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CASE REPORT

PRESTIGIO RING: "A 59-year-old HIV-1 positive, highly treatment-experienced woman failing darunavir/ritonavir plus raltegravir"

Laura Labate[1], Bianca Bruzzone[2], Vincenzo Spagnuolo[3], Maurizio Zazzi[4], Maria Mercedes Santoro[5], Antonio Di Biagio[1,6], Antonella Castagna[3,7]

[1]*Department of Health Sciences (DISSAL), University of Genoa, Italy;*
[2]*Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences, Genoa, Italy;*
[3]*Infectious Diseases Unit, San Raffaele Hospital, Milan, Italy;*
[4]*Department of Medical Biotechnologies, University of Siena, Italy;*
[5]*Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy;*
[6]*Infectious Diseases Unit, Policlinico San Martino Hospital - IRCCS, Genoa, Italy;*
[7]*Infectious Diseases Unit, Vita-Salute San Raffaele University, Milan, Italy*

SUMMARY

Management of heavily treatment experienced (HTE) people with HIV remains a challenge. Tailored antiretroviral therapy (ART) is needed in this fragile population who almost invariably harbor viral quasispecies with resistance-associated mutations (RAMs). The reference method for HIV genotypic resistance testing (GRT) has long been Sanger sequencing (SS), but next-generation sequencing (NGS), following recent progress in workflow and cost-effectiveness, is replacing SS because of higher sensitivity. From the PRESTIGIO Registry, we present a case of a 59-year-old HTE woman who failed darunavir/ritonavir plus raltegravir at low-viremia levels due mainly to high pill burden and poor adherence. NGS-GRT was performed on HIV-RNA at failure and the results were compared to all past SS-GRT data available (historical genotype). In this case, NGS-GRT did not detect any minority drug-resistant variants. After discussing several therapeutic options, the treatment was changed to dolutegravir 50 mg twice daily plus doravirine 100 mg once a day, based on clinical history, adherence issues, and pill burden, as well as the historical SS-GRT and the latest NGS-GRT results. At six months follow-up visit, the patient had HIV-RNA below 30 copies/ml and CD4+ T cell count increased from 673 cells/ mm^3 to 688 cells/ mm^3. Close follow-up of this patient is ongoing.

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CASE PRESENTATION

A 59-year-old, highly treatment-experienced (HTE) woman with human immunodeficiency virus-1 (HIV-1) subtype B infection, followed at the HIV outpatient clinic in San Martino Hospital, Genoa, since 1994 was enrolled in the PRESTIGIO registry (Galli L, OFID 2022) in February 2022. At the time of diagnosis, CD4+ T cell count was 152 cell/mm^3, CD4+/CD8+ ratio was 0.3, and plasma HIV-RNA was >1 million copies/ml. The patient was diagnosed with AIDS due to a progressive multifocal leukoencephalopathy (PML) which has left a significant gait instability; at the last follow-up in October 2022, brain MRI showed multiple areas of altered cortico-subcortical signal at the vertex, which were already present on previous MRIs.

In 2009, the patient underwent hysterectomy because of cervical squamous cell carcinoma; in 2016, due to concomitant HCV infection evolving into liver cirrhosis, she was effectively treated with daclatasvir/sofosbuvir/ ribavirin for 12 weeks.

She is currently unemployed, lives alone and receives in-home care because of psychomotor neurological deficits. The patient's body weight and height are 43 kg and 150 cm, respectively, with a BMI of 19.1 and a waist circumference of 73 cm. The patient has a history of smoking, previous alcohol and intravenous heroin use, and is currently on methadone (80 mg once daily). Due to persistent dyslipidemia, she is treated with ezetimibe. In addition, she takes proton pomp inhibitors (PPI) for gastroesophageal reflux, ferrogluconate, foline, D3 vitamin, laxatives, alprazolam 0.5 mg once daily, and duloxetine 30 mg once

Key words:
Highly treatment-experienced, next generation sequencing, HIV drug resistance, low-level viremia, darunavir, raltegravir.

Corresponding author:
Laura Labate
E-mail: laura.labate92@gmail.com

daily. The ASCVD score is 6.1% at 10 years and the hip fracture Frax Score is 1.4%

In 1995, she started antiretroviral therapy (ART) with zidovudine (AZT) as monotherapy, followed by several ART regimens including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PI), and integrase inhibitors (INI) (*Table 1*).

