

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

Objective: To assess changes in HIV RNA and their relationship to disease progression.

Design and setting: Delta was a randomized double-blind trial comparing zidovudine (ZDV) monotherapy with ZDV plus didanosine (ddI) or ZDV plus zalcitabine (ddC). Participants had AIDS (with CD4 cell counts above $50 \times 10^6/l$), AIDS-related complex, or were asymptomatic with CD4 cell counts below $350 \times 10^6/l$. The trial included both ZDV-naive and ZDV-experienced participants.

Participants: A total of 1280 participants in the Delta trial whose serum samples had been stored at -70°C and who had a minimum of one sample taken before the start of treatment and at least one later sample.

Methods: HIV-1 RNA quantification was performed using the nucleic acid sequence-based amplification HIV-1 RNA quantitative assay with a cut-off of 800 copies/ml.

Results: Reductions in HIV RNA by treatment group were consistent with the clinical results; in ZDV-naive participants the maximum median fall occurred at 4 weeks for all three groups (ZDV, $0.54 \log_{10}$ copies/ml; ZDV-ddI, $1.38 \log_{10}$ copies/ml; ZDV-ddC, $1.31 \log_{10}$ copies/ml). On average the reductions were smaller in ZDV-experienced participants but the difference between the monotherapy and combination arms was very similar in ZDV-naive and experienced participants. Baseline HIV RNA levels, adjusted for CD4 cell counts were highly predictive of time to virological response (HIV RNA < 800 copies/ml); HIV RNA nadirs achieved were predictive of survival. Viral load rebound following response was independent of treatment group and previous ZDV therapy.

Conclusions: Virological changes in response to treatment are of value in assessing prognosis and the activity of new therapies; in particular, there is a strong association between the minimum HIV RNA achieved in the first 16 weeks and subsequent clinical response. CD4 cell counts are independently predictive of response.

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Introduction

Since quantification of HIV viral load by molecular techniques has become widely available its predictive value for clinical outcome has been demonstrated in participants in many studies [1–10]. Guidelines now include viral load as part of the criteria for initiating antiretroviral therapy, and change in viral load is

being used routinely in making treatment decisions. Indeed, some clinicians advocate that only treatments that result in viral load falling below the lower limits of detection of current assays should be used. In almost all of the studies reported to date the RNA results used in the analyses are restricted to baseline values or only reflect changes within the first 8 weeks of therapy.

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Thus, a number of important questions remain. For example, how rapidly is the minimum HIV RNA level (nadir) achieved, and therefore, when should decisions to change therapy be considered if the initial response was unsatisfactory? How good a predictor of clinical outcome is the HIV RNA nadir or the time to viral load rebound? How does the ability of HIV RNA to predict survival compare with that of the CD4 cell count? Are the baseline and later assessments of HIV RNA independently predictive of clinical outcome? The answers to these and other questions may vary depending on the treatment regimens employed.

This study assesses changes in HIV RNA and CD4 cell counts and their relationship to survival as well as disease progression in an extended virology study of over 1200 participants in the Delta trial [11], which compared monotherapy with zidovudine (ZDV) with combination therapy of ZDV plus either didanosine (ddI) or zalcitabine (ddC), and showed substantial and highly significant benefit from the combination regimens both for survival and disease progression. Follow-up and sample collection was continued irrespective of whether participants continued on the allocated regimens. This study provides a unique opportunity to answer some of the key questions both in treatment-naive individuals and in those who had prior ZDV therapy.

Population and methods

Delta was a randomized double-blind trial comparing ZDV alone (600 mg daily) with ZDV plus ddI (400 mg daily) or ZDV plus ddC (2.25 mg daily). Participants had AIDS (with CD4 cell counts $> 50 \times 10^6/l$), AIDS-related complex, or were asymptomatic with CD4 cell counts below $350 \times 10^6/l$. The trial included both those with no reported prior ZDV (Delta 1) and those with at least 3 months of prior ZDV (Delta 2).

