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Emerging resistance mutations in PI-naive patients failing an atazanavir-based regimen (ANRS multicentre observational study)

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Background: Atazanavir is a PI widely used as a third agent in combination ART. We aimed to determine the prevalence and the patterns of resistance in PI-naive patients failing on an atazanavir-based regimen.

Methods: We analysed patients failing on an atazanavir-containing regimen used as a first line of PI therapy. We compared the sequences of reverse transcriptase and protease before the introduction of atazanavir and at failure [two consecutive viral loads (VLs) >50 copies/mL]. Resistance was defined according to the 2014 Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS) algorithm.

Results: Among the 113 patients, atazanavir was used in the first regimen in 71 (62.8%) patients and in the first line of a PI-based regimen in 42 (37.2%). Atazanavir was boosted with ritonavir in 95 (84.1%) patients and combined with tenofovir/emtricitabine or lamivudine ($n = 81$) and abacavir/lamivudine or emtricitabine ($n = 22$). At failure, median VL was 3.05 $\log_{10}$ copies/mL and the median CD4+ T cell count was 436 cells/mm^3. The median time on atazanavir was 21.2 months. At failure, viruses were considered resistant to atazanavir in four patients (3.5%) with the selection of the following major atazanavir-associated mutations: I50L ($n = 1$), I84V ($n = 2$) and N88S ($n = 1$). Other emergent PI mutations were L10V, G16E, K20I/R, L33F, M36I/L, M46I/L, G48V, F53L, I54L, D60E, I62V, A71T/V, V82I/T, L90M and I93L/M. Emergent NRTI substitutions were detected in 21 patients: M41L ($n = 2$), D67N ($n = 3$), K70R ($n = 1$), L74I/V ($n = 3$), M184V/I ($n = 16$), L210W ($n = 1$), T215Y/F ($n = 3$) and K219Q/E ($n = 2$).

Conclusions: Resistance to atazanavir is rare in patients failing the first line of an atazanavir-based regimen according to the ANRS. Emergent NRTI resistance-associated mutations were reported in 18% of patients.

Introduction

Although many antiretroviral drugs have been developed, PIs remain the treatment of choice for HIV-1 therapy because their exceptionally high potency means that the emergence of antiretroviral resistance is rare.[1] Indeed when administered with low doses of ritonavir, PIs offer a high genetic barrier against the selection of drug-resistant variants of HIV and are therefore especially reliable options for patients for whom poor antiretroviral adherence is anticipated.[2,3] Based on high rates of discontinuation owing to adverse events among patients treated with atazanavir/ritonavir in ACTG 5257 (a randomized trial comparing the efficacy of atazanavir/

ritonavir-, darunavir/ritonavir- and raltegravir-based therapy),[4] atazanavir/ritonavir was reclassified as an 'alternative' to darunavir/ritonavir in the most recent iteration of the European[5] and French guidelines,[6] and in the Department of Health and Human Services (DHHS) guidelines in certain clinical situations.[7] However, boosted atazanavir combined with tenofovir/emtricitabine or tenofovir/lamivudine or abacavir/lamivudine remains recommended as the initial ART in pregnant women in DHHS perinatal guidelines.[8] Atazanavir nevetheless remains widely used as a third agent in combination ART. Indeed, atazanavir is a potent, well-tolerated, once-daily PI with a resistance profile that is generally different to that of other drugs in the same class.[9,10] Atazanavir boosted with ritonavir is more potent, with the ability to treat infection caused by resistant HIV-1 strains without causing significant toxicity.[11] Therefore, we aimed to determine emergent resistance mutations in the protease and reverse transcriptase (RT) genes and to describe mutational patterns of resistance in PI-naive patients failing an atazanavir-based regimen in a real-life clinical setting over a follow-up period of 10 years.

Methods

Between the years 2005 and 2015, a national multicentre observational retrospective study was conducted in France involving patients exhibiting virological failure with a boosted or unboosted atazanavir-based regimen. All 17 participating laboratories belong to the Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS) AC11 network. Patients were defined as failing on atazanavir/ritonavir or atazanavir when two consecutive >50 copies/mL HIV-1 viral loads (VLs) were measured. We analysed 190 genotypic tests from patients failing on an atazanavir-containing regimen used as a first line of PI therapy. Genotype results were available both at failure and before atazanavir or atazanavir/ritonavir initiation for 113 patients. In this group, we compared the sequences of RT and protease genes before the introduction of atazanavir and at failure. In accordance with the 2014 ANRS list,[12] a resistance mutation was considered to be selected if it was not present in previous genotypes. Drug resistance was defined according to the 2014 ANRS algorithm and the genotypic susceptibility score (GSS) of treatment at baseline was calculated on antiretrovirals currently available ($n = 18$) as follows: 1 for a susceptible drug, and 0 for a resistant or possibly resistant drug. Major atazanavir-associated resistance mutations—I50L, M84V, N88S—were defined according to the IAS-USA list.[13]

The following factors associated with selection of at least one PI resistance mutation were studied: age, gender, prior ART, subtypes, baseline HIV-1 VL, baseline CD4, associated antiretrovirals, baseline GSS, ART duration, first line of ART or first line of PI therapy, boosted atazanavir or not, and the presence of an RT M184I/V mutation at baseline.

