

Effectiveness of Antiretroviral Regimens Containing Abacavir with Tenofovir in Treatment-Experienced Patients: Predictors of Virological Response and Drug Resistance Evolution in a Multi-Cohort Study

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Abstract

Background: In treatment-naïve patients, a combination antiretroviral therapy (cART) containing tenofovir (TDF) and abacavir (ABC) with lamivudine leads to unacceptably high virological failure rates with frequent selection of reverse transcriptase mutations M184V and K65R. We explored the efficacy of at least 16 weeks of ABC + TDF-containing cART regimens in 307 antiretroviral-experienced HIV-1-infected individuals included in observational databases.

Methods: Virological failure was defined as an HIV RNA > 400 copies/ml after at least 16 weeks of treatment. Patients had received a median of three prior cART regimens. Of these, 76% concomitantly received a potent or high genetic barrier regimen (with at least one protease inhibitor [PI]) or non-nucleoside reverse transcriptase inhibitor or thymidine analogue) while a third non-thymidine nucleoside analogue was used in the remaining patients.

Results: The 1-year estimated probability of virological failure was 34% in 165 patients with HIV RNA > 400 copies/ml at ABC + TDF regimen initiation. Independent predictors of virological failure were the absence of a potent or high genetic barrier cART, the higher number of cART regimens experienced, and the use of a new drug class. In the subset of 136 patients for whom there were genotypic resistance test results prior to ABC + TDF initiation, the virological failure (1-year estimated probability 46%) was independently predicted by the higher baseline viral load, the concomitant use of boosted PI, and the presence of reverse transcriptase mutation M41L. In 142 patients starting ABC + TDF therapy with HIV RNA ≤ 400 copies/ml, virological failure (1-year estimated probability 17%) was associated only with the transmission category. In a small subset of subjects for whom there were an available paired baseline and follow-up genotype (n = 28), the prevalence of most nucleoside analogue reverse transcriptase inhibitor resistance mutations decreased, suggesting a possible low adherence to treatment. No selection of K65R was detected.

Conclusion: The virological response to ABC + TDF-containing regimens in this moderately-to-heavily treatment-experienced cohort was good. Higher viral load and the presence of M41L at baseline were associated with worse

virological responses, while the concomitant prescription of drugs enhancing the genetic barrier of the regimen conveyed a reduced risk of virological failure.

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Introduction

The advent of potent combination antiretroviral therapy (cART) has dramatically changed the prognosis of HIV-1 infection and AIDS [1]. Not every combination of antiretroviral drugs can be used, however, because of drug–drug interactions, limited potency, or toxicity issues. According to the most updated guidelines [2], the first-line cART regimens of choice are based on two nucleoside analogue reverse transcriptase inhibitors (NRTIs), often referred to as the “backbone”, and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor with ritonavir boosting (PI/r). The case for the

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The Appendix provides the names of other members of the MASTER cohort.

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use of co-formulated two-NRTI backbones, including tenofovir (TDF) plus emtricitabine (FTC) and abacavir (ABC) plus 3TC is based on considerations of convenience and efficacy.

The choice of an optimal NRTI backbone can be particularly challenging because of the complex interplay between cross-resistance and potential hypersusceptibility as well as toxicity issues within this class. For several reasons, some NRTI-based combinations are contraindicated [3–6]. ABC and TDF are among the latest approved NRTIs. In an estimate on the impact of newly approved drugs on the AIDS-free survival of patients between 1998 and 2001, the use of ABC was a predictor of therapeutic success, equivalent to the use of lopinavir/ritonavir [7]. In the same study, TDF was also associated with a lower risk of clinical progression, although its importance was probably underestimated due to the low number of patients treated at that time. The potency and efficacy of TDF were subsequently well established [8, 9].

Both ABC and TDF are also well tolerated, particularly in the long term, allow for once-daily administration, are active against a number of NRTI-resistant strains, and select for a limited number of NRTI-resistance mutations [10–12]. Despite this, the Tonus trial, which studied the ABC + 3TC + TDF combination in 38 patients in a once-daily regimen, had to be stopped prematurely because of poor results: 12 (33%) of 36 enrolled patients experienced virologic failure by week 24 [13]. A number of reasons have been suggested to explain this poor outcome, with the strongest evidence pointing to the low genetic barrier to resistance of this association [14, 15].

