Simplification to high-genetic-barrier 2-drug regimens in People Living with HIV harboring four-class resistance enrolled in the PRESTIGIO Registry

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RUNNING HEAD 2DR in PLWH with 4-class drug-resistant HIV-1

TRANSPARENCY DECLARATIONS

D.C., L.G., A.C.[3], S.M., M.F. have none to declare.

N.G. has been advisor for Gilead Sciences, AbbVie and Janssen-Cilag and has received speakers' honoraria from Gilead Sciences, ViiV, Bristol-Myers Squibb, Merck Sharp.

L.C. reports HIV grants from Janssen, MSD, Gilead and ViiV for lectures in the last three years.

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LETTER TO THE EDITOR

Today, most people living with HIV (PLWH) with access to ART successfully achieve virological suppression. Nevertheless, many efforts are still needed to face unsolved problems such as drug-resistance (HIVDR), ART-related toxicities and comorbidities, to ensure PLWH the best quality and expectancy of life.

Four-class drug-resistance (four-class HIVDR), including NRTIs, NNRTIs, PIs, and integrase strand transfer inhibitors (InSTIs), can dramatically hinder the effectiveness of ART. To date, the prevalence of four-class HIVDR on a global scale is largely unknown: according to a prospective study carried on in North Carolina, it affects around 1% of PLWH, with a slight increase observed since 2007.[1] Italian epidemiological data are consistent, with a prevalence of four-class HIVDR varying from 1% (2008-2013) to 3% and 2% (2014-2016 and 2017-2018, respectively) of plasma Genotypic Resistance Tests (GRT) of a cohort of 6,802 ART-experienced patients.[2] These rates, despite steadily low, represent a life-threatening issue and a considerable risk for transmission when viral suppression cannot be reached.

PRESTIGIO (www.registroprestigio.com) is an Italian registry that involves 40 Italian Infectious Diseases units and records demographic, clinical and laboratory data, GRTs, and treatments of PLWH with a documented four-class HIVDR; if InSTI resistance test was not available, a documented virological failure to an InSTI was accepted as a proof of resistance to the class, in accordance with previous studies in which prior class exposure was inferred as high risk of having resistance, e.g. the OPTIONS trial).[3] The purpose is to collect information about burden of disease, survival, and efficacy and safety of salvage regimens.

The protocol of the PRESTIGIO Registry was approved by the Ethic Committees of the participating centers; all patients provided written informed consent to be enrolled.

Hence, we conducted an observational retrospective study with the aim to define the features and the outcomes of PLWH harboring four-class HIVDR enrolled in the PRESTIGIO Registry, who simplified their antiretroviral therapy to a high-genetic-barrier 2-drug regimen (2DR), while with HIV-RNA <50 copies/mL (pre-specified inclusion criteria). Simplification might have occurred for different needs, commonly observed in real life among people treated with complex antiretroviral regimens: toxicity reduction, avoidance of adverse events or drug-drug interactions, and adherence improvement. A high-genetic-barrier 2DR was defined as a regimen including at least one high-genetic-barrier compound among LPV/r 400/100 mg BID, DRV/r 600/100 mg BID or 800/100 mg QD, or DTG 50 mg BID.The start of the 2DR was considered as baseline (BL). The follow-up accrued from BL to viral failure (VF) or treatment failure (TF), loss to follow-up, death or data freezing (December 31, 2019). VF was defined as HIV-RNA >50 copies/mL in two consecutive determinations or a single determination >50 copies/mL followed by ART modification or a single determination >1000 copies/mL. TF was defined as VF and/or any modification of the 2DR. The total Genotypic Susceptibility Score (GSS) [0=fully resistant; 1=intermediate; 2=fully susceptible] on cumulative GRT on RNA and on pro-viral DNA (when available), was calculated as the sum of the scores for the individual drugs included in the 2DR; DRV was scored according to the original Tibotec score;[4] ETR, RPV and DTG by means of the Stanford HIV Drug Resistance Database (Version 8.8 update 2019/02/13, available at https://hivdb.stanford.edu/): for each drug, "susceptible" or "potential low-level" were scored as 1 point, "low-level", "intermediate" or "high-level resistance" as 0 points; the viral tropism was estimated by the Geno2Pheno algorithm. Patients' features were described as median (interquartile range, IQR) for continuous variables or proportions for categorical variables. Changes from baseline were tested by the Wilcoxon signed-rank test. All analyses were conducted using SAS statistical software version 9.4 (Statistical Analyses System Inc, Cary, NC, USA).

