

HIV-1 reverse transcriptase sequence in plasma and cerebrospinal fluid of patients with AIDS dementia complex treated with Abacavir

E. Randall Lanier, Glenn Sturge^a, Daniel McClernon, Stephen Brown^b, Mark Halman^c, Ned Sacktor^d, Justin McArthur^e, Joseph H. Atkinson^e, David Clifford^f, Richard W. Price^g, David Simpson^h, Gabriel Torresⁱ, Jose Catalan^j, Karen Marder^k, Chris Power^l, Colin Hall^m, Carmen Romeroⁿ and Bruce Brew^o

Objective: To assess HIV-1 RNA levels and the relationship between HIV-1 reverse transcriptase (RT) genotype from plasma and cerebrospinal fluid (CSF) during treatment with abacavir (Ziagen, ABC) or placebo in combination with stable background therapy (SBG) in subjects with AIDS dementia complex (ADC) (study CNA3001).

Design: One-hundred and five HIV-1 infected adults with ADC were randomized to receive either ABC (600 mg twice daily) or ABC-matched placebo (twice daily) in addition to SBG for 12 weeks.

Methods: Plasma and CSF were collected for population sequencing at baseline and week 12 (CSF optional). Sequences were analyzed for mutations associated with resistance to nucleoside reverse transcriptase inhibitors (NRTI).

Results: Sixty out of sixty-seven subjects with baseline plasma HIV-RT sequence data harbored virus with ≥ 1 NRTI-associated mutations; 50 out of 67 had the M184V mutation. At week 12, more subjects in the ABC group had plasma HIV-1 RNA ≤ 400 copies/ml than the SBG group (46% versus 13%, $P = 0.002$). Non-response to ABC was associated with multiple baseline zidovudine (ZDV)/stavudine (d4T)-associated mutations. Baseline RT mutation patterns differed in 14 out of 21 (67%) paired samples from plasma and CSF. Four subjects experienced $> 1 \log_{10}$ copies/ml reductions in CSF HIV-1 RNA, two in the absence of reductions in plasma HIV-1 RNA and two with undetectable plasma HIV-1 RNA at baseline.

From the From Glaxo Wellcome, Research Triangle Park, North Carolina, ^{a,b}TRP/DAIDS/NIAID/NIH, ^aBethesda, Maryland and ^bAIDS Research Alliance, West Hollywood, California, USA, the ^cDepartment of Psychiatry, Saint Michael's Hospital, Toronto, Ontario, Canada, ^dJohns Hopkins University School of Medicine, Department of Neurology, Baltimore, Maryland, the ^eHIV Neurobehavioral Research Centre, San Diego, California, the ^fWashington University Medical Centre, Department of Neurology, St. Louis, Missouri, ^gSan Francisco General Hospital, San Francisco, California, ^hNeurophysiology, Mount Sinai Medical Centre, ⁱSt. Vincent's Hospital, New York, USA, ^jImperial College, Psychological Medicine Unit, London, UK, ^kColumbia University, Sergievsky Center, New York, USA, ^lSection of Neurology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, ^mUniversity of North Carolina School of Medicine, Department of Neurology, Chapel Hill, North Carolina, USA, ⁿGlaxo Wellcome, Clinical Research Department, Madrid, Spain, ^oDepartment of Neurology and Centre for Immunology, National Centre in HIV Epidemiology and Clinical Research, St Vincent's Hospital, Sydney, Australia.

Requests for reprints to: E. Randall Lanier, Glaxo Wellcome, 5 Moore Drive, Research Triangle Park, NC 27709, USA.

Received: 1 June 2000; revised: 21 December 2000; accepted: 9 January 2001.

Conclusions: Substantial decreases in plasma and CSF HIV-1 RNA following addition of ABC were not precluded by baseline HIV-1 NRTI-associated mutations, including the M184V mutation, but non-responders commonly harbored multiple ZDV/d4T-associated mutations. HIV-1 RNA responses and RT genotype appear to be discordant between CSF and plasma in some subjects. © 2001 Lippincott Williams & Wilkins

AIDS 2001, 15:747–751

Keywords: HIV-1, reverse transcriptase, genotype, cerebrospinal fluid, HIV-1 RNA, abacavir

Introduction

Abacavir (ABC, Ziagen) is a uniquely activated HIV reverse transcriptase (RT) inhibitor/substrate that produces marked and sustained reductions of plasma HIV-1 RNA [1,2]. ABC had previously been shown to cross into the cerebrospinal fluid (CSF; 10–25% of plasma levels) and to have anti-HIV activity in all cell types tested, including monocytes and macrophages [2,3]. These properties suggested that ABC may be a useful agent as part of a combination regimen for the treatment of AIDS dementia complex (ADC) and led to the initiation of study CNA3001. The primary efficacy measure was changes in neuropsychological performance (reported separately); the study also evaluated the relationship between plasma and CSF HIV-1 RT-genotype in subjects with ADC treated with ABC-containing therapy.