The cumulative, or historical, genotype (HG) resulting from two Sanger sequencings (SS) (Trugene HIV-1 Genotyping test) performed in February 2004 and in March 2008 showed the following resistance associated mutations (RAMs): for the NRTIs, M184V and the thymidine analogue mutations (TAMs) M41L, D67N, T215Y; for the NNRTIs, K103N; for the PIs, L33F, M46I, I54V, V82A and L90M. In 2011, after different, unsuccessful ART regimens, she started darunavir/ritonavir (DRV/r) 600/100 mg twice daily plus raltegravir (RAL) 400 mg twice daily. Since then, probably also thanks to better adherence to therapy, the patient achieved and maintained viremia <50 copies/ml up to September 2022, except for two blips (one with 101 copies/mL in 2018 and another with 65 copies/mL in 2020).

On September 13, 2022, plasma HIV-RNA was 156 copies/ml and remained detectable (196 copies/ml) at the next test on October 4, 2022, meeting the definition of virological failure according to Italian guidelines (HIV/AIDS Italian Expert Panel 2017). The patient reported poor adherence to ART with DRV/r and RAL. An HIV drug resistance testing with Next Generation Sequencing (NGS) technology was performed on the 196-HIV RNA copy plasma sample. Viral RNA was extracted by using the EZ1&2 Virus Mini Kit v2.0 on the EZ1 automated platform. The NGS library was prepared by using the commercial kit AD4SEQ HIV-1 Solution v2 (Arrow Diagnostics S.r.l) and sequenced on the iSeq100 platform (Illumina). FastQ files were analyzed by SmartVir (SmartSeq S.r.l.) software, which returns a report with all mutations identified and RAMs according to Stanford HIVdb classification.

Considerations on genotypic resistance testing (GRT)

Our patient failed the DRV/r plus RAL treatment at low-level viremia (<200 copies/ml). Treatment guidelines (EACS Guidelines, 2022) recommend a GRT at these viremia levels, although a reduced rate of success is expected. Italian guidelines, for instance, give an AII recommendation for performing a resistance test at virological failure with 50 to 200 HIV-RNA cop-

Table 1 - *Previous antiretroviral therapy, reason of discontinuation and viro-immunovirological features.*

Date of ART regimen start	*Date of ART regimen change*	*ART*	*Reason for discontination*	*HIV-RNA log copies/ mL at time of start ART regimen*	*CD4+T cells/mm³ at time of start regimen change*	*HIV-RNA log copies/ mL at time of ART regimen change*	*CD4+T cells/mm³ at time of regimen change*
17/07/1995	11/07/199	AZT	Choice of patient	3.60	152	4.38	150
11/07/1996	27/03/1997	3TC+AZT	Virological failure	4.38	150	4.38	132
27/03/1997	08/05/1998	3TC+D4T	Toxicity: neuropathy	4.38	132	3.49	149
08/05/1998	05/05/1999	3TC+D4T +IDV	Choice of patient	3.49	149	4.70	317
05/05/1999	20/01/2000	D4T+NFV+NVP	Choice of patient	4.70	317	4.15	225
20/01/2000	11/08/2000	3TC+ABC+D4T+NFV	Pill burden	4.15	225	4.74	329
11/08/2000	26/11/2001	3TC+ABC+D4T	Virological failure	4.74	329	3.57	234
26/11/2001	02/04/2004	D4T+LPV/r	Toxicity: neuropathy	3.57	234	3.75	176
02/04/2004	28/06/2006	3TC+TDF+LPV/r	Pill burden	3.75	176	3.75	40
28/06/2006	22/01/2007	3TC+LPV/r	Immunological failure	3.75	40	1.60	14
22/01/2007	21/03/2008	3TC+LPV/r+TDF	Simplification	1.60	14	1.60	50
21/03/2008	05/06/2008	EFV+TDF	Toxicity: insomnia	1.50	50	1.50	217
05/06/2008	29/06/2011	3TC+DRV/r+RAL+TDF	Simplification	1.50	217	1.50	343
29/06/2011	04/11/2022	DRV/r+RAL	Virological failure	1.70	343	2.30	673

3TC=lamivudine; ABC=abacavir; AZT=zidovudine; d4T=stavudine; TDF/TAF=tenofovir; EFV=efavirenz; ETR=etravirine; NVP=nevirapine; DRV=darunavir; IDV=indinavir; LPV=lopinavir; NFV=nelfinavir; r=ritonavir. Virological failure is defined by HIV-RNA>2.30 log.

ies/ml (HIV/AIDS Italian Expert Panel 2017). GRT at low-level viremia is important because resistance may be detected, although the rate increases with increasing viremia (Bruzzone *et al.*, 2014; Santoro *et al.*, 2014; Sudderuddin *et al.*, 2023).

