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MAJOR ARTICLE

A Low Level of Darunavir Resistance–Associated Mutation Emergence in Patients With Virological Failure During Long-term Use of Darunavir in People With HIV. The ANRS CO3 Aquitaine Cohort

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Background. Ritonavir-boosted darunavir (DRV/r) is a protease inhibitor (PI) indicated for the treatment of naïve and pretreated HIV-infected patients since 2007. Our study aims to describe DRV/r-treated patients experiencing virological failure (VF) documented with HIV resistance testing.

Methods. Data from patients belonging to the ANRS CO3 Aquitaine Cohort treated with a regimen including DRV/r between February 2007 and December 2015 were analyzed. Baseline characteristics of patients experiencing VF (defined by 2 consecutive plasma viral loads >50 copies/mL) were compared with those without VF. We then described factors associated with VF as emergence of IAS DRV resistance–associated mutations (RAMs).

Results. Among the 1458 patients treated at least once with a DRV/r-based regimen, 270 (18.5%) patients experienced VF during follow-up, including 240 with at least 1 genotype resistance test (GRT). DRV RAMs were detected in 29 patients (12%). Among them, 25/29 patients had ≥2 DRV RAMs before DRV/r initiation, all of whom had experienced VF during previous PI treatments. For 18/29, DRV/r was maintained after VF, and controlled viremia was restored after modification of DRV-associated antiretroviral molecules or increased DRV dose. Finally, only 6/29 patients selected new DRV RAMs after DRV/r initiation. All of these experienced previous VFs while on other PIs.

Conclusions. These results highlight the efficacy and robustness of DRV/r, as the emergence of DRV RAMs appeared in <0.4% of patients receiving a DRV/r-based regimen in our large cohort.

Keywords. darunavir; HIV-1; genotype; mutation rate.

HIV infection has changed from a fatal to a chronic, manageable infection where patients live longer while receiving antiretroviral therapy (ART). Development of HIV-1 protease inhibitors (PIs) was a turning point in clinical management, as their use as a component of highly active antiretroviral therapy dramatically reduced the morbidity and mortality associated with HIV disease [1].

Ritonavir-boosted darunavir (DRV/r) is a potent PI indicated for the treatment of naïve and pretreated HIV-infected patients since 2007. DRV/r was approved for 800/100-mg once-daily (QD) dosing in treatment-naïve individuals, then in treatment-experienced individuals without DRV resistance-associated mutation (RAM) [2–4]. Twice-daily dosing (600/100 mg twice a day) was recommended in treatment-experienced patients, based on an analysis of subjects with triple-class ARV experience with 1 or more primary PI RAMs [5].

Although recent European guidelines for the treatment of people with HIV (PWH) favor the use of an unboosted integrase strand transfer inhibitor with a high genetic barrier (DTG or BIC) as the preferred third agent for treatment of naïve infected people, DRV is still recommended in this indication [6, 7]. Dual therapy associating DRV with 3TC can be proposed as a switch strategy as well as DRV/r monotherapy after at least 2 years of sustained virological suppression according to French guidelines [8, 9]. In patients experiencing virological failure (VF) and those with poor adherence, DRV/r

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remains a preferred molecule due to its high genetic barrier and low cross-resistance with other PIs [10]. In France, the ANRS Multivir study performed in 2014 on patients experiencing VF showed that 1.6% of viruses were not susceptible to any PI [11]. Resistance genotypes performed for ACTG A5288 screening revealed that susceptibility to DRV was preserved in 97% of PWH experiencing second-line ART containing PIs in resource-limited settings [12]. Due to this low prevalence of DRV resistance among highly antiretroviral-experienced HIV-infected patients, DRV is a key molecule in salvage or simplification strategies [13, 14].

The efficacy and safety of DRV/r have been extensively demonstrated in different clinical trials [15]. Here, we aimed to study the long-term virological response in a large cohort of HIV-infected patients starting a DRV/r-containing regimen according to their baseline PI resistance and to describe the virological failures and DRV RAMs occurring in the very long term and in real-life settings.