Methods

Study design and subject population

CNA3001 was a phase III randomized, placebo controlled, double-blind study conducted at 13 sites in Australia, Canada, the USA and the UK. Enrollment was restricted to HIV-1 seropositive adults, aged 18 to 65 years with Stage 1 or 2 (mild to moderate) ADC according to the MSK scale [4], who had received the same antiretroviral treatment for a minimum of 8 weeks prior to study entry. In addition to their current antiretroviral treatment, subjects were randomized to receive either ABC (600 mg twice daily) or ABC-matched placebo (twice daily) for 12 weeks.

Plasma and CSF HIV-1 RNA

Plasma HIV-1 RNA was evaluated by Amplicor HIV-1 MONITOR v1.0 (Roche Molecular Systems, Somerville, NJ, USA), lower limit of detection $2.6 \log_{10}(400)$ copies/ml. CSF HIV-1 RNA (optional except at baseline) was quantified using NASBA HIV-1 QT technology (Organon Teknika, Duham, NC, USA) [5], limit of detection 2.0 (100) copies/ml.

Genotype analysis

The HIV-1 coding region was amplified from plasma samples extracted by NASBA HIV-1 QT technology (Organon Teknika) using the rTth XL RT-PCR kit (Perkin-Elmer, Foster City, CA, USA). Purified cDNA was sequenced using the PRISM FS dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and resolved on an ABI 373 DNA sequencer. Data were aligned and analyzed using the Sequencher program. Where mixed viral populations were present, a ratio of mutant to wild-type electropherogram peak size greater than 70% was designated as mutant.

Results

Study population

A total of 105 subjects was randomized into the study but three subjects from each group withdrew prior to the baseline evaluation, hence data were collected from 49 subjects from the ABC + stable background therapy (SBG) group and 50 subjects from the SBG alone group (intent-to-treat population). The two groups were balanced in baseline parameters with the exception of plasma HIV-1 RNA. Median baseline plasma HIV-1 RNA for subjects in the ABC + SBG group was $3.72 \log_{10}$ copies/ml (range, 2.6–5.85 \log_{10} copies/ml) versus $4.50 \log_{10}$ copies/ml (range, 2.6–6.12 \log_{10} copies/ml) for subjects in the SBG alone group. A total of 10 out of 44 (23%) of subjects in the ABC + SBG group and four out of 45 (9%) in the SBG alone group had baseline plasma HIV-1 RNA ≤ 400 copies/ml, although the difference was not significant ($P = 0.084$). The median CD4 cell count was $150 \times 10^6/l$ and $188 \times 10^6/l$, median baseline summary neuropsychological score -2.3 and -1.8 for the ABC + SBG and SBG alone groups respectively.

All subjects, except one, were receiving treatment with between one and five antiretroviral agents (ART) at study entry; approximately half were receiving triple ART, but the exact duration of each therapy prior to the 8 week protocol requirement was not available.

The most commonly used therapies were lamivudine (81%), zidovudine (ZDV; 56%) and indinavir (38%).

Antiviral efficacy

By Week 12, the majority of subjects in the study had improvements in virological and neuropsychological measures, regardless of treatment group.

The percentage of subjects with plasma HIV-1 RNA ≤ 400 copies/ml was significantly higher in the ABC + SBG treated group than in the SBG alone group (46% versus 13%; $P = 0.002$). Following additional analyses accounting for baseline detectability status, the results at week 12 still favored the ABC + SBG group ($P = 0.029$).

The CSF HIV-1 RNA levels were similar between treatment groups at baseline; 56% of subjects in each group with available CSF samples had levels < 100 copies/ml (25/45 for the ABC + SBG group and 19/34 for the SBG alone group). Of the CSF samples available at week 12, the majority had HIV-1 RNA values below the limit of detection with 83% (19/23) < 100 copies/ml in the ABC + SBG group and 75% (12/16) < 100 copies/ml in the SBG alone group.

Genotype versus HIV-1 RNA response

Due primarily to the unexpectedly low HIV-1 RNA copy number at baseline, only 67 plasma and 26 CSF samples were successfully genotyped.

Most subjects (60/67, 90%) harbored virus in plasma with RT-mutations associated with resistance to one or more of the nucleoside reverse transcriptase inhibitors (NRTI) and 21 out of 26 (81%) of virus samples obtained from CSF had similar genetic mutations associated with NRTI phenotypic resistance. The incidence of mutations associated with ZDV and stavudine (d4T) was similar in virus from plasma (76%) and CSF (73%), but there was an apparent difference in

the incidence of M184V between the compartments (75% for plasma and 50% for CSF).

