# Predicting in vitro resistance phenotypes for HIV protease and integrase inhibitors using sequences from treated and non-treated individuals

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## Background

- The emergence of antiviral drug resistance is a significant obstacle facing the global management and treatment of HIV.
- Here, a simple strategy for HIV drug resistance phenotype prediction is developed for protease and integrase inhibitors based on using only the ratios of the observed prevalence of all mutations from previously treated vs. non-treated individuals
- Predicted resistance phenotypes were then compared with recombinant virus phenotypes

## Calculating the resistance score

- The Stanford HIV database was used to retrieve subtype B HIV protease and integrase sequences (table 1).

| | Protease | Integrase |
|---|---|---|
| Treated | 14,978 | 1,003 |
| Non-treated | 42,159 | 5,735 |
| Total | 57,137 | 6,738 |

Table 1. Sequence counts for the Stanford HIV database (August 2017)

- Prevalence ratios were calculated for each position from treated and non-treated individuals
- Positions selected during treatment would therefore be expected to have higher prevalence ratios and likely to be associated with resistance
- The corresponding prevalence ratio for each amino acid in an individual's sequence is logged and the simple sum of these values is defined as the resistance score (LPR score, Fig. 1)

$$\text{LPR score} = \log_{10}(n1 * n2 * n3 \ldots)$$

**FIGURE 1.** Equation summarizing the proposed resistance score (LPR score), where for each sequence, the prevalence ratio for each corresponding amino acid (n) is logged and summed.

- LPR scores between treated and non-treated individuals are significantly different ($p < 0.01$)

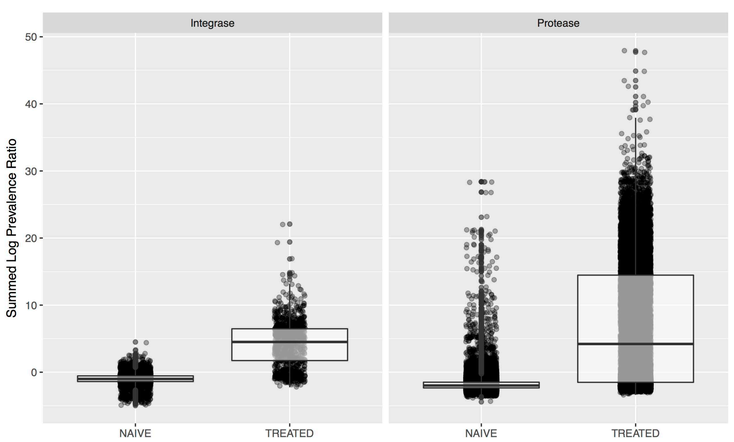


**Figure 2**. Summed log prevalence ratios (LPR) for protease and integrase sequences from the Stanford HIV database

- The LPR score was then assessed for its ability to predict drug susceptibility using Monogram or BC CfE data

## Phenotypic assay prediction

- For all available samples with matching phenotypes (n = 591) the LPR score was then compared with an *in vitro* phenotype assay (Monogram PhenoSense) for 8 protease inhibitors (Fig. 3)

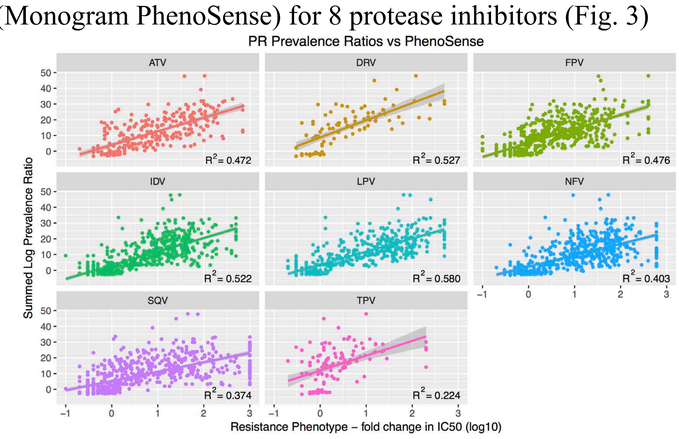


**FIGURE 3**. Summed log prevalence ratios (LPR) compared with the PhenoSense scores for each protease inhibitor using a linear model with 95% confidence intervals.

