

# Achieving virological control in pan-resistant HIV-1 infection: A case series



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## Summary

**Background** HIV-1 pan-resistance refers to a reduced susceptibility to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and integrase strand transfer inhibitors. Although still anecdotal, its management remains a concern both for affected people living with HIV (PLWH) and for public health.

**Methods** We described genotypic resistance testing (GRT) of three PLWH with a documented poor virological response to previous antiretroviral therapies, who started ibalizumab, an anti-CD4 monoclonal antibody, combined with an optimized background therapy. Both historical and most recent GRT on plasma RNA and peripheral blood mononuclear cell DNA were interpreted according to the Stanford HIVDb version 9.0 (last updated on 22 February, 2021). After the switch to a regimen including the monoclonal antibody, HIV-1 RNA has been quantified biweekly (PCR Cobas<sup>®</sup> HIV-1 test 6800 Systems, Roche Diagnostics). Follow-up was censored at data freezing (16 January, 2021).

**Findings** We report findings from heavily treatment-experienced PLWH with a pan-resistant HIV-1 infection, who achieved virological control once introduced injections of ibalizumab, that is free from cross-resistance with all the antiretroviral drugs available and ensures patient adherence due to a close monitoring attributable to the route of administration, combined with recycled enfuvirtide and an optimized background regimen, selected on the basis of an accurate evaluation of resistance mutations.

**Interpretation** In these cases, this new approach has revealed to be a turning point in achieving virological control.

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**Keywords:** HIV; Anti-HIV agents; HIV fusion inhibitors; HIV drug resistance; Multiple; Viral load; Ibalizumab; Case report

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## Introduction

Antiretroviral therapy (ART) has revolutionized the course of HIV infection, reducing mortality and improving the quality of life for people living with HIV (PLWH).<sup>1</sup> However, the emergence of HIV-1 drug resistance has raised concerns because it represents a major determinant of treatment failure.<sup>2</sup> For that purpose, international guidelines recommend to define a tailored therapy based on genotypic resistance testing (GRT) in PLWH with virological failure (VF), also considering

new drugs, such as ibalizumab (IBA) and fostemsavir, in people with limited options.<sup>3,4</sup>

Although the prevalence of three and four-class resistance has been estimated about 5–10% in Europe and < 3% in North America with a high rate of morbidity and mortality, HIV-1 pan-resistance is still anecdotal and refers to a limited susceptibility to five antiretroviral classes, including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and integrase strand transfer inhibitors (InSTIs), suggesting that it could be a challenge for individual management as well as for public health.<sup>5–8</sup> Emerging evidence of HIV-1 resistance has stressed the need to develop drugs

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## Research in context

### *Evidence before this study*

References were identified in PubMed with the search terms “HIV drug resistance”, “HIV-1” and “multi-drug resistance” or, “multi-drug class resistance”, “ibalizumab”, “fostemsavir”, and “enfuvirtide” from 1985 until June, 2021. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this paper.

Over the last four decades, while life expectancy of most people living with HIV infection has been significantly improved through highly-active antiretroviral therapy, a minority of people have developed multidrug resistance, and in anecdotal cases pan-resistance, defined as a limited susceptibility to the main four antiretroviral classes. To the best of our knowledge, three cases of pan-resistance to each of the five available antiretroviral classes, including entry inhibitors, have been reported. Pan-resistance represents a life-threatening condition for the individual as well as for public health, owing to the risk of pan-resistant virus transmission from people who do not achieve virological suppression. In this context, taking into account historical genotypes and performing resistance testing when clinically appropriate is highly recommended by international guidelines, with the aim of building an antiretroviral regimen capable of the best achievable control. In the absence of fully active drugs, the development of molecules with innovative mechanisms of action as well as an accurate management of therapeutic tools are crucial for the survival of these people.

### *Added value of this study*

Here we report a series of three people with pan-resistance including reduced susceptibility to entry inhibitors. All of them were previously enrolled in the phase 3 BRIGHTE trial involving subjects with multidrug resistant HIV-1 infection and limited treatment options. After the aforementioned subjects developed protocol-defined virological failure related to non-adherence and associated to a reduced susceptibility to the investigational HIV-1 attachment inhibitor fostemsavir, we proposed a new strategy to achieve virological control. Our approach combines a deep assessment of the historical and current resistance mutations in order to tailor an optimized background therapy with the use of the first monoclonal antibody blocking HIV-1 entry ibalizumab together with the recycling of enfuvirtide.

### *Implications of all the available evidence*

*Implications of all the available evidence:* The control of the HIV epidemic cannot disregard the management of people with multidrug resistant and pan-resistant infections. Our experience shows that it is feasible to achieve virological control in people living with pan-resistant HIV-1 infection, thus reducing their risk of morbidities, mortality and pan-resistant HIV-1 transmission.

with novel mechanisms of action, such as fostemsavir, an attachment inhibitor, lenacapavir, a capsid inhibitor, islatravir, a nucleoside reverse transcriptase translocation inhibitor, and IBA, an anti-CD4+ humanized monoclonal antibody that blocks the entry of HIV-1 by binding to CD4+ cell-surface receptors.

We present a case series where we combined both a deep assessment of the historical and current resistance mutations to tailor an optimized background regimen (OBR) including any drug with a residual antiretroviral activity and a new therapeutic approach based on the use of IBA; it represented an “induction treatment” to get virological control; once this target was achieved, a “maintenance treatment” was proposed, with the main aim to promote good tolerability and adherence.

## Methods

### Ethics

The treatment here reported received the authorization of the Italian Medicines Agency (AIFA), which supplied ibalizumab by means of the 5% Fund, providing support for the use of orphan drugs for the treatment of rare diseases and potential life-saving drugs, not yet available on the market, for particular and serious diseases. AIFA authorization was then notified to the Ethics Committee of IRCCS San Raffaele Scientific Institute, as recommended for retrospective studies. The research here described was conducted in accordance with the World Medical Association Declaration of Helsinki. Each subject read and subscribed a written informed consent for treatment, collection and use of data or samples, and being included in scientific publications.

