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


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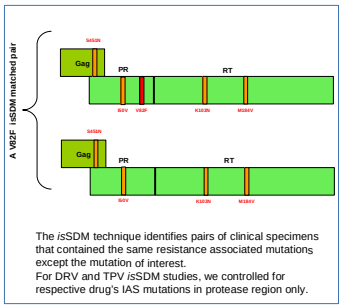
## BACKGROUND

- Darunavir/r (DRV) and Tipranavir (TPV) are next-generation protease inhibitors (PI) that have shown activity against many HIV-1 strains with multiple PI resistance associated mutations.
- International AIDS Society (IAS) guidelines for mutations associated with DRV are commonly used to determine resistance to DRV and TPV.
- 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

- Using a database of matched phenotype and genotype (N > 50,000 for DRV and TPV), univariate and multivariate analysis was performed to derive mutations strongly associated with resistance to DRV and TPV.
- Fisher's Exact Test (FET) was performed to identify significant associations between presence or absence of a given mutation with the phenotypic drug susceptibility, as measured by fold change in IC50 (FC) being over or under the respective clinical cutoff. Results for most impactful mutations are shown in Table 1.
- The impact of each mutation was further evaluated using a novel technique, *in-silico* site directed mutagenesis (*is*SDM), which identifies paired samples with matched amino acids at relevant resistance positions, but differ at a single mutation of interest. Table 1 shows the comparison of FC between these *is*SDM pairs.
- For DRV resistant samples (N=2141), temporal trends were evaluated over the period 2006-2009.

### Figure 1: *In silico* Site Directed Mutagenesis

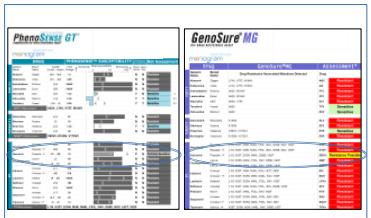



The *is*SDM technique identifies pairs of clinical specimens that contained the same resistance associated mutations except the mutation of interest.
For DRV and TPV *is*SDM studies, we controlled for respective drug's IAS mutations in protease region only.

### Table 1: DRV and TPV fold-change for *is*SDM pairs, with statistics derived from Fisher's Exact Test

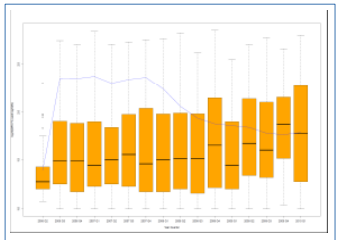
| Mutation | Drug | *is* SDM: Median Fold-Change when Mutation Present | *is* SDM: Median Fold-Change when Mutation Absent | FET Odds Ratio | FET *p-value* (with Bonferroni correction) |
|---|---|---|---|---|---|
| E35N | DRV | 2.12 | 1.72 | 5.51 | <0.001 |
| I47A | DRV | 3.23 | 2.84 | 3.54 | <0.001 |
| V82L | DRV | 2.76 | 0.89 | 4.67 | <0.001 |
| E35N | TPV | 3.11 | 1.40 | 7.48 | <0.001 |
| I47A | TPV | 7.67 | 1.80 | 3.85 | 0.005 |
| V82L | TPV | 4.38 | 1.44 | 24.70 | <0.001 |
| L10F | DRV | 10.35 | 5.88 | 4.01 | <0.001 |
| G48M | DRV | 7.22 | 3.45 | 3.03 | <0.001 |
| V82F | DRV | 17.02 | 2.73 | 4.73 | <0.001 |
| I54S | TPV | 2.72 | 2.54 | 5.26 | <0.001 |
| I84A | TPV | 5.85 | 1.98 | 10.90 | 0.005 |
| I84C | TPV | 9.04 | 1.99 | 10.98 | <0.001 |
| I50V | DRV | 22.05 | 15.86 | 10.57 | <0.001 |
| I54L | DRV | 18.11 | 8.17 | 8.96 | <0.001 |
| L76V | DRV | 8.39 | 3.50 | 6.03 | <0.001 |
| I50V | TPV | 8.73 | 2.11 | 9.18 | <0.001 |
| I54L | TPV | 1.50 | 3.81 | 0.80 | <0.001 |
| L76V | TPV | 1.75 | 2.89 | 1.12 | 0.5 |

### Figure 2: PhenoSense GT™ and GenoSure™ MG patient report



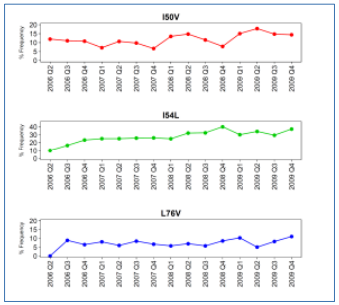

DRV Fold-Change = 15 with no occurrence of IAS DRV mutations. Presence of E35N and V82F as novel resistance associated mutations.

### Figure 3: Temporal trend in DRV fold-change amongst DRV resistant samples

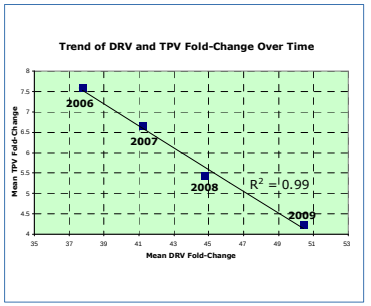


Distribution of DRV fold changes by quarter shown by box plots amongst DRV resistant samples. The blue line represents the log10(N) per box plot. N is decreasing only in DRV resistant samples and not in total samples tested.

### Figure 4: Temporal trends in 3 DRV IAS mutations that have sensitizing effect on TPV amongst DRV resistant samples




### Figure 5: Increase in DRV FC within DRV resistant samples is correlated with decline in TPV FC over time




## 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.


### ACKNOWLEDGEMENTS

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