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A Critical Analysis of the Pharmacology of AZT and its Use in AIDS

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Summary

The triphosphorylated form of the nucleoside analogue 3'-azido-3'-deoxythymidine (Zidovudine, AZT) is claimed to interrupt the HIV replication cycle by a selective inhibition of viral reverse transcriptase, thereby preventing the formation of new proviral DNA in permissive, uninfected cells. Given that initial HIV infection of an individual instigates abundant HIV replication from inception until death, and that the life of infected T-cells is only several days, the administration of AZT should lead both in vitro and in vivo (i) to decreased formation of proviral DNA; and thus (ii) to decreased frequencies of 'HIV isolation' (detection of p24 or reverse transcription or both) in stimulated cultures/cocultures of T-cells from seropositive individuals; (iii) to decreased synthesis of HIV p24 and RNA ('antigenaemia', 'plasma viraemia', 'viral load') ultimately resulting in low or absent levels of all three parameters; and (iv) to a perfect and direct correlation between all these parameters. A critical analysis of the presently available data shows that no such evidence exists, an outcome not unexpected given the pharmacological data on AZT. HIV experts all agree that only the triphosphorylated form of AZT (AZTTP) and not the unphosphorylated form administered to patients, nor its mono- or diphosphate, is the active agent. Furthermore, the mechanism of action is the ability of AZTTP to halt the formation of HIV-DNA (chain termination). However, although this claim was posited from the outset, AZT underwent clinical trials and was introduced as a specific anti-HIV drug many years before there were any data proving that the cells of patients are able to triphosphorylate the parent compound to a level considered sufficient for its putative pharmacological action. Notwithstanding, from the evidence published since 1991 it has become apparent that no such phosphorylation takes place and thus AZT cannot possess an anti-HIV effect. However, the scientific literature does elucidate: (i) a number of biochemical mechanisms which predicate the likelihood of widespread, serious toxicity from use of this drug; (ii) in vitro data proving that AZT has significant antibacterial and antiviral properties which confound interpretation of its effects when administered to patients. Based on all these data it is difficult if not impossible to explain why AZT was introduced and still remains the most widely recommended and used anti-HIV drug.

Introduction

Any drug used in the treatment of patients suffering from an infectious disease relies upon evidence obtained from both *in vitro* and *in vivo* studies proving beyond reasonable doubt that:

1. The patients are infected with a specific microbial agent and the agent is the cause of the disease.
2. The drug inhibits the agent or its biological effects.
3. The drug is non-toxic or its toxicity is less detrimental than its benefits.

The claim that HIV plays a causative role in AIDS has been questioned by many individuals¹⁻¹⁸. In fact, there is considerable doubt that the presently available data prove that AIDS patients, those at risk or other individuals are infected with a unique retrovirus HIV^{3,7,8,10-12,17-19}. None the less, for the purpose of the present discussion it will be assumed that such laboratory tests as 'HIV isolation', 'plasma viraemia', 'p24', 'p24 antigenaemia', 'HIV RNA' and 'proviral' 'HIV DNA' are all HIV specific and thus are proof of infection with a unique, exogenously acquired retrovirus, HIV.

The retroviral theory of AIDS asserts that the cycle of HIV replication begins with fusion of HIV to permissive cells and the introduction of HIV into the cell. Inside the cell the viral RNA is reverse transcribed into DNA, which is then inserted into the cellular DNA as the 'HIV provirus'. The process of reverse transcription is catalysed by an enzyme said to be viral specific known as reverse transcriptase. Subsequently, the DNA provirus is transcribed into viral RNA, which in turn is translated into viral proteins. Finally, RNA and proteins are assembled into viral particles which are released from the cell membrane, whereupon the newly produced viral particles infect fresh cells and the

replicative cycle repeats. Although the previous conviction was that the production of HIV from proviral DNA involved prolonged virological latency, at present HIV experts assert 'high-level viral replication from the time of initial infection until death'; that is, HIV infected T-cells are killed from inception^{20,21}. According to the HIV experts, AZT in its triphosphorylated form is a selective inhibitor of viral reverse transcriptase, inhibiting the generation of proviral HIV DNA and thus interrupting the cycle of new cellular infection while leaving intact the production of virus from cells already infected. Since virus production from infected T-cells is soon exhausted by their short lifespan ('half-life of about 1.6 days'), it can be predicted that the administration of AZT will be followed by a rapid reduction in all HIV parameters ('HIV isolation', 'plasma viraemia', 'p24', 'p24 antigenaemia', 'HIV RNA' and 'proviral' 'HIV DNA') and indeed to the complete absence of infected T-cells.

AZT is the first drug introduced to treat HIV infection and still remains the most frequently used drug for this purpose. The design, execution and interpretation of the clinical studies of AZT, administered either alone or in combination with other drugs, have been questioned by many authors. From the time of its introduction into clinical practice, John Lauritsen and Peter Duesberg have thoroughly and critically analysed the clinical trials of AZT and have consistently argued that the drug has no clinical benefits but is severely toxic – 'Poison by Prescription'²², 'AIDS by prescription'¹. Recently, many other authors have expressed doubts in relation to the trials and the clinical usefulness of AZT^{23,24}. Because of this, the clinical data will not be further analysed here, and instead the present analysis will concentrate on evaluating the data which are said to affirm AZT as an anti-retroviral agent.

AZT is a thymidine analogue in which the 3'-hydroxy (-OH) group is replaced by an

azido ($\equiv\text{N}$) group. The 3'-hydroxy group is absolutely necessary for the triphosphorylated nucleotides to be attached to the growing DNA chain. Because in AZT this group is missing, once AZT becomes attached to the DNA chain, no further growth can take place; that is, 'the DNA chain is terminated'²⁵. For AZT, as for the natural nucleotides, to be attached to the DNA chain – that is, to act as a DNA chain terminator – it must first be triphosphorylated. However, although AZT given to patients is not triphosphorylated, it is said that AZT, like the natural nucleotides, is triphosphorylated by cells. Since AZT has been used routinely in clinical practice for over 10 years, one would expect that at present there would be ample evidence which proves that cells are able to metabolise AZT to its active form to levels sufficient to inhibit the replication of HIV both *in vitro* and *in vivo* and that the drug indeed inhibits the replication of HIV.