### Cases

Three PLWH followed at IRCCS San Raffaele Scientific Institute (Milan, Italy) were considered for this retrospective study. At the start of IBA combined with the OBR (baseline), a validated in-house method was used to identify mutations in the reverse transcriptase, protease and integrase genes; HIV-1 RNA was extracted from patients' plasma samples using QIAamp viral RNA kit (Qiagen, Valencia, CA). Synthesis and amplification of cDNA were performed in a single step by using the commercial SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen) and specific external primers. Outer and inner primers were designed to amplify overlapping fragments within the *pol* region in order to obtain genes separately, providing an increased efficiency of the amplification. We also performed GRT on peripheral blood mononuclear cell (PBMC) HIV-1 DNA, using an “home-made” protocol. HIV-1 DNA was extracted from PBMCs by QIAamp DNA Viral Mini kit, (Qiagen); HIV-1 DNA levels were

determined by a Taqman real-time quantitative PCR assay result PCR-products were sequenced by using the BigDye terminator v.3.1 cycle sequencing kit (Applied Biosystems), and an automated sequencer (ABI 3130 XL). GRT on plasma HIV-RNA and PBMC HIV-DNA were interpreted according to the Stanford University HIV Drug Resistance Database version 9.0 ([hivdb.stanford.edu/hivdb/by-sequences/](http://hivdb.stanford.edu/hivdb/by-sequences/), last updated on 22 February, 2021).<sup>9</sup> We also analyzed previous historical GRT and plasma levels of viro-immunological markers collected from diagnosis to data freezing.

### Study medications

IBA was administered intravenously at a 2000 mg loading dose followed by an 800 mg maintenance dose every 14 days. Enfuvirtide (ENF) was injected subcutaneously at a 90 mg twice daily (td) dose until a stable virological control was reached. The OBR consisted of approved antiretrovirals at the recommended dosage, and the regimen was tailored based on GRT-driven susceptibility prediction, clinical and pharmacological history.

### Follow-up

Since baseline, HIV-1 RNA had been quantified biweekly or monthly using PCR Cobas<sup>®</sup> HIV-1 test 6800 Systems, Roche Diagnostics; undetectable viral load was defined as HIV-1 RNA < 50 copies/mL. HIV-1 coreceptor usage was determined as formerly described.<sup>10</sup> The follow-up was censored at data freezing (16 January, 2021). HIV-1 RNA and CD4+ cell count collected before and after baseline are reported in [Figures 1–3](#).

### Role of funders

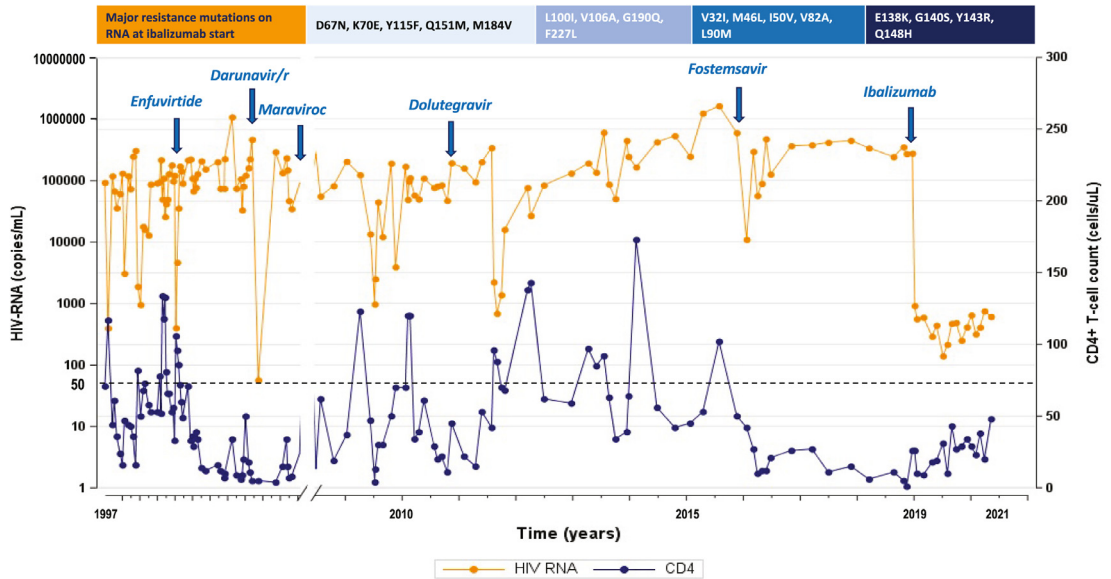
This research was supported by internal funding.

### Results

Case 1 is a 62-year-old man known for a sexually-transmitted HBV and HIV-1 subtype B co-infection since 1986 and treated with ART from 1990. Opportunistic infections, such as esophageal candidiasis and neurotoxoplasmosis, recurrently occurred due to a poor immune recovery (CD4+ T-cell count < 100 cells/ $\mu$ L). He never maintained a stable virological suppression, partly due to a scarce drug adherence. In 2016, he met eligibility criteria for enrollment into the BRIGHTIE trial (NCT02362503) as part of the non-randomised cohort; he started a regimen with fostemsavir (FTR) plus an OBR including atazanavir/cobicistat (ATV/c) 300/150 mg once daily (od) plus maraviroc (MVC). A prior treatment failure under MVC was reported, with R5 tropism at screening. A modest drop in HIV-1 RNA ranging from 294,300 copies/mL at start of FTR+OBR to a

