AZT MONOTHERAPY AND VIRAL LOAD

This is a letter rejected by the Medical Journal of Australia in 2002

The nucleoside analogue 3'-azido-3'-deoxthymidine (Zidovudine, AZT) is claimed to interrupt the HIV replication cycle through selective inhibition of viral specific reverse transcriptase, thereby preventing the formation of new proviral DNA. It is accepted[1] that only triphosphorylated AZT (AZTTP) but not the unphosphorylated or mono- or diphosphate is the active agent. The natural impermeability of cells to nucleotides[2] predicts that administration of phosphorylated AZT may prove problematic. Therefore it is essential to demonstrate that the unphosphorylated, therapeutically inactive pro-drug taken by patients results in intracellular concentrations of AZTTP sufficient to exert its putative pharmacological action. According to Furman *et al*,[3] *in vitro* under the most ideal conditions, the AZTTP IC_{50} value for viral reverse transcriptase is 0.7 μM using the synthetic (not the HIV RNA) primer-template poly(rA).oligo$(dT)_{12\text{-}18}$. Compared to the *in vitro* conditions addressed by researchers, *in vivo* conditions are more complex and likely to require higher concentrations of the active drug. For example, *in vivo* AZTTP has to compete with the naturally occurring nucleotides for incorporation into HIV DNA. However, AZT underwent clinical trials and was introduced as a specific anti-HIV drug many years before there were data documenting the extent to which cells are able to triphosphorylate the parent compound to the active moiety. The *in vivo* scientific data published so far indicate that even the degree of triphosphorylation required *in vitro* does not take place (Table 1). Thus, AZT cannot possess anti-HIV effects.

This conclusion is supported by the published data on serial measurements of plasma HIV-RNA, "viral load". According to the British HIV Association guidelines for antiretroviral treatment, "If the viral load has not fallen by about 1 log 8-12 weeks after treatment initiation consideration should be given to modify therapy".[4] Saag, Shaw, Coombs and their associates state: "A three-fold or greater sustained reduction (>0.5 log) of the plasma HIV RNA levels is the minimal response indicative of an antiviral effect...return of HIV RNA levels to pretreatment values (or to within 0.3 - 0.5 log of the pretreatment value), confirmed by at least two measurements, is indicative of drug failure".[5] On this basis all the data to date document that treatment with AZT cannot be regarded as anything other than "drug failure" (Figure 1).

Since intracellular metabolism produces insignificant concentrations of the active, triphosphorylated compound, as further evidenced by the failure of AZT to decrease HIV-RNA, one must question why AZT remains the most widely used anti-HIV drug either alone or in combination. This includes use as a sole agent for the prevention of mother-to-child transmission, especially given the drug is not devoid of toxicities.[6]

Table 1

Measurement of AZT triphosphorylation in humans

In none of these studies does AZTTP reach the concentration estimated ideally *in vitro* of 0.7 μM

Year	Peak Concentration of Triphosphorylated AZT	*Reference*
1991	0.5 pmol/10^6 cells	Kuster H, et al. J Infect Dis; 164: 773–776
1991	56 pmol/10^7 cells (5.6 pmol/10^6 cells)	Toyoshima T, et al. Analytical Bioch; 196: 302–307
1992	0.14 pmol/10^6 cells	Slusher JT, et al. Antimic Agents & Chemoth; 36: 2473–2477
1994	326 fmol/10^6 cells (0.326 pmol/10^6 cells)	Robbins BL, et al. Antimicrob Agents Chemother; 38: 115–121
1994	0.06 pmol/10^6 cells	Barry MG, et al. AIDS; 8: F1 – F5
1996	95 fmol/10^6 cells (0.095 pmol/10^6 cells)	Rodman JH, et al. J Infec Dis; 174: 490-499
1996	0.069 pmol/10^6 cells	Peter K, et al. J Pharm & Biomed Anal; 14: 491 – 499
1996	0.042 pmol/10^6 cells (average)	Peter K and Gambertoglio JC. Clin Pharmacol Ther; 60: 168–176
1996	0.07 pmol/10^6 cells	Barry MG, et al. AIDS; 10: 1361–367
1998	0.046 pmol/10^6 cells, in mononuclear cells from lymph nodes. 0.085 pmol/10^6 cells in PBMC	Peter K et al. AIDS; 12: 1729–1731
1998	0.07 pmol/10^6 cells	Robbins BL, et al. Antimicrob Agents Chemother; 42: 2656-2660
1998	160 fmol/10^6 cells (average) (0.16 pmol/10^6 cells)	Fletcher CV, et al. Clin Pharmacol Ther 64: 331–338
1999	329 fmol/10^6 cells (0.329 pmol/10^6 cells)	Rodman JH et al. J Infec Dis; 180:1844-50
1999	193 fmol/10^6 cells (0.193 pmol/10^6 cells)	Font E, et al. Antimicrob Agents Chemother; 43: 2964-8
2000	0.32 pmol/10^6 cells	Wattanagoon Y, et al. Antimicrob Agents Chemother; 1986-1989