GRT by SS has long been effective in supporting ART changes. However, during the last few years, NGS has gradually replaced SS. While NGS set at 20% threshold correlates well with SS (Lee *et al.*, 2020), NGS can detect RAMs at a prevalence as low as 0.1% (Tzou *et al.*, 2018; Siamoglou *et al.*, 2022). However, solid evidence on the clinically relevant NGS cut-off is still lacking and most clinical applications of NGS have used thresholds between 5 and 10% (Tzou *et al.*, 2018; Trabaud *et al.*, 2017). Similarly, in our clinical case an NGS threshold of 5% was used.

Moreover, when a therapy change is planned, the evaluation of Historical (or cumulative) Genotype (HG) offers a more comprehensive evaluation of the burden of resistance compared to the latest available genotype (Garcia *et al.*, 2011; Zaccarelli *et al.*, 2009; Armenia *et al.*, 2022). In our case, before discussing therapeutic management, both the mutations listed in HG and those detected through NGS-GRT at the latest time point were considered.

Evaluation of drug resistance detected by NGS compared to prior resistance

In our test, coverage was >100x (100-93-100), aligned reads were 119,764, used reads were 117,994, the NGS cut-off was set at 5%. No RAM was detected in the interval 5-20%; only resistant variants with a prevalence >20% were detected, most of them with a prevalence above 90% (*Figure 1*).

All the PI RAMs listed in the HG were detected by the latest NGS-GRT analysis, in agreement with DRV

Figure 1 - *Comparison of historical GRT with SS and GRT at time of switch with NGS on HIVRNA.*

	HG GRT	NGS GRT
Drugs		Date: 04/10/2022 ART: DRV/r+RAL HIV-RNA log copies/ml: 2.2 CD4+ T cells count: 673 cells/mm^3
NRTI	**RAMs:** M41L, D67N, M184V, T215Y	**RAMs:** D67N (80%), T215C/S (50%)
3TC		
ABC		
AZT		
D4T		
DDI		
FTC		
TAF		
TDF		
NNRTI	**RAMs:** K103N	**RAMs:** None
DOR		
EFV		
ETR		
NVP		
RPV		
PI	**RAMs:** L10F, L33F, M46I, I54V, V82A, L90M	**RAMs**: L10F (97%), L33F (97%), M46I (97%), I54L/V (72%/25%), V82A (98%), L90M (96%)
ATV/r		
DRV/r		
FPV/r		
IDV/r		
LPV/r		
NFV		
SQV/r		
TPV/r		
INSTI	Not Available	**RAMs:** N155H (94%)
RAL		
EVG		
DTG		
BIC		
CAB		

The color code represents the susceptibility of each drug according to the GRT interpretation obtained by the HIVDb algorithm version 9.4 (https://hivdb.stanford.edu): in red, high-level resistance; in orange, intermediate resistance; in yellow, low-level resistance; in dark green, potential low-level of resistance; in bright green, no resistance.

NRTI= Nucleoside Reverse Transcriptase Inhibitor; NNRTI= Non-Nucleoside Reverse Transcriptase Inhibitor; PI=protease inhibitor; INSTI= integrase strand-transfer inhibitor; RAMs= resistance associated mutations; 3TC=lamivudine; ABC=abacavir; AZT=zidovudine; d4T=stavudine; ddI=didanosine; FTC=emtricitabine; TDF/TAF=tenofovir; EFV=efavirenz; ETR=etravirine; NVP=nevirapine; RPV=rilpivirine; DOR=doravirine; ATV=atazanavir; DRV=darunavir; FPV=fosamprenavir; IDV=indinavir; LPV=lopinavir; NFV=nelfinavir; SQV=saquinavir; TPV=tipranavir; r=ritonavir; DTG=dolutegravir; EVG=elvitegravir; RAL=raltegravir; BIC=bictegravir; CAB=cabotegravir. GRT, genotypic resistance testing; H, historical; NGS, next generation sequencing; SS, Sanger sequencing.

pressure. Specifically, NGS-GRT showed the presence of the primary RAMs L33F, G73S, M46I, V82A, L90M in 97% of the viral population. The only difference between HG and NGS-GRT was the evolution of I54V, detected as the only species by SS but as an I54L/V mixture (72%/25%) by NGS, with the 54L variant outgrowing the previously dominating 54V. Of note, the I54L mutation is a typical DRV RAM and the transition from I54V to I54L showed that the patient had started to fail to this antiretroviral as well (de Meyer *et al*., 2008; Delaugerre *et al*., 2010). Altogether, these PI RAMs cause partial resistance to tipranavir/ritonavir (TPV/r) and DRV/r and resistance to all other PIs.