During the Delta trial, serum samples were collected before the start of treatment, at 4-week intervals to week 24, then at 8-week intervals to week 64, and thereafter every 16 weeks. Participants from centres whose samples had been stored at -70°C were eligible for inclusion in the extended virology study, provided they had a minimum of one serum sample taken before the start of treatment and at least one later sample. In addition, for logistical reasons a small number of centres (including the Italian centres) were not included. Serum samples collected and stored at, or close to, the scheduled timepoints were assayed for HIV-1 RNA load at one of two sites in The Netherlands and the United States using the nucleic acid sequence-based amplification (NASBA) HIV-1 RNA quantitative assay (Organon Teknika, Boxtel, The Netherlands) [12]. For

the purposes of analysis, a cut-off value for the assay of 800 copies/ml ($2.9 \log_{10}$ copies/ml) was assigned, although lower values were observed in some assay runs. A previous evaluation of NASBA in comparison with the Quantiplex branched DNA assay (version 1.0; Chiron, Emeryville, California, USA) and the Amplicor Monitor assay (Roche Diagnostic Systems, Branchburg, New Jersey, USA) showed a generally close equivalence of performance between NASBA and the other assays [13]. Within the present study, a comparison was made of 116 plasma samples with the corresponding stored serum samples. The results obtained from the plasma using the Roche assay were very similar to those in serum using NASBA, with a mean difference in HIV-1 RNA (serum - plasma) of $-0.0005 \log_{10}$ copies/ml (SD, $0.4968 \log_{10}$).

Statistical analysis

The log-rank, Kaplan-Meier and Cox proportional hazards regression methods were used in the analysis of time to death. The effect of treatment, baseline HIV-1 RNA and baseline CD4 cells on the following virological endpoints were considered: (i) response, defined as \log_{10} RNA $\leq 2.90 \log_{10}$ copies/ml (i.e., 800 copies/ml), and (ii) failure (viral load rebound), defined as \log_{10} RNA $> 2.90 + 2 \text{ SD } \log_{10}$ copies/ml (i.e., $> 3.50 \log_{10}$ copies/ml or 3185 copies/ml), where SD was 0.30 (within-person SD), calculated using data from a subset of 147 participants, a random selection from those participants with more than one baseline RNA measurement (before starting treatment).

Time to failure was assessed in participants responding within the first 16 weeks and having at least one subsequent sample before 52 weeks (i.e., within the permitted time interval for a 48-week sample, see below); 16 weeks was chosen because almost all patients who were going to respond had done so by then, and the remainder were usually outliers without samples in the early weeks of treatment. Participants with baseline RNA levels below the level defining virological response were excluded from the analysis. Those not achieving a virological response within 16 weeks of starting treatment were excluded from time-to-failure analyses. Follow-up was right-censored at 52 weeks in time-to-response analyses and at 100 weeks for time to failure. For the purpose of the analysis of events, patient follow-up was divided into intervals of 4 weeks up to week 24 and 8 weeks thereafter, centred at the scheduled collection times. The number of observed events in each interval was assumed to follow a Poisson distribution with rate dependent on interval, treatment group, prior ZDV experience, baseline CD4 cell count and baseline RNA level. Events were assumed to occur at the midpoint of the period between the start of the interval and the observed event time. If, however, one or more scheduled samples immediately prior to the event was

missing, a proportion of the event and its associated follow-up was attributed to each interval for which the RNA result was not recorded. Survival curves were calculated from estimated rates using standard methods.

All comparisons between treatment groups were carried out as randomized and all except time to failure, which was restricted to those responding, were analysed on an intention-to-treat basis. As would be expected, on-treatment analyses yielded very similar results to those undertaken on an intention-to-treat basis during the initial weeks of treatment. Subsequently, the virological response, measured by the mean \log_{10} HIV RNA and the proportion below the cut-off, was marginally better if restricted to those still on allocated treatment.

Population

A total of 9436 samples were available for quantification, but in 34 samples no valid result was available. The analyses are therefore based on 9402 samples from 1280 participants; 145 (1.5%) of the samples were less than 500 μ l in volume and the calculation of viral load was adjusted proportionately. Over half of the participants had at least one sample available during 1–12, 13–24, 25–48 and 49–96 weeks; 95.5% had at least one sample available in the first 12 weeks. The median number of samples available was seven (interquartile range, 4–8); only 49 participants had no more than one post-baseline sample.