METHODS

Study Design and Patients

The ANRS CO3 Aquitaine Cohort is an open, prospective hospital-based cohort of HIV-1-infected adults under routine clinical management. This cohort was initiated in 1987 at the Bordeaux University hospital and involves 10 other public hospitals of the Aquitaine region in Southwestern France. The present study includes patients aged 18 years or older who were treated at least once with DRV/r between February 1, 2007, and December 31, 2015, and for whom at least 1 T-CD4 lymphocyte measure and 1 plasma HIV viral load (pVL) measure were available in the year following the introduction of DRV/r. Patients who initiated DRV/r before inclusion in the ANRS CO3 Aquitaine Cohort were not included in the present analysis.

Patient Consent Statement

All patients sign an informed consent to be included in the cohort. The ANRS CO3 Aquitaine Cohort received approval from the Ethics Committee of the CHU de Bordeaux (Comité de Protection des Personnes).

Data Analysis

Data were collected at baseline (at DRV/r initiation date) and at all subsequent clinic visits or hospitalizations. Demographic characteristics (age, gender), comorbidities, mode of infection, hepatitis B and C serological status, AIDS stage, previous antiretroviral treatments, and previous virological failure were considered. Virological success (VS) was defined as plasma viral load (pVL) <50 copies/mL after DRV/r initiation. Virological failure (VF) was defined (i) for ARV-naïve PWH as 2 consecutive pVL >50 copies/mL or 1 pVL >1000 copies/mL after 1 pVL <50 copies or no pVL <200 copies/mL at 6 months or <50 copies/mL at 1 year after DRV/r starting; (ii) for ARV-experienced PWH switching to DRV while VS as 2 consecutive pVL >50 copies/mL or 1 pVL >1000 copies/mL; (iii) for ARV-experienced PWH switching to DRV while failing therapy as 2 consecutive pVL >50 copies/mL or 1 pVL >1000 copies/mL after 1 pVL <50 copies or no pVL <50 copies/mL at 6 months.

DRV Resistance

HIV-1 genotype resistance testing (GRT) was performed from plasma HIV RNA or proviral DNA (if viral load was <120 copies/mL) according the ANRS consensus method (http://www.hivfrenchresistance.org) as previously described [16]. All genotypes were reviewed according to the 2019 IAS and ANRSv29 mutation lists to determine PI drug resistance-associated mutations (RAMs): V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V. The resistance to DRV, depending on the dose (≥3 mutations for 600/100 mg twice daily or ≥2 RAMs for 800/100 mg once daily) was determined according to the ANRS algorithm, version 29. The Genotypic Susceptibility Score (GSS) was used to estimate the resistance to the prescribed treatment and was calculated as the sum of fully active drug belonging to the antiretroviral treatment.

Statistical Analysis

The Aalen-Johansen estimator was used to estimate the cumulative incidence of VF and VS up to M36, considering DRV/r discontinuation, loss to follow-up, and death as a competing risk. Comparisons of patients' characteristics at baseline and at VF were carried out by the Student *t* test for quantitative variables and by the chi-square test for qualitative variables. All patients were compared at baseline according to VF, and patients with VF were compared according to presence of DRV RAMs at DRV initiation. *P* values <.05 were considered statistically significant. All analyses were performed with SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Virological Outcome and Patient Characteristics

A total of 1458 PWH were treated at least once with a DRV/r-containing regimen. All patients received DRV boosted with ritonavir because cobicistat was not available in France. Among them, 212 were ARV naïve, 654 were ARV experienced but virologically suppressed, and 592 experienced virological failure at the time of DRV/r initiation. The median duration of follow-up (interquartile range [IQR]) was 24.7 (9.1–49.0) months in an undertreatment approach.

We estimated the probability of VF for these 3 groups (Figure 1). Overall, VF was observed for 270 patients (18.5%). The cumulative incidence of VF at 36 months for the DRV/r-based regimen was 6.8% (95% CI, 3.6%–11.3%), 7.1% (95% CI, 5.1%–9.5%), and 22.0% (95% CI, 18.5%–25.6%) for ARV-naïve patients, ARV-experienced but VS patients,