nadir of 56,358 copies/mL was described at study week 4, CD4+ count of 12 cells/ $\mu$ L peaked at 27 cells/ $\mu$ L at week 8. Drug accountability indicated non-adherence to FTR+OBR. He met protocol-defined VF (PDVF) criteria at day 172 with emergent gp160 amino acid substitutions at M426 M/L, and temsavir (TMR) half-maximal inhibitory concentration (IC50) fold change (FC) increased from 0.37 to 17.0 ([Table 1](#), Case 1). Before baseline, GRT both in plasma HIV-RNA and PBMC HIV-DNA were performed, revealing a CXCR4-tropic virus and high-level resistance to all the drugs tested, except for intermediate resistance to darunavir/ritonavir (DRV/r) and potential low-level resistance to tipranavir/r (TPV/r), according to the presence of the major resistance mutations V32I, M46L, I50V, V82AS, L90M ([Table 1](#), Case 1). At baseline, HIV-1 RNA was 275,000 copies/mL and CD4+ 9 cells/ $\mu$ L (0.9%). IBA was associated to DRV/r 600/100 mg td (because of a potential residual activity), recycled ENF 90 mg td, and emtricitabine/tenofovir alafenamide (FTC/TAF) 200/10 mg od, because several studies support the evidence that NRTIs can retain efficacy despite the predominance of variants bearing the M184V/I mutation, according to the hypothesis that maintaining the M184V/I mutation can represent an advantage in terms of viral replication.<sup>11–14</sup> Case 1 showed a viremia ranging between 100 and 600 copies/mL from week 10. At week 34, ENF was stopped as planned, because an improved stable virological control was reached, and dolutegravir (DTG) 50 mg td was added. At week 76, HIV-1 RNA was 948 copies/mL and CD4+ count 30 cells/ $\mu$ L (2.5%).

Case 2 is a 56-year-old man with a sexually-transmitted subtype B HIV-1 infection treated since 1995. Over time, he developed a severe lipodystrophy, relapsing skin lesions caused by *Molluscum contagiosum*, atypical mycobacteriosis, and a life-threatening multi-drug resistant (MDR) *Pseudomonas aeruginosa* pneumonia. He never achieved stable virological suppression or CD4+ cell recovery, consistently < 100 cells/ $\mu$ L. In 2016, he was enrolled into the non-randomized cohort of the BRIGHTIE trial, where investigational FTR was added to an initial OBR of DTG 50 mg td, DRV/r 600/100 mg td and FTC/tenofovir disoproxil fumarate (TDF) 200/300 mg od. Viral load and CD4+ cells did not meaningfully improve (the nadir viral load was 13,354 copies/mL at week 16). Drug accountability indicated non-adherence to FTR+OBR. He met PDVF criteria at week 36 with emergent M426L and IC50 FC increase from 7.56 at baseline to > 4670 ([Table 1](#), Case 2). He was withdrawn from the BRIGHTIE study after he developed *Cytomegalovirus* pneumonia. Baseline GRTs on PBMC HIV-DNA predicted susceptibility to doravirine (DOR), not yet available in our country at that time, low-level resistance to rilpivirine (RPV), potential low-level resistance to etravirine (ETR) and intermediate resistance to efavirenz (EFV), but high-level resistance was predicted on past plasma HIV-RNA to every NNRTI, except for

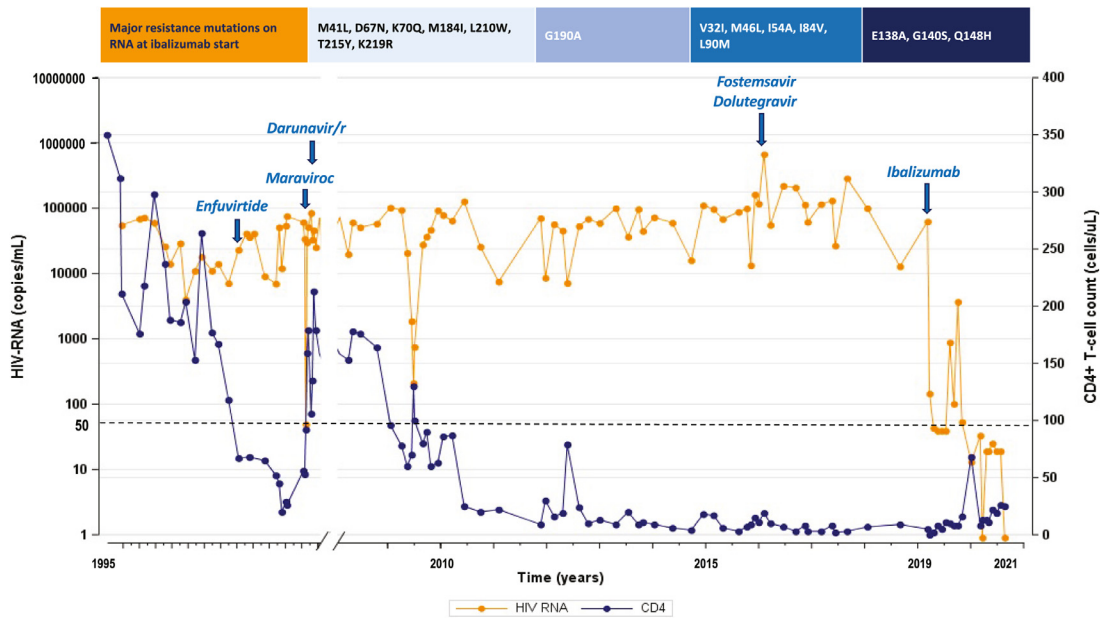


**Figure 1.** On the top: major resistance mutations on HIV-RNA at ibalizumab start (according to Stanford HIVDb version 9.0, last updated on 2021-02-22), from the right to the left on NRTI, NNRTI, PI, INSTI. Graphic: HIV-RNA (orange line) and CD4+ T-cell count (blue line) trend represented by all the available values since the first years after HIV-1 infection diagnosis to ibalizumab start (blue arrow on the right), and then monthly until data freezing. Antiretroviral dosages: enfuvirtide 90 mg iv twice daily (td), darunavir/ritonavir 600/100 mg td, maraviroc 300 mg td, dolutegravir 50 mg td, fostemsavir 600 mg td, ibalizumab administered iv at a 2000 mg loading dose followed by 800 mg every 14 days.

