Detection of Minority HIV-1 Drug-Resistant Variants Moderately Improves the Prediction of Salvage Antiretroviral Therapy Outcomes

C Pou[1], M Noguera-Julian[1], S Pérez-Álvarez[1], F García[2]; R Delgado[3]; D Dalmau[4], M Álvarez-Tejado[5], C Rodríguez[1], JR Santos[2], B Clotet[1,2], R Paredes[1,2]

[1]IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain,[2]Hospital Universitario San Cecilio, Granada, Spain, [3]Hospital 12 de Octubre, Madrid, Spain, [4]Hospital Universitari Mútua de Terrassa, Spain, [5]Roche Diagnostics, SL, Spain, [6]HIV Unit, Hospital Universitari Germans Trias i Pujol , Badalona, Spain

P.123
Christian Pou
PhD Student
irsiCaixa
cpou@irsicaixa.es

Background

Ultrasensitive HIV drug resistance tests like 454 Sequencing (Roche Diagnostics/454 Life Sciences) allow the detection of low-frequency resistant variants which are often missed by Sanger sequencing genotypic tests. Detection of minority drug-resistant mutants more than doubles the risk of virological failure in antiretroviral naïve patients. However, the clinical relevance of detecting minority HIV-1 drug resistant variants in antiretroviral treatment (ART)-experienced subjects remains uncertain.

Methods

OBJECTIVE

•To analyze the association between baseline detection of resistance mutations through 454 sequencing and the virological outcome of salvage antiretroviral therapy; in comparison with Sanger sequencing.

DESIGN

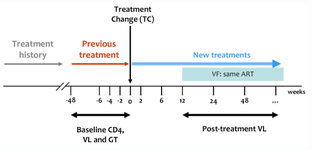
• Retrospective; multicenter; cohort study performed in 4 centers from Badalona, Madrid, Terrassa and Granada, Spain (Clinicaltrials.gov ID: NCT01346878).

SUBJECTS

•ART-experienced adults.
•Initiating salvage ART including PI/r, raltegravir (RAL) or etravirine (ETR).
•HIV-1 RNA (VL) ≥5000 copies/mL and 1 mL of plasma available for testing within 12 months before treatment change (TC).
•Clinical follow-up available through at least 48 weeks after TC.
•Good adherence to therapy in clinical records.

Figure 1. Definition of Treatment Change Episode (TCE) and VF

*TCE was defined as the change of the antiretroviral drug after a determination of CD4 cells count, viral load and genotype by Sanger. **Virological Failure** was defined as 2 consecutive measurements of VL>200 c/mL 2week 12 (3rd month) after a treatment change with no changes on ART.*



MEASUREMENTS

•Virological failure (VF) was defined as 2 consecutive VL measurements >200 copies/mL at least 90 days after TC, while the subject remained on the same ART initiated.

•Sanger and 454 sequencing of PR/RT were used to calculate Genotypic Sensitivity Scores (GSS-Sanger and GSS-454; respectively) using the HIVDB Sierra interface to HIVDB/ANRS/REGA algorithms.

•A score of 1 was added per each RAL, MRV and T20. Here, we only present results with HIVdb GSS. ANRS and REGA provided similar findings.

•The ability of GSS-Sanger and GSS-454 to predict VF was assessed by:

- ROC curves considering clinically meaningful independent variables. All possible simple logistic regression models were fitted setting the VF definition as the response variable. The AUC of GSS-454 and GSS-Sanger ROC curves were compared by Delong's test based on U-statistics theory and asymptotic normality.
- Survival analysis of time to VF, including:
  - Kaplan Meier curves, using the Log-rank test (same weight to all observations) and Peto-Peto (independent of censoring pattern) to test for statistically significant differences.
  - Univariate and multivariate Cox semiparametric regression models of baseline parameters associated with VF. The multivariate Cox model was constructed using covariates with a p value <0.1 in the univariate analysis.

Results

146 subjects were included in the study (Table 1). Pre-TC genotypes were obtained in a median of 48 (0;135) days before TC. Virological outcomes were evaluable in 138 individuals. 41% developed VF.

