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Proof-of-Principle Evaluation of Predictive Performance for Therapy Outcome of Baseline Estimated Fitness and Genetic Barrier towards Resistance in a Clinical Cohort of HIV-1 Treated Patients

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BACKGROUND

- We have previously developed a computational method to reverse engineer, from clinical genotypes only, the **fitness landscape** experienced by HIV **under treatment** (Deforche K et. al., XV Intl. Drug Res. Workshop, Sitges, 2006) and applied it to NFV and the combination of AZT and 3TC
- We demonstrated that the fitness landscape correlates with in vitro resistance phenotype (Theys K et. al., CROI 2007), and allows prediction of **genetic barrier** through simulated evolution (Deforche K et. al., 5th Eur. Drug Res. Workshop, 2007)

OBJECTIVE

To evaluate, in an independent clinical cohort, a potential new genotypic interpretation for AZT+3TC+NFV based on the estimated fitness landscapes, for virological response (at 3 and 6 months) of regimens containing these antiretroviral drugs. We used 3 commonly used interpretations systems (Rega, ANRS and Stanford) as comparators.

METHODS

Patient Population (n = 95, Table 1)

- Patients were selected from the EuroSIDA cohort who started a new treatment with AZT+3TC+NFV and for whom a genotype was available at any time before initiation of this treatment.
- For these patients, two outcomes were studied:
  - Change in viral load (ΔVL) at 3 months (n = 62, patients without a viral load in the 2-4 months window were excluded)
  - Treatment failure (yes/no) at 6 months (n = 95), where failure was defined by:
    i) a viral load >500 copies/ml in the time window ranging between 5 and 7 months from starting therapy
    or
    ii) discontinuation of AZT, 3TC or NFV before month 6

Genotypic Factors

- Several genotypic factors were computed from the estimated Fitness Landscapes for NFV, AZT, and combination AZT+3TC:
  - Estimated fitness (**EF**) during treatment
    - Higher replication capacity or higher resistance contribute to higher viral fitness during treatment
  - As measures of genetic barrier, the expected number of mutations **(#MR)** or "*generations*" **(#GR)** before developing a major resistance mutation. Major resistance mutations according to antiretroviral drugs were defined as below:
    NFV: 30N, 88S or 90M
    AZT: 41L or 215Y/F
    AZT+3TC: 41L, 184V or 215Y/F

    Therefore the smaller the value of #MR (e.g. for AZT) the closer is a patient's dominant virus to develop full resistance to AZT. A *generation* corresponds to a simulated generation in the ideal Wright-Fisher model that was used to simulate evolution on the fitness landscape. Therefore #GR, compared to #MR, reflects also the time needed to develop the mutations (with less time for a mutation with higher selective advantage).
  - Also as measures of genetic barrier, the expected number of mutations **(#MHF)** or generations **(#GHF)** before exceeding a fitness threshold (average fitness of a strain with at least one major resistance mutation)
- For comparison, genotypic susceptible scores (GSS) separately for NFV, AZT, 3TC, and for the combination AZT+3TC (by summing the individual scores for AZT and 3TC) were computed using rule-based interpretation systems ANRS v14, Rega 6.4.1, and HIVDB 4.2.6. GSS was defined as 0 for a resistant, 0.5 for intermediate, and 1 for susceptible interpretation. (Figure 1)

Statistical analyses

- Unadjusted analyses of correlation between each set of genotypic predictors (GSS, EF, #MHF and #MR) and the outcomes were performed:
  - Linear regression of ΔVL at 3 months, adjusted only for pre-treatment viral load
  - ROC analysis of treatment failure (VL>500 copies/ml or treatment switch) at 6 months:
    GSS: $GSS_{AZT} + GSS_{3TC} + GSS_{NFV}$
    EF: $\log EF_{AZT+3TC} + \log EF_{NFV}$
    MR: $\#MR_{AZT+3TC} + \#MR_{NFV}$
    MHF: $\#MHF_{AZT+3TC} + \#MHF_{NFV}$
- Adjusted analyses of correlation, adjusted for baseline characteristics shown in Table 1 were performed:
  - Linear Regression for ΔVL at 3 months
  - Logistic Regression for odds of treatment failure at 6 months

Table 1

Characteristics of the study population (n = 95)