$1\mu mol = 10^{-6}$ mole; 1 pmol = 10^{-12} mole; 1 fmol = 10^{-15} mole; 1 pmol/10^6 cells $\approx 1\ \mu M$

REFERENCES

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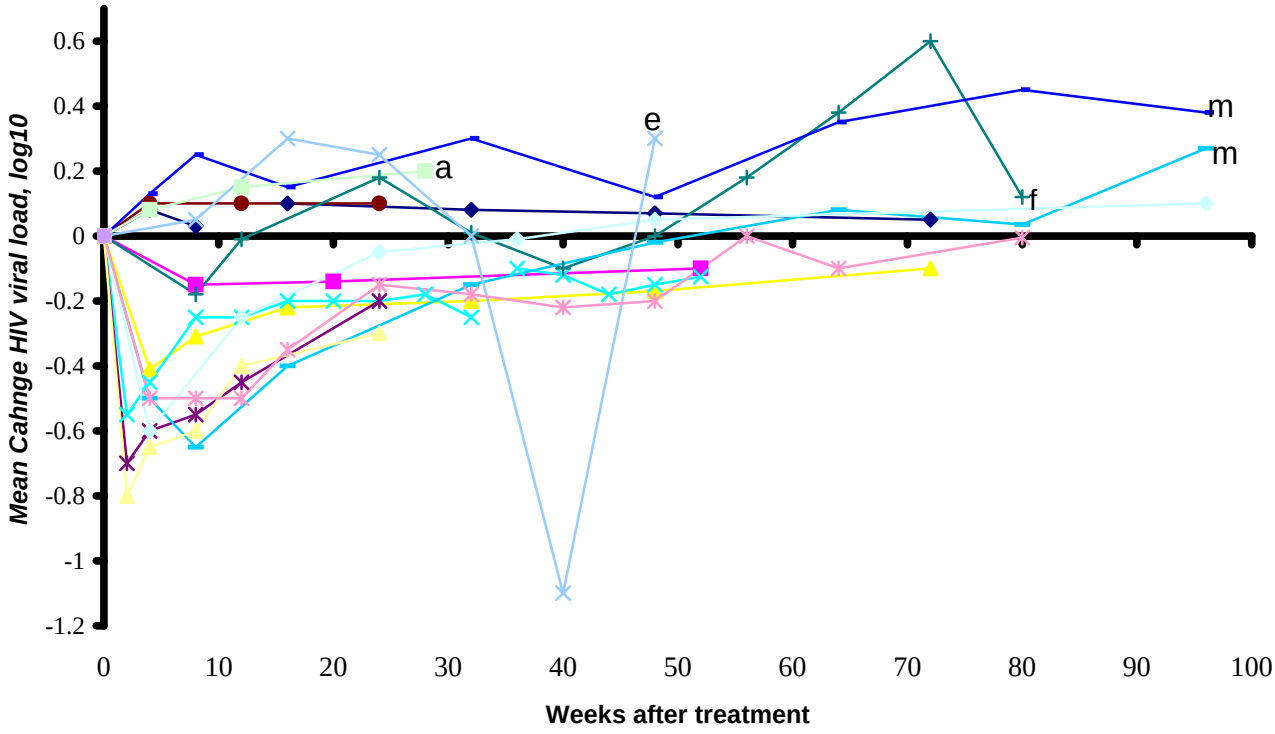
2. Leibman KC, Heidelberger C. The metabolism of P^{32} labelled ribonucleotides in tissue slices and cell suspensions. J Biol Chem 1955;216:823-830.
3. Furman PA, Fyfe JA, St Clair MH, Weinhold K, Rideout JL, Freeman GA, et al. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. Proc Nat Acad Sci USA 1986;83:8333-7.
4. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. Lancet 1997;349:1086-1092.
5. Saag MS, Holodniy M, Kuritzkes DR, WA OB, Coombs R, Poscher ME, et al. HIV viral load markers in clinical practice. Nature Medicine 1996;2:625-9.

Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Alfonso H, Page BAP, Causer D, et al. Mother to Child Transmission of HIV and its Prevention with AZT and Nevirapine. Publisher: The Perth Group; Perth, Western Australia. 2001. pps 214. www.aidspanelreport.com/newperthpaper.htm

FIGURE 1.

Changes of HIV viral load induced by AZT. Combined data.



In subsequent correspondence with the editor we had reason to separate studies of AZT naïve and experienced patients. Here are the data.

	Study	No. patients	Classification
a)	Eron JJ et al. NEJM 1995;333:1662-9	**85**	11-20% BOTH
b)	De Jong MD, et al. PNAS 1996;93:5501-6	**24**	N
c)	Katlama C, et al. JAMA 1996;276:118-25	**129**	N
d)	Katlama C, et al. JAMA 1996;276:118-25	**129**	N
e)	Staszewski S et al. JAMA 1996;276:111-7	**223**	E
f)	Carr A, AIDS 1996;10:635-41	**49**	E
g)	O'Brien WA, et al. NEJM 1996;334;426-31	**270**	N
h)	O'Brien WA, et al. NEJM 1996;334;426-31	**270**	N
i)	Katzenstein D, et al. NEJM 1996:1091-8	**1067**	N
j)	Bakshi SS, et al. J Infect Dis 1997;175:1039-50	**250**	E
)	Bruisten SM et al. AIDS Res & Hum Retr 1998;12:1053-8	**42**	N
l)	Delta Committee. AIDS, 1999:57-65	**298**	N
m)	Delta Committee. AIDS, 1999:57-65	**113**	E
n)	Lillo FB, et al. AIDS 1999;13:791-6	**28**	BOTH