Among 99 PLWH enrolled in the PRESTIGIO Registry with HIV-RNA <50 copies/mL, ten (10%) received a high-genetic-barrier 2DR. They were mainly males (90%) with a median age of 54 (51-56) years, a history of HIV infection for 26 (23-29) years, a CD4+ cell count nadir of 126 (73-214) cells/µL and 18.5 (14.3-20.0) years of ART exposure. At BL, they had been virologically suppressed for 197 (124-495) days, with a median CD4+ cell count of 446 (375-724) cells/µL and a median CD4+/CD8+ ratio of 0.45 (0.38-0.48). Cumulative GRT on RNA and, when available, on proviral DNA, GSS, treatment data and virological outcomes are shown in Table 1. Throughout a median follow-up of 25.4 months (2.3-26.7), 9 patients maintained virological suppression, while one patient with a GSS=0, who started 2DR after withdrawal of FTC/TDF because of proteinuria and oedema, had VF; after VF, a regimen including DTG 50 mg bid, DRV/r 600/100 mg bid, FTC/TAF 200/10 mg qd was started with prompt virological control. One patient discontinued the 2DR after 97 weeks of virological suppression, because of potential drug-drug interactions with anti-HCV drugs.

No other serious adverse event was reported after starting 2DR, neither relevant changes in hematological, renal and hepatic parameters or lipid and glucose levels (data not shown). Through 2DR, no significant increase occurred in CD4+ cell count [+59 cells/µL (-34;+157); p=0.114], CD4+% [+1.95% (-1.48;+4.0); p=0.169] and CD4+/CD8+ ratio [+0.08 (0;+0.18); p=0.052].

To explain the inconsistency between the high mutational burden on cumulative RNA-GRTs and the good virological response to 2DRs, for 8 patients we additionally analyzed the proviral DNA-GRT. This evaluation was performed using samples collected before starting 2DR in one case, during the treatment in 5 cases and after stopping 2DR in 2 cases (Table 1).

Armenia et al, in a recent study, reported that resistance detected in proviral DNA predicts virological rebound (VR) after switching therapy in virologically suppressed patients; patients with an intermediate or full resistance showed by DNA-GSS had a higher adjusted hazard of

experiencing VR and patients with the same condition on both GSSs (from plasma and PBMCs) had the highest probability of experiencing virological rebound.[5]

In our sample, the only GSS=0 both in RNA and DNA was scored in the patient who had VF, while all other available DNA-GSSs were ≥1, greater than (in 5/7 patients) or equal to (in 2/7 patients) the corresponding cumulative RNA-GSS. The outcome for most of the patients is consistent with the more favourable DNA resistance profile, together with the high-genetic barrier of 2DRs.

We can argue that the ideal management of hard-to-treat PLWH cannot be standardized but requires a comprehensive analysis of multiple different factors to pursue the goals of precision medicine.[6,7] With the limits of a small sample and a retrospective observational design, we observed that a high-genetic-barrier 2DR might represent an effective option in selected PLWH with four-class HIVDR who need simplification for any reason. Our results suggest that, when available, a proviral DNA-GSS before the time of switch may offer complementary information to cumulative RNA-GSS for the selection of a simplification strategy in patients with long treatment history and prior virological failures. The value of proviral DNA-GSS information at the time of switch for such population should be further investigated.

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AUTHORS' CONTRIBUTION

A.C.[1,2] and G.N. provided scientific input to study design and supervised the creation of the manuscript.

D.C. wrote the first draft of the manuscript.

D.C., N.G., A.C.[3] L.C., R.G., S.R., S.M., G.C., M.F., M.M.S. M.Z., A.C.[1,2] contributed to the development of the study design and provided the data in the eCRF.

M.M.S. took care of patient's specimens.

L.G. performed the statistical analyses.

All the authors evaluated the study results, reviewed and edited the manuscript and gave the final approval for the final version of the manuscript submitted for publication.

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TABLE 1. Resistance profile, therapeutic characteristics and outcome of people living with HIV (PLWH) harboring four-class resistance enrolled in the PRESTIGIO Registry who started a 2-drug regimen (2DR) with HIV-RNA <50 copies/mL.