A. Anti-HIV Effects of AZT – in Vitro

The introduction of AZT to treat HIV infected individuals is based on two studies conducted by researchers from the National Cancer Institute, Duke University Medical Center, and the Wellcome Research Laboratories. In the first study, reported by Mitsuya *et al.* in October 1985²⁶, the effects of AZT on two HIV parameters, p24 and reverse transcriptase (RT), were investigated in cell cultures. It was concluded that AZT 'was a selective and potent inhibitor of human T-cell lymphotropic virus type III'. In the second study, published by Furman *et al.* in November 1986²⁷, the only HIV parameter studied was reverse transcriptase. These authors reported that 'The reverse transcriptase was much more sensitive to inhibition than was the DNA polymerase α of H9 cells. The

IC_{50} values for the viral reverse transcriptase were $0.7\ \mu\text{M}$ with poly(rA).oligo(dT)₁₂₋₁₈ and $2.3\ \mu\text{M}$ with activated calf thymus DNA as primer-templates. In contrast, an IC_{50} value of $260\ \mu\text{M}$ was determined for azidothymidine triphosphate with the H9 DNA polymerase α when activated calf thymus DNA was used as primer-template.' Based on the evidence from these two *in vitro* studies, the authors introduced AZT into clinical practice. In fact the first two clinical trials of AZT were commenced before the publication of the Furman *et al.* study. However:

1. *In vitro* data cannot be extrapolated *in vivo*. The authors themselves emphasised 'that the activity of an agent against viruses *in vitro* does not ensure that the agent will be clinically useful in treating viral diseases. Toxicity, metabolic features, bioavailability, and other factors could negate the clinical utility of a given agent'. Because of this, the introduction of a drug in clinical practice is usually preceded by experiments to gain such data in animals. Such data on AZT, in addition to providing information on the anti-HIV effects of the drug, may have also provided useful information on the bioavailability, cellular triphosphorylation and toxicity of AZT. However, the first data on the bioavailability of AZT were not obtained until the first clinical trial, where it was found that the maximum plasma concentration was reached about 1 h after the oral administration of AZT and was $1.5\text{--}2\ \mu\text{mol/l}$ with a $2\ \text{mg/kg}$ dose, and $4\text{--}6\ \mu\text{mol/l}$ with a dose of $10\ \text{mg/kg}$. The 'plasma disappearance had a half-life of approximately 1 h'. By giving $10\ \text{mg/kg}$ of AZT every 4 h, 'plasma drug levels were maintained above $1\ \mu\text{mol/l}$ '²⁸. At present, such a dose would be considered prohibitively toxic by most, if not all, HIV/AIDS researchers. None the less, these authors reported

that 'Treatment was not limited by side-effects, the commonest of which were headaches and depression of white-cell counts.' No data on the triphosphorylation of AZT were obtained.

2. In their 1985 paper, Mitsuya *et al.* reported that 'a substantial level [14,000 cpm] of reverse transcriptase activity could be detected in the supernatants of normal PBM exposed to HTLV_{III}B in the absence of A509U [AZT]...Inhibition was observed at doses as low as 0.005 μ M and was marked [8,000 cpm] at 0.05 μ M. Complete inhibition was achieved at doses of 0.5 μ M and more'. However:

- (a) although the authors stated that 'the unphosphorylated compound [AZT] does not inhibit reverse transcriptase *per se*' and the AZT used was not triphosphorylated, no data were presented that the AZT used in their experiments ('Structure I', 'A509U') was triphosphorylated by the cells;
- (b) in the two studies, AZT was introduced at the time of infection of the cultures, while patients are infected for many months or years before treatment.
- (c) one year after the publication of the two studies researchers from Yamaguchi University and Hokkaido University, Japan, reported that AZT 'did not show any effect in the HTLV-III-producing cell line Molt-4/HTLV-III', which was infected before the introduction of AZT²⁹.
- (d) in a study published in 1987 by researchers from the University of North Carolina, H9 and Jurkat cells were pretreated with concentrations of AZT ranging from 0.5 to 100 μ M, infected and maintained in drug-containing medium. Discussing their findings and those of others, the au-

thors wrote: 'Although AZT may be primarily a competitive inhibitor for RT, acts as a chain terminator, and perturbs nucleoside triphosphate pools within the cells, our results showed that complete DNA copies of the viral genome were formed in the presence of AZT. Since further steps in the virus life cycle (e.g. production of mRNA and progeny viral RNA) dependent on cellular RNA polymerase were not affected by the drug, virus production could then ensue. These proposed effects of the drug on aspects of the viral replication cycle are supported by a report that virus production is not suppressed in cells already producing HIV....Whether virus spread occurs by cell-free virus or by cell-to-cell contact, cultures treated with 25 μ M AZT eventually produced as much virus as the non-drug-treated infected cultures. These results were confirmed by the detection of unintegrated viral DNA in drug-treated H9 cultures when they began producing virus at high levels. The unintegrated viral DNA in these drug-treated cultures was present in quantities similar to those in non-drug-treated infected cultures'³⁰.

3. As mentioned, in the 1986 Furman *et al.* paper, the IC₅₀ AZT 'values for the viral reverse transcriptase were 0.7 μ M with poly (rA).oligo(dT)₁₂₋₁₈ and 2.3 μ M with activated calf thymus DNA as primer-templates'. These results were obtained by using 'purified HIV reverse transcriptase'. However, the ability of AZT to inhibit 'purified' enzyme does not prove the same effect will be observed on RT present in cells or in the viral particle.
4. HIV reverse transcriptase was 'purified' as follows: '750 ml of culture fluid harvested from HIV-infected H9 cells was

centrifuged at 18,000 rpm for 90 min in an R19 rotor (Beckman) to pellet virus. Enzyme was extracted by incubating the virus pellet in buffer A [50 mM Tris.HCl, pH 7.9/0.25% Nonidet P-40/20 mM dithiothreitol/50% (vol/vol) glycerol] containing 1 mM EDTA, 500 mM KCl, and 0.5% deoxycholate. The enzyme was partially purified by passing the extract through a DEAE-cellulose column (3 x 10cm) previously equilibrated with buffer A. Fractions containing enzyme activity were dialysed against buffer B [50 mM Tris.HCl, pH 7.9/50 mM NaCl/1 mM EDTA/1 mM dithiothreitol/20% glycerol] and were further purified by phosphocellulose chromatography. The peak fractions were pooled and dialysed against buffer B containing 50% glycerol. To the dialysed enzyme, bovine serum albumin was added to give a final concentration of 1 mg/ml. The enzyme was characterised as HIV reverse transcriptase based on its cation, salt, pH, and template requirements (5). However, by this method it is not possible to say that one has a purified HIV enzyme or any purified enzyme, viral or cellular. As far as the claim of characterisation as 'HIV reverse transcriptase' is concerned, Reference 5, cited by the authors, is a paper published by Jay Levy and his colleagues in 1985 where they present results which 'indicate specific characteristics of the RT of ARV', namely 'The RNA-dependent DNA polymerase of the AIDS-associated retrovirus (ARV) gives highest activity with the synthetic template, poly(rA).oligo(dT) and prefers Mg²⁺ over Mn²⁺ as a divalent cation', '100–200 mM KCl' as the monovalent cation, 'the major peak occurring at pH 8.0. A change in 0.2 pH units from 8.0 in either direction did not dramatically affect the reaction sensitivity'³¹. However, all cellular DNA polymerases can use Mg²⁺ as

the divalent cation and KCl as a monovalent cation and can be active at the pH of 8.0. As far as the template poly(A).oligo(dT) is concerned, it is sufficient to mention that:

- (a) the template-primer A(n).dT₁₅ can be transcribed not only by RT but by all the cellular DNA polymerases, α , β and γ ³². In fact, in 1975, an International Conference on Eukaryotic DNA polymerases, which included Baltimore and Gallo³³ defined DNA polymerase γ , 'a component of normal cells'³⁴, 'found to be widespread in occurrence'³², whose activity can be increased by many factors including PHA stimulation³⁵, as the enzyme which 'copies A(n).dT₁₅ with high efficiency but does not copy DNA well'³³;
- (b) in a paper published in 1984 by French researchers including Barre-Sinoussi, Montagnier and Chermann, it was shown that cellular DNA polymerases can also use Mg²⁺ as a divalent cation, KCl as a monovalent cation, including 200 mM KCl and a pH of 7.8. They also showed that enzymes from non-infected lymphocytes (especially DNA polymerase β) also used poly(rA).oligo(dT) as template primer³⁶.
- (c) Thus it is impossible to claim that the 'purified' enzyme which was inhibited by the drug was 'HIV reverse transcriptase', and not a cellular reverse transcriptase or any of the other cellular DNA polymerases. Indeed, given the facts that:
 - (i) the existence of 'HIV reverse transcriptase' was proven following the demonstration of reverse transcription of a particular synthetic RNA template-primer;
 - (ii) the same template-primer,

under the same experimental conditions, can be reverse transcribed by cellular DNA polymerases;

one can plausibly argue that at present no proof exists for the existence of a specific retroviral enzyme.