Characteristic	
Age, years, median (IQR)	39 (32-44)
Female, n(%)	38 (40%)
HIV Exposure, n(%)	
Homosexual	32 (34%)
IDU	29 (31%)
Heterosexual	31 (33%)
Other	3 (3%)
Viral load, log copies/ml, median (IQR)	4.6 (3.5 – 5.3)
CD_4 count, cells/?l, median (range)	183 (90 – 326)
Number of drugs previously failed, n(%)	
0	46 (48%)
1 – 3	26 (27%)
4 – 6	17 (18%)
› 6	6 (6%)
Previous failure of, n(%)	
AZT	31 (33%)
3TC	37 (39%)
NFV	11 (12%)

Figure 1

Genotypic susceptible scores according to the Rega interpretation system
(ANRS en HIVDB were similar)

Table 2

Genotypic factors computed from the estimated fitness landscapes, at time of starting AZT+3TC+NFV

Factor	NFV	AZT	AZT+3TC
log (EF)	1.7 (1.4 – 2.3)	1.0 (0.9 – 1.4)	1.0 (0.9 – 1.8)
#MR	3.0 (2.6 – 3.5)	4.8 (0.8 – 5.9)	1.7 (0.0 – 2.2)
#MHF	2.9 (1.1 – 3.6)	6.5 (3.6 – 7.2)	5.6 (2.3 – 6.0)
#GR	90 (80 – 107)	252 (0 – 321)	67 (0 – 91)
#GHF	86 (31 – 105)	330 (145 – 372)	197 (57 – 223)

Table 3

Univariable statistical analysis of genotypic predictors for viral load change at 3 months (corrected for baseline viral load).

Factor *(per 1 higher)*	Decrease in VL @ 3M	*p* value
GSS_{AZT}	*1.54 (0.62 – 2.45)*	*0.001*
GSS_{3TC}	0.29 (-0.72 – 1.31)	0.57
GSS_{NFV}	-0.49 (-1.76 – 0.78)	0.44
$\log EF_{AZT+3TC}$	*-0.38 (-0.76 – -0.02)*	*0.04*
$\log EF_{NFV}$	-0.49 (-1.12 – 0.13)	0.12
$\#MR_{AZT+3TC}$	*0.40 (0.06 – 0.74)*	*0.02*
$\#MR_{NFV}$	-0.08 (-0.41 – 0.25)	0.64
$\#MHF_{AZT+3TC}$	*0.15 (0.00 – 0.30)*	*0.05*
$\#MHF_{NFV}$	0.17 (-0.08 – 0.41)	0.18

RESULTS

- Table 1 shows the baseline characteristics of our study population.
  - About half of the patient population (48%) was treatment naïve.
  - Of the patients with treatment experience, 63% had failed previously on treatment with AZT, 75% with 3TC, and 22% with NFV.
- Figure 1 shows the genotypic susceptibility as predicted by Rega and Table 2 shows variation in genotypic predictors computed from the estimated fitness landscapes.

Unadjusted analyses

- Table 3 shows the predictive performance for 3 months viral load reduction, for each genotypic factor within each of the four separate univariable models (GSS, EF, #MHF, and #MR)
  - One unit greater susceptibility to AZT according to Rega was associated with 1.54 greater viral load reduction from pre-therapy levels (p=0.001)
  - Greater estimated fitness (EF) for AZT+3TC was associated with a smaller viral load reduction (0.38 log per log EF higher, p=0.04)
  - Greater estimated fitness (EF) for NFV was also associated with a smaller viral load reduction (0.49 log per log EF higher) although the association was not significant (p=0.12)
  - Higher genetic barrier (greater #MR and #MHF) for AZT+3TC correlated with 0.40 (p=0.02) and 0.15 (p=0.05) greater viral load reduction
- Figure 2 shows ROC curves for predicting failure at 6 months, for each of the four models
  - Area under ROC tended to be higher for EF and #MHF compared to GSS or #MR, but they were not significantly different (p=0.61)
  - The better performance of EF and #MHF compared to GSS was due to a larger increase in sensitivity when specificity dropped to values below 70%. Indeed, out of 55 patients with a GSS=3, 24 (43%) did not experience treatment failure and 31 (56%) did experience treatment failure. For these patients, variation in EF and #MHF still tended to associate with treatment failure, although at lower levels of specificity