The aim of our study was to investigate the efficacy of the ABC + TDF combination as part of cART regimens in treatment-experienced patients and as predictors of virological failure of this NRTI association.

Patients and Methods

Study Population and Data Collection

Patients included in this study were selected from three Italian cohorts: the clinical cohort of the Department of Infectious Diseases of the Catholic University in Rome, Italy (UCSC), the ARCA cohort (<http://www.hivarca.net>), a multi-center database of HIV-infected individuals undergoing drug resistance testing [16], and the MASTER cohort, a multi-center clinical database [17]. Eligible individuals had to be treatment experienced, have baseline viral loads and complete treatment histories available, and be able to start any ABC + TDF-containing cART (i.e., the combination of at least three antiretroviral drugs) and continue it for at least 16 weeks with virological follow-up data available. Pre-baseline and follow-up resistance test results, treatment history, and HIV RNA and CD4 outcomes were retrieved from the individual databases and merged centrally. Drug resistance mutations were classified according to the IAS–USA table (version fall 2006; International AIDS Society–USA, San Francisco, CA) [18]. The genotypic susceptibility score (GSS) of drugs accompanying ABC and TDF was determined by the Stanford University HIV Drug Resistance Database (Stanford HIVDB) algorithm (ver. 5.0.0) [19]. The

interpretations provided were translated into numeric values; each drug in the regimen was assigned a susceptibility score ranging from 0 to 1. We arbitrarily chose to translate intermediate resistance into high-level resistance (score of 0), and potential low-level resistance into susceptible (score of 1). ABC + TDF-including regimens were defined as potent or having a high genetic barrier when a thymidine analogue or a NNRTI or a PI was present, as opposed to being defined as weak or possessing a low genetic barrier in the absence of these concomitant drugs when a non-thymidine analogue NRTI was used as the third drug.

Statistical Analysis

Viral load values were log-transformed before calculations. Continuous variables were compared with the Student's *t*-test, categorical variables with the χ^2 -test. The rates of virological failure, defined as an HIV RNA > 400 copies/ml after at least 16 weeks of continuous ABC + TDF treatment, were analyzed by survival analysis, using an intention-to-treat criterion and ignoring the changes of the concomitant drugs. For patients not meeting the virological failure definition, follow-up was censored at the interruption of TDF and/or ABC or at the last available viral load measure. One-year estimates of virological failure were calculated by the Kaplan–Meier method. Predictors of virological failure were analyzed using univariable and multivariable Cox regression. All analyses were performed using SPSS ver. 13.0 (SPSS, Chicago, IL).

Results

Patient Characteristics and Antiretroviral Exposure

307 were eligible for inclusion in the study, 69% of whom were males, and the median age of the patient cohort at baseline was 41 years (interquartile range [IQR] 38–45 years). HIV transmission modes were heterosexual contacts in 32% of cases, male homosexual contacts in 16% of cases, and iv drug use in 40% of cases. 39% of individuals had a history of previous AIDS-defining events. The median CD4+ T cell count was 373 (IQR 232–608) cells/ μ l, and the median plasma HIV-1 RNA was 2.93 (IQR 1.69–4.34) \log_{10} copies/ml. 54% of individuals were hepatitis C virus (HCV) antibody positive. Overall, patients had experienced a median of three cART regimens (IQR 3–4), and 30% had experienced mono-dual NRTI therapy prior to cART. Previous drug exposure included PIs (53% of patients), NNRTIs (21%), TDF (24%), and ABC (25%). Concomitantly with ABC and TDF, 35% of patients received boosted PIs, 5% received unboosted PIs, 18% received NNRTIs, 25% received thymidine analogues, 44% received lamivudine or emtricitabine, and 8% received didanosine (ddl). A new drug class was used in 11% of cases. 76% of the patients received “potent or high genetic barrier” regimens, defined as the concomitant use of a PI and/or an NNRTI and/or a thymidine analogue, while 24% received “weak or low genetic barrier” regimens, defined as the concomitant use of a non-thymidine nucleoside analogue. Patient characteristics of the various analysis subgroups are summarized in table 1.