5. Even if the 'purified' enzyme which transcribed poly(rA).oligo(dT)₁₂₋₁₈ and 'activated calf thymus DNA' was HIV RT, just because the drug inhibited the transcription of this primer-template it does not mean that it will have the same or similar effect when the template is the HIV genome. That the template-primer to be transcribed plays a significant role is best illustrated by the finding that 'The IC₅₀ values for the viral reverse transcriptase were 0.7 μM [of triphosphorylated AZT] with poly (rA).oligo(dT)₁₂₋₁₈ and 2.3μM with activated calf thymus DNA as primer template'.
6. Because 'Azidothymidine triphosphate inhibited HIV reverse transcriptase ≈ 100 times better than it inhibited the H9 polymerase α, with activated calf thymus DNA as template', the authors of the first two *in vitro* studies concluded that AZT was 'a selective' inhibitor of HIV RT. However, polymerase α is not the only cellular DNA polymerase. For some unknown reason, these authors did not present data on the two other cellular DNA polymerases, polymerase β and γ. However, in 1990 Mitsuya and his associates, discussing the effects of nucleoside analogues in general, wrote: 'Several 2',3'-dideoxynucleoside 5'-triphosphates have been extensively studied and have higher affinities for HIV reverse transcriptase than for cellular DNA polymerase α, although cellular DNA polymerases β and γ (mitochondrial DNA polymerase) appear to be sensitive to the dideoxynucleoside 5'-triphosphates. The activity against mitochond-

rial DNA polymerase might explain certain side effects, such as a toxic mitochondrial myopathy in individuals receiving long-term AZT therapy'³⁷.

B. Phosphorylation of AZT

In determining the inhibition of the HIV RT by AZT, Furman *et al.*, in addition to not using cells or even 'pure HIV' but 'purified' enzyme, also did not use AZT in the form administered to patients. Instead, they used the triphosphorylated form of AZT (AZTTP), the only form of AZT accepted to have an anti-retroviral effect. (For their experiments 'The mono-, di-, and triphosphates of azidothymidine were prepared from azidothymidine by published methods'.) Apparently, to overcome this predicament, they conducted experiments to prove that cells are capable of phosphorylating AZT to AZTTP. For this, 'H9 cells were infected with HTLV-III_B' and incubated with 50μM AZT for 24 h either during infection or 'through the replication cycle of the virus', that is at days 3, 6 and 9 after infection. One non-infected H9 culture was also cultured with the same concentration of AZT for 24 h. The phosphorylated derivatives of AZT were measured using High-Performance Liquid Chromatography (HPLC). They reported that 'High concentrations of azidothymidine monophosphate were detected in the uninfected and the HIV-infected H9 cells, whereas the levels of the diphosphate and triphosphate were low. By 24 h these phosphorylated derivatives had accumulated maximally.... Increasing the time that the cells were exposed to the drug did not result in higher levels of phosphorylated derivatives'. The level of AZTTP reported was 1.5 pmol per 10⁶ cells (1.8 μM) in the non-infected culture and 0.9 (1.1); 1.0 (1.3); 1.7 (2.0); and 0.9 (1.1) in the four infected cultures. In other words, the level of AZT phosphorylated to AZTTP by the H9

cells was not sufficient to induce even a 50% inhibition of the 'purified HIV RT' when the non-synthetic, 'activated calf thymus DNA' was used as template-primer. To determine the decrease in the levels of the phosphorylated derivatives of azidothymidine, after removal of the drug from the incubation medium at day 5 after infection, cells were incubated for 24 h with 50 μM AZT, after which the cells were washed and the incubation was continued in a drug-free culture medium. The level of AZTTP was determined at time 0, 0.5, 1, 2 and 4 hours after removal of the drugs and was reported as being 7.2, 5.2, 1.9, 1.7 and 1 μM respectively. As can be seen, the level of AZTTP reported in the H9 cells not only did not decrease after the cells were washed as one would expect but, at least for the first 2 h, was if anything higher than when the drug was present.

Even if one cell line phosphorylates AZT to levels of AZTTP sufficient to inhibit the HIV RT, it does not mean that other cell lines will be able to produce the same effect. Indeed, by 1988 researchers from the US National Institutes for Health, including Samuel Broder, in collaboration with researchers from Belgium showed that the phosphorylation of AZT to AZTTP was dependent on the type of cell as well as the length of time during which the cells are incubated with the drug. The human lymphocyte ATH8 and human lymphoblast Molt/4F cells were incubated with 5 μM AZT for 5, 24 and 48 h. The level of AZTTP was 0.6, 0.4 and 0.2 μM in the Molt/4F cells at 5, 24 and 48 h. The respective levels in the ATH8 cells was 0.2; 0.1; < 0.1 μM ³⁸.

In a study published in 1991 by researchers from Sweden, resting and PHA stimulated PBMC from 31 healthy individuals and 5 HIV seropositive individuals were incubated with different concentrations of either radioactive or non-radioactive AZT. The phosphorylated AZT metabolites were quantified by HPLC. The authors reported:

'It was only possible to measure the di- and triphosphorylates when the cells had been labelled with radioactive AZT, while the monophosphate was detectable by ultra-violet (UV) absorbance even after incubation with non-labelled AZT'. The stimulated PBMC were incubated with 0.08, 0.16, 0.8 and 1.6 μM AZT. The quantity of AZTTP found in these cultures was: 0.12 ± 0.06 , 0.17 ± 0.09 , 0.20 ± 0.15 and 0.32 ± 0.19 nmol/ 10^9 cells, respectively (nmol/ 10^9 cells \equiv pmol/ 10^6 cells). For the non-stimulated PBMC the results for only two concentrations of AZT, 0.8 and 1.6 μM , are reported. In these cultures the AZTTP was found to be 0.002 ± 0.001 and 0.003 ± 0.002 nmol/ 10^9 cells, respectively. In other words, cells which are stimulated form approximately 100 times the amount of the triphosphorylated compound compared with cells which are unstimulated.