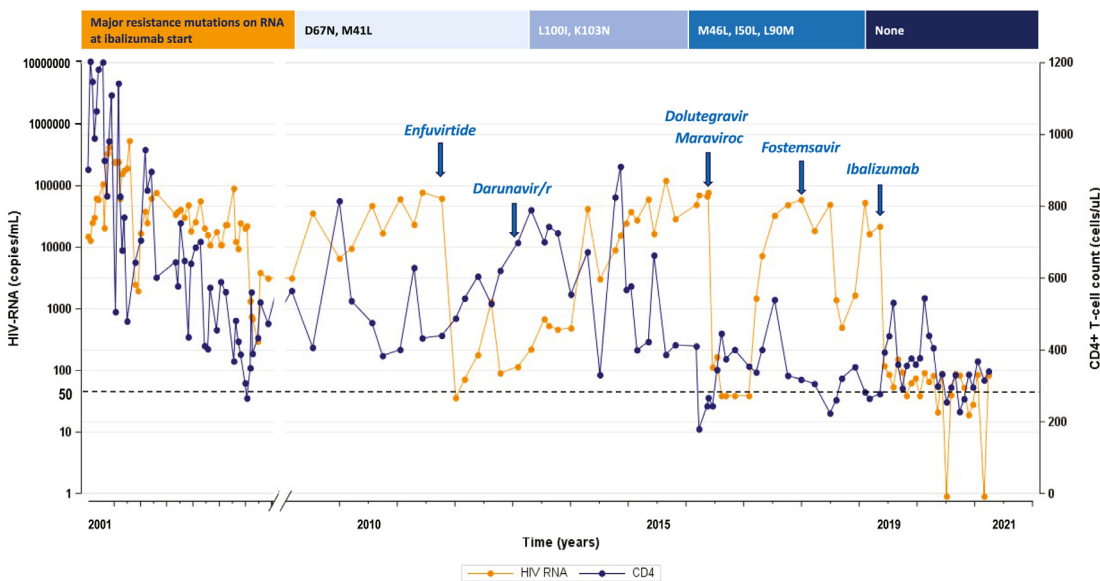
intermediate resistance to DOR (Table 1, Case2). An intermediate resistance to DRV/r was highlighted both in cumulative and baseline plasma HIV-RNA and PBMC HIV-DNA (according to the presence of the major resistance mutations V32I, M46L, I54A, I84V, L90M). High-level resistance to the other available drugs was reported, including CXCR4 tropism. Past GRTs on plasma HIV-RNA documented a G36E and L34M mutation for ENF; viral susceptibility to ENF was not evaluated at baseline. At baseline, he had HIV-1 RNA 62,600 copies/mL and CD4+ 5 cells/μL (0.7%). On the basis of the described resistance prediction, we built an OBR including DRV/r 600/100 mg td, FTC/TAF/RPV 200/25/25 mg od, ENF 90 mg td. Because of the very limited therapeutic options, we added MVC to target residual R5 variants and maximize the antiviral activity of OBR.<sup>15</sup> Case 2 viremia reached values permanently < 100 copies/mL from week 4. A transient virological rebound was observed between week 24 and 34 (3690 copies/mL), correlating with ENF interruption because of reduced tolerance. However, upon restarting ENF he achieved complete virological suppression until once again stopped ENF at week 72, due to painful nodules at injection sites. At week 76, HIV-1 RNA was < 50 copies/mL and CD4+ count 25 cells/μL (2.5%).

Case 3 is a 24-year-old male with a mother-to-child transmitted subtype F HIV-1 infection, on ART since 1997 after being diagnosed with *Pneumocystis jirovecii* pneumonia and disseminated *Cytomegalovirus* infection.

Despite several antiretroviral regimens, he never achieved a stable virological control. In 2016, he met the criteria to be enrolled into the randomised cohort of the BRIGHT trial, thus he received investigational FTR 600 mg td added to an initial OBR consisting of ATV/r plus DTG td. ENF resulted fully active at the time of enrollment, but the patient refused its use as injectable drug. DTG was the only OBR agent assessed as “fully active”, although previous exposure and a documented raltegravir (RAL) resistance. During BRIGHT, he reached HIV-1 RNA < 40 copies/mL between study weeks 12 and 36, with increase in CD4 count from 180 to 540 cells/μL. At week 60, he met PDVF criteria. Drug accountability indicated non-adherence to FTR +OBR. Pre-enrollment GRT revealed a polymorphic amino acid replacement on M426L and M434V which was detected also at week 60, while TMR IC50 FC increased from 181 at baseline to 288 at PDVF (Table 1, Case 3). He was discontinued from BRIGHT at week 156 due to lack of efficacy likely attributed to underlying non-adherence to treatment. Before baseline, plasma HIV-RNA and PBMC HIV-DNA GRT were performed, showing a resistance profile to most of the drugs in each antiretroviral class (Table 1, Case 3). GRTs on PBMC HIV-DNA showed susceptibility to FTC/lamivudine (3TC) and intermediate resistance to TDF/TAF and abacavir (ABC), while high-level resistance resulted from previous plasma HIV-RNA tests. Intermediate resistance to DOR and ETR resulted from all the



**Figure 2.** On the top: major resistance mutations on HIV-RNA at ibalizumab start (according to Stanford HIVDb version 9.0, last updated on 2021-02-22), from the right to the left on NRTI, NNRTI, PI, InSTI. Graphic: HIV-RNA (orange line) and CD4+ T-cell count (blue line) trend represented by all the available values since the first years after HIV-1 infection diagnosis to ibalizumab start (blue arrow on the right), and then monthly until data freezing. Antiretroviral dosages: enfuvirtide 90 mg iv twice daily (td), darunavir/ritonavir 600/100 mg td, maraviroc 300 mg td, dolutegravir 50 mg td, fostemsavir 600 mg td, ibalizumab administered iv at a 2000 mg loading dose followed by 800 mg every 14 days.



**Figure 3.** On the top: major resistance mutations on HIV-RNA at ibalizumab start (according to Stanford HIVDb version 9.0, last updated on 2021-02-22), from the right to the left on NRTI, NNRTI, PI, InSTI. Graphic: HIV-RNA (orange line) and CD4+ T-cell count (blue line) trend represented by all the available values since the first years after HIV-1 infection diagnosis to ibalizumab start (blue arrow on the right), and then monthly until data freezing. Antiretroviral dosages: enfuvirtide 90 mg iv twice daily (td), darunavir/ritonavir 600/100 mg td, maraviroc 300 mg td, dolutegravir 50 mg td, fostemsavir 600 mg td, ibalizumab administered iv at a 2000 mg loading dose followed by 800 mg every 14 days.