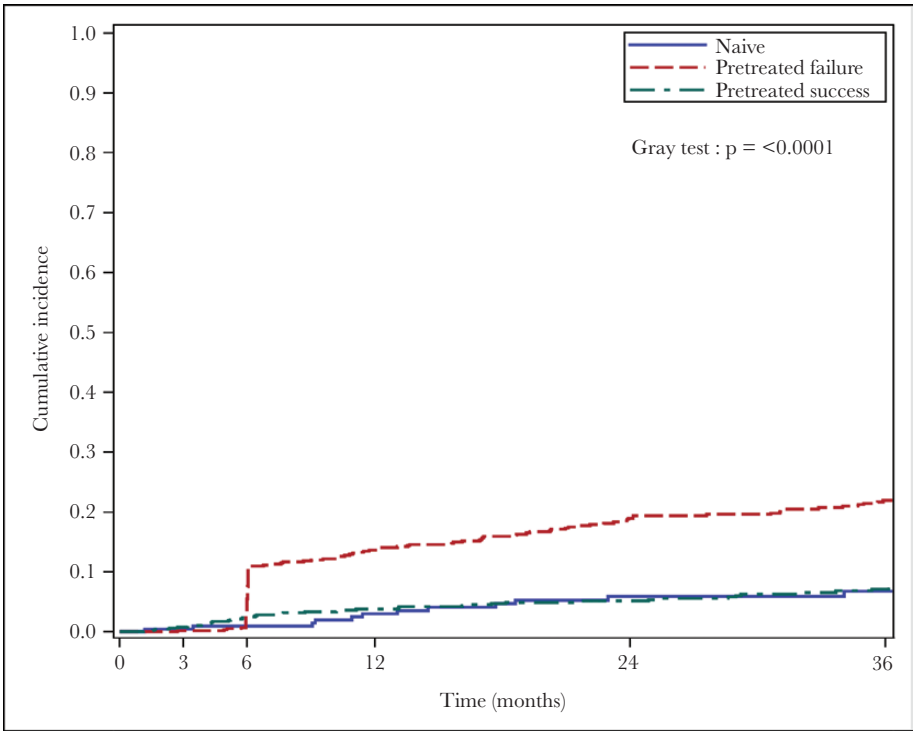

Figure 1. Aalen-Johanssen estimates of cumulative incidence of virological failure according to status at DRV/r initiation, considering DRV/r discontinuation, loss-to-follow-up, and death as a competing risk.

and ARV-experienced but VF patients, respectively. At VF, the median CD4 cell count (IQR) was 384 (204–584) cells/mL, and the HIV-1 pVL (IQR) was 399 (95–7594) copies/mL. Likely, the cumulative incidence of VS calculated at 36 months for ARV-naïve patients, ARV-experienced but VS patients, and ARV-experienced but VF patients treated with a DRV/r-based regimen was 89.4% (95% CI, 84.4%–92.9%), 81.8% (95% CI, 78.6%–84.5%), and 67.2% (95% CI, 63.3%–70.8%), respectively.

Baseline patient characteristics according to their virological response to the DRV/r-based regimen are described in Table 1. Compared with patients achieving a controlled viremia on a DRV/r-based regimen, patients experiencing VF during follow-up have a lower nadir CD4 cell count (IQR) (154 [53–152] vs 208 [101–337] cells/mL; $P < .0001$), patients had received more previous regimens (6 [3–10] vs 5 [1–9]; $P = .0118$), the baseline CD4 cell count was lower (327 [164–538] vs 456 [308–668] cells/mL; $P < .0001$), and HIV RNA >50 copies/mL was most frequently observed.

Triple therapy with 2 nucleoside reverse transcriptase inhibitors (NRTIs) was the most prescribed combination therapy. The most frequent NRTI associations were emtricitabine/tenofovir (79.2%) and lamivudine/abacavir (15.3%). Among the 866 ARV-naïve and ARV-experienced but virologically suppressed patients, 74.8% received triple therapy with 2 NRTIs (75.4% of no VF patients vs 69.2% of VF patients), 10.4% DRV/r dual therapy (10.2% of no VF patients vs 12.8% of VF patients), and 3% DRV/r monotherapy (2.9% of no VF patients vs 3.8% of VF patients).