They also measured the half-life of AZT phosphorylated metabolites. For this the cells were cultured with AZT for 4 h after which the drug was washed and the cells cultured in drug-free medium. The half-lives of AZTMP, AZTDP and AZTTP in stimulated cells were 2.3 ± 0.7 h, 2.5 ± 0.6 h and 2.8 ± 0.6 h, respectively. 'The half-life of intracellular AZTMP in resting PBMC was also measured and was determined to be 1.5 ± 0.2 h.... Because of the low incorporation of radioactivity in the azidothymidine di- and triphosphate pools of the resting PBMC it was impossible to determine the half-life of these two metabolites....An approximately 20% variation in the amount of product found in stimulated cells from different individuals was found....The corresponding variation in resting PBMC was 50%....The intra-individual variation measured in subjects analysed repeatedly at 2-4 different occasions was also around 20%'.
'

They reported the following results from five seropositive individuals: 'PBMC from 5 HIV+ patients (1 classified as asymptomatic, 3 as ARC, and 1 as AIDS, respectively) were

incubated with AZT...we found stimulation by PHA of the PBMC only in the asymptomatic case. These cells thus accumulated AZTMP, AZTDP and AZTTP (18, 0.2 and 0.01 nmol/10⁹ cells, respectively, after incubation for 4 h with 1.6 μM AZT)...In the ARC and AIDS cases no stimulation was observed after 72 h. Resting PBMC from all 5 patients accumulated azidometabolites (1.6 μM AZT gave 0.05–0.42 nmol AZTMP/10⁹ cells), which would correspond to what was found with PBMC from HIV subjects'. No mention is made of the level of AZT diphosphate or AZTTP in the cells from ARC and AIDS patients³⁹.

Even if all human cells phosphorylated AZT to AZTTP with high efficiency under *in vitro* condition, it does not follow that the same effect would be observed *in vivo*. In other words the finding *in vitro* cannot be extrapolated to the situation *in vivo*. In fact, it is paramount that such evidence be obtained from AIDS patients and HIV seropositive individuals, not healthy volunteers. Indeed, given that:

- (a) The toxicity of AZT was recognised long before the AIDS era;
- (b) It is recognised that the antiretroviral effect of AZT is conferred only by its triphosphorylated form;

it is inconceivable to contemplate the introduction of AZT in clinical practice before there is proof that AZT is triphosphorylated in HIV positive individuals to a level necessary to inhibit viral RT. Yet this seems to be the case, since the first results of *in vivo* phosphorylation of AZT did not appear until the 1990s. Even then, although the then available *in vitro* evidence showed that no relationship existed between AZT concentration and the level of phosphorylated AZT or the total AZT phosphates level, or the triphosphate levels, for some unknown reason researchers from well known institutions such as the University of Cincinnati and Division of AIDS, National Institute of

Allergy and Infectious Diseases, Bethesda, Maryland, continued to report total AZT phosphorylates and not its active form, AZTTP^{40–42}.

In 1991, Takuo Toyoshima and his colleagues from the University of Tokyo and Research Institute, Sankyo Co., pointed out that 'for the better understanding of pharmacokinetics of AZT, it is necessary to gain an insight into the metabolism of AZT, especially into the intracellular concentrations of AZT-TP', but 'concentrations of these metabolites in peripheral blood mononuclear cells have not been measured in patients with acquired immunodeficiency syndrome'. They performed both *in vitro* and *in vivo* experiments. For the *in vitro* experiments they used the MT-4 and Molt-4 cell lines. For the *in vivo* experiments, 'A patient with AIDS and an asymptomatic carrier (AC) received 200 mg of AZT orally, and blood samples (15 ml each) were drawn 1 and 4 h after ingestion of the drug'. Using the MT-4 cell line they found that 'intracellular concentrations of AZT-MP increased as concentrations of AZT in the medium were elevated; a concentration of 6770 pmol/10⁷ cells was attained when 10 μM of AZT was present in the medium. Concentrations of AZT-DP and AZT-TP were one to two orders of magnitude lower than those of AZT-MP, and seemed to level off when the concentrations of AZT were higher than 5 and 2 μM, respectively.

'In MT-4 and Molt-4 cells incubated with 5 μM AZT, concentrations of AZT-MP increased time dependently, while the AZT-DP/AZT-MP ratios decreased with time'. They concluded, 'These data suggest that high dose of AZT may not necessarily increase intracellular concentration of AZT-TP'. From their experiments they reported that 'Concentrations of AZT-MP in PBMCs from a patient with AIDS and an AC were 260 and 500 pmol/10⁷ cells after 1 h, and 260 and 240 pmol/10⁷ cells after 4 h, respectively.

Those of AZT-DP were measured in an AC only; 6.5 pmol/10⁷ cells after 1 h, and 3.9 pmol/10⁷ cells after 4 h. Those of AZT-TP were 56 and 15 pmol/10⁷ cells after 1 h, and 17 and 13 pmol/10⁷ cells after 4 h, for a patient with AIDS and an AC, respectively⁴¹.

In the same year Herbert Kuster and his colleagues from the University Hospital Zurich wrote: 'Serum pharmacokinetics of AZT have been studied extensively: however, no data about the extent and kinetics of *in vivo* phosphorylation are available. To date the intracellular anabolism of AZT and of other dideoxynucleosides has been examined only *in vitro* using radiolabeled compounds. A detailed knowledge about the phosphorylation is important for several reasons. First, there is a documented variability of AZT phosphorylation in various cell systems, and data from *in vitro* experiments cannot necessarily be extrapolated to the *in vivo* situation. Second, interindividual differences in drug metabolism are well known in clinical medicine for a variety of compounds.... Finally, a better understanding of the *in vivo* pools and pharmacokinetics of intracellular AZT-TP might lead to improved drug schedules for individual patients. Thus we developed a method to measure the intracellular anabolites of AZT in whole blood from patients treated with this drug'.

They also performed both *in vitro* and *in vivo* experiments. For the *in vitro* experiments IL-2 stimulated PBMC from a healthy HIV-negative individual were incubated with ³H labelled and unlabelled AZT. The following findings were reported: 'In PBMC cultured in the presence of 2 µmol/l [³H] AZT for 24 h, concentrations of AZT-MP, AZT-DP and AZT-TP were 193, 1.3, and 2.0 pmol/10⁶ PBMC, respectively, after ficoll-hypaque density-gradient centrifugation. If cells were harvested by simple centrifugation, concentrations of 215, 1.7 and 1.6 pmol/10⁶ PBMC were found. PBMC of the same donor treated under identical conditions but with un-

labelled AZT yielded concentrations of 198 pmol/10⁶ PBMC for AZT-MP, 1.8 pmol/10⁶ PBMC for AZT-DP, and 2.4 pmol/10⁶ PBMC for AZT-TP by RIA'.

In the *in vivo* study: 'Blood samples were obtained from three patients on long term oral therapy with 250 mg of AZT every 12 h. AZT nucleotides were determined before and 1, 2, and 4 h after administration of the drug. No phosphorylation products were found before administration. Intracellular concentrations of AZT-MP after 1–2 h were 0.9–1.4 pmol/10⁶ PBMC and then declined to 0.3–1.1 pmol/10⁶ PBMC after 4 h. AZT-DP and AZT-TP reached concentrations of 0.3–0.5 pmol/10⁶ PBMC after 1–2 h and could not be detected after 4 h in any of the three patients'⁴⁴.