	Class	Sample, time	Major resistance mutations	Drug	Cumulative	Baseline	Plasma HIV-RNA	PBMC HIV-DNA	
Case 1	NRTI	Plasma HIV-RNA, historical	D67N, K70E, L74IL, Y115F, Q151M, M184MV, K219EK	ABC	High-Level	High-Level	High-Level	na	RESISTANCE PREDICTION
		PBMC HIV-DNA, historical	na	FTC	High-Level	High-Level	High-Level	na	
		Plasma HIV-RNA, baseline	D67N, K70E, Y115F, Q151M, M184V	3TC	High-Level	High-Level	High-Level	na	
		PBMC HIV-DNA, baseline	na	TDF/TAF	High-Level	High-Level	High-Level	na	
	NNRTI	Plasma HIV-RNA, historical	L100IL, G190Q, F227L	DOR	High-Level	High-Level	High-Level	na	
		PBMC HIV-DNA, historical	na	EFV	High-Level	High-Level	High-Level	na	
		Plasma HIV-RNA, baseline	L100IL, V106AV, G190Q, F227FL	ETR	High-Level	High-Level	High-Level	na	
		PBMC HIV-DNA, baseline	na	NVP	High-Level	High-Level	High-Level	na	
	PI	Plasma HIV-RNA, historical	M46L, I50V, V82AS, L90M	RPV	High-Level	High-Level	High-Level	na	
		PBMC HIV-DNA, historical	na	DRV/r	Intermediate	Intermediate	Intermediate	na	
		Plasma HIV-RNA, baseline	V32I, M46L, I50V, V82A, L90M	LPV/r	High-Level	High-Level	High-Level	na	
		PBMC HIV-DNA, baseline	na	TPV/r	Potential Low-Level	Potential Low-Level	Potential Low-Level	na	
	InSTI	Plasma HIV-RNA, historical	E138K, G140S, Y143CRHY, Q148H	BIC	High-Level	High-Level	High-Level	High-Level	
		PBMC HIV-DNA, historical	E138EK, G140GS, Y143YCHR, Q148QH	DTG	High-Level	High-Level	High-Level	High-Level	
		Plasma HIV-RNA, baseline	E138K, G140S, Y143R, Q148H	EVG	High-Level	High-Level	High-Level	High-Level	
		PBMC HIV-DNA, baseline	E138EK, G140GS, Y143YCHR, Q148QH	RAL	High-Level	High-Level	High-Level	High-Level	
Entry Inhibitors	Plasma HIV-RNA, historical	CXCR4 (range FPR 0.2-6.7%); gp41: no mutations	MVC	Not effective	Not effective	Not effective	Not effective		

Table 1 (Continued)

	Class	Sample, time	Major resistance mutations	Drug	Cumulative	Baseline	Plasma HIV-RNA	PBMC HIV-DNA		
Case 2		PBMC HIV-DNA, historical	CXCR4 (FPR 1.7%); gp41: no mutations							
		Plasma HIV-RNA, baseline	CXCR4 (FPR 1.7%); gp41: na	ENF	Susceptible	na	Susceptible	Susceptible		
		PBMC HIV-DNA, baseline	CXCR4 (FPR 1.7%); gp 41: na							
		Plasma HIV-RNA, BRIGHT baseline	no mutations	FTR	Reduced susceptibility to TMR <sup>a</sup>	na	Reduced susceptibility to TMR <sup>a</sup>	na		
	NRTI	Plasma HIV-RNA, BRIGHT PPDF	M426M/L							
		Plasma HIV-RNA, historical	M41L, D67N, K70Q, M184I, L210W, T215Y, K219R	ABC	High-Level	High-Level	High-Level	High-Level	High-Level	RESISTANCE PREDICTION
	PBMC HIV-DNA, historical	M41L, D67N, K70Q, M184I, L210W, T215Y, K219R	FTC	High-Level	High-Level	High-Level	High-Level	High-Level		
	Plasma HIV-RNA, baseline	M41L, D67N, K70Q, M184I, L210W, T215Y, K219R	3TC	High-Level	High-Level	High-Level	High-Level	High-Level		
	PBMC HIV-DNA, baseline	M41L, D67N, K70Q, M184I, L210W, T215Y, K219R	TDF/TAF	High-Level	High-Level	High-Level	High-Level	High-Level		
	NNRTI	Plasma HIV-RNA, historical	K101KE, Y181V, G190A	DOR	Intermediate	Susceptible	Intermediate	Susceptible		
		PBMC HIV-DNA, historical	G190A	EFV	High-Level	Intermediate	High-Level	Intermediate		
		Plasma HIV-RNA, baseline	G190A	ETR	High-Level	Potential Low-Level	High-Level	Potential Low-Level		
		PBMC HIV-DNA, baseline	G190A	NVP	High-Level	High-Level	High-Level	High-Level		
	PI			RPV	High-Level	Low-Level	High-Level	Low-Level		
		Plasma HIV-RNA, historical	V32I, M46L, I54A, I84V, L90M	ATV/r	High-Level	High-Level	High-Level	High-Level		
		PBMC HIV-DNA, historical	V32I, M46L, I54A, I84V, L90M	DRV/r	Intermediate	Intermediate	Intermediate	Intermediate		
		Plasma HIV-RNA, baseline	V32I, M46L, I54A, I84V, L90M	LPV/r	High-Level	High-Level	High-Level	High-Level		
		PBMC HIV-DNA, baseline	V32I, M46L, I54A, I84V, L90M	TPV/r	High-Level	High-Level	High-Level	High-Level		

Table 1 (Continued)