The analysis included 913 (43%) of the 2124 participants in Delta 1 and 367 (34%) of the 1083 participants in Delta 2. The participants included were from Australia and New Zealand ($n = 51$), France ($n = 747$), The Netherlands ($n = 235$), and the United Kingdom

and Ireland ($n = 247$). The characteristics of participants are shown in Table 1 separately for the ZDV-naive (Delta 1) and ZDV-experienced (Delta 2) participants. Although the study was limited to certain centres (because of the requirements stated above) randomization was by centre and there was no significant difference between those included and those not included with respect to age, sex, risk group, disease stage and CD4 cell count at entry. A smaller proportion of those included had prior experience with ZDV treatment (29% compared with 37%; $P < 0.001$).

The median follow-up time was 31.3 months and 262 deaths were reported. The survival benefit of combination therapy found in the total study population [11] was also seen in the extended virology population (log-rank test $\chi^2 = 10.14$, 2 degrees of freedom, $P = 0.006$).

Results

Predictive effect of baseline HIV RNA and CD4 cell count

There was a marked increase in death rates with increasing baseline HIV RNA and decreasing baseline CD4 cell count ($P < 0.001$ for both trends; data not shown). The absolute risk of death in those with less than 5000 copies/ml was very similar to those with 5000–10 000 copies/ml: 7% (nine out of 122) and 8% (seven out of 91), respectively. However, death rates increased progressively with higher baseline HIV RNA to a maximum of 31% (128 out of 414) in those with $\geq 100\ 000$ copies/ml. Death rates by baseline CD4 cell counts increased from 3% (eight out of 255) in those

Table 1. Comparison of treatment groups in the extended virology study.

| | Delta 1 | | | Delta 2 | | | Total [n (%)]* |
|------------------------------------|---------|---------|---------|---------|---------|---------|-------------------|
| | ZDV | ZDV-ddI | ZDV-ddC | ZDV | ZDV-ddI | ZDV-ddC | |
| Male | 241 | 250 | 257 | 102 | 122 | 107 | 1079 (84) |
| Mean (SD) age (years) | 36 (8) | 37 (10) | 36 (9) | 38 (8) | 39 (9) | 37 (9) | 37 (9) |
| Prior ZDV | | | | | | | |
| None | 298 | 304 | 311 | 0 | 0 | 0 | 913 (71) |
| < 6 months | | | | 18 | 27 | 26 | 71 (6) |
| 6–12 months | | | | 33 | 31 | 21 | 85 (7) |
| 12–24 months | | | | 30 | 36 | 42 | 108 (8) |
| ≥ 24 months | | | | 32 | 41 | 30 | 103 (8) |
| Disease stage | | | | | | | |
| Asymptomatic/ARC 4E | 173 | 166 | 189 | 52 | 55 | 49 | 684 (53) |
| ARC | 89 | 96 | 82 | 39 | 56 | 40 | 401 (31) |
| AIDS | 36 | 42 | 40 | 22 | 24 | 30 | 195 (15) |
| HIV-1 RNA (\log_{10} copies/ml) | | | | | | | |
| % Positive | 98 | 97 | 98 | 99 | 99 | 98 | 98 |
| Mean | 4.67 | 4.71 | 4.74 | 4.56 | 4.56 | 4.49 | 4.66 |
| SD | 0.70 | 0.67 | 0.70 | 0.65 | 0.68 | 0.67 | 0.69 |
| CD4 cell count ($\times 10^6/l$) | | | | | | | |
| Mean | 216 | 214 | 214 | 186 | 179 | 183 | 206 |
| SD | 106 | 110 | 108 | 116 | 107 | 120 | 111 |
| Deaths | 56 | 40 | 40 | 47 | 39 | 40 | 262 (20) |
| Total | 298 | 304 | 311 | 113 | 135 | 119 | 1280 (100) |

*Unless otherwise indicated. ZDV, Zidovudine; ddI, didanosine; ddC, zalcitabine, ARC, AIDS-related complex.

with cell counts of $\geq 300 \times 10^6/l$ to 56% (131 out of 236) in those with counts below $100 \times 10^6/l$. A Cox proportional hazards analysis, stratified by allocated treatment and prior ZDV demonstrated that the risk of death increased independently with both increasing HIV RNA load and decreasing CD4 cell count ($P < 0.001$ for both).