Parameters	Patients with baseline HIV RNA \leq 400 copies/ml (n = 142)	Patients with baseline HIV RNA > 400 copies/ml (n = 165)	Patients with baseline resistance genotype (n = 136)
Male (%)	70	69	72
Risk factor IDU (%)	43	39	15
MSM (%)	15	13	39
Heterosexual (%)	28	33	33
Age, years (median, IQR)	41 (37–44)	41 (38–45)	42 (39–47)
Prior cART regimens (median, IQR)	3.5 (3–4)	3.5 (3–4)	3 (2–4)
Prior mono-dual NRTI treatment (%)	67	76	72
Prior TDF (%)	19	33	24
Prior ABC (%)	33	21	28
Associated regimen NNRTI (%)	16	18	24
Non-boosted PI (%)	4	6	4
Boosted PI (%)	42	38	37
Thymidine analogues (%)	32	29	21
Potent/high genetic barrier cART (%)	74	79	74
Use of a new class (%)	10	15	18
HIV RNA (log ₁₀ copies/ml, median, IQR)	1.69 (1.69–1.78)	4.19 (3.46–4.92)	3.43 (1.72–4.64)
CD4 counts (cells/mm ³ , median, IQR)	352 (199–545)	342 (203–511)	370 (215–654)
Prior AIDS (%)	21	16	23
Anti-HCV + serostatus (%)	44	52	55

IDU: Injecting drug users; MSM: men who have sex with men; IQR: interquartile range; cART: combination anti-retroviral therapy; NRTI: nucleosidic reverse transcriptase inhibitor; TDF: tenofovir; ABC: abacavir; NNRTI: non-nucleosidic reverse transcriptase inhibitor; PI: protease inhibitor; HCV: hepatitis C virus

Virological Failure Rates and Its Predictors in the Different Groups of Patients

81 virological failures (26.4%) were observed during a median follow-up of 284 days (IQR 180–515), resulting in a failure rate of 24.6 per 100 person-years of follow-up. Virological failure occurred in 33.3% (n = 55) of cases in the group of patients starting ABC + TDF regimens with an HIV RNA load > 400 copies/ml (n = 165), with a 1-year estimated probability of virological failure of 34%. The univariable analysis demonstrated an association between age and the use of a new drug class with a higher risk of virological failure, whereas the number of previous cART regimens experienced and the concomitant use of a boosted PI or of any potent or high genetic barrier cART were associated with a lower risk. The independent predictors of virological failure were potent or high genetic barrier cART, the number of cART regimens experienced, the use of a new drug class, and age (see Table 2).

In the subgroup of patients (n = 142) who were switched to the ABC + TDF regimen with a viral load < 400 copies/ml, virological failure occurred in 18.3% (n = 26) of cases, resulting in a 1-year estimated probability of viral rebound of 17.0%. The univariable analysis revealed that risk factor (sex vs others) and CD4 cell count at baseline were significantly associated with the risk of virological failure. The only independent predictor of virological failure was risk factor for HIV acquisition (see Table 3).

Details of HIV-1 Resistance Genotyping Results

The results of a genotypic resistance test performed prior to the initiation of the ABC + TDF-containing cART regimen was available for a subset of 136 patients. NRTI resistance mutations were present in 91% of these patients, NNRTI resistance mutations in 34%, and major PI resistance mutations in 65%.

A breakdown of the prevalence of individual NRTI resistance mutations and mutational groups is given in figure 1a. Among patients who experienced virological treatment failure with the ABC + TDF regimen, 31 had genotypic resistance test results at failure, of whom 28 also had a baseline genotypic test result. The 31 resistance genotypes at ABC + TDF regimen failure contained NRTI-resistance mutations (74% of cases), thymidine-associated mutations (TAMs; 56%: 31% type 1 and 25% type 2), K65R and K70E (each 3%), and L74V (9%), respectively. The prevalence of individual mutations in the 28 patients with paired pre- and post-ABC + TDF genotypes is shown in figure 1b. None of the L74V and K70E mutations detected during follow-up were present in the baseline sample. The patient with a K65R mutation at follow-up did not have a baseline genotype, while neither of the baseline K65R cases were detected in the post-ABC + TDF sample (see Figure 1b). In this subgroup of patients with available genotypic resistance test results before baseline (n = 136), virological failure occurred in 50% (n = 68) of cases: the estimated 1-year probability of virological failure was 46%. Univariable analysis revealed

