

Introduction

Considerations for antiretroviral drug selection include potency, toxicity (short and long term), and pill burden/dosing schedule. In addition, selection of drug therapies should include consideration of pre-existing resistance and cross-resistance, and whether potential emergent resistance might limit subsequent therapies.

Thymidine analog sparing NRTI backbones or all-NRTI regimens do not select for thymidine analog mutations at initial failure; however they may rapidly select "multi-nucleoside resistance" patterns typically involving combinations of K65R, L74V and M184V/I mutations. We evaluated phenotypic effects of these mutations in a large database of clinical samples to help define the range of cross-resistance, and potential drug sequencing implications.

Methods

Phenotypic susceptibilities (mean, median, range, and % above cutoff) to NRTIs of viruses in the ViroLogic database containing K65R, L74V, M184I, and M184V were generated at ViroLogic by published methods (Petropoulos, 2000). All database queries were performed at ViroLogic, South San Francisco, CA.

Queries were carried out on samples existing within the database in August, 2003. The total number of samples with Phenotype and Genotype in the starting file (with repeats) was 21,623. Repeats for individual patients were removed before searching for matching phenotypes in this query, resulting in 17,633 samples with no patient repeats within, or between, groups.

Samples with mixtures at the positions of interest or other nucleoside analogue associated mutations (NAMs) (41L, 67N, 69X, 70R, 75X, 115F, 151M, 210W, 215FY, 219X; X = any non-wt amino acid) were excluded. Data is presented here as median fold change (MFC) in the drug concentration that inhibits virus production by 50% (IC50) vs. reference virus NL4-3, and as % above cutoff (AC). Clinical cutoffs were used for ABC=4.5, ddl=1.7, 3TC=3.5, d4T=1.7 and TDF=1.4, while biological cutoffs were used for ddC=1.7 and ZDV=1.9. Hypersusceptibility is an assay-based biological cutoff, and is defined as 2.5 fold increased susceptibility (e.g., 0.40 if NL4-3 = 1.0). TDF designates tenofovir, excepting with respect to the clinical cutoff where TDF designates tenofovir disoproxil fumarate.

Clinical cutoffs are based on clinical trial results, and represent the fold change which is considered clinically significant for patients. The biological cutoff for a given drug is currently defined for the ViroLogic Phenosense assay as the 99th percentile of wildtype virus (Parkin, 2004).

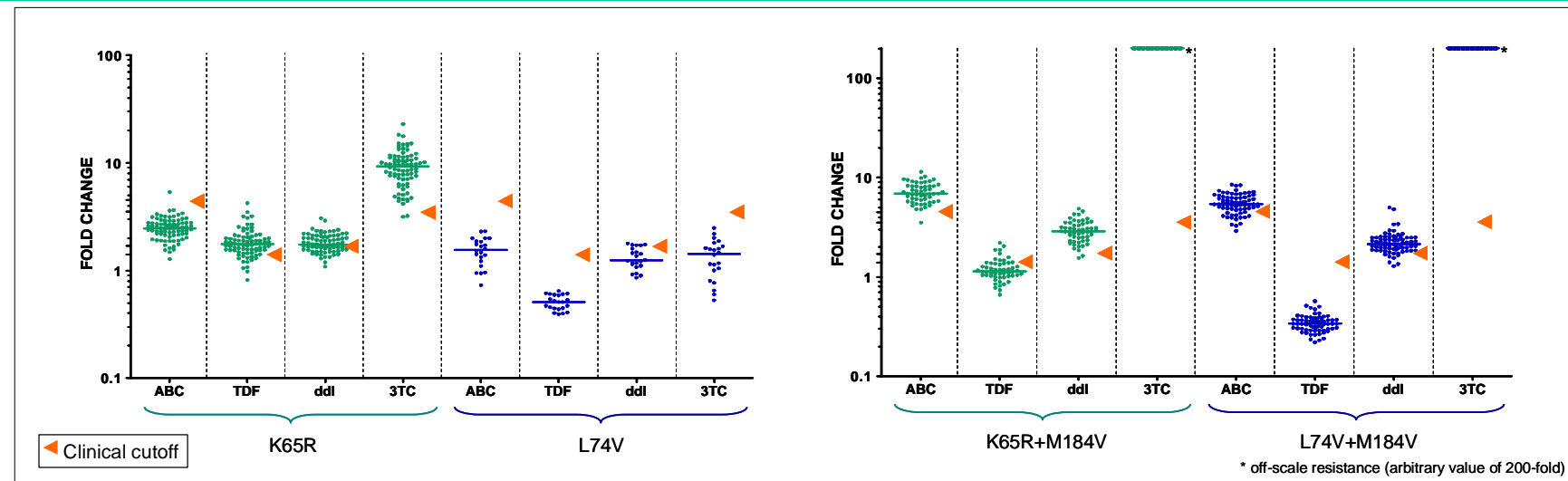
Table 1. Median fold changes (MFC's) in IC50s for designated NRTI's in clinical isolates containing NRTI associated mutations K65R, L74V, and/or M184V/I. The percent of isolates above the cutoff (AC) is shown in brackets.