Drug Resistance Mutations and Virological Failure

Among the 270 patients who experienced VF, baseline and/or VF genotype resistance testing (GRT) was available for 240 of them, including 222 with GRT performed before DRV/r initiation and 18 at VF only. Among the 222 patients with GRT before DRV/r initiation, 140 had also a GRT at VF. To describe the relationship between VF and the presence of DRV RAMs, we focused our analysis on the presence of DRV RAMs before DRV-based treatment and at failure. In the 222 baseline resistance analysis, only 5 were performed from proviral DNA. Finally, 25 GRTs performed at baseline (on 222 samples, before DRV/r initiation) expressed ≥2 DRV RAMs (conferring HIV resistance according to the ANRS algorithm, version 29). Four additional GRTs (on 158 samples) expressed >2 DRV RAMs on GRT at VF. The characteristics of these 29 patients with viruses harboring ≥2 DRV RAMs are presented in Table 2. They were receiving more drugs (3 or more in 55.1% of patients vs 22.3% for the group without RAMs), had received more previous therapeutic combination regimens containing PIs (IQR) (7 [4–12] vs 3 [1–5]; $P < .0001$), and had experienced more previous VF on PI treatment (96.6% vs 60.7%; $P = .0001$). The dose of DRV/r at VF was 1200 mg/200 mg per day for 26 of the 29 patients with DRV RAMs.

Table 1. Characteristics of Patients at Baseline Starting a Darunavir/Ritonavir-Containing Regimen According to the Virological Response

Characteristics	No Virological Failure (n = 1188)	Virological Failure (n = 270)	*P* Value
Age, median [IQR], y	47.4 [40.8–53.5]	46.1 [39.2–52.0]	.0938
Male sex, No. (%)	861 (72.5)	187 (69.3)	.2888
Route of transmission, No. (%)			.0166
Men who have sex with men	503 (42.3)	92 (34.1)	
Heterosexual sex	396 (33.3)	94 (34.8)	
Injection drug use	200 (16.8)	51 (18.9)	
Others	89 (7.5)	33 (12.2)	
Years since HIV diagnosis, median [IQR]	14.8 [6.3–20.5]	14.9 [8.6–19.8]	.1871
AIDS stage, No. (%)	292 (24.6)	76 (28.1)	.2230
CD4 count, median [IQR], cells/mm^3	456 [308–668]	327 [164–538]	<.0001
CD4 nadir, median [IQR], cells/mm^3	208 [101–337]	154 [53–252]	<.0001
Patient status, No. (%)			<.0001
Naïve	195 (16.4)	17 (6.3)	
Pretreated success	593 (49.9)	61 (22.6)	
Pretreated failure	400 (33.7)	192 (71.1)	
HIV RNA <50 cp/mL, No. (%)	596 (50.2)	61 (22.6)	<.0001
Baseline therapeutic combination, No. (%)			.0396
2 NRTI + DRV/r	804 (67.7)	161 (59.6)	
DRV/r-based dual therapy	124 (10.3)	31 (11.5)	
DRV/r monotherapy	27 (2.3)	5 (1.9)	
Others	233 (19.7)	73 (27)	
No. of previous therapeutic combinations, median [IQR]	5 [1–9]	6 [3–10]	.0118

Abbreviations: DRV/r, darunavir/ritonavir; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor.

In the 25/29 patients with viruses carrying ≥2 DRV RAMs before DRV/r initiation, the number of DRV RAMs was 2, 3, and 4 or more in 12, 7, and 6 patients, respectively (Table 2). When DRV RAMs were reanalyzed according to French resistance rules (ie, including the number of RAMs and the DRV/r dosing), we observed that susceptibility to DRV/r at initiation was preserved in 11 cases and that intermediate to full resistance was retained for 14 patients.

At the time of VF, additional DRV RAMs were selected by 6 of those 29 patients including 4 with 1 previous DRV RAM and 2 with 2 previous DRV RAMs (Table 3). All patients who acquired RAMs after DRV/r initiation were ARV-experienced with VF, treated with DRV/r 600/100 twice daily. The delay between DRV/r initiation and VF of these 6 patients varied between 12 and 55 months. Five patients were treated with 2 NRTIs plus DRV 600 mg with ritonavir 100 mg twice daily, with 1 having additional raltegravir. One patient had 3TC plus raltegravir plus DRV 600 mg with ritonavir 100 mg twice daily (lamivudine). Baseline GSS showed that the NRTI backbone was inefficient in patients 1 and 4. At failure, RAMs were detected only on protease. Four of the 6 patients experiencing VF had viruses with <2 DRV RAMs (ie, without any DRV resistance) before DRV initiation (Tables 2 and 3). The PI RAM L76V was preexisting for 2 of the 6 patients, whereas patients 1 and 4 had viruses harboring I84V and I54L, respectively.