Statistical analysis

Quantitative variables were summarized by means of median and IQR and discrete variables by sample size and percentage. Comparisons between groups were performed using either exact Fisher or Kruskal–Wallis tests. The analysis was done with SAS (version 9.4).

Results

Since 2005, from 17 ANRS centres we have recorded 3197 patients treated with an atazanavir-containing regimen used as the first line of PI treatment (associated with two NRTIs) (no available data for one centre). Among these patients, 558 (17.4%) experienced virological failure, and genotypic data at failure were available for 190 of them (34.1%). We studied the selection of resistance mutation in a group of 113 patients who had available genotypes both at baseline and at failure. In this group, atazanavir was used in the first regimen for 71 (62.8%) patients and in the first line of a PI-based regimen for 42 (37.2%) (Table 1). Atazanavir was boosted with ritonavir in 95 (84.1%) patients and combined with tenofovir/lamivudine or emtricitabine ($n = 81$, 71.7%) and abacavir/lamivudine or emtricitabine ($n = 22$, 19.5%). At failure, the median VL was 3.05 $\log_{10}$ copies/mL and the median CD4+ T cell count was 436 cells/mm^3. The median time on atazanavir was 22 months. Fifty-one percent of patients were infected with subtype B virus. Another 77 patients for whom only genotype at failure was available showed similar characteristics (data not shown).

Table 1. Characteristics of patients at failure ($N = 113$)

Male, *n* (%)	82 (72.6)
Age (years), median (IQR)	44 (37–52)
CD4+ cell count (cells/mm^3), median (IQR)	436 (289–639)
Plasma HIV-1 VL ($\log_{10}$ copies/mL), median (IQR)	3.05 (2.50–4.02)
Duration of ART (months), median (IQR)	25 (10–67)
Duration of atazanavir treatment (months), median (IQR)	22 (10–35)
Patients with boosted atazanavir, *n* (%)	95 (84.1)
Subtype B, *n* (%)	58 (51.3)
First line of ART, *n* (%)	71 (62.8)
First line of PI therapy, *n* (%)	42 (37.2)
NRTI backbone, *n* (%)	
abacavir/lamivudine or emtricitabine	22 (19.5)
tenofovir/lamivudine or emtricitabine	81 (71.7)
Baseline resistance or possible resistance to at least one NRTI, *n* (%)	20 (17.7)
M184I/V at baseline, *n* (%)	14 (12.4)

At failure, virus-selected resistance mutation to atazanavir/ritonavir was seen in 4/113 patients (3.5%) with the ANRS algorithm with the selection of major atazanavir-associated resistance mutations: I50L ($n = 1$), I84V ($n = 2$) and N88S ($n = 1$). Other emergent PI mutations (L10V, G16E, K20I/R, L33F, M36I/L, M46I/L, G48V, F53L, I54L, D60E, I62V, A71T/V, V82I/T, L90M and I93L/M) were selected in 15 patients (4 presenting major atazanavir-associated mutations). Emergent NRTI substitutions were seen in 21 patients: M41L ($n = 2$), D67N ($n = 3$), K70R ($n = 1$), L74I/V ($n = 3$), M184V/I ($n = 16$), L210W ($n = 1$), T215Y/F ($n = 3$) and K219Q/E ($n = 2$). In summary, we showed selection of NRTI and/or PI resistance mutations in viruses of 29 out of 113 patients (25.7%), whose characteristics are described in Table 2.

At failure, of the 190 available genotypes, 103 patients (54.2%) presented a virus with at least one PI resistance mutation, and 65 patients (34.2%) with at least one RT resistance mutation (23.8% and 19.3% with an NRTI and/or an NNRTI resistance mutation, respectively). The percentage of patients harbouring a virus resistant to atazanavir/ritonavir was 3.7% (which was in accordance with our result of 3.5% in 113 patients); no patients harboured a virus resistant to darunavir/ritonavir. The percentage of patients harbouring virus resistant to abacavir, tenofovir and lamivudine or emtricitabine was 4.3%, 0.5% and 21.5%, respectively.