Parameters	Univariable analysis		Multivariable analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Use of a high genetic barrier or potent association	0.47 (0.26–0.85)	0.012	0.43 (0.22–0.84)	0.013
Age (per year more)	1.05 (1.02–1.08)	0.003	1.04 (1.01–1.08)	0.010
Prior use of mono-dual therapy	1.56 (0.80–3.08)	0.20	–	–
Sex	1.14 (0.63–2.60)	0.68	–	–
Risk factor (sexual vs other)	1.15 (0.89–1.50)	0.30	–	–
Prior use of TDF	1.44 (0.80–2.50)	0.20	–	–
Prior use of ABC	0.55 (0.26–1.16)	0.12	–	–
Number of cART regimens experienced	0.74 (0.59–0.94)	0.01	0.63 (0.53–0.90)	0.006
Use of new class of drugs	2.49 (1.35–4.60)	0.004	3.70 (1.83–7.44)	<0.001
Concomitant use of NNRTI therapy	1.18 (0.61–2.28)	0.63	–	–
Non-boosted PI therapy	0.87 (0.50–1.53)	0.63	–	–
Boosted PI therapy	0.54 (0.30–0.98)	0.04	–	–
Baseline CD4 (per 100 cells/mm ³ higher)	0.90 (0.77–1.02)	0.11	–	–
Viral load at baseline (per log ₁₀ higher)	1.23 (0.89–1.70)	0.21	–	–

HR: Hazard ratio; CI: confidence interval

Parameters	Univariable analysis		Multivariable analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Use of a high genetic barrier or potent association	1.79 (0.75–4.26)	0.19	–	–
Age (per year more)	0.94 (0.88–1.00)	0.07	–	–
Prior use of mono-dual therapy	1.60 (0.53–4.84)	0.40	–	–
Sex	0.56 (0.23–1.37)	0.21	–	–
Risk factor (sexual vs other)	0.41 (0.21–0.79)	0.008	0.44 (0.21–0.90)	0.02
Prior use of TDF	0.77 (0.26–2.29)	0.64	–	–
Prior use of ABC	0.80 (0.27–2.42)	0.70	–	–
Number of cART regimens experienced	1.07 (0.88–1.31)	0.51	–	–
Use of new class of drugs	0.91 (0.31–2.70)	0.87	–	–
Concomitant use of NNRTI therapy	1.01 (0.30–3.47)	0.99	–	–
Non-boosted PI therapy	1.38 (0.56–3.35)	0.48	–	–
Boosted PI therapy	1.44 (0.50–4.11)	0.50	–	–
Baseline CD4 (per 100 cells/mm ³ higher)	1.11 (1.00–1.23)	0.05	1.08 (0.96–1.21)	0.17
Viral load at baseline (per log ₁₀ higher)	1.00 (0.99–1.00)	0.73	–	–

that the concomitant use of boosted-PI (inhibitor) therapy, the baseline viral load, the prior use of mono- or dual-NRTI therapy, prior ABC use, and the presence of the NRTI-resistance mutation M41L were significantly associated with the risk of virological failure. Other mutations considered in this analysis were those with a prevalence of > 5%, namely D67N, K70R, L74V, V118I, M184I/V, L210W, T215Y, and K219E/Q; none of these was shown to be associated with virological failure. The association with M41L withheld Bonferroni correction for multiple comparisons ($p = 0.033$). The independent predictors of virological failure were the concomitant use of boosted-PI (inhibitor) therapy, the presence of the NRTI-resistance mutation, M41L, and a higher baseline viral load (see Table 4).

Discussion

Abacavir and TDF are theoretically attractive drugs for use as a backbone in cART regimens because of their good general tolerability and low impact on lipid metabolism and lipodystrophy [20, 21]. They have also shown good activity in fixed-dose formulations with 3TC or FTC [22] and have proved to be useful in treatment-experienced patients [8, 9]. Moreover, the combined use of ABC and TDF as a backbone is also supported by the lack of interactions between the two drugs in *in vitro* studies [23, 24].