Class	Sample, time	Major resistance mutations	Drug	Cumulative	Baseline	Plasma HIV-RNA	PBMC HIV-DNA	
InSTI	Plasma HIV-RNA, historical	E138A, G140S, Y143H, Q148H	BIC	High-Level	High-Level	High-Level	High-Level	
	PBMC HIV-DNA, historical	E138A, G140S, Q148H	DTG	High-Level	High-Level	High-Level	High-Level	
	Plasma HIV-RNA, baseline	E138A, G140S, Q148H	EVG	High-Level	High-Level	High-Level	High-Level	
	PBMC HIV-DNA, baseline	E138A, G140S, Q148H	RAL	High-Level	High-Level	High-Level	High-Level	
Entry Inhibitors	Plasma HIV-RNA, historical	CXCR4 (range FPR 0.4-2.68%); gp41: G36E, L34M	MVC	Not effective	Not effective	Not effective	Not effective	
	PBMC HIV-DNA, historical	CXCR4 (FPR 5.8%); gp41: no mutations						
	Plasma HIV-RNA, baseline	CCR5 (FPR 69.8%); gp41: no mutations	ENF	Potential resistance	Susceptible	Potential resistance	Susceptible	
	PBMC HIV-DNA, baseline	CXCR4 (FPR 3.1%); gp41: no mutations						
	Plasma HIV-RNA, BRIGHT baseline	no mutations	FTR	Reduced susceptibility to TMR <sup>b</sup>	na	Reduced susceptibility to TMR <sup>b</sup>	na	
	Plasma HIV-RNA, BRIGHT P <sub>2</sub> DF	M426L						
Case 3 NRTI	Plasma HIV-RNA, historical	M41L, D67N, T69Si, M184IMV, L210W, T215Y, K219EK	ABC	High-Level	Intermediate	High-Level	Intermediate	RESISTANCE PREDICTION
	PBMC HIV-DNA, historical	M41L, D67N, L210W, T215H, K219E	FTC	High-Level	Susceptible	High-Level	Susceptible	
	Plasma HIV-RNA, baseline	M41L, D67N	3TC	High-Level	Susceptible	High-Level	Susceptible	
	PBMC HIV-DNA, baseline	M41L, D67N, L210W, T215H, K219E	TDF/TAF	High-Level	Intermediate	High-Level	Intermediate	
NNRTI	Plasma HIV-RNA, historical	L100IL, K103N	DOR	Intermediate	Intermediate	Intermediate	Intermediate	
	PBMC HIV-DNA, historical	L100IL, K103N	EFV	High-Level	High-Level	High-Level	High-Level	
	Plasma HIV-RNA, baseline	L100I, K103N	ETR	Intermediate	Intermediate	Intermediate	Intermediate	
		L100I, K103N	NVP	High-Level	High-Level	High-Level	High-Level	

Table 1 (Continued)



Class	Sample, time	Major resistance mutations	Drug	Cumulative	Baseline	Plasma HIV-RNA	PBMC HIV-DNA
PI	PBMC HIV-DNA, baseline		RPV	High-Level	High-Level	High-Level	High-Level
	Plasma HIV-RNA, historical	M46L, I50V, V82A, L90M	ATV/r	High-Level	High-Level	High-Level	High-Level
	PBMC HIV-DNA, historical	M46L, I50L, V82VA, L90M	DRV/r	Low-Level Resistance	Susceptible	Low-Level	Susceptible
	Plasma HIV-RNA, baseline	M46L, I50L, L90M	LPV/r	High-Level	High-Level	High-Level	High-Level
InSTI	PBMC HIV-DNA, baseline	M46L, I50L, V82VA, L90M	TPV/r	Susceptible	Susceptible	Susceptible	Susceptible
	Plasma HIV-RNA, historical	Y143CHRY, S147G, N155H	BIC	Intermediate	Intermediate	Intermediate	Intermediate
	PBMC HIV-DNA, historical	S147G, N155H	DTG	Intermediate	Intermediate	Intermediate	Intermediate
	Plasma HIV-RNA, baseline	none	EVG	High-Level	High-Level	High-Level	High-Level
Entry Inhibitors	PBMC HIV-DNA, baseline	S147G, N155H	RAL	High-Level	High-Level	High-Level	High-Level
	Plasma HIV-RNA, historical	CCR5 (range FPR 50.5-87.8%); gp41: L34M	MVC	Not effective	Not effective	Susceptible	Not effective
	PBMC HIV-DNA, historical	CXCR4 (FPR 1.8%); gp41: L34M					
	Plasma HIV-RNA, baseline	CCR5 (FPR 86.2%); gp41: L34M	ENF	Susceptible	Susceptible	Susceptible	Susceptible
	PBMC HIV-DNA, baseline	CXCR4 (FPR 1.8%); gp41: L34M					
	Plasma HIV-RNA, BRIGHT baseline	M426L	FTR	Reduced susceptibility to TMR <sup>c</sup>	na	Reduced susceptibility to TMR <sup>c</sup>	na
	Plasma HIV-RNA, BRIGHT PDVF	M426L					

**Table 1: Case 1-2-3. On the left: major resistance mutations according to Stanford HIVDb version 9.0 (last updated on 2021-02-22) detected on plasma HIV-RNA and peripheral blood mononuclear cell (PBMC) HIV-DNA, cumulative (all the available tests, baseline included) versus baseline (test collected just before ibalizumab plus Optimized Background Therapy starting). NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; InSTI, Integrase strand transfer inhibitor; FPR, false positive rate; PDVF, protocol-defined virological failure; na, not available.**

On the right: resistance prediction according to Stanford HIVDb version 9.0 (last updated on 2021-02-22) for the drugs of common use for each class, distinguished based on time (cumulative plasma + PBMC GRTs versus baseline plasma + PBMC GRTs) and sample (cumulative plasma GRTs versus cumulative PBMC GRTs). ABC, abacavir; FTC, emtricitabine; 3TC, lamivudine; TDF/TAF, tenofovir disoproxil fumarate/tenofovir alafenamide; DOR, doravirine; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine; ATV/r, atazanavir/ritonavir; DRV/r, darunavir/r; LPV/r, lopinavir/r; TPV/r, tipranavir/r; BIC, bictegravir; DTG, dolutegravir; EVG, elvitegravir; RAL, raltegravir; MRV, maraviroc; ENF, enfuvirtide. (a,b,c) Viral susceptibility to temsavir (TMR) at baseline and after PDVF was reported as fold change (FC) in half-maximal inhibitory concentration (IC<sub>50</sub>) and were (a) 0.37 and 17; (b) 7.56 and >4669.6; (c) 181 and 288, respectively.