Table 2. Therapeutic and virological characteristics of the 29 patients with emergence of resistance mutations

Patient	ART exposure	Time to failure (months)	VL at failure (copies/mL)	Emergent mutations at virological failure	
				RT	PI
1	first line	16.1	54		M36I, I62V
2	experienced	24.8	160	L74I	
3	first line	no data	234	M41L	
4[a]	experienced	46.6	125	M184V	
5	first line	33.8	415	D67N, K70R, M184V, L210W	
6	experienced	32.2	414	D67N, M184V	L33F, F53L, I54L, D60E, A71V, V82T, **I84V**, L90M
7	first line	39.8	479	M184I	
8	first line	10.8	501	M184I	
9	first line	6.0	562	M184I	
10	first line	34.2	636		M36L
11	first line	4.6	769	M184V	
12	first line	66.7	800	M184I	M36L
13	first line	2.9	831	M184V, M184I	
14	experienced	44.6	921	K219Q	A71V
15	experienced	45.8	1000	L74V	L10V, A71V
16	experienced	6.7	1086	M184V	
17	first line	9.8	1949	M184I	I93M
18	experienced	8.0	2085	M184V	
19	first line	23.3	2155		I62V
20	experienced	29.8	2357		M36L, V82I
21	experienced	48.4	3810	M184V	
22	experienced	21.2	4603	L74V, M184V	
23	experienced	20.5	5790		D60E
24	first line	23.3	6684	M184V, T215F	L33F, **I50L**
25[a]	experienced	65.7	6948		G16E
26	first line	27.6	9135		K20I
27	first line	6.2	37 000	D67N, M184V, T215F, K219E	K20R, M36I, G48V, I54L I62V, A71V, G73T, V82T, **I84V,** I93L
28	experienced	22.8	43 000	M41L, T215Y	
29	first line	6.5	232 200		M46I, A71T, **N88S**

Bold formatting indicates major atazanavir-associated mutations according to the IAS-USA list,[13] and underlining indicates A71V.
[a]Received unboosted atazanavir.

Factors associated with the selection of at least one PI resistance mutation (according to the 2014 ANRS algorithm) were having taken zidovudine ($P = 0.05$) and didanosine ($P = 0.02$) in previous treatment, and having taken abacavir associated with an atazanavir-containing regimen ($P = 0.02$). Having M184I/V at baseline did not affect the selection of a PI resistance mutation at failure.

Discussion

Our retrospective study analysed over 10 years of resistance in PI-naive patients failing on an atazanavir-based regimen. In this French cohort, virological failure, defined as two consecutive VLs >50 copies/mL, occurred with 17.4% of patients, confirming previous findings from atazanavir/ritonavir clinical trials.[14–16]

At failure, 25.7% of our patients presented a virus with NRTI and PI resistance mutations, but only 3.5% selected major PI mutations to atazanavir/ritonavir: I50L ($n = 1$), I84V ($n = 2$) and N88S ($n = 1$); 23.8% selected NRTI mutations (M184I/V, $n = 16$).

In the CASTLE clinical trial,[15] 6% of virological failures occurred in the atazanavir/ritonavir arm. Two patients taking atazanavir/ritonavir showed emergence of non-polymorphic PI resistance mutations on treatment. In one patient, the N88S substitution, associated with atazanavir resistance, emerged. The second patient receiving atazanavir/ritonavir with emergent non-polymorphic PI resistance mutations had six PI mutations at baseline, and rebounded rapidly at week 24 after suppression to an HIV RNA of <50 copies/mL.

Our proportion of virological failure (17.4%) is also concordant with an observational European cohort based on 517 patients.[17] Indeed, in that earlier study 85 (16.4%) patients presented virological failure and 9 of them (22.4%) had treatment-emergent minor PI resistance mutations, but contrary to our study no patient had treatment-emergent major PI resistance mutations. The ACTG 5257 study[4] showed similar results: in the atazanavir arm, 95 patients (16%) presented a virological failure without PI resistance detected, and NRTI resistance was detected in 8 patients (10.8%).

Our study focused on mutation both at failure and before atazanavir or atazanavir/ritonavir initiation, and permitted both described and undescribed mutations to be highlighted. We showed the occurrence of new selected substitutions (A71V/T) in 5 patients among the 29 virological failures with resistance. After several months of culture, in atazanavir-resistant variants, the mutation A71V was selected *in vitro* in association with other mutations.[18] In the CASTLE study, one patient in the atazanavir/ritonavir group had major and minor PI emerging substitutions and ultimately failed at week 67. This patient had baseline T12A/S, I13I/V, M36I, N37D, I62V, L63P, A71A/T, I72V and I93L, with an atazanavir fold change of 0.78.[14]

In an unplanned atazanavir-based treatment interruption, one A71V and one A71I were selected among emergent resistance mutations.[19]

In conclusion, development of atazanavir resistance is low (3.5%) in patients failing the first line of an atazanavir-based regimen, and all patients remained susceptible to darunavir/ritonavir. In this population of naive and NRTI-experienced patients, emergence of NRTI resistance in 14% of patients is not negligible. Evidence of new selected substitutions (A71V/T) in the protease gene with atazanavir/ritonavir-based ART has enabled the algorithms for the interpretation of French ANRS atazanavir/ritonavir resistance to be updated to 2015.[12]

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