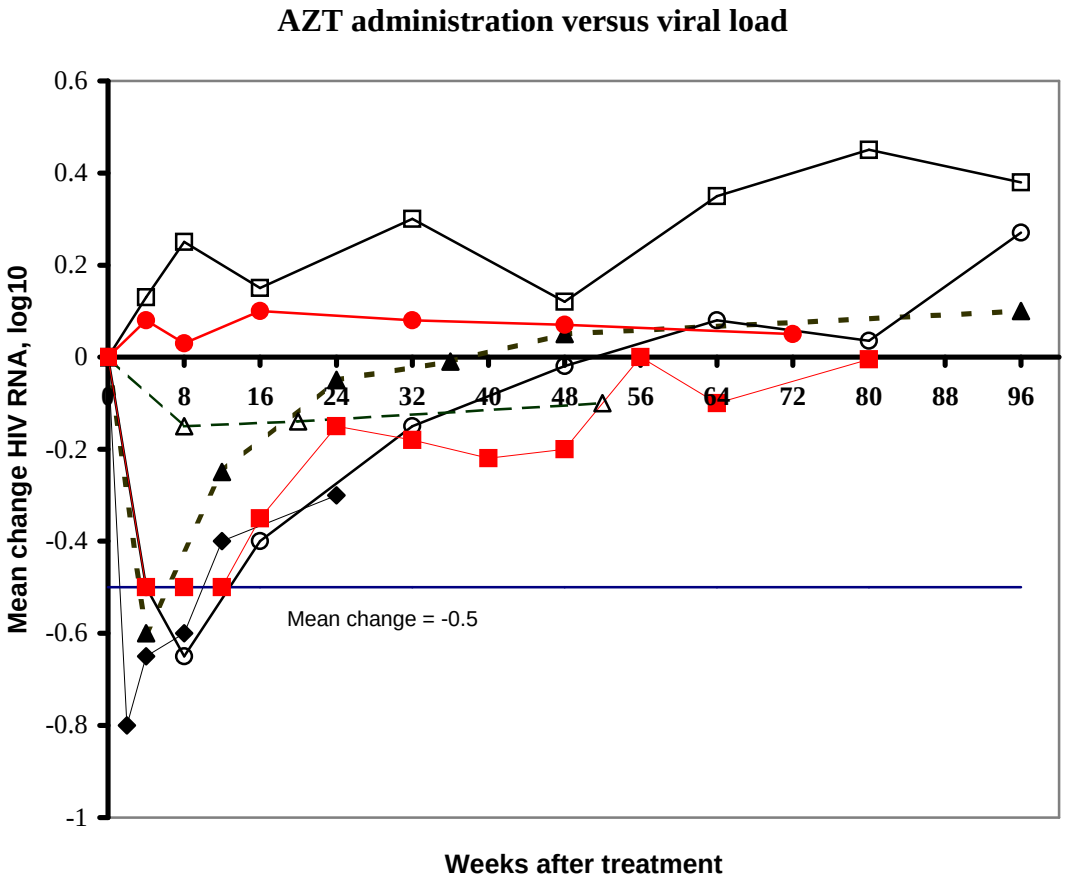
N= naïve; E= experienced. BOTH= N + E
(d) =© but viral load measured using RT Roche vs immune capture assay.

Below are graphed the viral load results as experienced only and naïve only. We have removed graphs (a) and (n) because both AZT naïve and experienced patients were reported. (Although (n) has at maximum 20% experienced patients). (c) was also excluded because viral load was measured using an immune capture assay.

In regard to these graphs one should note:

(a) None of the studies report a reduction in viral load of 1 log as indicative of drug failure by the British HIV Association;
(b) most of the studies do not demonstrate a reduction in viral load > 0.5 log. Only 3 [(b, d, g] of the 11 and this was not sustained for more than a few weeks;
(c) eight studies did not reduce the viral load more than 0.5 log and of these two (2/8) were small studies: (f, 49 patients and n, 28 patients). One study was AZT experienced and one contained both types of patients;
(d) one of the three studies that did transiently reduce viral load > 0.5 log was small (24 patients and AZT naïve);
(e) the largest studies (h) and (i) with 270 and 1067 patients respectively and AZT naïve did not reduce the viral load at any time;
(f) results obtained by the Delta Committee from 113 patients and AZT experienced result if anything in a greater reduction in viral load than three studies obtained from naïve patients. But none of the studies is significant;
(g) two of the experienced group studies are comparable with the results obtained in the two largest naïve patient groups, that is, in none of these studies does the viral load decline;
(h) apart from one entry in study (j) no experienced group had worse results than those obtained in the largest and longest naïve group.