The pattern of acquired resistance was different, but the V32I mutation was found for 4 out of the 6 (66.7%) patients. Patient 5 had only 1 V32I additional RAM, which should not affect the virological response to DRV prescribed at 1200-mg dosing. Protease sequencing showed that I54M + I84V were added to the unique baseline DRV RAM L76V in patient 3. In patient 1, the V32I and L33F mutations were newly identified at failure, and patients 2 and 6 had viruses with 1 additional DRV RAM (V32I and T74P, respectively) at failure. These additional mutations increased the total number of DRV RAMs to 3 and then increased the level of resistance to DRV/r.

For patient 4, 3 DRV RAMs (V32I + L33F + I84V) were selected in addition to the baseline I54L, conferring complete resistance to DRV, even at a 1200-mg dose. No data on plasma DRV plasma concentrations during follow-up were available.

Among these 6 patients whose plasma virus has selected resistance mutations, the follow-up showed that DRV/r was maintained for 4 patients, with modification of DRV/r-associated antiretroviral molecules for 3 of them. Three of these 4 patients achieved an undetectable HIV-1 pVL; 1 with modification of DRV/r-associated molecules experienced another VF and died.

The long-term virological follow-up of the 29 patients with DRV RAMs showed that DRV/r was maintained after VF for 18 (62.1%) of them (9 with the same treatment, 8 with

Table 2. Characteristics of Patients at Baseline Starting a Darunavir/Ritonavir-Containing Regimen According to VF DRV RAMs

	No DRV RAMs	DRV RAMs	
	(n = 211)	(n = 29)	*P* Value
Age, median [IQR], y	45.5 [39.0–51.8]	46.6 [41.3–55.0]	.3505
AIDS stage, No. (%)	60 (28.4)	9 (31.0)	.7719
Years since HIV diagnosis, median [IQR]	14.5 [7.0–19.6]	16.6 [12.1–20.6]	**.0165**
CD4 count, median [IQR], cells/mm^3	293 [130–533]	283 [180–422]	.7384
CD4 nadir, median [IQR], cells/mm^3	147 [49–248]	71 [33–206]	.2026
HIV RNA <50 cp/mL, No. (%)	45 (21.3)	3 (10.3)	.1656
Patient status, No. (%)			.0765
Naïve	17 (8.1)	0 (0.0)	
Pretreated success	45 (21.3)	3 (10.3)	
Pretreated failure	149 (70.6)	26 (89.7)	
Baseline therapeutic combination, No. (%)	211	29	**.0044**
2 NRTI + DRV/r	131 (62.1)	10 (34.5)	
Dual therapy	28 (13.3)	3 (10.3)	
DRV/r monotherapy	5 (2.4)	0 (0.0)	
Others	47 (22.3)	16 (55.1)	
No. of previous therapeutic combinations, median [IQR]	5 [2–10]	10 [7–15]	**<.0001**
Including PI, median [IQR]	3 [1–5]	7 [4–12]	**<.0001**
Previous PI treatment, No. (%)			
Saquinavir	44 (20.9)	15 (51.7)	**.0003**
Indinavir	54 (25.6)	19 (65.5)	**<.0001**
Nelfinavir	50 (23.7)	17 (58.6)	**.0001**
Amprenavir	11 (5.2)	16 (55.2)	**<.0001**
Lopinavir	112 (53.1)	26 (89.7)	**.0002**
Atazanavir	111 (52.6)	6 (20.7)	**.0013**
Tipranavir	4 (1.9)	13 (44.8)	**<.0001**
Fosamprenavir	29 (13.7)	17 (58.6)	**<.0001**
Previous VF on PI treatment	128 (60.7)	28 (96.6)	**.0001**
No. of DRV RAMs before DRV/r start[a]			
0	170	0	
1	23	4	
2	-	12	
3	-	7	
4 or more	-	6	

Abbreviations: DRV, darunavir; DRV/r, darunavir/ritonavir; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RAM, resistance-associated mutation; VF, virological failure.

