs where clinical outcome data are not available.

### **Methods**

Population-based trough (C<sub>min</sub>) concentrations, LCCO for atazanavir (ATV), ritonavir-boosted ATV, lopinavir (LPV/r), and indinavir were gathered from data in the literature. Protein binding adjustment factors were determined experimentally. Average wild-type virus IC<sub>95</sub> values were derived from over 3000 recently tested samples lacking recognized resistance mutations. The inhibitory quotient (IQ<sub>95</sub>, C<sub>min</sub> divided by the protein-binding adjusted IC<sub>95</sub>) for viruses with an IC<sub>95</sub> corresponding to the known LCCO was calculated and applied to other PIs in order to back-calculate the unknown LCCO.

### **Results**

The mean experimental IQ<sub>95</sub> was 2.5 and ranged from 1.3 (ATV) to 4.2 (LPV/r). Either the lower estimate or the mean was used to predict LCCO for other PI regimens. The predicted LCCO values for boosted amprenavir, saquinavir, and tipranavir using an IQ<sub>95</sub> of 1.3 were 4.4, 1.0, and 4.0, respectively; using an IQ<sub>95</sub> of 2.5, the values were 2.3, 0.5, and 2.1, respectively. Recently experimentally-derived estimates for the LCCO using clinical trial data for these PIs are 4.0, 2.3, and 2.0, respectively. Similar LCCO results were obtained using IC<sub>50</sub> values; the mean IQ<sub>50</sub> value at the LCCO was 12 (range: 6-22).

### **Conclusions**

LCCO predictions were close to and validate experimentally derived values. Therefore, PK-driven estimates of LCCO for RTV-boosted PIs and perhaps other drugs may have utility until appropriate clinical outcome data are available.

## **Distribution Of Susceptibility Among HIV-1 Clinical Samples Submitted For Enfuvirtide (ENF) Resistance Testing**

**E. P. COAKLEY**, J. WHITCOMB, W. HUANG, R. PESANO, T. BUI, C. PETROPOULOS;  
Monogram Biosciences, South San Francisco, CA.

**Introduction:** ENF was the first entry inhibitor approved for the treatment of HIV infection. To date, limited surveillance data defining phenotypic ENF susceptibility in clinical practice has been reported.

**Methods:** ENF susceptibility was evaluated using PhenoSense HIV Entry; a single-cycle assay that evaluates pseudovirions containing patient derived HIV envelope proteins. Resistance was defined as an IC<sub>50</sub> fold change (FC) exceeding the 99th percentile of the distribution of baseline viruses within the TORO dataset (n=220).

**Results:** 131 unique samples submitted for routine ENF susceptibility testing were evaluated. The observed distribution of ENF susceptibilities described a continuous range from 0.4 FC to greater than 1576 FC. 37% and 63% of the viruses were classified as susceptible and resistant (FC>6.5), respectively. The mean FC (median, range) of ENF resistant isolates was 262 (150.5, 6.74-1576) and susceptible isolates was 2.3 (1.6, 0.4-6.4). The mean (median, range) HIV pol replication capacity (RC) was 53.7% (43%, 0.8%-138% (n=54)). Retrospective coreceptor tropism profiling (n=131) demonstrated that 51% were R5, 4% were X4 and 41% were dual/mixed tropism. No relationship between tropism and ENF susceptibility was observed. Among ENF resistant isolates the mean (median) FC was 262 (151) in this clinical dataset (n=82) which is higher than the reported mean (median) FC of 125 (63) for resistant isolates among the TORO protocol defined endpoint group (n=178), p=0.00004.

**Conclusions:** These data represent an initial description of phenotypic ENF susceptibility in clinical practice. The observed distribution in ENF susceptibility is broad. Among clinical isolates defined as ENF resistant, the IC<sub>50</sub> FC are higher than those previously described for resistant isolates within the TORO trial datasets. Coreceptor tropism profiles are similar to those previously reported in experienced populations.

## **Modulation of HIV-1 Co-Receptor Tropism and Susceptibility to Co-Receptor Inhibitors by Regions outside of the V3 Loop: Effect of gp41 Amino Acid Substitutions.**

W. Huang, J. Toma, S. Fransen, N. Parkin, J. Whitcomb, and C. Petropoulos  
Monogram Biosciences, South San Francisco, CA USA

**Background:** Major genetic determinants of co-receptor (CR) tropism and CR inhibitor resistance map to the V3 region of the HIV-1 envelope. However, little is known about the influence of other regions of envelope on CR usage. To explore the effects of changes outside V3 on tropism and sensitivity to CR inhibitors, we analyzed patient derived, clonal variants with identical V3 sequence but differing phenotypes.

**Methods:** Multiple gp160 envelope clones were isolated from a patient plasma sample using RT-PCR and cloning into an expression vector. CR use and susceptibility to CXCR4 and CCR5 inhibitors were determined using a single cycle pseudovirion assay (PhenoSense HIV Entry). Chimeric envelope genes were generated using different clones from the same patient to further examine the influence of different regions of gp160 on phenotype.

**Results:** We identified R5, dual and X4 tropic envelope clones from the virus population. Dual-tropic and R5-tropic clones with identical V3 sequences were identified. There were at least 10 amino acid differences throughout gp160 outside of the V3 loop between the R5- and dual-tropic clones. Analysis of envelope chimeras showed that sequence changes in C1, V1/V2, C3 and V4 of gp120 did not affect tropism. In contrast, the gp41 sequences from the dual-tropic clones were able to confer the ability to use CXCR4. Furthermore, these gp41 sequences were also associated with decreased sensitivity of dual tropic clones to CXCR4 and CCR5 inhibitors.

**Conclusions:** Envelope variants with different tropism and sensitivity to CXCR4 or CCR5 inhibitors but having identical V3 sequences contain alternative genetic determinants important for HIV-1 entry. These results reinforce the complexity of genetic determinants of CXCR4 tropism and drug resistance. Based on these and similar data from other patients we have developed a model to explain how R5 tropic HIV acquires the ability to efficiently utilize CXCR4.