Plasma HIV-1 RNA response at week 12 in relation to the baseline plasma virus genotype is presented in Table 1.

The majority of subjects in the ABC + SBG (23/33) and in the SBG alone (27/34) groups had virus with the M184V mutation at baseline, often accompanied by other NRTI-associated mutations. The presence of the M184V and up to two ZDV/d4T-associated mutations did not preclude antiviral efficacy of ABC therapy or of SBG alone. The most common genotype associated with no response to ABC included multiple ZDV and d4T-associated mutations. More non-responders (10/16, 63%) than responders (1/6, 17%) to ABC had multiple (more than three) ZDV/d4T-associated mutations. The most common baseline HIV-1 RT mutations among non-responders were M41L, K70R, M184V, L210W and T215Y with each being present in baseline virus for at least 50% of non-responders. The most common baseline HIV-1 RT mutations among responders to ABC were M41L, K70R, M184V and T215Y. The M184V was present at baseline for 11 out of 16 (69%) of non-responders and five out of six (83%) of responders to ABC, suggesting it has little predictive value for response to ABC. The single subject with baseline virus containing only the M184V mutation had a 1.7 \log_{10} copies/ml decrease (to ≤ 400 copies/ml) in plasma HIV-1 by week 12 of ABC + SBG therapy.

Plasma and CSF comparison

Levels of HIV-1 RNA in CSF and plasma tended to fluctuate together, although there were some discordant responses. CSF and plasma viral genotypes were different in 14 out of 21 (67%) of baseline pairs. Most differences were 'minor' changes in ZDV/d4T-mutation profiles, such as the presence or absence of

Table 1. Response at week 12 to treatment by baseline plasma genotype.

Baseline reverse transcriptase genotype	ABC + SBG group (n = 33)		SBG alone group (n = 34)	
	Non-detectable ^a viral load (baseline)	Response ^b or with non-detectable viral load	Non-detectable ^a viral load (baseline)	Response ^b or with non-detectable viral load
No mutations	4/6 67%	5/6 83%	1/1 100%	0/1 0%
1–2 mutations	2/6 33%	3/6 50%	0/10 0%	4/10 40%
≥ 3 mutations	6/21 29%	8/21 38%	2/23 9%	7/23 30%
Any mutation, except M184V	0/4 0%	0/4 0%	0/6 0%	1/6 17%
M184V \pm any mutation	7/23 30%	11/23 48%	2/27 7%	10/27 37%
1–2 ZDV/d4T mutations – M184V	0/1 0%	0/1 0%	0/1 0%	0/1 0%
≥ 3 ZDV/d4T mutations – M184V	0/3 0%	0/3 0%	0/3 0%	1/3 33%
1–2 ZDV/d4T mutations + M184V	4/10 40%	6/10 60%	2/10 20%	3/10 30%
≥ 3 ZDV/d4T mutations + M184V	4/11 36%	4/11 36%	0/11 0%	4/11 36%
Total	10/33 30%	16/33 48%	2/34 6%	12/34 35%

^aLower limit of detection, 400 copies/ml. ^bPatients achieving $\geq 0.5 \log_{10}$ copies/ml decrease in viral load. ABC, Abacavir; SBG, stable background therapy; ZDV, zidovudine; d4T, stavudine.

Table 2. Subjects with notably different virus populations in plasma versus cerebrospinal fluid (CSF).

Subject	Week	CSF change in HIV-1 RNA copies/ml		Week	Plasma change in HIV-1 RNA copies/ml	
		(log ₁₀ copies/ml)	CSF reverse transcriptase genotype		(log ₁₀ copies/ml)	Plasma reverse transcriptase genotype
ABC + SBG group						
4942	0		215 (mix), 219	0		69, 70, 184, 219
SBG group						
229	-		-	0		41, 184, 210, 215
	6	+ 0.67	Wild-type	4	+ 0.25	41, 184, 210, 215
232	0		Wild-type	0		41, 65, 67, 184I, 210, 215, 219
	6	- 2.23	41, 67, 184I, 215	-	- 0.57	-
	12	- 3.46	41, 67, 184I, 210, 215	12	- 0.4	41, 65, 67, 184I, 210, 215, 219
290	0		41, 215	0		70, 184
345	0		41, 69S[S-G/T], 210, 215	0		41, 62A/V, 69S[S-T/A], 74M/V, 184M/V, 210, 215
	6	- 0.91, < 400	41, 69S[S-G], 210, 215	6	- 0.34	41, 62mix, 69S[S-T], 74V, 184M/V, 210, 215
	12	- 0.91, < 400	41, 69S[S-G/T], 210, 215	12	+ 0.29	41, 62V, 69S[S-T], 74V, 184V, 210, 215
612	0	-	41, 215	0		184
	-		-	12		41, 184, 215
5001	0		210L	0		184

L210W or K219Q in a similar background of M41L, D67R and T215Y. Some of the most notable differences are shown in Table 2. At baseline, six subjects in the SBG alone group and one in the ABC + SBG group had virus with the M184V mutation in plasma, but not in CSF.

