

M184V selection in suppressed subjects at week 24 may not be associated with treatment outcome (NZTA4002)

Introduction

Drug resistance can be one of the main causes of virologic failure to antiretroviral therapies in HIV-1 infected individuals. Results from two studies demonstrate that key resistance mutations to indinavir or zidovudine were infrequent in subjects failing triple therapy^{1,2}. In contrast, resistance to lamivudine (3TC) is common in subjects failing antiretroviral therapies such as Combivir® plus indinavir and Combivir® plus abacavir^{2,3}.

We recently presented data on protease (PRO) and reverse transcriptase (RT) sequences for 47 treatment-naïve subjects enrolled in NZTA4002 (see below) who experienced virologic failure⁴. Confirming the data described above, thymidine analog mutations occurred in only 4% of virologic failures, while M184V, the substitution conferring 3TC resistance, occurred in 51% of the subjects experiencing virologic failure.

Many investigators have asked whether the M184V mutation is the cause of failure with antiretroviral therapies that include 3TC, or if this mutation is a marker of ongoing, even if extremely low, viral replication in the presence of 3TC.

In order to determine if the selection of M184V during virologic suppression was predictive of subsequent treatment failure, we attempted to sequence the reverse transcriptase of all of the subjects enrolled in NZTA4002 who were virologically suppressed at 24 weeks.

Methods

NZTA4002 was a multi-center, randomized, controlled, open-label study in which 302 antiretroviral therapy-naïve, HIV-1-infected adults with screening plasma HIV-1 RNA $\geq 5,000$ copies/mL and CD4+ lymphocyte counts ≥ 50 cells/mL were stratified by screening plasma HIV-1 RNA \leq or $>50,000$ copies/mL, and then randomized to receive one of two antiviral therapies for 64 weeks:

- Triple Therapy: Combivir® (3TC 150 mg/ZDV 300 mg) bid plus nelfinavir 750 mg tid, or
- Quadruple Therapy: Combivir® bid, amprenavir (APV) 1200 mg bid plus abacavir 300 mg bid.

Plasma HIV-1 RNA was determined using the NucliSens® assay (Organon Teknika), with a 40-copies-per-milliliter lower limit of quantitation. Virologic response was defined as ≤ 120 copies/mL by Week 24. Virologic failure was defined as the inability to respond, i.e., HIV-1 RNA ≤ 120 copies/mL by Week 24 or a confirmed viral rebound >120 copies/mL following virologic response. Treatment failure included subjects who failed virologically, met a protocol-defined toxicity endpoint, or withdrew early from the study for any reason.

The Patient Medication Adherence Questionnaire (PMAQ Version 1.1) was used to assess self-reported medication adherence. In the PMAQ, subjects indicated on a 5-point scale the number of days in which all doses of a medication were missed during the previous 4 weeks, separately for nucleoside reverse transcriptase inhibitors (NRTIs) and for protease inhibitors (PIs). Adherence data were collected at study weeks 4, 12, 24, 48, and 64.

For this genotypic analysis, protease and reverse transcriptase sequences were obtained from plasma HIV-1 RNA in responders at Week 24. Sequencing was performed using the TruGene™ HIV-1 assay (Visible Genetics Inc)⁵.

To examine the impact of the M184V mutation, we explored the association with treatment regimen (Quadruple or Triple) and subjects' status in the study (success or failure). Fisher's exact test was used to compare proportions from 2 x 2 frequency tables. Exact confidence intervals for the difference in proportions were constructed using StatXact™. Logistic regression analysis was performed using SAS. All computations were performed on a UNIX computer platform.

Results

A total of 179 subjects (103 enrolled in the Triple Therapy and 76 in the Quadruple Therapy) were virologic responders (<120 cp/mL on or before Week 24) and eligible for analysis. Sequencing was successfully performed on the samples from 119 subjects: 71 (69%) on Triple Therapy and 48 (63%) on Quadruple Therapy. Of these, 61 (84%) of Triple Therapy subjects and 40 (81%) of Quadruple Therapy subjects, respectively, had HIV-1 RNA <40 cp/mL, while 8 (11%) and 15% for the T and Q regimens, respectively, had HIV-1 RNA between 40 and 120 cp/mL. The results below are based on the genotypic analysis of samples from these 119 subjects.

All but one subject did not have M184V at baseline, but M184V was identified in 21.8% (26/119) of subjects at Week 24. A summary of the baseline and Week 24 mutations are provided in Tables 1 and 2.