Predictive value of HIV RNA and CD4 cell count at week 8

The predictive value of the HIV RNA and CD4 cell count at 8 weeks for subsequent survival is shown in Table 2. The risk of death increased significantly with increasing HIV RNA load and decreasing CD4 cell count ($P < 0.001$ for both). In a multivariate Cox model in which both baseline and week 8 values of HIV RNA were included, only week 8 HIV RNA was significantly associated with the risk of death. In contrast, in a similar model, baseline and week 8 CD4 cell counts were each highly significant after adjustment for the other. Although the log hazard of death decreased linearly with \log_{10} RNA at week 8 the association with CD4 cell count at week 8 declined linearly only up to cell counts of $200 \times 10^6/l$, there was no evidence of further reduction in risk of death thereafter. When all four factors, baseline and week 8 HIV RNA and CD4 cell count, were included in the model only baseline HIV RNA was not significantly associated with subsequent death.

Change in HIV RNA over time

In the ZDV-naive population (Delta 1) the maximum median fall in HIV RNA occurred by 4 weeks for all

Table 2. Risk of death by quartiles of HIV RNA and CD4 cell count at week 8 relative to the group with the lowest CD4 cell count and highest HIV RNA (based on zidovudine-naive and experienced participants).

| HIV RNA (copies/ml) | CD4 cells ($\times 10^6/l$) | | | |
|---------------------|-------------------------------|-----------|-----------|-----------|
| | ≥ 276 | 200–275 | 118–199 | < 118 |
| < 900 | 0.01 (1) | 0.03 (3) | 0.01 (1) | 0.13 (4) |
| 900–6999 | 0.02 (2) | 0.02 (1) | 0.05 (2) | 0.25 (11) |
| 7000–37999 | 0.06 (4) | 0.08 (6) | 0.24 (17) | 0.55 (25) |
| ≥ 38000 | 0.26 (6) | 0.29 (14) | 0.43 (23) | 1.00 (67) |

The number of deaths occurring after 8 weeks is shown in parentheses.

three arms (0.54, 1.38 and 1.31 \log_{10} copies/ml for ZDV, ZDV-ddI and ZDV-ddC, respectively; Table 3). Thereafter, the median HIV RNA increased towards baseline levels. Maximum median HIV RNA falls in the Delta 2 participants were lower than for Delta 1 (0.75 \log_{10} copies/ml for ZDV-ddI, 0.70 \log_{10} copies/ml for ZDV-ddC; median HIV RNA did not decline in the ZDV monotherapy arm).

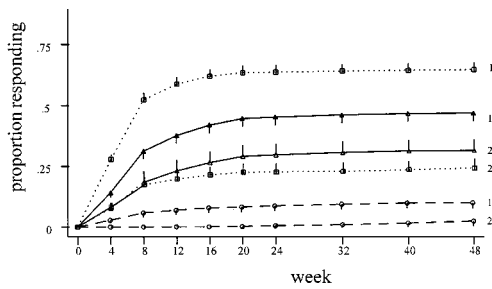
Time to virological response

The proportions responding (i.e., achieving HIV RNA < 800 copies/ml) in the first 52 weeks in Delta 1 were 61% (179 out of 294) on ZDV-ddI, and 45% (137 out of 304) on ZDV-ddC ($P < 0.001$ for ZDV-ddI versus ZDV-ddC). In Delta 2, the proportion of responders was lower: 23% (31 out of 133) on ZDV-ddI, and 30% (35 out of 117) on ZDV-ddC (non-significant difference). In the monotherapy arms, the proportions responding were 10% (28 out of 291) and 2% (two out of 111) in Delta 1 and 2, respectively. Fig. 1 shows the cumulative proportion responding over time separately

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.



1 DELTA 1

| | | | | |
|-----------------|-----|-----|-----|-----|
| --- ○ ZDV | 291 | 250 | 233 | 217 |
| — △ ZDV+DDC | 304 | 179 | 146 | 133 |
| □ ZDV+DDI | 294 | 108 | 94 | 85 |

2 DELTA 2

| | | | | |
|-----------------|-----|----|----|----|
| --- ○ ZDV | 111 | 99 | 84 | 71 |
| — △ ZDV+DDC | 117 | 81 | 66 | 57 |
| □ ZDV+DDI | 133 | 98 | 84 | 70 |

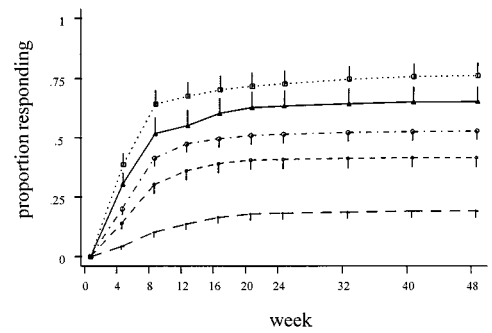
Fig. 1. Cumulative virological response (with 1 SE) by treatment arm.

for participants in Delta 1 and Delta 2. Almost all those who responded did so by week 16.