[a]The presence of DRV RAMs was not determined for 18 patients due to missing genotypic resistance testing.

modification of DRV/r-associated molecules, and 1 with increased DRV/r dose) and that controlled viremia (pVL < 50 cp/mL) was restored. Finally, DRV/r was stopped for 8/29 (27.6%) patients, 6 of whom were DRV resistant (20.7%) and 3/29 (10.3%) of whom died while on the DRV/r regimen (Supplementary Data).

DISCUSSION

Clinical studies have described the long-term efficacy and safety of DRV/r in randomized trials with screened patients, but there are few data available on DRV resistance in real-life settings. In our study, we estimated the probability of virological failure and the emergence of DRV resistance–associated mutations in patients included in the large ANRS CO3 Aquitaine Cohort.

We showed that VF occurred for 18.5% of patients treated with a DRV-based regimen. Cumulative incidence rates of VS at 36 months in ARV-naïve patients and ARV-experienced patients who were virologically controlled were 89.4% and 81.8%, respectively. These results were close to those provided by the FHDH, which studied DRV/r use in France between 2012 and 2016 [17]. The FHDH showed that the 4-year cumulative incidence of VS was 80.9% and 87.4% for ARV-naïve PWH and ARV-experienced patients who were virologically controlled, respectively. In a Spanish cohort of 173 PWH who initiated DRV/r between 2007 and 2015, the rate of virological suppression in naïve patients was 63.6% at 144 weeks [18]. In the ARTEMIS trial, 68.8% of ARV-naïve patients randomized to receive DRV/r achieved a pVL <50 cp/mL at week 192 [2]. The low rate of VF in naïve patients observed in our study might

Table 3. Evolution of Resistance Profile From Baseline to Virological Failure for 6/29 Patients who Selected DRV RAMs

Patient	ARV Regimen at VF	Time	GSS[a]	HIV-1 VL, cp/mL	PI Mutations	DRV Discontinuation (Modified Treatment)
1	3TC + ABC + DRV/r	D0	1	753 100	10I 54V 63P 71V *84V* 90M	Yes (RAL + ETR + DDI)
		VF M12	0.5	140	10I *32I 33LF* 46I 54V 63P 71V *84V* 90M	
2	3TC + ABC + DRV/r	D0	2.5	1382	10I 20R 24I 36I 46L *54L* 62V 63T 71V 74S 82I *84V* 85V	No (3TC + ABC + DRV/r)
		VF M16	0.5	320	10I 20R *32I* 36I 46L *54L* 62V 63T 71V 74S 82I *84V* 85V	
3	3TC + RAL + DRV/r	D0	2	13 688	10I 46I 58E 63P *76V*	No (RAL + ETR + DRV/r)
		VF M31	1.5	1844	10I 46I *54M* 58E 63P 71TA *76V* 82I *84V*	
4	TDF + FTC + DRV/r	D0	2	62	15V 20T 36I *54L* 63P 71IV *74S* 90M	Yes (TDF + FTC + DTG)
		VF M48	1.5	192	10F 15V 20T *32I 33F* 36I *54L* 71I 73S *84V* 90M	
5	TDF + FTC + DRV/r	D0	1	17 958	10I 15V 46I 54V 58E 60E 62V 63T 71V *76V* 77I 82F 90M	No (RAL + ETR + DRV/r)
		VF M53	1	1200	10I 15V *32I* 46I 54V 58E 60E 62V 63T 71V *76V* 77I 82F 90M	
6	TDF + FTC + DRV/r + RAL	D0	2	55 787	10F 15V *33F* 46I *50V* 63P 82°	No (ETR + DDI + DRV/r)
		VF M55	0.5	1008	10F 15IV *33F* 46I *50V* 63P *74P* 82°	

All these patients were ARV experienced treated with a salvage regimen containing DRV BID. Neither ARV-naïve nor ARV-experienced but virologically suppressed patients had virus with DRV additional RAMs at VF.

Abbreviations: ARV, antiretroviral; BID, twice a day; D0, baseline; DRV, darunavir; DRV/r, darunavir/ritonavir; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RAM, resistance-associated mutation; VF, virological failure; VF M, delay to VF in months.

[a]Genotypic susceptibility score was determined according to the ANRS, version 29, algorithm (0 resistant; 0.5 intermediate resistance; 1 susceptible). DRV RAMs are underlined. DRV RAMs accumulated at VF are shown in bold.

be related to the lower VL at DRV initiation. Indeed, the median (IQR) pVL was 61 018 (16 152–175 500) copies/mL, and 61.7% of patients had a baseline VL <100 000 copies/mL (data not shown).