Table 1 • Summary of baseline mutations for responders at Week 24

Triple Therapy (n = 71)		Quadruple Therapy (n = 48)			
	Frequency	Percent	Frequency	Percent	
RT mutations					
L210W	3	4.2	L210W	0	
M184V	0		M184V	1	2.1
T215F	0		T215F	1	2.1
T215S/C	1	1.4	T215S/C	0	
M41L	3	4.2	M41L	2	4.2
PRO mutations					
D30N/D	0		D30N/D	0	
I50V	0		I50V	0	
L90M/L	0		L90M/L	1	2.1

*Mutations are not all inclusive

Fifteen (21%) subjects on Triple Therapy had M184V at Week 24 and 11 (23%) subjects on Quadruple Therapy had M184V at Week 24. There was no significant association between treatment regimen and having M184V at Week 24 ($P = 0.83$).

Of 26 subjects having M184V at Week 24, 12 (46%) eventually experienced treatment failure; of 93 subjects without M184V at Week 24, 28 (30%) eventually experienced treatment failure (Figure 1). This difference (16%) was not statistically significant ($P = 0.16$, 95% CI from -6% to 41%).

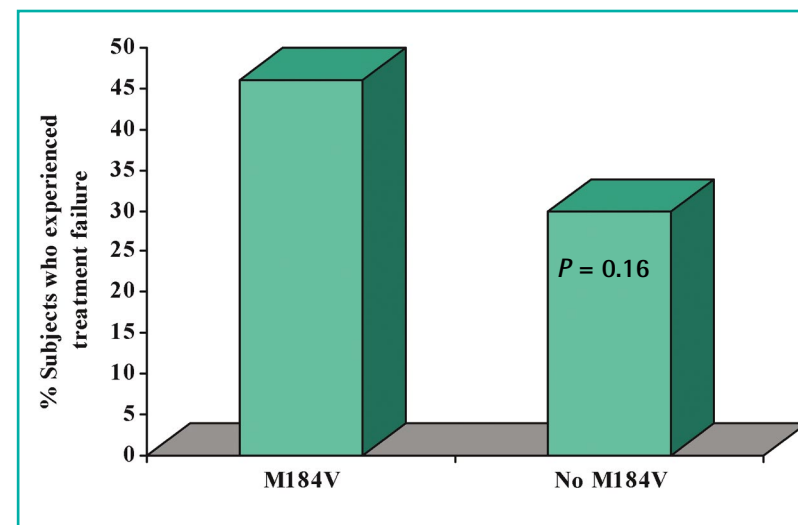
Table 2 • Summary of Week 24 mutations for responders

Triple Therapy (n = 71)		Quadruple Therapy (n = 48)			
	Frequency	Percent	Frequency	Percent	
Mutation Pattern*					
Wild type at RT	52	73.2	Wild type at RT	32	66.7
Wild type at PRO	26	36.6	Wild type at PRO	9	18.8
Unable to amplify PRO	1	1.4	Unable to sequence PRO region	1	2.1
RT mutations					
M184M/V	15	21.1	M184V	11	22.9
M41L	2	2.8	M41L	3	6.2
D67N	2	2.8	D67N	1	2.1
K70R	1	1.4	K70R	2	4.2
L210W	2	2.8	L210W	0	
T215Y/F	6	8.4	T215Y/F	3	6.2
K219K/Q/E	1	1.4	K219K/Q/E	3	6.2
PRO mutations					
D30N/D	1	1.4	D30N/D	0	
I50V	0		I50V	0	
I54V	1	1.4	I54V	0	
L63P/L	29	40.8	L63P/L	25	52.1
L90L/M	3	4.2	L90L/M	6**	12.5

*Mutations are not all inclusive

**Three subjects originally randomized to amprenavir switched to nelfinavir by Week 24

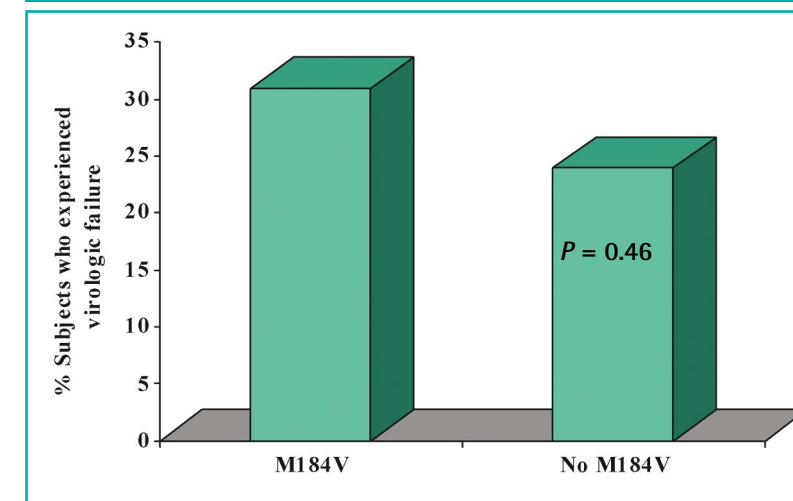
Figure 1 • M184V at Week 24 and treatment failure



When only virologic failures are considered, 31% (8/26) of subjects with M184V at Week 24 eventually experienced virologic failure, while for those without M184V at Week 24, 24% (22/93) eventually experienced virologic failure (difference: 7%; $P = 0.46$, 95% CI from -13% to 32%) (Figure 2).