Table 1. Characteristics of the cohort

Gender, %		Age at diagnosis, years, median (IQR)	29 (24;33)
Female	26	Age at TCE, years, median (IRQ)	43 (38;47)
Male	74	Time since diagnosis, years, median (IRQ)	15 (11;14)
Mode of infection, %		Follow-up, years, median (IQR)	13 (9;15)
HTS	14	Number of previous drugs, median (IQR)	13 (9;17)
MSM	25	CD_4, cells/mm³, median (IQR)	
IVDU	36	Nadir	39 (26;177)
Transfusion	1	Baseline	232 (104;388)
Unknown	24	Baseline VL, c/mL, median (IQR)	39905 (17,000; 100,665)
Prior AIDS, %	64		

HTS, heterosexual; MSM, men who have sex with men; TCE, Treatment Change Episode; IVDU, Intravenous drug use; IQR, interquartile range.

Figure 2. HIVdb GSS distribution for Sanger and 454

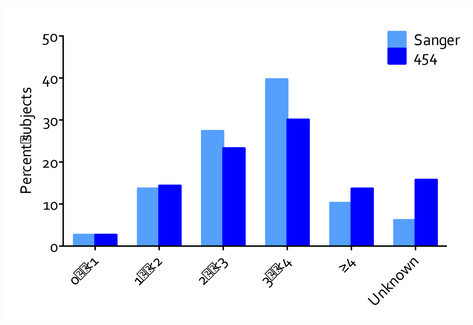
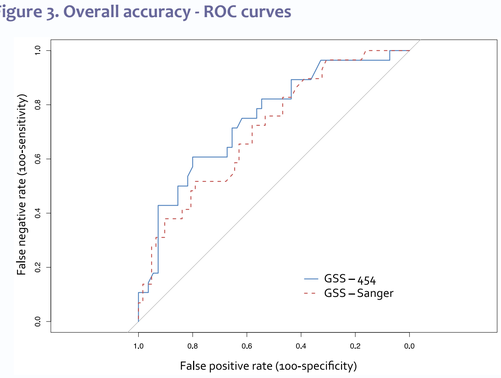
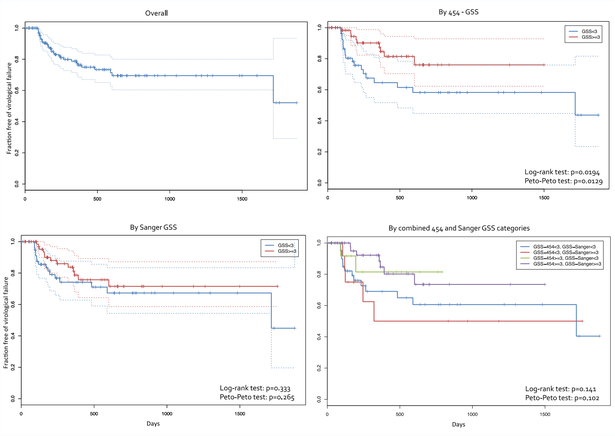


Figure 3. Overall accuracy - ROC curves



- The ROC curve AUCs were (Delong's test; p=0.6514)
  - GSS-454: 74.6% (95%CI: 63.4 – 85.8)
  - GSS-Sanger: 71.0% (95%CI: 59.8 – 82.1)
- After checking for significance and colinearity among variables, each regression model included, in addition to the respective GSS, previous AIDS diagnosis and number of previous antiretrovirals.

Figure 4. Kaplan-Meier Curves of time to virological failure



Subjects with GSS-454 <3 were significantly more likely to develop VF than those with GSS-454 ≥3. No differences in VF were observed between different categories of GSS-Sanger. After combining the 454 and Sanger GSS score categories, GSS-454 <3 also seemed to discriminate VF better than Sanger GSS scores.