ID	Cumulative Major* NRTI RNA resistance mutations	Cumulative Major* NNRTI RNA resistance mutations	Cumulative Major* PI RNA resistance mutations	Cumulative Major* InSTI RNA resistance mutations	GSS RNA	Last available Major* DNA resistance mutations	GSS DNA	Pre-2DR	Weeks of VS before 2DR	Type of 2DR	Weeks of FU under 2DR	Outcome
15	M41L M184V L210W T215D	K101P K103S	V32I M46I I47V L76V V82A I84V L90LM	Q148H	0	**NRTI:** M41L L210W T215D **NNRTI:** K101P K103NS **PI:** V32VI V82VA I84IV L90LM **InSTI:** G140GS Q148QH	0	DRV/r 600/100 mg bid FTC/TDF 200/245 mg qd DTG 50 mg bid	495	DTG 50 mg bid DRV/r 600/100 mg bid	10	VF
27	L74V	L100I K103N V179VI Y181YC	L90M	G140S Q148H	1	**NRTI:** none **NNRTI:** none **PI:** none **InSTI:** G140GS	2	DRV/r 600/100 mg bid MVC 150 mg bid DTG 50 mg bid	1288	DTG 50 mg bid DRV/r 600/100 mg bid	97	TF
82	M41LM 74LV M184V 210LW T215FNSTY K219KT	K103KN Y181C M230L	L32I M46IM I54L I84IV L90LM	G140GS Q148HQ	0	**NRTI:** NA **NNRTI:** NA **PI:** NA **InSTI:** none	1	DRV/r 600/100 mg bid RPV/ FTC/TAF 200/25/25 mg qd DTG 50 mg bid	1459	DTG 50 mg bid DRV/r 600/100 mg bid	57	VS
89	D67G K70R M184V T215NSY K219E	K103N Y181C	M46I I47V I54V I84V L90M	N155H	1	**NRTI:** D67G K70R M184V T215NSY K219E **NNRTI:** none **PI:** I84V L90M **InSTI:** none	1	ABC/3TC 600/300 mg qd DRV/r 600/100 mg bid DTG 50 mg bid	199	DTG 50 mg bid DRV/r 600/100 mg bid	184	VS
108	D67N K70R M184V K219HQ	Y188CY	L90M	G140S Q148H	1	**NRTI:** none **NNRTI:** none **PI:** none **InSTI:** G140GS	2	DRV/r 800/100 mg qd RPV 25 mg qd DTG 50 mg bid	122	DTG 50 mg bid RPV 25 mg qd	105	VS
120	M41L D67N K70KR L74IV M184V 210W T215TNSY K219KQ	K103N Y181C	V32I M46I I47V I54M V82A I84V L90M	Y143YCHR N155H	0	**NRTI:** D67DN K70KR T215TS K219KQ **NNRTI:** none **PI:** none **InSTI:** none	2	DRV/r 600/100 mg bid FTC/TDF 200/245 mg qd DTG 50 mg bid	272	DTG 50 mg bid DRV/r 600/100 mg bid	226	VS
127	M184V T215DFS	G190A	M46I I54V V82A	N155H	2	**NRTI:** K70KR M184MV T215X **NNRTI:** none **PI:** M46MI L24LI **InSTI:** N155NH	2	FTC/TDF 200/245 mg bid ETR 200 mg bid	136	DTG 50 mg bid DRV/r 800 mg bid+100 mg qd	116	VS
139	M41L D67N M184V T215Y K219N	L100I K103N	D30N, N88D	G140S Q148H	1	**NRTI:** M41ML D67DN M184MV T215TNSY K219KN **NNRTI:** L100LI K103KN V108VI **PI:** none **InSTI:** none	2	DRV/r 600/100 mg bid DTG 50 mg bid ENF 90 mg bid	124	DTG 50 mg bid DRV/r 600/100 mg bid	136	VS
415	D67N K70R M184V T215FIS K219E	K101E Y181C G190S	V32I M46I I54M V82A I84V L90M	E138K Y143G	1	**NRTI:** NA **NNRTI:** NA **PI:** NA **InSTI:** NA	NA	ABC/3TC 600/300 mg qd MVC 150 mg bid DTG 50 mg bid	0	DTG 50 mg bid MVC 150 mg bid	239	VS
446	M41L M184V L210W T215CSY	K101I K103N	L90M	VF to EVG/c	2	**NRTI:** NA **NNRTI:** NA **PI:** NA **InSTI:** NA	NA	FTC/TDF/EVG/c 200/245/150/150 mg qd	195	DRV/r 600/100 mg bid ETR 200 mg bid	88	VS

Table 1. Resistance profile, therapeutic characteristics and outcome of people living with HIV (PLWH) harboring four-class resistance enrolled in the PRESTIGIO Registry who started a 2-drug regimen (2DR) with HIV-RNA <50 copies/mL. ID: Identification number inside the Prestigio Registry. Major*: Major HIV-1 drug resistance mutations according to Stanford HIV Drug Resistance database updated Feb 4, 2019. Abbreviations: BL, baseline; InSTIs, integrase strand transfer inhibitors; GSS, Genotypic Susceptibility Score was estimated according to the original Tibotec score for DRV, according to prediction of viral tropism by Geno2Pheno algorithm for MVC, according to resistance ≥ low level resistance defined by Stanford HIVdb for ETV, RPV and DTG. 0=fully resistant; 1=intermediate; 2=fully susceptible. GRT: Genotypic Resistance Test; b/a: before/after; s/e: start/end; VS, virological suppression; FU, follow-up; VF, virological failure; TF, treatment failure; NA, not available.