- Resistance for PhenoSense was defined using manufacturer specific cutoffs: ATV−2.2, DRV-10, FPV-4, IDV-10, LPV-9, NFV-3.6, SQV-1.7, TPV-2
- The ability of the LPR score to predict in vitro phenotypes was assessed with ROC (Fig.4) and precision recall curves (Table 2)

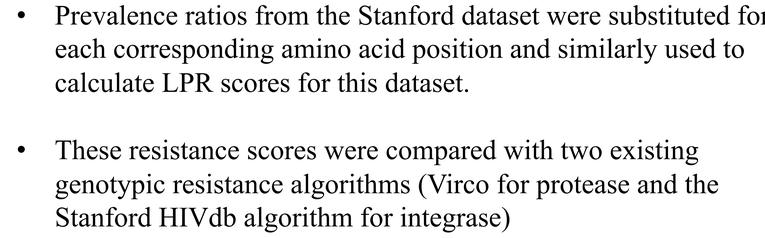


**FIGURE 4.** ROC curve assessing the predictive potential of the LPR score and treatment status for the Stanford protease sequence dataset as defined from PhenoSense

- To account for the imbalanced nature of the dataset (493 treated/ 98 non-treated), it was split into a training (75%) and test (25%) dataset 100 times
- At each cycle, ROC curves were generated and sensitivity, specificity were calculated (Table 2). Precision-recall curves were generated using the entire dataset.

| Sensitivity | Specificity | AUROC | Prec | Recall | AUPRC |
|---|---|---|---|---|---|
| 0.95 +- 0.01 | 0.84 +- 0.02 | 0.94+-0.02 | 0.95 | 0.93 | 0.95 |

**TABLE 2.** Summary of the results for 100 receiver operating characteristic (ROC) and a precision/recall (PR) curve for PhenoSense susceptibility prediction. Values were generated using an LPR threshold of 2.79. Sensitivity was required to be at least 0.95 for a threshold to be applied. AUROC – area under the ROC, AUPRC – Area under the PR, PREC – Precision

## Genotypic assay prediction

- The capacity of the LPR score to perform as a genotypic assay was assessed using an independent dataset composed of 33,600 protease and 3,298 integrase sequences from HIV infected individuals in British Columbia
- Prevalence ratios from the Stanford dataset were substituted for each corresponding amino acid position and similarly used to calculate LPR scores for this dataset.
- These resistance scores were compared with two existing genotypic resistance algorithms (Virco for protease and the Stanford HIVdb algorithm for integrase)
- Resistance for Virco was defined as greater than: ATV-2.5, DRV-10.0, FPV-1.5, IDV-2.3, LPV-6.1, NFV-2.2, SQV-3.1, TPV-1.5, whereas resistance for the Stanford HIV DB required values greater than 2 for all drugs

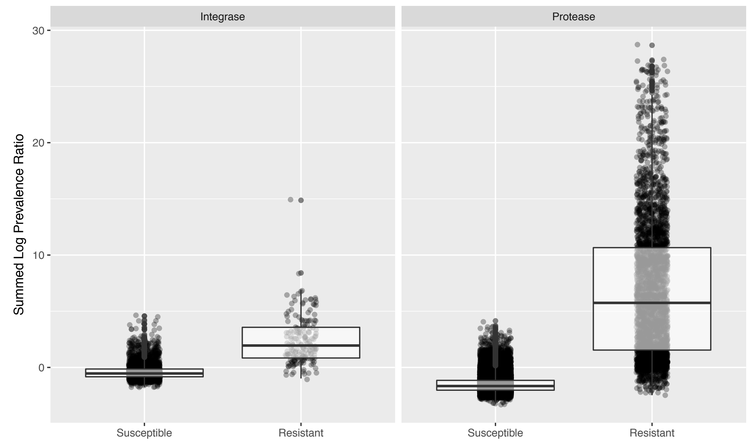


**FIGURE 4**. Summed log prevalence ratios compared with resistance predictions for Virco (Protease) and Stanford HIV DB (Integrase)

- There was a significant relationship between the protease resistance scores and Virco resistance predictions (Fig. 4/5)