In 1992, researchers from Johns Hopkins University, Baltimore, stressed that the '*In vitro* studies of Zidovudine (ZDV) phosphorylation may not accurately reflect the *in vivo* dose–response relationship, which is crucial to determining the relationship between ZDV exposure, efficacy, and toxicity.... Quantification of intracellular levels of ZDV-TP, which is the active metabolite, and defining the time course of ZDV-TP formation and degradation, are of paramount importance for understanding the relationships between intracellular levels of ZDV-TP and antiviral activity'. Commenting on their own work and that of other researchers, the authors wrote: 'Attempts at measuring ZDV and its phosphorylated anabolites have been reported by Toyoshima *et al.*, who utilised a high-pressure liquid chromatography (HPLC) system with column switching and UV detection. Kuster *et al.*, using a coupled HPLC-radioimmunoassay (RIA) method, also measured ZDV and ZDV phosphates in HIV-infected patients. These methods however have not been thoroughly validated and they lack the sensitivity (limit of detection, 0.1 pmol/10⁶ peripheral blood mononuclear cells [PBMC]) needed for the study of the

time course of ZDV anabolism. Our study describes the development and validation of a specific and sensitive assay for measurement of ZDV and its phosphorylated anabolites from PBMCs of ZDV-treated HIV-infected patients'.

For their *in vitro* assay they used the Molt-4 cell line and PBMC, which they cultured with 2 μ M of AZT. The levels of ZDV-TP were 1.6 ± 0.7 pmol/ 10^6 cells in the Molt-4 cells and 0.011 ± 0.002 pmol/ 10^6 cells in the PBMC. *In vivo* they studied six infected patients who were receiving ZDV. 'The duration of previous ZDV therapy at the time of the study ranged from 1 to 8 months'. Two hours after a 300 mg oral dose, 'The mean concentrations (\pm standard deviation) of parent and of mono-, di-, and triphosphates were 0.15 ± 0.08 , 1.4 ± 1 , 0.082 ± 0.02 , and 0.081 ± 0.13 pmol/ 10^6 PBMC, respectively (1 pmol/ 10^6 PBMC represents a concentration of approximately 1 μ M). Concurrent serum ZDV concentrations were between 1.3 and 7.1 μ M'⁴⁵.

In a study published in 1994, 'ZDV-TP in PBMCs and plasma ZDV concentrations were measured in 12 HIV-infected adult volunteers receiving ZDV at St Jude Children's Research Hospital. All 12 volunteers studied were administered a single 100- or 500-mg oral dose of ZDV. Plasma ZDV concentrations and intracellular ZDV-TP levels were determined at 1, 2, 4, and 6 h after administration of the drug'. The authors reported that: 'Median intracellular ZDV-TP levels ranged from 5 to 57 and 42 to 92 fmol/ 10^6 cells in volunteers administered 100 and 500 mg of ZDV, respectively'⁴⁶ (1 fmol \equiv 10^{-3} pmol).

Michael Barry and his associates from the University of Liverpool's Department of Pharmacology and Therapeutics published two papers, one in 1994 and the other in 1996. In the first study five seronegative volunteers and 12 HIV-positive patients were given 250 mg AZT and blood samples were taken at 0, 1, 2, 4 and 6 h following drug administration. 'Three patients were asympto-

matic [Centers for Disease Control and Prevention (CDC) group II] and nine had AIDS'. In the seronegative volunteers the mean ZDV-TP levels were 0.04, 0.03, 0.02 and 0.06 pmol/ 10^6 cells at 1, 2, 4 and 6 h respectively. In the patients the corresponding values were: 0.05, 0.06, 0.06 and 0.04 pmol/ 10^6 cells. Commenting on their findings, the authors wrote: 'A concentration-dependent block in the formation of ZDV-DP and ZDV-TP from ZDV-MP has been observed in activated PBMC. These *in vitro* findings are consistent with the results we obtained in 12 HIV-seropositive patients administered ZDV 250 mg, where ZDV-MP was the main metabolite found in PBMC.... Interestingly, the concentrations of ZDV-TP in both HIV-seropositive patients and seronegative volunteers were comparable. In both groups there were subjects in whom ZDV-TP levels *could not be detected*. Although a more sensitive assay would be useful it is difficult at present to envisage an RIA with a detection limit much below that achieved in this and previous studies'⁴⁷ [our italics].

The 1996 study was designed to determine 'The effect of ZDV dose on the formation of intracellular phosphorylated metabolites', which 'may help define the optimum daily dose of ZDV, *which is still unknown*' [our italics]. Ten 'patients (ZDV-experienced) received, in random order, two dose regimens: ZDV 300 mg twice daily (600 mg per day) and ZDV 100 mg three times daily (300 mg per day) for 6 days. Therefore, all patients were at steady state ZDV therapy on attending the department for pharmacokinetic study on day 7. The study days were separated by at least 14 days.... On the study day patients arrived at 0800 h after an overnight fast. They ingested 100 or 300 mg ZDV at 0900 h according to the dose regime,' and blood was taken at 0, 1, 2, 4, 6 and 12 h after drug administration. When they compared the maximum concentration of ZDV in the plasma (C_{\max})

and the area under the ZDV concentration time curve (AUC_{0-12h}) for the two doses, they found that: 'The 300 mg dose produced an increase in C_{max} (2.59 ± 0.52 versus 0.7 ± 0.14 $\mu\text{mol/l}$) and AUC_{0-12h} (4.59 ± 0.79 versus 1.42 ± 0.51 $\mu\text{mol/l} \times \text{h}$)'. The time at which C_{max} was obtained, T_{max} , was not significantly different.

'For total intracellular ZDV phosphate metabolites the AUC_{0-12h} was doubled (7.64 ± 3.67 versus 3.71 ± 1.83 $\text{pmol}/10^6$ cells \times h) in patients taking 300 mg compared with 100 mg. The AUC_{0-12h} for ZDV-MP was significantly increased at the higher dose (6.47 ± 3.14 versus 2.77 ± 1.70 $\text{pmol}/10^6$ cells \times h)...However, there was marked intersubject variability in the AUC_{0-12h} for ZDV-DP (0.52 ± 0.32 versus 0.56 ± 0.57 $\text{pmol}/10^6$ cells \times h) and ZDV-TP (0.42 ± 0.42 versus 0.61 ± 0.81 $\text{pmol}/10^6$ cells \times h) with wide 95% confidence intervals on the differences in mean values, following ZDV 100 and 300 mg, respectively'. The mean C_{max} and T_{max} for AZT-TP were almost the same for both doses and were approximately 0.07 $\text{pmol}/10^6$ cells and 2 h respectively.