For ARV-experienced PWH switching to DRV/r while failing therapy in the FHDH cohort, the 4-year cumulative incidence of VS varied from 63.2% to 81.8% according to their baseline pVL [17]. The rate of virological suppression in treatment-experienced patients was 79.1% at 144 weeks in the study of Pernas and colleagues [18]. The efficacy observed in clinical trials varied between 55% and 89% depending on the previous combination ART—especially PI—experience of included patients [5, 19–21]. In our study, the probability of virological success at 36 months was quite similar (67.2%). This subgroup of patients had baseline characteristics similar to those of the patients included in the POWER 1 and 2 trials [5]; they started DRV/r in a more advanced clinical stage and were highly pretreated.

Among the 240 patients who presented VF during follow-up and with at least 1 GRT available (including 222 patients with GRT at inclusion and 158 at VF), we identified only 29 patients with ≥2 DRV RAMs. Twenty-five of these 29 patients had ≥2 preexisting RAMs at inclusion, conferring intermediate to high resistance to DRV/r, depending on the dosing. Half had been treated with complex therapeutic combination therapies that could be considered salvage therapies. Many previous PI treatments, often associated with VF experiences, were reported, suggesting that DRV resistance was the result of cross-resistance with other molecules belonging to the PI class [22]. Indeed, half of the patients had been treated with (fos)amprenavir, which shares mutational resistance patterns with DRV, due to their close molecular structures, explaining the preexisting DRV RAMs [23].

It has been shown that the prevalence of emerging DRV RAMs at previous failure in PI-experienced patients depends on the number of baseline DRV mutations, which is also a determinant of DRV response [24]. This explains why DRV-based treatments failed to control pVL in patients with preexisting DRV/r RAMs. Nevertheless, follow-up showed that controlled viremia was finally obtained with DRV/r, associated with the same or an optimized backbone, for 18 of 29 patients. This suggests that VF was mostly due to adherence problems and/or that DRV retained its antiviral activity despite RAMs. The presence of the V82A, which is associated with a better virological response to DRV/r, could have contributed to improved activity of DRV in viruses harboring this classic major PI resistance mutation [24]. Altogether, our data indicate that DRV/r could provide a sustained virological response even in patients with preexisting PI RAMs, as previously shown [5].

We observed a very limited emerging resistance to DRV. Only 6 patients who were heavily pretreated, out of 1458 (0.4%), developed DRV RAMs while on DRV. These results are

consistent with findings previously described in clinical trials (TITAN and POWER 1 and 2 studies). Similar data from the UK Collaborative HIV Cohort combined with the HIV Drug Resistance Database showed that 2.8% of participants developed emergent DRV RAMs [25]. All of the patients with additional DRV RAMs at failure had baseline genotypes indicating the presence of at least 1 DRV RAM. The patterns of resistance showed the well-identified mutations: V32I, L33F, I84V, I54M/L, 76V, 54L, 50V, T74P. The V32I critical mutation was selected in 4/6 of patients with emerging DRV RAMs, in association with the A71V, which compensated for compromised viral fitness by acquisition of V32I [26]. However, the number of these additional RAMs was limited and should have moderately increased the level of DRV/r resistance. In the last guidelines, the World Health Organization recommend the use of DRV/r as an alternative second-line regimen after a preferred second-line regimen with dolutegravir or PI atazanavir or lopinavir as the third agent [27]. We believe that according to our results and the safety of DRV/r it should be the preferred choice for PI instead of atazanavir or lopinavir to prevent the emergence of PI RAMs and to bolster the resistance profile. In addition, DRV/r is a therapeutic-based strategy available in pregnant women, infants, and adolescents.

CONCLUSIONS

The low rate of RAM selection at DRV failure confirmed the high genetic barrier of this PI molecule and its efficacy against resistant viruses. Ritonavir-boosted DRV might be considered as a highly valuable therapeutic option for those patients who have failed several ART regimens but also as the preferred PI choice in settings where genotypic resistance testing cannot be routinely performed.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Additional files. Description of the 29 patients with baseline DRV RAMs.

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