



# **Presentations at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy**

**Boston, MA  
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## Executive Summary

Presentations involving Monogram Biosciences at the 50th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy which met in Boston in September 2010 included the following topics: Novel protease mutations associated with darunavir and tipranavir, HIV-1 coreceptor determination using a cell-associated DNA assay, and a look at the impact of integrase inhibitor resistance associated mutations on the performance of the Abbott RealTime HIV-1 Viral Load Assay.

### Presentations involving Monogram Biosciences include the following:

- Identification of Novel Mutations Strongly Associated with Darunavir (DRV) and Tipranavir (TPV) Resistance and Their Trends in a Commercial Database
- Phenotypic Determination of HIV-1 Coreceptor Tropism Using Cell-associated DNA Derived from Blood Samples
- Integrase Inhibitor-Resistance Associated Mutations (RAM) in Clinical Specimens: No Impact on Performance of the Abbott RealTime HIV-1 Viral Load Assay

## Identification of Novel Mutations Strongly Associated with Darunavir (DRV) and Tipranavir (TPV) Resistance and Their Trends in a Commercial Database

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**Background:** We used data mining techniques to identify novel resistance mutations for DRV and TPV. Further we examined temporal, phenotypic and genotypic trends in the Monogram Biosciences' HIV-1 clinical database.

**Methods:** Mutations were identified by performing correlative analyses among samples with both genotypic and phenotypic data;  $N > 50,000$  for DRV and TPV. The impact of each mutation was evaluated using a novel technique, *in-silico* site directed mutagenesis (*isSDM*), which identifies paired samples with matched amino acids at relevant resistance positions, but differ at a single mutation of interest. The comparison of fold change (FC) between pairs allows an inference to be made on the importance of that mutation to drug resistance. For DRV resistant samples ( $N=2141$ ) temporal trends were evaluated over the period 2006-2009.

**Results:** Novel mutations with the strongest association with DRV and TPV resistance include: E35N, I47A, and V82L for both DRV and TPV; L10F, G48M, and V82F for DRV only; I54S and I84A/C for TPV only. Temporal trend analysis demonstrated declines in the overall prevalence of DRV and TPV resistance from 2006-2009. Amongst DRV resistant samples, the mean DRV FC increased from 38 to 50. Notably, the increase in DRV FC correlated with a reduction in the mean TPV FC from 7.6 to 4.3 ( $R^2=0.99$ ), and was associated with increases in the frequency of mutations I50V (11% $\Rightarrow$ 15%), I54L (17% $\Rightarrow$ 33%), and L76V (5% $\Rightarrow$ 9%). These mutations were previously reported to confer DRV resistance but enhance TPV susceptibility; this finding was confirmed in our analysis.

**Conclusions:** Continued monitoring of large databases is essential to detect emerging trends in drug resistance and to identify novel mutations that improve the accuracy of genotypic interpretation algorithms. The increase over time in mutations associated with DRV resistance and enhanced TPV susceptibility may be relevant to drug sequencing strategies.

Abstract H-912

## Author's Summary:

### Background:

- IAS guidelines are commonly used to evaluate resistance to DRV and TPV, which are next generation protease inhibitors (PI) that have shown activity against many resistant HIV-1 strains.
- We used data mining techniques to identify novel resistance mutations for DRV and TPV.
- Furthermore, we examined phenotypic and genotypic resistance patterns over time by surveying Monogram's patient testing database.

### Methods:

- Novel mutations for DRV and TPV were identified thru correlative analysis performed on Monogram's large database of phenotypic and genotypic results for clinical specimens.
- *In-silico* site directed mutagenesis (*isSDM*) analysis was performed to further evaluate the impact of these novel mutations.
- Temporal trends in fold-change and resistance associated mutation for these drugs were examined.

### Results:

- Novel mutations with the strongest association with DRV and TPV resistance include:
  - **E35N, I47A, and V82L** for both DRV and TPV
  - **L10F, G48M, and V82F** for DRV only
  - **I54S and I84A/C** for TPV only
- Temporal trend analysis demonstrated declines in the overall prevalence of DRV and TPV resistance from 2006-2009.
- Amongst DRV resistant samples, the mean DRV FC increased from **38** to **50**.
- The increase in DRV FC correlated with a reduction in the mean TPV FC from **7.6** to **4.3** ( $R^2=0.99$ ).
- During the same time period, increases in the frequency of these mutations were observed:
  - **I50V** from 11% to 15%
  - **I54L** from 17% to 33%
  - **L76V** from 5% to 9%
- These mutations were previously reported to confer DRV resistance but enhance TPV susceptibility; this finding was confirmed in our analysis.