Discussing their findings, the authors wrote: 'Consistent with previous reports, we found a weak correlation between plasma concentration of ZDV and intracellular metabolites. Total phosphorylation appears to be a saturable process, and therefore increases in plasma ZDV concentration do not result in parallel increases in total phosphate concentrations....As ZDV-TP inhibits viral reverse transcriptase, its measurement (or more precisely the ratio of ZDV-TP to thymidine triphosphate) is more likely to provide satisfactory dose-response relationships for ZDV. In this study, the AUC_{0-12h} for ZDV-TP did not differ significantly following the 100 or 300 mg ZDV dose....With the evidence that saturation of ZDV phosphorylation occurs after administration of 100 mg ZDV and with the half-life of intracellular phosphates being approximately 4 h, the

ability of the lower 100 mg dose to produce similar active drug, ZDV-TP and lower ZDV-MP (potentially toxic) suggests that ZDV 100 mg 8-hourly may be preferable to ZDV 300 mg 12-hourly....However, we also recognise that the antiviral effect of ZDV is ultimately dependent on the ratio of ZDV-TP to thymidine triphosphate, and we are aiming in future studies to measure the levels of both triphosphate anabolites'⁴⁸.

In an article published in *Nature Medicine* 1997, one reads that 'Azidothymidine triphosphorylate (AZT-TP) inhibits the viral RT by competing with endogenous thymidine triphosphorylate (TTP). The extent of inhibition, therefore, depends as much on the interplay of AZT-TP and TTP concentrations as on the concentrations of their respective intermediates, and the degree to which they themselves serve as substrates for the two kinases. Although AZT is converted to AZT-MP with nearly the same efficiency as the thymidine is converted to TMP, the conversion of AZT-MP to AZT-TP is less than one percent the efficiency of the TMP to TTP conversion....The end result is an accumulation of high concentrations of the inactive AZT-MP but not of the active AZT-TP'⁴⁹. Lately, several research groups have put forward proposals to account for the inability to achieve 'effective concentration of AZT-TP within cell sufficient to suppress HIV replication'⁵⁰⁻⁵², while others have reported that the herpes simplex virus type 1 thymidine kinase improves AZT triphosphorylation and suggested that 'gene transfer might be envisioned for genetic pharmacomodulation of antiviral drugs'⁵³.

Comments

Whatever the reason(s), the fact remains that, for AZT to have an anti-HIV effect, it must be triphosphorylated²⁸, but this is insignificant *in vivo*. In addition, the

triphosphorylated form is deemed responsible for its toxicity^{1,2}.

In their 1986 paper Philip Furman and his research colleagues from the National Cancer Institute, Duke University and Wellcome Laboratories²⁷ reported that, under ideal conditions, 'The IC₅₀ values for the viral reverse transcriptase were 0.7 μM with poly(rA).oligo(dT)₁₂₋₁₈, and 2.3 μM with activated calf thymus DNA as primer-templates'. In their first clinical trial²⁸ they acknowledged that 'a minimal level for an *in vitro* antiviral effect' is 'above 1 μmol/l' of AZTTP. However, such levels of AZT triphosphorylation are not obtained even under ideal, *in vitro* conditions, and the level of AZT triphosphorylation *in vivo* is even lower. This means that, as has been generally accepted to date, neither the well known toxic effects of AZT nor any antiretroviral effects can be due to its action as a DNA chain terminator. The question then is, how does AZT produce its toxic effects as well as its anti-HIV effects, if any?

Although AZT is not efficiently triphosphorylated it is very efficiently monophosphorylated. The mono-phosphorylation of AZT could act as an inhibitor of phosphorylation of cellular constituents, including cellular nucleotides. Indeed, in 1986 Furman and his associates showed that, *in vitro*, exposure of cells to 50 μM of AZT for 72 h led to a decrease of approximately 95% in dTTP and dCTP and a decrease of approximately 63% in dGTP. This decrease in the triphosphorylated nucleotides in its turn will lead to decreased cellular DNA synthesis. In the presence of such a profound, global reduction in the concentrations of the naturally occurring nucleotides, one would predict untoward effects on many tissues, especially those with the most rapid cellular turnover including the gut and the bone marrow. Indeed, 'a characteristic feature of zidovudine therapy is an elevated MCV [mean corpuscular red cell volume]⁵⁴, and 'The

antiviral agent zidovudine (AZT), used for treating the human immunodeficiency virus (HIV), often causes severe megaloblastic anaemia⁵⁵, anaemia 'caused by impaired DNA synthesis'⁵⁵.

It is a well known fact that AZT inhibits mitochondrial DNA (mtDNA) replication. However, since the level of AZT triphosphorylation is negligible, this effect cannot be due to AZT acting as a DNA chain terminator. In their effort to explain the AZT mitochondrial toxicity, researchers from the University of Nagoya studied the mtDNA of mice given either 1 mg/kg/day or 5 mg/kg/day of AZT orally for 4 weeks. Their findings, published in 1991, 'suggest that the oxygen damage of mtDNA is the primary cause of mitochondrial myopathy with AZT therapy...oxidative damage of mtDNA can be accumulated during even short period of AZT administration'. They concluded: 'The animal model of mitochondrial myopathy with AZT administration reported here seems to be useful for elucidating the mechanism of mtDNA mutations leading to myopathy. However, for AIDS patients, it is urgently necessary to develop a remedy substituting this toxic substance, AZT'⁵⁶.

The cellular toxicity of AZT was extensively studied by researchers from the State University of New York. In 1996, summarising their findings, they wrote: 'Prior to the commencement of the present study, although strong evidence existed that many ddNs, including AZT, could inhibit mtDNA replication, we had not yet substantiated our hypothesis that such inhibition would result in the impairment of oxidative phosphorylation....Nor had we yet demonstrated a cause-and-effect relationship between the AZT inhibition of mtDNA replication (or its consequence, an impairment of oxidative phosphorylation) on the one hand and an inhibition of cell growth on the other. Thus, the possibility had not been eliminated that AZT was exerting some general cytotoxic ef-

fect on the cell, which resulted in an inhibition of cell growth, and this, in turn, was leading to an inhibition of mtDNA replication....We noticed that the beginning of the AZT-induced inhibitory effect on cell growth occurred at a relatively short time after AZT addition to the medium, a period of time too short to account for the effect to have been brought about by an inhibition of mtDNA replication. This observation led to studies of the early metabolic events that occur upon exposure of the cells to AZT'.

In these studies the authors found that: 'mitochondria isolated from cells grown in the presence of pharmacological levels of AZT (5µM) for 5 days and tested for their ability to carry out oxidative phosphorylation showed a marked decrease in ability to synthesize ATP...Further studies of this phenomenon in which the frequency of sampling the medium was in hours rather than days...showed early changes in O₂ uptake, lactate synthesis, ATP level, and number of mitochondria per cell. Some of these changes, particularly that of ATP level, were observable as early as 3 h after exposure to AZT and, judging from the precipitous decline of the ATP/cell curve between 0 and 3 h, may have begun earlier than that. The 3 h time interval, equivalent to only 7% of the doubling time of the AZT-treated cells, is far too short a period of time to account for the effect brought about by an inhibition of mtDNA replication'⁵⁷.

In a study published in 1997, researchers from several French institutions 'compared the effects of AZT, ddI and ddC on proliferation, differentiation, lipid accumulation, lactate production and mitochondrial enzyme activities in cultured human muscle cells'. They reported that: 'All 3 compounds induced a dose-related decrease of cell proliferation and differentiation. AZT seemed to be the most potent inhibitor of cell proliferation. AZT, ddI and ddC induced cytoplasmic lipid droplet accumulations, increased lactate

production and decreased activities of COX (complex IV) and SDH (part of complex II)' (COX=cytochrome *c* oxidase; SDH=succinate dehydrogenase). Summarising their findings they wrote: 'In conclusion, AZT, ddI and ddC all exert cytotoxic effects on human muscle cells and induce functional alterations of mitochondria possibly due to mechanisms other than the sole mtDNA depletion'⁵⁸.