In Delta 1 the proportion of participants with HIV RNA below 800 copies/ml reached a maximum of 52% at 4 weeks for the ZDV–ddI arm and 36% at 16 weeks for the ZDV–ddC arm. At 48 weeks almost 30% and at 96 weeks about 20% of participants on the combination arms still had HIV RNA levels below cut-off. In Delta 2, the maximum proportion below cut-off was about 20% for both combination arms, between weeks 4 and 16 for the ZDV–ddI arm and between weeks 4 and 32 for the ZDV–ddC arm.

Because a substantial proportion of participants on the combination arms, in particular those allocated to ZDV–ddI, achieved HIV RNA levels below the limit of detection of the assay and their viral load was considered to be 800 copies/ml on those occasions, the mean viral load overestimates the true value. To attempt to correct for this, the mean viral load has been estimated assuming that \log_{10} HIV RNA is normally distributed. The revised estimates, as expected, suggest that the ZDV–ddI arm achieved lower mean HIV RNA values than the ZDV–ddC arm: for example, for Delta 1 at 16 weeks the estimated mean HIV RNA was 3.03 \log_{10} copies/ml for ZDV–ddI, 3.46 \log_{10} copies/ml for ZDV–ddC, and 4.44 \log_{10} copies/ml for the ZDV arm. This compares with 3.51 \log_{10} copies/ml, 3.66 \log_{10} copies/ml and 4.45 \log_{10} copies/ml, respectively (Table 3), when the results are censored at 800 copies/ml.

Baseline HIV RNA levels and CD4 cell counts were highly predictive of time to response, as defined in Methods, in both Delta 1 (Figs 2 and 3) and Delta 2. After adjusting for baseline CD4 cell counts, HIV RNA remained highly predictive in both study groups, ($P < 0.001$ for each comparison). However, CD4 cell



Baseline RNA

| | | | | |
|-------------------|-----|-----|-----|-----|
| □ 1: <5,000 | 59 | 19 | 15 | 10 |
| — △ 2: 5,000– | 56 | 21 | 17 | 16 |
| --- ○ 3: 10,000– | 256 | 125 | 109 | 96 |
| --- △ 4: 50,000– | 192 | 114 | 98 | 92 |
| --- ○ 5: ≥100,000 | 326 | 258 | 234 | 220 |

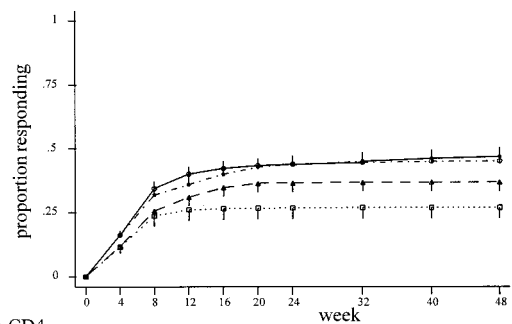
Fig. 2. Cumulative virological response (with 1 SE) by baseline HIV RNA in Delta 1.

counts adjusted for baseline HIV RNA levels were predictive of time to response in Delta 2 but not in Delta 1 ($P = 0.01$ and 0.8 , respectively).

Viral load rebound

The proportions of responders who had not failed by 48 weeks were 60% (12 out of 20) on ZDV, 51% (99 out of 196) on ZDV–ddI, and 55% (80 out of 145) on ZDV–ddC. There was no difference in failure rates between the ZDV–ddI and ZDV–ddC arms ($P > 0.9$ after adjustment for baseline HIV RNA and CD4 cell counts, global test). The proportions failing were very similar for Delta 1 and Delta 2 at 161 (52%) out of 307 and 30 (56%) out of 54, respectively.