NAÏVE

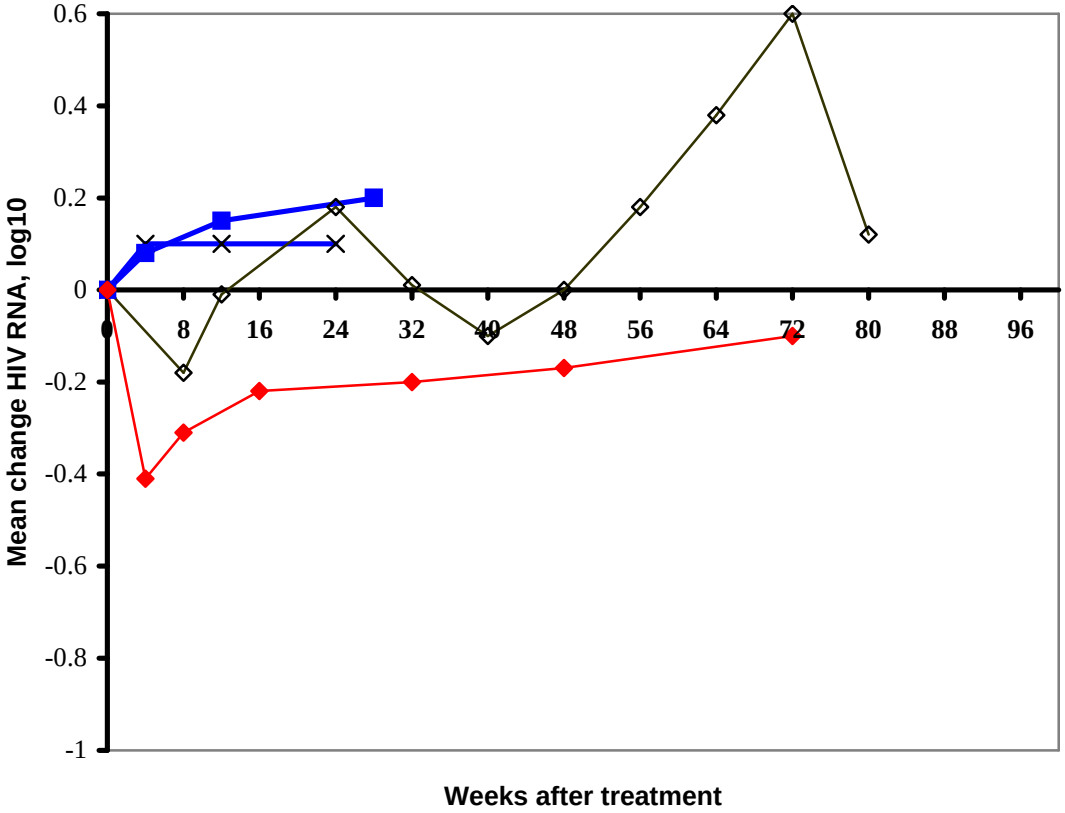


b)	De Jong MD, et al. PNAS 1996;93:5501-6
c)	Katlama C, et al. JAMA 1996;276:118-25
d)	Katlama C, et al. JAMA 1996;276:118-25
g)	O'Brien WA, et al. NEJM 1996;334;426-31
h)	O'Brien WA, et al. NEJM 1996;334;426-31
i)	Katzenstein D, et al. NEJM 1996:1091-8
k)	Bruisten SM et al. AIDS Res & Hum Retr 1998;12:1053-8
l)	Delta Committee. AIDS, 1999:57-65

EXPERIENCED

a. Carr A, et al. AIDS, 1996:635-641; b. Katlama C. et al. JAMA, 1996:118-25; c. Staszewski S, et al. JAMA, 1996:111-117; d. Delta Committee. AIDS, 1999:57-65; e. Lillo F. AIDS, 1999:791-6; f. Bakshi SS, et al. J Infect Dis 1997:1039-50; g. Bruisten SM, et al. AIDS Res & Hum Retr 1998:1053-8; h. De Jong MD, et al. PNAS 1996:5501-6; i. Katzenstein D, et al. NEJM 1996:1091-8; l. Eron JJ, et al. NEJM 1995:1662-9; m. O'Brien WA. NEJM 1996:426-31.

AZT administration versus viral load



e)	Staszewski S et al. JAMA 1996;276:111-7	
f)	Carr A, AIDS 1996;10:635-41	
j)	Bakshi SS, et al. J Infect Dis 1997;175:1039-50	
m)	Delta Committee. AIDS, 1999:57-65	

STUDIES

(a) Eron JJ, Benoit SL, Jemsek J, MacArthur RD, Santana J, Quinn JB, et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. New England Journal of Medicine 1995;333:1662-9.