available tests, as well as low-level resistance to DRV/r and susceptibility to TPV/r. Further, intermediate resistance emerged in all GRTs for DTG and bicitgravir (BIC). A CCR5-tropic virus was identified in plasma HIV-RNA, while PBMC HIV-DNA revealed a CXCR4-tropic virus. Only a minor L34M mutation was detected for ENF, described as strongly associated with CXCR4 tropism and a marginal reduction in ENF susceptibility. At baseline, he had HIV-1 RNA 21,966 copies/mL and CD4+ 278 cells/ $\mu$ L (13.6%). We built an OBR which included DTG 50 mg td, MVC 300 mg td, FTC/TAF/RPV od, and ENF 90 mg td. Case 3 reached HIV-1 RNA levels steadily below 200 copies/mL following a single loading dose of IBA, frequently achieving HIV-1 RNA < 50 copies/mL. At week 12, ENF was stopped because of stable viral control, and DRV/r 600/100 mg td was introduced. At week 98, HIV-1 RNA was 84 copies/mL with CD4+ count 341 cells/ $\mu$ L (13.2%).

## Discussion

We described three cases of people living with a pan-resistant HIV-1 infection, who reached virological control after introducing IBA and recycling ENF in association with an OBR based on current and historical GRT. All the PLWH had a mild increase of CD4+ cell count once started IBA and none of them has developed opportunistic infections during the observation period; our findings are consistent with data on IBA previously published in the setting of MDR infection.<sup>16</sup> IBA has been introduced as a rescue therapy for PLWH with MDR resistance; hence, its cost has been considered affordable if its use is restricted to heavily treatment-experienced (HTE) PLWH who have limited treatment options.<sup>17</sup> Moreover, the choice of a directly observed therapy received biweekly in the hospital has certainly allowed reinforcing adherence to oral and subcutaneous ART regularly, even during the SARS-CoV-2 pandemic. In addition, local discomfort due to ENF subcutaneous injection was the only reported adverse event related to OBR and did not affect their compliance.

A personalized OBR strategy was tailored both using historical and current plasma HIV-RNA GRT and also performing HIV-DNA GRT to detect resistance mutations harboured in PBMC. Furthermore, the assessment of resistance detected in HIV-DNA GRT has proved to have a potential role in predicting VF.<sup>18,19</sup> Despite the evidence of cumulative resistance to all the antiretroviral classes approved, each of the individual cases achieved undetectable viral load and maintain a significantly improved viral control after failing a prior ART regimen that included FTR, which induced viral suppression in one case. The exhaustion of FTR activity and its causes need to be more investigated. In the BRIGHT trial, treatment failure was secondary to gp120 substitutions previously associated with a reduced phenotypic susceptibility to TMR, the active

FTR-derived compound.<sup>20,21</sup> However, a suboptimal adherence may have contributed in failing FTR. It has been recently shown that these amino acid substitutions do not alter the mechanism of action of drugs that interferes with HIV-1 entry, such as IBA or MVC.<sup>22</sup> So, the sequential use of different entry inhibitor agents, in particular FTR, a pre-attachment inhibitor that affects gp120, and IBA, a post-attachment inhibitor, has not impaired IBA efficacy. However, mutations in the gp120 glycosylation site have been documented to decrease susceptibility to IBA.<sup>23</sup>

The approach we describe includes ENF recycling, that has demonstrated to improve survival in PLWH with a MDR infection.<sup>11</sup> Other benefits of combining IBA and ENF for a short period are based both on synergistic activity *in vitro* and lack of cross-resistance between two drugs.<sup>24,25</sup> ENF was started together with IBA with the aim to be discontinued when a stable HIV-1 RNA control was achieved, due to high rate of injection site reactions.

In conclusion, it is feasible and effective to use a GRT-driven strategy in clinical practice for people living with a pan-resistant HIV-1 infection. The recycling of ENF associated to a new drug free from cross-resistance with all the antiretrovirals available, including other entry inhibitors, and a close monitoring attributable to a route of administration that has ensured patient adherence, has revealed to be a turning point in achieving virological control in heavily-treated PLWH who have experienced repeated ART failure on any number of prior regimens over a 20–30 year period.

## Contributors

All authors have seen and approved the content and have contributed significantly to the work and thus fulfill the criteria for Authorship. AC conceived the study and contributed to writing of the manuscript. DC and CM contributed to conceive and design the study, interpretations, and wrote the manuscript. DC and CM collected and updated data. DC, CM and LG verified data. LG and AP realized the graphics and contributed to the writing of the manuscript. LG, NG, VS, and MF contributed to the interpretation of the results and reviewed the manuscript.

## Declaration of interests

DC, CM, LG, AP and MF declare no competing interests. VS has received grants from a Gilead Sciences fellowship program, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events and support for attending meetings and/or travel from Gilead Sciences and ViiV Healthcare. NG has received consulting fees from Janssen-Cilag and ViiV Healthcare, support for attending meetings and/or travel from ViiV Healthcare and participated on a Data

Safety Monitoring Board or Advisory Board for ViiV Healthcare. AC has received consulting fees, honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events, support for attending meetings and/or travel from Merck Sharp & Dohme, Gilead Sciences, Janssen-Cilag, ViiV Healthcare, and Theratechnologies.

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### Data sharing statement

All data of the three cases described in the manuscript are present in the paper; deidentified virological and immunological data used in the figures are available to researchers through the public repository “San Raffaele Open Research Data Repository” - ORDR (<https://ordr.hsr.it/research-data/>; 10.17632/jbwfm2vs7d.1).