The predictive effect of baseline HIV RNA on cumulative failure rates by time after adjusting for baseline CD4 cell count is shown in Fig. 4. After 12 weeks, there was a clear separation between those with $\geq 10\,000$ HIV RNA copies/ml and those with lower levels ($P < 0.001$), but little evidence that within these



Baseline CD4

| | | | | |
|-----------------|-----|-----|-----|-----|
| □ 1: <100 | 139 | 95 | 80 | 75 |
| --- △ 2: 100– | 271 | 163 | 146 | 137 |
| --- ○ 3: 200– | 287 | 162 | 149 | 137 |
| --- △ 4: ≥300 | 192 | 118 | 98 | 86 |

Fig. 3. Cumulative virological response (with 1 SE) by baseline CD4 cell count in Delta 1.

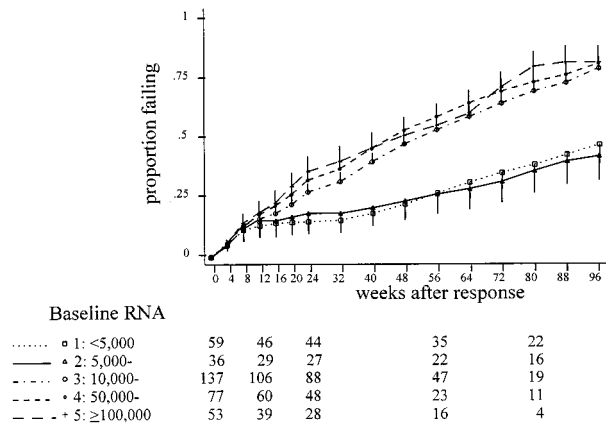


Fig. 4. Viral load rebound (with 1 SE) by baseline HIV RNA (adjusted for baseline CD4 cell count) in Delta 1 and Delta 2.

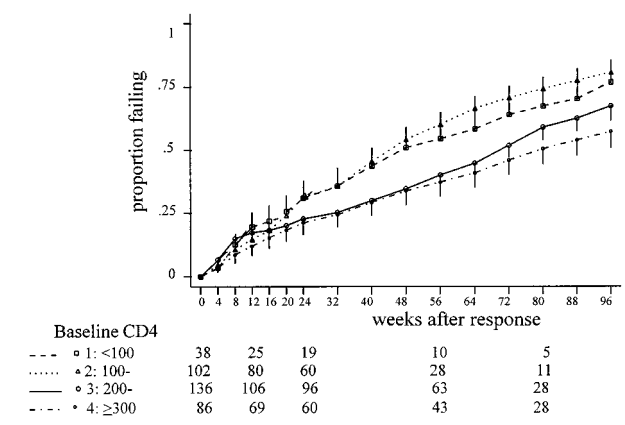


Fig. 5. Viral load rebound (with 1 SE) by baseline CD4 cell count (adjusted for baseline HIV RNA) in Delta 1 and Delta 2.

Table 4. Percentage of participants with a virological response who maintained their response at 48 weeks by baseline HIV RNA and CD4 cell count (based on zidovudine-naive and experienced participants).

| HIV RNA copies/ml | CD4 cells ($\times 10^6/l$) | | | | Total | 95% CI |
|-------------------|-------------------------------|-------------|-------------|------------|--------------|--------|
| | ≤ 100 | 100–199 | 200–299 | ≥ 300 | | |
| ≥ 50000 | 7/17 (41) | 12/45 (27) | 22/40 (55) | 16/28 (57) | 57/130 (44) | 35–53 |
| 10001–49999 | 6/17 (35) | 16/36 (44) | 28/52 (54) | 17/32 (53) | 67/137 (49) | 40–58 |
| ≤ 10000 | 1/4 (25) | 13/21 (62) | 34/43 (79) | 19/26 (73) | 67/94 (71) | 61–80 |
| Total | 14/38 (37) | 41/102 (40) | 84/135 (62) | 52/86 (60) | 191/361 (53) | 48–58 |
| 95% CI | 22–54 | 31–50 | 53–70 | 49–71 | | |

CI, Confidence interval.

groupings baseline HIV RNA levels were predictive of failure. A similar pattern was observed for CD4 cell counts (Fig. 5), the main difference being between those with CD4 cell counts of $\geq 200 \times 10^6/l$ and those with lower counts.