### Conclusions:

- Among DRV resistant samples, a trend has emerged: DRV fold-change is increasing, and this increase correlates with a decrease in TPV fold-change, and appears to be associated with selection of mutations strongly associated with DRV resistance that have sensitizing effect on TPV.
- Continued monitoring of large databases is essential to detect emerging trends in drug resistance and to identify novel mutations that improve the accuracy of genotypic interpretation algorithms.

## Phenotypic Determination of HIV-1 Coreceptor Tropism Using Cell-associated DNA Derived from Blood Samples

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**Background:** Agents that block the ability of HIV to utilize the CCR5 coreceptor are available for use in the clinic. Determining the coreceptor tropism of a patient's virus prior to CCR5 antagonist therapy is recommended. Current tropism assays are routinely performed using envelope (*env*) sequences derived from plasma viral RNA and thus cannot be used to evaluate patient samples with undetectable or low plasma viral loads (VL). To address this limitation, we have adapted a pseudovirion-based tropism assay to utilize *env* sequences derived from blood cell-associated DNA.

**Methods:** Plasma, PBMC and whole blood samples were obtained from 32 recent or chronic infections (SCOPE cohort). Tropism of plasma samples was determined using the Trofile assay. Cell-associated DNA was extracted from PBMC and/or whole blood samples and amplified *env* sequences were processed through the Trofile assay. Real time PCR was used to determine HIV copy number in cell-associated DNA samples. A cell line containing a single proviral copy was used to determine assay sensitivity.

**Results:** Tropism was determined in 31/32 PBMC samples and in 10 viremic plasma samples. The remaining 22 plasma samples had low or undetectable VL. Tropism determinations were concordant in the 10 paired plasma and PBMC samples (8 R5, 2 DM). In the undetectable or low VL samples, 1 sample failed to amplify, 11 were classified as R5 and 10 as DM or X4. The assay was capable of successfully detecting tropism in 100% of samples containing 10 copies of cell-associated HIV DNA.

**Conclusions:** Full length *env* sequences can be efficiently amplified from cell-associated HIV-1 DNA extracted from patient blood samples to determine coreceptor tropism. The ability to test tropism using cell-derived HIV DNA may be applicable to determining the suitability of CCR5 antagonist use in patients with low or undetectable plasma viral load.

Abstract H-921

## **Integrase Inhibitor-Resistance Associated Mutations (RAM) in Clinical Specimens: No Impact on Performance of the Abbott RealTime HIV-1 Viral Load Assay**

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**Background:** IN-inhibitor (INI) resistance-associated mutations were monitored to evaluate their impact on the Abbott RealTime HIV-1 assay (RealTime HIV-1, Abbott Molecular).

**Methods:** In a blinded study, 67 plasma samples previously tested for INI resistance (Monogram Biosciences) were tested in RealTime HIV-1 and Roche Monitor v1.5(v1.5) tests. Sequences were analyzed to identify RAL resistance-associated mutations and mismatches within the RealTime HIV-1 primer/probe sites.

**Results:** VL were obtained for 67 samples in RealTime HIV-1 (66 quantified, 1 detected <1.6 log<sub>10</sub>) and 67 in v1.5. Fifty-five of 66 quantified samples (83.3%) were within 0.5 log<sub>10</sub> copies/ml between tests; 64 (97%) within 1.0 log<sub>10</sub>. Relative to v1.5, RealTime HIV-1 quantified 2 samples 1.1 and 1.8 log<sub>10</sub> lower. Correlation (R<sup>2</sup>) was 0.8117 for 66 samples quantified within the dynamic range of both tests. Based on BlandAltman analysis the mean bias (RealTime HIV-1 minus v1.5) was -0.24 log<sub>10</sub>. IN sequence was available for all 67 samples. Log differences (RealTime HIV-1 vs. v1.5) ranged from -1.80 to 0.23 and -0.64 to 0.83, respectively, for 42 RAL susceptible and 24 RAL resistant specimens (15 with resistance-associated mutations in the RealTime HIV-1 forward primer site). Paired t-test analysis of VL values for RAL resistant specimens indicated at the 5% alpha level the mean difference between assays is not significantly different from 0.25 log<sub>10</sub> copies/ml (t=1.06, p=0.3018).

**Conclusions:** Analysis of RAM for RAL showed that very few are localized to the RealTime HIV-1 assay target region. The RealTime HIV-1 assay has the capacity to tolerate IN inhibitor selected mismatches with no apparent impact on assay performance. Study of a larger cohort is needed to confirm these findings.

*Abstract H-927*