In our study of 307 antiretroviral-experienced patients, we observed a virological failure rate of 24.6 per 100 patient-years of follow-up. This is in contrast with the

The more favorable outcome in our observational study of ABC + TDF-containing regimens is likely due to the higher potency and genetic barrier of the regimens that were used: 76% of the patients concomitantly received a PI and/or a NNRTI and/or a thymidine analogue. These combinations were likely more effective in preventing the development of drug resistance and, therefore, virological failure than the above-mentioned three-NRTI regimens. The incidence of virological failure rate was 34.9 per 100 patient-year of follow-up in the group of patients receiving weak or low genetic barrier regimens. This incidence is higher than that in the group of patients receiving a potent/high genetic barrier regimen (21.6 per 100 patient-year, log rank $p = 0.033$) and similar, although a little low, to the results of the TONUS and ESS3009 trial. Among the reasons investigated to explain their data, the investigators of the Tonus trial emphasized the fact that just two point mutations (M184V and K65R), each selected by at least two of the NRTIs used, were required to produce resistance to the whole regimen [13–15]. Another reason for the high rate of virological failure may have been the physiological compartmentalization of nucleoside and nucleotide analogues and the subsequent diversity of distribution and activation among the CD4 cells [13]. If this were the case, the use of another drug class or of a thymidine analogue with a different activation pathway could have prevented this phenomenon and, ultimately, the viroimmunological failure in our patients. These hypotheses are supported by the fact that the use of a “potent” association was an independent negative predictor of virological failure in our study.

The cART regimens performed even better in the subset of patients who switched to ABC + TDF while on a successful cART regimen, with just 17% of failures estimated at the 1-year follow-up. This is not an unexpected result because lower potency is generally required for simplification regimens, and the performances of several regimens are generally better in such settings. In the subjects with a viral load > 400 copies/ml, the use of a new drug class resulted in a higher risk of failure. We interpret this result as a possible consequence of channeling bias. Patients receiving a new drug class are probably those with a higher degree of present or past drug resistance.

In the subgroup of 136 patients whose genotypic resistance test results were available at or before baseline, the NRTI resistance mutation M41L was found to be independently predictive of virological failure. This is not an unexpected finding, given the deleterious effect of this type 1 TAM on the *in vivo* activity of both ABC and TDF [11, 26] as well as on that of ddI and thymidine analogues [27]. It is interesting to note that the ABC + TDF-failing patients selected a low proportion of isolates containing the K65R mutation. The K65R mutation, which is typically selected by TDF and more rarely by ABC and ddI

[10, 25], was present in two samples at baseline and reverted in both cases; it was also detected at failure in only one patient (3%) who was not tested for drug resistance at baseline. On the other hand, the NRTI resistance mutation L74V, typically selected by ABC and ddI, was found in 9% of cases at failure, while it was absent at baseline. These findings may be justified by the high prevalence of TAMs at treatment initiation: these show an antagonistic effect to the selection of K65R [12], while the selection of L74V is not affected. Although the comparison was possible in a limited number of cases, there was an overall reduction of NRTI resistance mutations in the follow-up genotype compared with the baseline genotype. This may have been related to the potency of both the backbone and the accompanying drugs in the regimen as well as derived from a low adherence to therapy in these patients failing treatment.

In conclusion, the results of this study indicate that ABC + TDF can be an effective NRTI backbone in treatment-experienced individuals if associated with an appropriate “third drug”, such as a thymidine analogue or a drug belonging to another drug class, and if a previously selected M41L resistance mutation is absent.

Appendix

Italian MASTER Cohort: G. Carosi (Chair), M. Puoti, C. Torti, E. Quiros-Roldan, G. Paraninfo, S. Casari, G. Cristini, F. Castelnuovo, I. El Hamad (Brescia); A. Antinori, G. Antonucci, A. Ammassari (Rome); A. Angarano, A. Saracino (Foggia); R. Cauda, A. De Luca (Rome); A. D’Arminio Monforte, P. Cicconi (Milan); F. Mazzotta, S. Lo Caputo, N. Marino (Florence); L. Minoli, R. Maserati, S. Novati, C. Tinelli (Pavia); F. Ghinelli, L. Sighinolfi (Ferrara); G. Pastore, N. Ladisa (Bari); T. Quirino (Busto Arsizio); F. Suter, F. Maggiolo (Bergamo); G. Carnevale, A. Pan (Cremona); A. Gori (Monza).

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