Table 4 shows the percentage of participants with a virological response who were still classified as responders at 48 weeks according to their baseline HIV RNA and CD4 cell count. Long-term virological response was independently associated with HIV RNA and CD4 cell counts at baseline ($P < 0.001$ for both associations). Those achieving a virological response by 56 days sustained that response for longer than those responding after 56 days (Fig. 6; $P = 0.004$ for comparison of time to failure in Delta 1 and Delta 2 combined, adjusted for baseline HIV RNA and CD4 cell count).

Table 5. Minimum HIV RNA (nadir) achieved and subsequent death (based on zidovudine-naive and experienced participants).

| Minimum HIV RNA to week 16 (copies/ml) | No. at risk | Deaths | Hazard ratio (95% CI) | P |
|--|-------------|--------|-----------------------|---------|
| < 800 | 390 | 18 | 1.0 | |
| 800–4999 | 268 | 27 | 2.4 (1.3–4.5) | 0.004 |
| 5000–9999 | 110 | 17 | 3.3 (1.7–6.7) | < 0.001 |
| 10000–49999 | 273 | 86 | 6.0 (3.4–10.7) | < 0.001 |
| ≥ 50000 | 181 | 97 | 9.8 (5.2–18.4) | < 0.001 |

CI, Confidence interval.

Association between maximum virological response and death

The relationship between the HIV RNA nadir achieved at any time within the first 16 weeks and subsequent death is shown in Table 5. The data suggested that the risk of death increased linearly with increase in the minimum \log_{10} HIV RNA (test for non-linearity, $P = 0.2$). The risk was increased 3.7-fold [95% confidence interval (CI), 2.0–5.6] for each \log_{10} increase in the minimum HIV RNA.

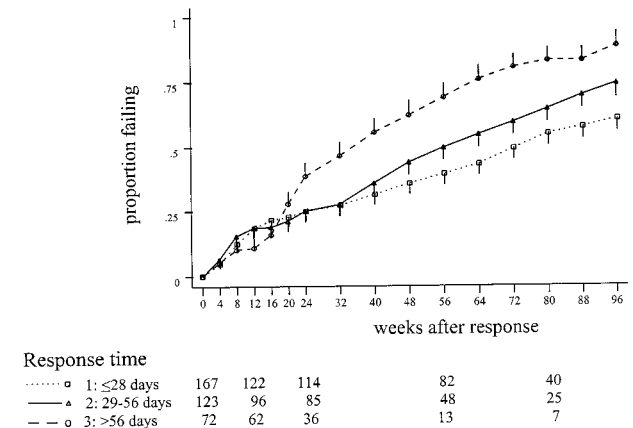


Fig. 6. Time to viral load rebound (with 1 SE) by time to virological response (adjusted for baseline HIV RNA and CD4 cell count) in Delta 1 and Delta 2.

Discussion

Although many studies have been reported that assess the prognostic value of viral load, most have been based on small numbers of patients and have not included changes in viral load in response to therapy. This includes the virological substudy of the Delta trial, which reported on viral load and resistance in 240 of the 3307 participants, none of whom had received previous antiretroviral therapy. The results reported here from the extended Delta virology study are based on over 1200 participants, a third of the participants in Delta [11], of whom almost one-third had received prior ZDV. This is one of the largest virological studies within a trial that showed substantial differences in survival between combination regimens and ZDV monotherapy and was itself large enough to demonstrate significant differences between the randomized groups in both survival and disease progression over an average follow-up of 2.5 years and with virological results up to 2 years. In the analyses reported here, no formal modelling was done to allow for missing HIV RNA values. Missing values were not likely to have affected the comparison between the treatment groups. However, within treatment groups, time to virological response was likely to have been underestimated if more patients with a poor prognosis dropped out than amongst those with a good response.