Adjusted analyses

- Predictive performance of genotypic predictors in 5 models for 3 months viral load reduction are shown in Table 4
  - Higher GSS for AZT correlated with greater viral load reduction
  - Other independent predictors of viral load reduction were pre-therapy viral load (mean reduction=0.61 per log higher, p ‹ 0.001) and previous virological failure on 3TC (mean reduction=-1.19 ,vs. no previous virological failure on 3TC, p=0.03)
- Predictive performance of genotypic predictors in 5 models for odds of failure at 6 months are shown in Table 5
  - Only higher estimated fitness in presence of NFV (EF_{NFV}) and lower genetic barrier to NFV resistance (MHF_{NFV}) correlated with increased risk for treatment failure (p=0.02)
  - In each model, age (OR=0.5 per 10 years younger, p=0.02), CD_4 count (OR=0.69 per 100 cells higher, p=0.03) and previous failure on 3TC(OR=16.86, vs. no previous virological failure on 3TC, p=0.006) were independently associated with risk for failure
  - In a model including both EF and MHF, no genotypic factor was predictive, indicating that EF_{NFV} and MHF_{NFV} are not independent predictors, although the results should be interpreted with caution as predictors were highly collinear (data not shown)

Predictive performance of genetic barrier predictors expressed as number of estimated generations (#GHF and #GR) was similar to that of those expressed as estimated mutations (#MHF and #MR), therefore we report only the results of #MHF and #MR. All three interpretation systems (Rega, ANRS, and HIVDB) performed similarly, therefore only one is reported.

CONCLUSION

- No genotypic predictor significantly outperformed any other (probably due to small sample size). In the analysis comparing methods according to their ability to classify patients with treatment failure, fitness landscape based predictors (EF and #MHF) showed an increased sensitivity (at specificity ‹ 70%) over expert interpretation systems for isolates which were scored by expert systems as fully susceptible. Larger number of patients are needed to repeat this exercise using a more rigid definition of treatment failure. It is indeed conceivable that a proportion of patients with viruses susceptible to AZT+3TC+NFV were classified as failure at 6 months because of discontinuation due to intolerance
- In all cases, previous treatment failure on 3TC was a better predictor for subsequent treatment failure than genotypic information. This may be due to the highly predictable emergence of 184I under the pressure of a 3TC-containing regimen, and the tendency to revert when patients are off 3TC

Figure 2

Areas under the ROC for a number of univariable models

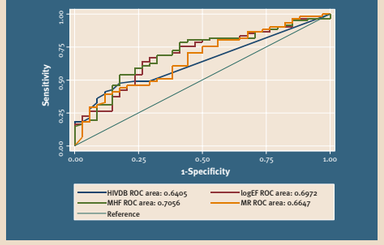

Table 4

Adjusted ΔVL at 3 months for genotypic predictors from fitting 5 linear regression models

Factor *(per 1 higher)*	Decrease in VL @ 3M	*p* value
$GSS_{AZT+3TC}$	0.47 (-0.20 – 1.14)	0.17
GSS_{NFV}	0.13 (-1.08 – 1.33)	0.84
GSS_{AZT}	*1.10 (0.01 – 2.19)*	*0.05*
GSS_{3TC}	-0.30 (-1.54 – 0.94)	0.63
GSS_{NFV}	0.44 (-1.19 – 2.07)	0.60
$\log EF_{AZT}$	-0.11 (-0.77 – 0.54)	0.73
GSS_{3TC}	-0.01 (-1.12 – 1.11)	0.99
$\log EF_{NFV}$	-0.53 (-1.24 – 0.19)	0.15
$\#MR_{AZT+3TC}$	0.23 (-0.22 – 0.68)	0.32
$\#MR_{NFV}$	-0.10 (-0.46 – 0.27)	0.60
$\#MHF_{AZT+3TC}$	0.05 (-0.15 – 0.26)	0.62
$\#MHF_{NFV}$	0.16 (-0.11 – 0.43)	0.25

Table 5

Adjusted OR of treatment failure at 6 months for genotypic predictors from fitting 5 logistic regression models

Factor *(per 1 higher)*	Odds Failure @ 6M	*p* value
$GSS_{AZT+3TC}$	0.85 (0.25 – 2.84)	0.79
GSS_{NFV}	0.53 (0.05 – 5.91)	0.60
GSS_{AZT}	1.01 (0.14 – 7.26)	0.99
GSS_{3TC}	0.71 (0.09 – 5.61)	0.74
GSS_{NFV}	0.57 (0.05 – 6.85)	0.65
$\log EF_{AZT}$	0.44 (0.13 – 1.49)	0.19
GSS_{3TC}	0.62 (0.10 – 3.87)	0.61
$\log EF_{NFV}$	*4.61 (1.33 – 15.99)*	*0.02*
$\#MR_{AZT+3TC}$	0.87 (0.41 – 1.48)	0.72
$\#MR_{NFV}$	0.74 (0.37 – 1.48)	0.40
$\#MHF_{AZT+3TC}$	1.23 (0.85 – 1.78)	0.28
$\#MHF_{NFV}$	*0.62 (0.41 – 0.93)*	*0.02*

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