Regarding INSTI resistance, NGS-GRT revealed the N155H RAM in 94% of the viral population, causing high-level resistance to RAL and elvitegravir (EVG) but having a minimal impact on dolutegravir (DTG), bictegravir (BIC) and cabotegravir (CAB). The dominant N155H is in agreement with the ongoing treatment with RAL, which has a low genetic barrier, despite being very potent. Since the patient was poorly compliant, discontinuous drug pressure may have favored the selection of N155H. Although a previous integrase GRT before RAL treatment start was not available, this RAM can reasonably be considered as newly onset, based on the evidence that INSTI RAMs in INSTI-naïve individuals is a rare event (Bailey *et al*., 2021)

Choice of the new antiretroviral regimen and follow-up

The following therapeutic options were discussed in the PRESTIGIO RING virtual seminar

1) No change in therapy, only close monitoring: this strategy may be supported by DHHS guidelines, which define virological failure at HIV-RNA >200 copies/ml. However, a recent and large observational study suggested that both blips and low-level viremia during ART are associated with an increased risk of subsequent virological failure (Elvstam *et al*., 2023).
2) Perform therapeutic drug monitoring to guide treatment: this choice was ruled out because lack of adherence with suboptimal uptake of ART was declared by the patients as a recognized issue in this case.
3) Intensify current therapy by adding an NRTI backbone: this strategy was ruled out because of the HG pattern.
4) Add to this therapy a new drug with a new mechanism of action: lenacapavir, the first-in-class HIV-1 capsid inhibitor, is not currently available in Italy; ibalizumab, a monoclonal antibody that blocks HIV-1 entry into CD4 cells, was difficult to obtain and highly expensive; fostemsavir, the first HIV-1 attachment inhibitor, may be a good add-on strategy, but in November 2022 it was not available at Genoa center. Moreover, some perplexities arose about the add-on strategy concerning the total pill burden.
5) Change antiretroviral regimen with dolutegravir 50 mg twice daily + doravirine 100 mg once daily. Although this regimen has not been studied in clinical trials, it can offer some advantages. It is a dual, high-genetic barrier, PI and NRTI sparing regimen, well tolerated and virologically effective (Saladini, JAC 2023; Denver *et al*., 2022) with a potential favorable impact on lipid profile (Mazzitelli *et al*., 2022). Moreover, the pill burden is reduced, likely strengthening adherence, and there are no proven interactions with the concomitant medications taken by the patient (duloxetine, alprazolam, and methadone). In addition, the patient had an increased cardiovascular risk (female, dyslipidemia, smoker) which might potentially benefit from the switch from PIs to doravirine. The concomitant intake of PPIs also favored the choice of doravirine over rilpivirine

Based on the integrated analysis of past virological failures, side effects, adherence issues, pill burden, and of course data from HG and current NGS-GRT, a two-drug regimen with dolutegravir 50 mg twice daily plus doravirine 100 mg once daily was chosen after a discussion of pros and cons with the patient.

At 4 weeks after changing therapy, HIV-RNA was below 50 copies/ml. At a six-month follow-up visit, the patient reported good ART tolerance and excellent compliance. HIV-RNA was maintained below 50 copies/ml and CD4+ T cell count increased from 673 cells/ mm^3 to 688 cells/ mm^3.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report. The patient was enrolled in the Italian PRESTIGIO Registry, and her case was discussed during the PRESTIGIO RING, a quarterly Italian virtual meeting where clinicians, virologists, pharmacologists and other experts participating in the PRESTIGIO Registry discuss clinical cases on HTE patients with multi-drug resistance, with the ultimate goal of optimizing management of this fragile population. The PRESTIGIO Registry is an observational, prospective, Italian, multicenter, annual collection of data on clinical, laboratory, treatment, and virological characteristics of people living with HIV and four-drug class resistance, approved in 2017 (NCT04098315). Prestigio Registry activities are supported by VIIV health Care, Gilead Sciences, Merck Sharp & Dohme (MSD).

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