### References

- Antiretroviral Therapy Cohort Collaboration. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. *Lancet HIV*. 2017;4:e349–e356.
- Godfrey C, Thigpen MC, Crawford KW, et al. Global HIV antiretroviral drug resistance: a perspective and report of a national institute of allergy and infectious diseases consultation. *J Infect Dis*. 2017;216:S798–S800.
- Günthard HF, Calvez V, Paredes R, et al. Human immunodeficiency virus drug resistance: 2018 recommendations of the international antiviral society-USA panel. *Clin Infect Dis*. 2019;68:177–187.
- European AIDS Clinical Society Guidelines for treatment of people living with HIV. EACS Guidelines version 10.1, October 2020. [https://www.eacsociety.org/files/guidelines-10.1\\_30032021.1.pdf](https://www.eacsociety.org/files/guidelines-10.1_30032021.1.pdf) Accessed 28th June, 2021.
- Judd A, Lodwick R, Noguera-Julian A, et al. Higher rates of triple-class virological failure in perinatally HIV-infected teenagers compared with heterosexually infected young adults in Europe. *HIV Med*. 2017;18:171–180.
- Lombardi F, Giacomelli A, Armenia D, et al. Prevalence and factors associated with HIV-1 multi-drug resistance over the past two decades in the Italian ARCA database. *Int J Antimicrob Agents*. 2020;28:106252.
- Galli L, Parisi MR, Poli A, et al. Burden of disease in PWH harboring a multidrug-resistant virus: data from the PRESTIGIO registry. *Open Forum Infect Dis*. 2020;7:ofaa456.
- Puertas MC, Ploumidis G, Ploumidis M, et al. Pan-resistant HIV-1 emergence in the era of integrase strand-transfer inhibitors: a case report. *Lancet Microbe*. 2020;1:e130–e135.
- Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucl Acids Res*. 2003;31:298–303.
- Muccini C, Galli L, Spagnuolo V, et al. Brief report: association between low HIV-1 DNA and western blot reactivity to HIV-1 pol in chronically infected individuals. *J Acquir Immune Defic Syndr*. 2019;82:373–376.
- Cossarini F, Galli L, Sagnelli C, et al. Survival of HIV-1 infected multidrug-resistant patients recycling enfuvirtide after a previous failure. *J Acquir Immune Defic Syndr*. 2009;51:179–184.
- Parades R, Sagar M, Marconi VC, et al. *In vivo* fitness cost of the M184V mutation in multidrug-resistant human immunodeficiency virus type 1 in the absence of lamivudine. *J Virol*. 2009;83:2038–2043.
- Ciaffi L, Koulla-Shiro S, Sawadogo AB, et al. Boosted protease inhibitor monotherapy versus boosted protease inhibitor plus lamivudine dual therapy as second-line maintenance treatment for HIV-1-infected patients in sub-Saharan Africa (ANRS12 286/MOBIIDIP): a multicentre, randomised, parallel, open-label, superiority trial. *Lancet HIV*. 2017 Sep;4(9):e384–e392.
- Charpentier C, Montes B, Perrier M, Meftah N, Reynes J. HIV-1 DNA ultra-deep sequencing analysis at initiation of the dual therapy dolutegravir + lamivudine in the maintenance DOLULAM pilot study. *J Antimicrob Chemother*. 2017;72(10):2831–2836. Oct 1.
- Cavarelli M, Mainetti L, Pignataro AR, et al. Complexity and dynamics of HIV-1 chemokine receptor usage in a multidrug-resistant adolescent. *AIDS Res Hum Retrovir*. 2014;30(12):1243–1250. Dec.
- Emu B, Fessel J, Schrader S, et al. Phase 3 study of ibalizumab for multidrug-resistant HIV-1. *N Engl J Med*. 2018;379:645–654.
- Millham LRI, Scott JA, Sax PE, et al. Clinical and economic impact of ibalizumab for people with multidrug-resistant HIV in the United States. *J Acquir Immune Defic Syndr*. 2020;83:148–156.
- Armenia D, Zaccarelli M, Borghi V, et al. Resistance detected in PBMCs predicts virological rebound in HIV-1 suppressed patients switching treatment. *J Clin Virol*. 2018;104:61–64.
- Canetti D, Galli L, Gianotti N, et al. Simplification to high genetic barrier 2-drug regimens in people living with HIV harboring 4-class resistance enrolled in the PRESTIGIO registry. *J Acquir Immune Defic Syndr*. 2020;84:e24–e28.
- Kozal M, Aberg J, Pialoux G, et al. Fostemsavir in adults with multidrug-resistant HIV-1 infection. *N Engl J Med*. 2020;382:1232–1243.
- Lataillade M, Lalezari JP, Kozal M, et al. Safety and efficacy of the HIV-1 attachment inhibitor prodrug fostemsavir in heavily treatment-experienced individuals: week 96 results of the phase 3 BRIGHT study. *Lancet HIV*. 2020;7:e740–e751.
- Ronald R, Gartland M, Li Z, et al. Clinical evidence for a lack of cross-resistance between temsavir and ibalizumab or maraviroc. *AIDS*. 2021. <https://doi.org/10.1097/QAD.0000000000003097>. Oct 7 Epub ahead of print. PMID: 34628442.
- Toma J, Weinheimer SP, Stawiski E, et al. Loss of asparagine-linked glycosylation sites in variable region 5 of human immunodeficiency virus type 1 envelope is associated with resistance to CD4+ antibody ibalizumab. *J Virol*. 2011;85:3872–3880.
- Zhang XQ, Sorensen M, Fung M, Schooley RT. Synergistic *in vitro* antiretroviral activity of a humanized monoclonal anti-CD4+ antibody (TNX-355) and enfuvirtide (T-20). *Antimicrob Agents Chemother*. 2006;50:2231–2233.
- Jacobson JM, Kuritzkes DR, Godofsky E, et al. Safety, pharmacokinetics, and antiretroviral activity of multiple doses of ibalizumab (formerly TNX-355), an anti-CD4+ monoclonal antibody, in human immunodeficiency virus type 1-infected adults. *Antimicrob Agents Chemother*. 2009;53:450–457.