From these analyses, there was not enough evidence to establish a statistically significant association between presence of the M184V in virologically suppressed subjects at Week 24 and eventual treatment or virologic failure.

Figure 2 • M184V at Week 24 and virologic failure



We also examined the association of M184V at Week 24 with baseline HIV-1 RNA. Twenty-four percent (17/70) of subjects with baseline HIV-1 RNA $<50,000$ copies/mL had M184V, while 18% (9/49) of subjects with baseline HIV-1 RNA $>50,000$ copies/mL had M184V. There was no statistically significant association between baseline HIV-1 RNA and the presence of M184V at Week 24 ($P = 0.50$) (Table 3). For those subjects enrolled in the Triple Therapy, 17% (8/48) with baseline HIV-1 RNA $<50,000$ copies/mL had M184V, and 30% (7/23) with baseline HIV-1 RNA $>50,000$ copies/mL had M184V. For those subjects enrolled in the Quadruple Therapy, 41% (9/22) with baseline HIV-1 RNA $<50,000$ copies/mL had M184V and 8% (2/26) with baseline HIV-1 RNA $\geq 50,000$ copies/mL had M184V.

Table 3 • Presence and absence of M184V at Week 24 compared to baseline HIV-1 RNA

	Baseline HIV-1 RNA	
	$<50,000$ copies/mL (n=70)	$\geq 50,000$ copies/mL (n=49)
M184V mutation	24% (17/70)	18% (9/49)
No M184V mutation	76% (53/70)	82% (40/49)

No association was found between self-reports of adherence at Weeks 4, 12, and 24 and the presence of the M184V mutation at Week 24 ($P = 0.35$, 0.78, and 0.94), nor was there any association between treatment and presence of the M184V mutation at Week 24 ($P = 0.83$). Self-reports of missing therapy after Week 24 were not associated with treatment failure ($P = 0.66$).

Discussion

3TC is a very potent antiretroviral drug which selects quickly and efficiently for the M184V mutation. This mutation causes approximately a hundred-fold increase in resistance to 3TC. Subjects who experience virologic failure on antiviral regimens including 3TC commonly express virus with the M184V mutation. However, it is unlikely that M184V is the cause of this failure since subjects with viruses containing the pre-existing M184V mutation respond at least as well to the addition of a third drug to their regimen as do subjects without pre-existing M184V⁶. In addition, clinical data have demonstrated that subjects whose virus contains the M184V mutation experience greater virologic suppression in the presence of antiretroviral therapy than subjects whose virus does not contain the M184V mutation⁷.

The majority of HIV-1 genotypic data has been obtained from subjects experiencing virologic failure due to the difficulties of sequencing samples from fully suppressed subjects. Recently, we described a technology that allows for sequencing of HIV-1 from subjects with low viremia⁸. Using this technology, 69% (119/179 = 66%) of samples from the NZTA4002 study with ≤ 120 copies/mL were sequenced and 22% of them were found to have the M184V mutation. There was no significant association between expressing M184V while virologically suppressed at Week 24 and exhibiting treatment or virological failure.

The M184V mutation appears to be a marker of 3TC therapy, rather than a cause of virologic failure of antiretroviral therapy regimens that include 3TC. As the present analysis suggests, in this study subjects with the M184V mutation at the time they are virologically suppressed are no more likely to experience subsequent virologic failure than those patients without the M184V mutation. Therefore it cannot be concluded that, the development of the M184V mutation was the cause of eventual virologic failure. In addition to the selection of resistant viruses, other potential causes of failure may include (1) incomplete adherence, (2) changes in bioavailability (e.g., decreased absorption), (3) drug-drug interactions, (4) changes in active drug supplies (decreased activation or active exocytosis), and (5) changes in host immune status. Deletion of 3TC from an antiretroviral regimen due to the presence of the M184V mutation denies the subject the benefits of M184V: reversal of zidovudine and d4T resistance⁹, increased fidelity (and resulting delay in accumulation of additional resistance mutations)⁹, and decreased fitness¹⁰.

Conclusions

- Nearly twenty-two percent of subjects virologically suppressed on Triple or Quadruple Therapy including 3TC express virus with the M184V mutation.
- The M184V mutation is not associated with subsequent virologic failure or treatment failure in subjects initially suppressed by Triple or Quadruple Therapy.
- There was no association between self-reported adherence and the presence of the M184V mutation in virologically suppressed subjects.
- Evolution of resistance mutations occurs in subjects despite highly suppressive therapy.

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