At present, evidence also exists which shows that AZT is rapidly reduced by compounds containing sulphhydryl (-SH); that is, AZT oxidises the -SH groups⁵⁹. Ample evidence also exists which shows that oxidation in general (and of -SH in particular) and decreased levels of ATP may lead to many laboratory and clinical abnormalities, including wasting, muscular atrophy, anaemia, damage to the liver and kidney, decreased cellular proliferation, cancer and immunodeficiency^{8,19}. Since patients who are at risk of AIDS are exposed to many oxidising agents⁸ and are known to have low -SH levels^{60,61} one would expect AZT to have particularly toxic effects in these individuals – and the sicker the patient the more toxic the drug. That this is the case was accepted by researchers from the National Cancer Institute, Wellcome Laboratories and Abbott Laboratories as far back as 1988: 'Azidothymidine, however, is associated with toxicities that can limit its use....These toxicities are particularly troublesome in patients with established AIDS; the use of azidothymidine is often limited in this population'⁶². Despite these caveats it is possible that, if a thymidine analogue is to be administered to patients with AIDS or to those at risk, at least part of its toxicity may be eliminated by substituting the 3'-OH group with a -SH-group instead of an azido (≡N) group. Yuzhakov *et al.* have performed such experiments and shown that the resulting compound inhibits 'HIV RT'⁶³.

C. Anti-HIV Effect of AZT

Since it is a fact that:

1. HIV experts agree that AZT produces its anti-HIV effects only by inhibiting the reverse transcription of the 'HIV RNA' into 'HIV proviral DNA';
2. The same experts also agree that only triphosphorylated AZT can inhibit the synthesis of proviral DNA;
3. The AZT given to patients is not triphosphorylated;
4. The triphosphorylation of AZT in HIV seropositive and AIDS patients, if any, is significantly lower than the concentration needed to inhibit RT even in the most ideal conditions;

the inescapable conclusion is that AZT, as given to patients, cannot have an anti-HIV effect. How is it then possible to reconcile this fact with the claim that HIV is an anti-HIV drug?

The only way of proving the antiretroviral effect of AZT is to determine its effect on HIV isolated from tissues of infected, treated patients. The correct procedure, used for over half a century to achieve isolation of retroviruses^{64,65}, requires:

1. Culture of putatively infected tissues.
2. Purification of specimens by density gradient ultracentrifugation.
3. Electron micrographs of particles exhibiting the morphological characteristics and dimensions of retroviral particles at the sucrose density gradient of 1.16 gm/ml containing nothing else, not even particles of other morphologies or dimensions.
4. Proof that such particles contain reverse transcriptase.
5. Analysis of the particles' proteins and RNA, and proof that these are unique.
6. Proof that 1-5 are properties only of pu-

tatively infected tissues and cannot be induced in control cultures.

7. Proof that such particles replicate into identical particles and are thus infectious.

This procedure has never been used to prove the antiretroviral effects of AZT, or for any other purpose, including proving the existence of HIV. Instead, the antiretroviral effects of AZT have been studied by observing its effects on:

1. 'HIV isolation', defined as detection of RT and the 'HIV p24' protein in stimulated cultures/cocultures of tissues obtained from treated patients. Most often, the effects on 'HIV isolation' are merely detection of just one of these phenomena.
2. 'HIV antigenaemia', by which is meant reaction of proteins present in patient sera with antibody to the 'HIV p24' protein.
3. Estimation of 'viral load', defined by HIV researchers as the quantity of 'HIV RNA' molecules in a sample of patient plasma, or detection of p24 in plasma cultures.

However, RT is not specific to retroviruses and p24 and 'HIV RNA' have never been shown to belong to a particle, viral or non-viral, much less to a unique retroviral particle, HIV^{7,10,12,17,18}. In fact, at present there is ample evidence which shows that these parameters are not HIV specific⁶⁶⁻⁷¹. This means that, even if AZT has an effect on these three parameters, such evidence cannot be considered as proof that AZT has an anti-HIV effect. If, on the other hand, there is no proof that AZT significantly effects these three parameters, then it would be impossible to claim that AZT has an anti-HIV effect.

1. HIV Isolation

By design, the role of AZT is not to inhibit HIV expression (activation) but to inhibit the

reverse transcription of the HIV-RNA into new proviral DNA. In other words, if AZT has anti-HIV effects, then the first thing one would observe is a decrease in the HIV-DNA which in its turn would lead to a decrease in the rate of HIV isolation.

In 1986 the researchers from the National Cancer Institute, Duke University and Wellcome Research Laboratories, published their results of the Phase I clinical trial of AZT in 19 patients with AIDS or AIDS related complex. 'All patients received test doses of AZT. They were then given AZT intravenously for 14 days according to the following regimens: 1 mg/kg every 8 h for patients 1–4 (regimen A), 2.5 mg/kg every 8 h for patients 5–10 (regimen B), 2.5 mg/kg every 4 h for patients 11–15 (regimen C), and 5 mg/kg every 4 h for patients 16–19 (regimen D). Each dose was administered over a period of 1 h. Patients 1, 2, 3 and 12 received additional intravenous doses for another 7–14 days. Except for patients 2 and 12 who were withdrawn from the study, the patients next received 4 weeks of oral therapy at twice the intravenous dose'.

For the four patients treated with regimen A, the authors reported: 'Virus detected while on IV therapy, but not at end of oral'; 'Virus detected sporadically'; 'Decreased virus during initial AZT administration'; 'Virus not detected on day 0 or on AZT'.

With regimen B: in one patient, 'Low levels of virus detected early, then negative'; another patient, 'Low levels of virus at entry, then virus not detectable'. In the remaining 4 patients, 'Virus detected throughout'. Regimen C: in 3 patients, 'Virus detected sporadically'; for one patient results were not available; and for another they reported, 'Virus detected during first 2 weeks, but not after'. With Regimen D: in 2 patients, 'Virus not detected on day 0 or on AZT'; for one, 'Virus detected on day 0 and day 7, but not after'; and for the other, 'Virus detected on day 0, but not on AZT'.

Discussing their results, they wrote: 'For most of the patients on regimens A–C, virus continued to be detected in cultures established during therapy but virus was not detected in cultures established from any of the 4 patients on regimen D after 2 weeks of therapy. [From 2 of these 4 patients, they could not isolate HIV even before AZT administration.] In 2 of these patients (nos 16 and 18) virus cultures established at entry had been positive, which suggests that the failure to isolate virus was related to the administration of AZT...One patient (no. 15) on regimen C, also became virus negative while on AZT'²⁸.