| drug | Mutations (N): | | | | | |
|------|------------------|-----------------|-------------------|-------------------|-------------------|-------------------|
| | K65R (82) | L74V (22) | K65R/M184V (54) | L74V/M184V (74) | M184V (1720) | M184I (27) |
| d4T | 1.3 [12%]* | 0.9 [0%] | 1.0 [0%] | 0.8 [0%] | 0.7 [0.1%] | 0.8 [0%] |
| ZDV | 0.5 [2.4%] | 0.3 [0%] | 0.4 [0%] | 0.3 [0%] | 0.4 [0.1%] | 0.2 [0%] |
| 3TC | 9.3 [98%] | 1.4 [0%] | 200 [100%] | 200 [100%] | 200 [100%] | 200 [100%] |
| ddl | 1.7 [60%] | 1.2 [23%] | 2.9 [96%] | 2.2 [91%] | 1.4 [11%] | 1.4 [26%] |
| ddC | 2.3 [91%] | 1.2 [9.1%] | 3.9 [100%] | 2.3 [86%] | 1.6 [41%] | 2.0 [70%] |
| ABC | 2.5 [1.2%] | 1.6 [0%] | 6.9 [98%] | 5.4 [78%] | 2.8 [1.6%] | 1.6 [0%] |
| TDF | 1.8 [83%] | 0.5 [0%] | 1.1 [19%] | 0.3 [0%] | 0.5 [0%] | 0.4 [0%] |

* MFC (%AC); **Bolded red** numbering indicates median fold change (MFC) is above assay cutoff (AC), and **bolded-italicized green** numbering indicates MFC is less than 0.40 (therefore, e.g., 0.38 is designated hypersusceptible and 0.42 is not)

- The median fold change (MFC) for ZDV was not above cutoff (AC) for any mutation or combination examined; all of these mutations and combinations were associated with increased susceptibility to ZDV. MFC versus d4T was not above cutoff for any of the mutations, but K65R alone decreased sensitivity.
- The MFC for 3TC was above cutoff for all patterns examined except L74V alone. The MFC for ddl was above cutoff with K65R as well as with both of the double mutation combinations, but below cutoff for L74V or M184V/I alone.
- The MFC for ABC was above cutoff only for the double mutation combinations. The MFC for TDF was above cutoff for the K65R mutation alone and near wild-type for K65R + M184V; virus with M184V/I and/or L74V was more sensitive to TDF than wild-type.

Figure 1. Scatter plots of clinical isolate fold resistance for single mutations and double mutation combinations versus ABC, TDF, ddl, and 3TC



- Data from Table 1 is shown here in scatter plot format, with each dot indicating the fold change versus reference virus for a single clinical isolate. Orange arrowheads in each column indicate the clinical cutoff for that drug.

Discussion

NRTI mutation profiles continue to evolve in the HIV patient population. We have compared *in vitro* derived phenotypic and genotypic data generated from clinical isolates containing K65R, L74V, and M184V/I, alone or in combination. Reproducibility of phenotypic susceptibility measurements with this assay using site-directed NRTI mutants (presented in poster 704), suggested that the spread in the data is due mostly to genetic variability of clinical isolates.

For K65R, the median fold change reached the clinical or biological cutoff for four of the seven NRTI's analyzed here; for L74V, the median fold change did not reach the clinical or biological cutoff for any of the NRTI's.

The median fold change for both the K65R/M184V and the L74V/M184V combinations was above cutoff for 3TC, ABC, ddC, and ddl. The NRTI susceptibilities of samples containing the double mutation combinations were significantly different from each other (p<0.0001 either by t-test comparison of means or Mann-Whitney test). Of the two double mutants examined, a greater percent of the K65R/M184V isolates were above cutoff for ABC, ddC, ddl, and TDF. In addition, the L74V/M184V combination increased susceptibility to TDF.

Conclusions

- All of the mutations examined demonstrated increased susceptibility to ZDV.
- K65R resulted in median fold change above cutoff for 3TC, ddl, ddC, and TDF.
- L74V did not result in median fold change above cutoff for any drugs, though 23% of the isolates were above cutoff for ddl.
- The K65R/M184V combination was associated with a greater percent of isolates above cutoff for ABC, ddC, ddl, and TDF, than the L74V/M184V combination.
- L74V and M184V alone or together were associated with increased susceptibility to TDF.

References

- Petropoulos CJ, et al. Antimicrob Agents Chemother. 2000 Apr;44(4):920-8
Parkin NT, et al. Antimicrob Agents Chemother. 2004 Feb;48(2):437-43.