366 patients. 85 received AZT monotherapy. Majority AZT naïve.
"From 11 to 20 percent of the patients in each group had previously received antiretroviral therapy (zidovudine only), and the median duration of that therapy in the four groups was three weeks or less (P = 0.22)".

(b) de Jong MD, Veenstra J, Stilianakis NI, Schuurman R, Lange JM, de Boer RJ, et al. Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for the loss of human immunodeficiency virus type 1 RNA load suppression by zidovudine. Proceedings of the National Academy of Sciences of the United States of America 1996;93:5501-6.

24 Dutch patients AZT naïve
"Serum HIV-1 RNA load and the relative amounts of HIV-1 RNA containing mutations at RT codons 41, 70, and 215 were assessed sequentially during a 2-year period of zidovudine treatment in 24 previously untreated HIV-1 infected individuals…A mean maximum decline in RNA load occurred during the first month, followed by a resurgence between 1 and 3 months, which appeared independent of drug-resistance". This study "excluded patients with MT2 isolates which are resistant to AZT".

© Katlama C, Ingrand D, Loveday C, Clumeck N, Mallolas J, Staszewski S, et al. Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group. Journal of the American Medical Association 1996;276:118-25.

129 patients. "To compare safety and efficacy of lamivudine-zidovudine combination therapy with zidovudine monotherapy in treating human immunodeficiency virus type 1 (HIV-1)-infected, antiretroviral therapy-naive patients".
Graph © is RNA measured with an immune capture assay.

(d) Katlama C, Ingrand D, Loveday C, Clumeck N, Mallolas J, Staszewski S, et al.
Same as © but viral load measured with an RT assay (Roche).

(e) Staszewski S, Loveday C, Picazo JJ, Dellarnonica P, Skinhoj P, Johnson MA, et al. Safety and efficacy of lamivudine-zidovudine combination therapy in zidovudine-experienced patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group. Journal of the American Medical Association 1996;276:111-7.

"Double-blind, randomized, Multicenter, comparative trial of 223 patients treated for 24 weeks". 73 patients in AZT arm all of who had received prior AZT treatment for between 105-2011 days.

(f) Carr A, Vella S, de Jong MD, Sorice F, Imrie A, Boucher CA, et al. A controlled trial of nevirapine plus zidovudine versus zidovudine alone in p24 antigenaemic HIV-infected patients. The Dutch-Italian- Australian Nevirapine Study Group. AIDS 1996;10:635-41.

49 patients. 24 received AZT monotherapy. All AZT experienced.

(g) O' Brien W, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S, et al. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. New England Journal of Medicine 1996;334:426-31.

270 patients, all AZT naïve and treatment deferred.

(h) O' Brien W, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S, et al.
Same as (g) but treatment immediate.

(i) Katzenstein DA, Hammer SM, Hughes MD, Gundacker H, Jackson JB, Fiscus S, et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. AIDS Clinical Trials Group Study 175 Virology Study Team. New England Journal of Medicine 1996;335:1091-8.

"No prior exposure to antiretroviral agents (N=1067)"

(j) Bakshi SS, Britto P, Capparelli E, Mofenson L, Fowler MG, Rasheed S, et al. Evaluation of pharmacokinetics, safety, tolerance, and activity of combination of zalcitabine and zidovudine in stable, zidovudine-treated pediatric patients with human immunodeficiency virus infection. AIDS Clinical Trials Group Protocol 190 Team. Journal of Infectious Diseases 1997;175:1039-50.

"A double-blind phase II trial compared zalcitabine (0.03 mg/kg/day) in combination with zidovudine (720 mg/m2/day) and zidovudine monotherapy in 250 clinically stable, previously zidovudine-treated, human immunodeficiency virus-infected children…At all time points, the virus load was lower in patients in the combination therapy arm, although statistical significance was not achieved…There was no difference in virologic response to combination therapy on the basis of length of prior zidovudine therapy". CHECK Helman this suggests something. What is it?