CSF HIV-1 RNA response at week 12 and baseline viral genotype are available for six subjects in the SBG alone group and five subjects in the ABC + SBG group. In the SBG alone group five out of six subjects had baseline CSF virus carrying more than three NRTI-associated mutations; three out of five had CSF HIV-1 RNA reductions at week 12.

In the ABC + SBG group three out of five baseline CSF isolates carried more than NRTI-associated mutations and two out of three had a reduction in CSF HIV-1 RNA at week 12 to < 100 copies/ml. Four subjects experienced > 1 log₁₀copies/ml reductions in CSF HIV-1 RNA, two in the absence of reductions in plasma HIV-1 RNA and two with undetectable plasma HIV-1 RNA at baseline, suggesting that ABC may have efficacy in the CSF even in the absence of detectable efficacy in the plasma.

Discussion

Given the current understanding of viral RNA responses to combination therapy and ABC cross-resistance [6,7], the inconclusive results from CNA3001 are not surprising. At the time this trial was designed, the potency of triple combination therapy was not fully appreciated and the high percentage of subjects with

undetectable virus at baseline (plasma and CSF) was unexpected. Likewise, the time for plasma HIV-1 RNA to reach nadir following initiation of a new combination ART is now known to be considerably longer than the entry criterion of 8 weeks allowed for this trial, providing the most likely explanation for the numerous HIV-1 RNA responses to placebo in both CSF and plasma.

However, this study confirmed that baseline HIV-1 RT mutations known to be associated with resistance to NRTI did not preclude substantial decreases in plasma and CSF HIV-1 RNA following addition of ABC to SBG. Multiple mutations associated with ZDV and d4T resistance appeared to predict a diminished likelihood of virologic response to ABC. Genome sequencing revealed the potentially important finding that CSF and plasma could harbor major populations of virus with different HIV-genotypes. HIV-1 RNA responses and RT genotype may be discordant between CSF and plasma.

These data support the contention that viral suppression in the periphery may be inadequate in the absence of viral suppression in the CNS. If this is true, it will be critical to include antiretroviral drugs that are active in the CNS in all combination therapies designed for durable and complete suppression of viral replication.

Sponsorship: Supported by Glaxo Wellcome.

References

1. Miller WH, Daluge SM, Garvey EP, et al. **Phosphorylation of**

- carbovir enantiomers by cellular enzymes determines the stereoselectivity of antiviral activity. *J Biol Chem* 1992, **267**: 21220–21224.
2. Faletto MB, Miller WH, Garvey EP, St Clair MH, Daluge SM, Good SS. **Unique intracellular activation of the potent anti-human immunodeficiency virus agent 1592U89.** *Antimicrob Agents Chemother* 1997, **41**:1099–1107.
 3. Daluge SM, Good SS, Miller WH: **Abacavir (1592), a second-generation nucleoside HIV reverse transcriptase inhibitor.** *International Antiviral News* 1998, **6**:122–124 (correction; p.156).
 4. Ho H-T, Hitchcock MJM. **Cellular pharmacology of 2',3'-dideoxy-2',3'-didehydrothymidine, a nucleoside analog active against human immunodeficiency virus.** *Antimicrob Agents Chemother* 1989, **33**:844–849.
 5. Parker WB, Shaddix SC, Bowdon BJ, *et al.* **Metabolism of carbovir, a potent inhibitor of human immunodeficiency virus type 1, and its effects on cellular metabolism.** *Antimicrob Agents Chemother* 1993, **37**:1004–1009.
 6. Tisdale M, Alnadaf T, Cousens D. **Combination of mutations in human immunodeficiency virus type 1 reverse transcriptase required for resistance to the carbocyclic nucleoside 1592U89.** *Antimicrob Agents Chemother* 1997, **41**:1094–1098.
 7. Lanier ER, Ait-Khaled M, Madison S, *et al.* **Analysis of possible predictors of response to abacavir (ABC) in antiretroviral-experienced adults; comparison of viral genotype, viral phenotype and patient treatment history.** *Sixth Conference on Retroviruses and Opportunistic Infections*. Chicago, January–February 1999 [abstract 34p].