In the extended virology study, as in the substudy, the difference between the treatment groups in changes in HIV RNA over time as well as in CD4 cell count were consistent with the difference in clinical outcome in both ZDV-naïve and pretreated participants. Although the extended study used the NASBA assay with a cut off of 800 copies/ml, the findings in ZDV-naïve individuals were similar to those in the substudy that used the Roche Amplicor assay with a cut-off of 400 copies/ml. 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. However, the reduction in viral load in the two combination groups was, if anything, greater than that seen in the ZDV monotherapy group in ZDV-naïve participants. The difference in viral load changes between the monotherapy and combination arms in the ZDV-experienced group and ZDV-naïve group were very similar (approximately $0.7 \log_{10}$ and $0.8 \log_{10}$ copies/ml, respectively), suggesting that the introduction of a new drug had a similar effect in ZDV-naïve and experienced participants.

The ability of therapies to reduce the HIV RNA below the limit of detection of the current assays is increasingly being used to define response. In ZDV-naïve participants, within the first 16 weeks of treatment the proportion achieving a virological response (< 800

copies/ml) was significantly lower in the ZDV monotherapy group than in the combination groups and significantly lower in the ZDV-ddC group than the ZDV-ddI group. In those participants who had already had ZDV, very few participants responded in the ZDV monotherapy group, but the proportions responding in the combination groups were greater than those in the ZDV monotherapy group in Delta 1. It is important to note that because the maximal response was achieved at different timepoints in individual participants, an analysis of the maximum response by participants shows greater median maximum response than the analysis of the median changes over time; the latter analysis may therefore underestimate the maximum achievable response, particularly if there is considerable variation between participants in the time when this is achieved. From these data we have demonstrated a strong linear association between the HIV RNA nadir achieved in the first 16 weeks and subsequent clinical response, the risk increasing 3.7-fold (95% CI, 2.0–5.6) for each \log_{10} increase in the minimum HIV RNA. The data provide no information on the relationship below the cut-off of 800 copies/ml used in this study.

If the ability to reduce viral load below detectable levels is to be used to assess new therapies, it is important to determine how rapidly this is likely to occur. Although other studies have attempted to look at this, they invariably use inappropriate statistical methods, based on Kaplan–Meier survival techniques, which require a more precise knowledge of the date of the event. Time to virological response based on HIV RNA levels is dependent on the availability of specimens and is affected by missing data; therefore, the interval censored methodology used here is more appropriate. Time to viral load rebound requires a similar approach.

Survival and disease progression were independently predicted by HIV RNA and CD4 cell count both at baseline and at 8 weeks, as was virological response (defined as HIV RNA below the limit of detection of the assay). However, when both baseline and 8-week values were assessed as predictors together, only the 8-week RNA was predictive, whereas both baseline and 8-week CD4 cells remained predictive. Although the ability to reduce the viral load initially is an important predictor, an equally if not more important measure of the activity of a therapy is likely to be the ability to sustain the virological response. Because the Delta trial included a prolonged follow-up both for HIV RNA and clinical outcome, the duration of response, measured as the time to viral load rebound (defined as an HIV RNA value of more than 2 SD above the lower limit of detection, i.e., $2.90 + 0.6 = 3.50 \log_{10}$ copies/ml), could be studied. In participants who responded, the time to failure did not appear to depend

on the treatment group or on whether participants had received prior ZDV. The durability of response was related to the baseline HIV RNA; over half the participants with HIV RNA less than 10 000 copies/ml had not failed compared with about one-quarter of those with higher levels. Similarly, baseline CD4 cell count predicted the duration of response, irrespective of viral load. Opravil and colleagues recently reported that baseline HIV RNA was strongly predictive of sustained suppression of HIV RNA (< 400 copies/ml) at 1 year in 1076 participants from six prospective trials treated with ZDV and lamivudine [14]. Thus, in the patients who were antiretroviral treatment-naïve, 72% (95% CI, 56–88) of those with less than 5000 copies/ml at baseline had sustained reductions of less than 400 copies/ml at week 48 compared with 14% (95% CI, 8–21) of those with 50 000–200 000 copies/ml and 1% (95% CI, 0–3) of those with more than 200 000 copies/ml at baseline. Baseline CD4 cell count was, however, not found to be independently predictive of long-term suppression.

This study adds a considerable body of data to the accumulating evidence that HIV RNA and CD4 cells and changes in the markers in response to treatment are valuable markers to assess prognosis and the activity of new therapies. However, the analysis did not address the issue of whether these markers can be used as surrogates for clinical outcome for the definitive evaluation of such therapies. Analyses to explore this have also been undertaken and will be reported separately.

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Appendix

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