(k) Bruisten SM, Reiss P, Loeliger AE, van Swieten P, Schuurman R, Boucher CA, et al. Cellular proviral HIV type 1 DNA load persists after long-term RT- inhibitor

therapy in HIV type 1 infected persons. AIDS Research and Human Retroviruses 1998;14:1053-8.

"In a set of 42 antiretroviral naive HIV-1 infected persons who were treated with either Zidovudine (AZT) monotherapy, or a combination of AZT + ddC (Zalcitabine) or AZT + ddI (Didanosine), the HIV-1 DNA load was measured by competitive polymerase chain reaction (PCR) and related to the HIV-1 RNA load in plasma, the CD4+ counts and to clinical markers".

(l) and (m) HIV-1 RNA response to antiretroviral treatment in 1280 participants in the Delta Trial: an extended virology study. Delta Coordinating Committee and Delta Virology Committee. AIDS 1999:57-65.HIV-1 RNA response to antiretroviral treatment in 1280 participants in the Delta Trial: an extended virology study Delta Coordinating Committee and Delta Virology Committee

The trial included both ZDV-naive and ZDV-experienced participants.

The characteristics of participants are shown in Table 1 separately for the ZDV-naïve (Delta 1) and ZDV-experienced (Delta 2) participants.

Table 3. HIV RNA viral load and percentage below lower level of detection at selected weeks*.

	Delta 1			Delta 2		
	ZDV (n = 298)	ZDV–ddI (n = 304)	ZDV–ddC (n = 311)	ZDV (n = 113)	ZDV–ddI (n = 135)	ZDV–ddC (n = 119)
HIV RNA copies/ml [mean (95% CI)]						
Absolute value						
Baseline	4.67 (4.60–4.75)	4.71 (4.64–4.79)	4.74 (4.66–4.82)	4.56 (4.44–4.68)	4.57 (4.46–4.68)	4.49 (4.37–4.61)
Week 4	4.26 (4.17–4.35)	3.36 (3.28–3.45)	3.60 (3.51–3.68)	4.64 (4.50–4.77)	3.93 (3.78–4.08)	4.00 (3.83–4.18)
Week 8	4.36 (4.27–4.45)	3.47 (3.38–3.57)	3.60 (3.51–3.68)	4.59 (4.46–4.73)	3.95 (3.80–4.10)	3.88 (3.71–4.05)
Week 16	4.45 (4.36–4.54)	3.51 (3.40–3.62)	3.66 (3.55–3.76)	4.66 (4.52–4.80)	3.93 (3.77–4.09)	3.92 (3.75–4.08)
Week 32	4.47 (4.36–4.57)	3.78 (3.66–3.90)	3.76 (3.65–3.87)	4.64 (4.50–4.79)	4.15 (3.94–4.29)	4.01 (3.83–4.20)
Week 48	4.50 (4.39–4.62)	3.92 (3.79–4.05)	3.86 (3.74–3.98)	4.63 (4.46–4.81)	4.17 (3.97–4.36)	4.06 (3.86–4.26)
Week 96	4.57 (4.41–4.72)	4.09 (3.91–4.26)	4.12 (3.97–4.27)	4.61 (4.39–4.84)	4.27 (4.04–4.51)	4.23 (3.97–4.49)
Minimum value at weeks 0–16	4.13 (2.72–5.54)	3.33 (1.94–4.72)	3.34 (2.19–4.69)	4.51 (3.22–4.73)	3.81 (2.32–5.30)	3.78 (2.23–5.33)
Maximum drop between 0 and 16 weeks	0.54 (–0.26–1.34)	1.38 (–0.23–2.99)	1.31 (0.06–2.56)	0.06 (–0.92–1.04)	0.75 (–0.39–1.89)	0.70 (–0.32–1.72)
Percentage below lower level of detection† (95% CI)						
Baseline	2 (0–5)	3 (2–6)	2 (0–5)	2 (0–6)	1 (0–5)	2 (0–6)
Week 4	7 (4–11)	52 (45–58)	28 (23–33)	0 (0–4)	20 (13–29)	18 (11–27)
Week 8	6 (1–9)	50 (44–57)	29 (24–35)	0 (0–4)	19 (12–28)	20 (12–30)
Week 16	4 (2–7)	51 (44–58)	36 (30–42)	0 (0–5)	21 (14–31)	18 (11–27)
Week 32	7 (4–11)	36 (29–42)	30 (24–36)	1 (0–8)	11 (5–20)	20 (12–30)
Week 48	6 (3–11)	28 (22–35)	28 (22–35)	2 (0–9)	16 (8–26)	13 (6–24)
Week 96	4 (1–10)	18 (11–27)	19 (13–27)	6 (0–3)	13 (5–26)	13 (5–26)