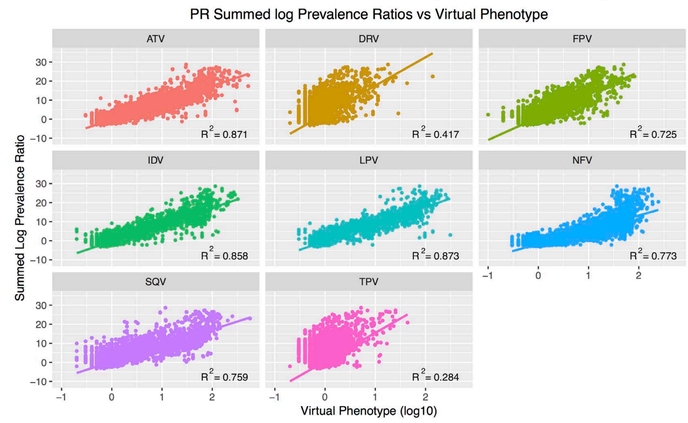


**FIGURE 5**. Summed log prevalence ratios compared with Virco scores for each protease inhibitor using a linear model with 95% confidence intervals.

| Gene | Drug | Threshold | # Res | Sensitivity | Specificity | AUROC | Precision | Recall | AUPRC |
|---|---|---|---|---|---|---|---|---|---|
| Pro | NFV | -0.29 | 3,171 | 0.95 +- 0.001 | 0.93 +- 0.005 | 0.98 +- 0.002 | 0.42 +- 0.018 | 0.95 +- 0.001 | 0.93 |
| | ATV | 2.54 | 2,037 | 0.95 +- 0.001 | 0.99 +- 0.001 | 1 +- 0.001 | 0.16 +- 0.016 | 0.95 +- 0.001 | 0.97 |
| | SQV | 3.78 | 1,518 | 0.95 +- 0.001 | 0.98 +- 0.001 | 0.99 +- 0.001 | 0.28 +- 0.008 | 0.95 +- 0.001 | 0.89 |
| | FPV | 1.87 | 1,729 | 0.95 +- 0.001 | 0.98 +- 0.001 | 0.99 +- 0.001 | 0.32 +- 0.011 | 0.95 +- 0.001 | 0.91 |
| | LPV | 5.61 | 1,374 | 0.95 +- 0.001 | 0.99 +- 0 | 1 +- 0.001 | 0.22 +- 0.008 | 0.95 +- 0.001 | 0.95 |
| | DRV | 9.77 | 51 | 0.97 +- 0.011 | 0.97 +- 0.004 | 0.99 +- 0.003 | 0.95 +- 0.007 | 0.97 +- 0.011 | 0.18 |
| | TPV | 1.23 | 708 | 0.95 +- 0.001 | 0.94 +- 0.006 | 0.98 +- 0.005 | 0.74 +- 0.02 | 0.95 +- 0.001 | 0.56 |
| | IDV | 2.79 | 2,052 | 0.95 +- 0.001 | 0.99 +- 0.001 | 1 +- 0.001 | 0.11 +- 0.005 | 0.95 +- 0.001 | 0.97 |
| | Total | -0.35 | 3,290 | 0.95 +- 0.001 | 0.93 +- 0.004 | 0.98 +- 0.002 | 0.40 +- 0.015 | 0.95 +- 0.001 | 0.93 |
| Int | DTG | -0.67 | 42 | 0.97 +- 0.003 | 0.45 +- 0.077 | 0.87 +- 0.05 | 0.02 +- 0.004 | 0.97 +- 0.003 | 0.59 |
| | EVG | -0.21 | 147 | 0.95 +- 0.002 | 0.62 +- 0.055 | 0.93 +- 0.02 | 0.11 +- 0.021 | 0.95 +- 0.02 | 0.34 |
| | RAL | -0.09 | 173 | 0.95 +- 0.002 | 0.71 +- 0.072 | 0.94 +- 0.01 | 0.16 +- 0.039 | 0.95 +- 0.002 | 0.62 |
| | Total | -0.21 | 178 | 0.95 +- 0.002 | 0.70 +- 0.08 | 0.94 +- 0.01 | 0.16 +- 0.04 | 0.95 +- 0.002 | 0.62 |

**TABLE 2.** Summary of the results for 100 ROC curves and an AUPRC curve assessing the ability of the resistance score to predict Virco (protease) and Stanford HIV db's (integrase) genotypic assay. Threshold – Optimal resistance score threshold, # Res – Number resistant, AUROC – area under the ROC curve, AUPRC – area under the PR curve. Sensitivity was required to be at least 0.95 for a threshold to be applied.

## Discussion

- The prevalence-ratio resistance score was shown to be a reasonably effective predictor of HIV drug resistance as compared with both phenotypic and genotypic assays and could be used in subsequent studies in optimizing HIV treatment strategies for drugs where phenotypes are not widely available