Table 2. Univariate and Multivariate Cox Models of Risk of Virological Failure

	UNIVARIATE			MULTIVARIATE		
	HR	95% CI	P-value	HR	95% CI	P-value
454 GSS						
≥ 3	1.00	-	-		Not included	
< 3	2.52	1.13-5.62	0.0241		Not included	
Sanger GSS						
≥ 3	1.00	-	-		Not included	
< 3	1.43	0.69-3.00	0.336		Not included	
Combined GSS categories						
454 ≥ 3 ; Sanger ≥ 3	1.00	-	-			
454 ≥ 3 ; Sanger < 3	1.09	0.23-5.25	0.9149	0.76	0.13-4.28	0.7609
454 < 3 ; Sanger ≥ 3	2.95	0.93-9.39	0.0665	2.75	0.73-10.30	0.1329
454 < 3 ; Sanger < 3	2.40	0.96-5.97	0.0621	1.98	0.72-5.46	0.1881
Centre						
Badalona	1.00	-	-	1.00	-	-
Madrid	2.25	0.99-5.13	0.054	1.93	0.78-4.78	0.1543
Terrassa	0.44	0.10-1.97	0.285	0.65	0.10-4.39	0.6579
Granada	0.90	0.34-2.38	0.828	0.50	0.11-2.21	0.3593
ART group						
PIr	1.00	-	-	1.00	-	-
ETR	3.19	0.41-24.69	0.266	11.38	1.09-119.12	0.0424
PIr + ETR	0.25	0.03-1.90	0.180	0.18	0.02-1.57	0.1218
RAL	1.43	0.47-4.36	0.525	0.73	0.14-3.84	0.7137
RAL + ETR	2.20	0.29-16.79	0.448	3.24	0.23-46.28	0.3854
RAL + PIr	0.63	0.24-1.63	0.345	0.66	0.21-2.02	0.4624
RAL + PIr + ETR	0.65	0.19-2.25	0.494	0.66	0.15-2.86	0.1218
Gender						
Female	1.00	-	-	-	-	-
Male	1.20	0.52-2.78	0.673	-	-	-
Transmission route						
Intravenous drug use	1.00	-	-	-	-	-
Heterosexual contact	0.81	0.23-2.87	0.745	-	-	-
Homo/bisexual contact	0.84	0.33-2.15	0.721	-	-	-
Mother-to-child	0.00	0.00-∞	0.998	-	-	-
Transfusion	0.00	0.00-∞	0.998	-	-	-
Unknown	2.07	0.87-4.93	0.101	-	-	-
AIDS						
No	1.00	-	-	-	-	-
Yes	1.26	0.52-3.07	0.615	-	-	-
Nadir CD_4+, cells/mm³						
> 350	1.00	-	-	-	-	-
> 200 – 350	0.79	0.14-4.33	0.787	-	-	-
≤ 200	1.23	0.29-5.19	0.781	-	-	-
Baseline CD_4+, cells/mm³						
> 350	1.00	-	-	-	-	-
> 200 – 350	0.57	0.20-1.62	0.289	-	-	-
≤ 200	0.89	0.41-1.93	0.775	-	-	-
Baseline HIV-1 RNA, copies/mL						
< 10000	1.00	-	-	-	-	-
10000 - < 100000	>100	0.00-∞	0.996	-	-	-
≥ 100000	>100	0.00-∞	0.996	-	-	-
# previous drugs, per each additional drug	1.08	1.00-1.16	0.0404	1.12	1.00-1.25	0.0413
Age, per each additional year	1.00	0.96-1.05	0.825	-	-	-

Conclusions

Detection of minority HIV-1 drug resistant variants moderately improves the prediction of salvage ART outcomes. Subjects with GSS-454 <3 are more likely to develop virological failure. 454 seems to discriminate outcomes better than Sanger. In the multivariate Cox proportional hazards model, however, the only variables significantly associated with increased risk of virological failure were receiving etravirine as the sole third agent, and the number of antiretroviral drugs the subject had been exposed to. Subgroup analyses as well as those accounting for additional maraviroc or T-20use are ongoing. Similar results were obtained when we used the ANRS or REGA rules to calculate GSS scores.

Acknowledgements

This study was supported through a grant from the *Centro de Desarrollo Tecnológico Industrial* of the Spanish Ministry of Science and Innovation, RP and CP were partially supported through 'CHAIN, Collaborative HIV and Anti-HIV Drug Resistance Network', Integrated Project no. 223131, funded by the European Commission Framework 7 Program and the 'Gala contra la sida – Barcelona 2011'.