*Results not available for all patients at each week. †800 copies/ml. ZDV, Zidovudine; ddI, didanosine; ddC, zalcitabine; CI, confidence interval.

"The results in participants who had already received at least 3 months of ZDV, as expected, showed very little change in viral load in the group randomized to ZDV monotherapy".

(n) Lillo FB, Ciuffreda D, Veglia F, Capiluppi B, Mastrorilli E, Vergani B, et al. Viral load and burden modification following early antiretroviral therapy of primary HIV-1 infection. AIDS 1999;13:791-6.

"Eight of the subjects had been enrolled in a placebo-controlled trial of ZDV between 1991 and 1995 [8]: four received placebo (group A) and four were treated with ZDV 250

mg X 2 (group B)…The parameters of viral replication and CD4 cell recovery were only slightly better in the patients receiving ZDV monotherapy than in the untreated patients, thus confirming that the course of the infection is hardly affected by the monotherapy".

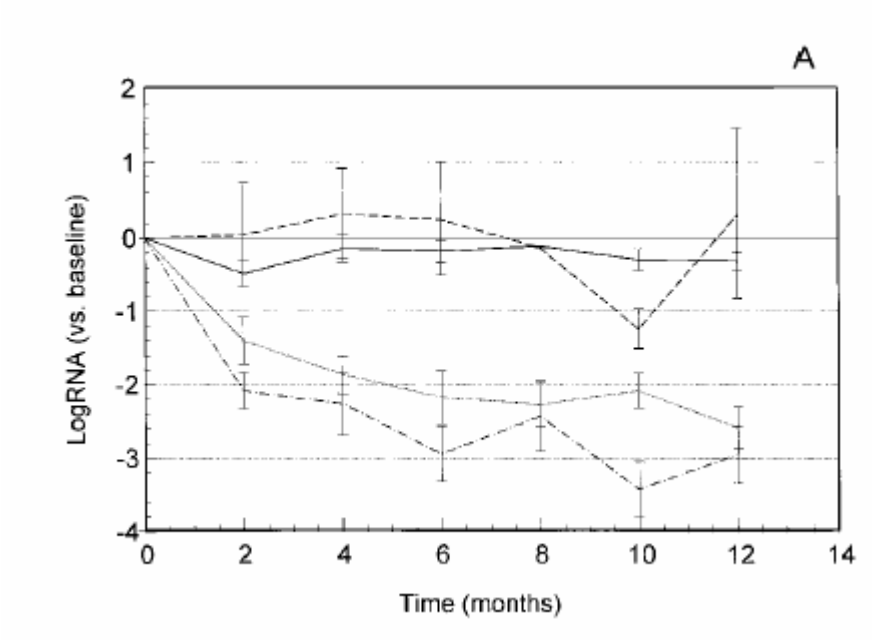


Fig. 2. HIV-RNA (panel A) variations during 1 year of follow-up. In all of the panels, the untreated patients are represented by a straight line (————), zidovudine (ZDV)-treated by a dashed line (----------), patients treated with ZDV + lamivudine (3TC) + saquinavir (SQV) by a dotted/dashed line (·····), and patients treated with ZDV + 3TC + SQV + ritonavir (RTV) by an irregularly dashed line (–·–·–·– –). Standard errors are shown for each parameter and time point.