On the basis of the findings in the Phase I clinical trial, a multicentre 'double-blind, randomised placebo-controlled trial intended to last 24 weeks...to evaluate the safety and efficiency of AZT in the treatment of a well-defined group of subjects with AIDS or AIDS-related complex' was conducted by Margaret Fischl and her associates. AZT was given to 145 patients, 250 mg every 4 h; 137 received placebo. Blood was collected, in addition to other tests, 'for detection of anti-HIV antibody by enzyme-linked immunosorbent assay, for measurement of serum p24 antigen levels (Abbot Laboratories, Chicago) and for isolation of HIV from peripheral-blood lymphocytes⁸'. Reference 8 in this extract is a paper by Levy and his colleagues, who apparently consider that just the detection of reverse transcription is synonymous with HIV isolation.

In this 'double-blind' study, 'Drug therapy was temporarily discontinued or the frequency of doses decreased to one capsule every eight hours or longer if severe adverse reactions were noted. The study medication was withdrawn if unacceptable toxic effects or a neoplasm requiring therapy developed. Subjects in whom an opportunistic infection developed were withdrawn from the study only if therapy with another experimental medication was required or if antimicrobial

therapy might have resulted in serious additive toxic effects....Twenty-seven subjects had completed 24 weeks of the study, 152 had completed 16 weeks, and the remainder had completed at least 8 weeks'.

Fischl and her colleagues reported that 'HIV was isolated at entry in 57 percent of the AZT group and 58 percent of the placebo group. No statistically significant differences in isolation rates were noted between the two groups during the study'. Discussing this finding, the authors wrote: 'The lack of a measurable effect on virus isolation from peripheral-blood lymphocytes may have been due to the activation of latent virus in cells by the culture techniques or by the failure of AZT to inhibit virus replication. Nevertheless, the ability to culture virus from many patients after several months of therapy indicates that such patients are still infectious and should be counseled to continue to follow appropriate practices to prevent the transmission of HIV'⁷².

In 1988, Antonella Surbone and her associates from the National Cancer Institute, Wellcome Research Laboratories, Abbott Laboratories and the Rush-Presbyterian-St. Luke's Medical Center treated 8 patients (4 with AIDS and 4 with ARC) with AZT and acyclovir. Patients received 100 mg AZT orally every 4 h for 7 days, followed by 100 mg of AZT and 800 mg of acyclovir orally every 4 h for an additional 9 weeks. 'In four patients, virus isolation was attempted at the initiation of therapy and during treatment. Human immunodeficiency virus could be detected by culture of mitogen-stimulated lymphocytes throughout the treatment period in Patient 6; virus was detected during treatment in Patients 3 and 4, who were negative at entry...and no virus could be detected at entry or during therapy in one patient'⁶².

In 1990, Ann Collier and her associates from several institutions in the USA, including the University of Washington and the

University of California, 'conducted a Phase II open-label, dose-escalating trial to evaluate the clinical and antiviral effects of zidovudine at low (300 mg daily, 28 subjects), medium (600 mg, 24 subjects), and high (1500 mg, 15 subjects) doses, either with or without acyclovir (4.8 g) by random assignment'. From 402 individuals screened they enrolled only 67. 'Most exclusions were due to the absence of HIV antigenemia or viremia or to ineligible CD4 counts [$<200/uL$]....The study was divided into three phases: an initial 12-week period, an elective extension phase of varying duration (from the end of the first 12 weeks until April 1989), and an 8-week crossover phase involving a new dose of zidovudine. During the crossover phase, the subjects who had received 300-mg or 600-mg doses of zidovudine were given 1500 mg per day, and those who had received 1500-mg doses were given 300 mg per day. The subjects randomly assigned to acyclovir received it throughout the study'. For some unknown reason, data on HIV isolation were given only for the first 12 weeks. 'Of the 38 subjects who had plasma viremia before entry [only] 25 had quantifiable titers. The mean (\pm SD) \log_{10} plasma titer on day 0 was 2.5 ± 0.9Mean plasma virus titers decreased by 1.80 during the first 12 weeks....No dose of zidovudine caused plasma viremia to disappear, but the magnitude of the decrease in plasma titers was similar for all doses of zidovudine....Thirty-nine of the 40 subjects who had peripheral-blood mononuclear cells cultured for HIV tested positive. The proportion with positive cultures was similar in all groups during the study'⁷³. (The authors fail to explain how it is possible to obtain a decrease in plasma viraemia with a drug like AZT which, by definition, inhibits only the quantity of proviral DNA and not the transcription of DNA into RNA; that is, any reduction in plasma viraemia is related to a decrease in HIV proviral DNA, the latter reflected by a decreased frequency of HIV isolation from cells.)

In a study published in 1997 by researchers from several institutions from the USA, 'Two groups of subjects were recruited on the basis of CD4 cell count, antiretroviral therapy, and lack of cell-free virus in plasma at entry. Group A consisted of HIV-1 infected subjects with >600 CD4 cells/ μ L before enrollment (n=30); group B subjects had initial CD4 cell counts of 400–550 (n=15). All group B subjects received zidovudine monotherapy (500–600mg/day) for \geq 6 months before enrollment and continued to receive zidovudine monotherapy for the duration of the study...At study entry, HIV-1 was isolated by the quantitative microculture method from 12 (86%) of 14 subjects in group B versus 15 (56%) of 27 in group A', although the patients from group B had received AZT. Furthermore, 'the titer of cell-associated virus increased over time', in group B but not in group A⁷⁴.

2. HIV DNA

According to the HIV model of AIDS pathogenesis, in the years following infection the concentration of infected mononuclear cells in the blood progressively increases, eventuating in very high levels of infected cells – that is, proviral DNA concentration, 'viral burden' – followed by viral expression and cellular death; that is, acquired immune deficiency. Given the general acceptance of this theory, one would assume that at present there is ample evidence to prove (i) the model; (ii) that AZT decreases the number of infected cells. However, these do not appear to be the case.

According to American researchers from the California State Department of Health, University of California and the Departments of Epidemiology and Biostatistics and Laboratory Medicine, University of San Francisco, 'Surprisingly, most of the data supporting the above model are based on

cross-sectional studies or short term follow-up studies of small numbers of patients.' To overcome this deficiency, these researchers tested the peripheral blood mononuclear cells (PBMC) of 9 rapid-, 9 intermediate- and 10 non-progressors whose date of seroconversion was not known at entry to the study using 'HIV-1 DNA *gag* polymerase chain reaction'. The same test was then repeated after five years. To their surprise, the number of infected PBMCs at entry was low in all groups, 73 (approx. 1–85)/10⁶PBMC, 160 (approx. 10–500)/10⁶PBMC, and 330 (approx. 10–1000)/10⁶PBMC in non-, intermediate- and rapid-progressors respectively. Even more surprising was their finding 'that there was little or no change in the concentration of HIV DNA positive cells from study entry to the 5-year follow-up visit for most subjects' in all three groups. In fact, 'the concentration of circulating HIV DNA positive cells' in at least two subjects from each group decreased in time, although none of the patients had anti-retroviral treatment. They also studied serial samples collected immediately before and after seroconversion for 18 subjects; samples were collected at 6-month intervals. 'In all subjects the concentration of HIV-1 infected PBMCs established shortly after seroconversion remained remarkably stable for up to 5 years', including in subjects whose CD4 cell counts declined (from 1049 to 46 cells/ml and 1063 to 276 cells/ml, one of whom developed PCP). In fact, on inspection of the graph depicting the results for the first 12 months for 15 of the subjects, it is easily seen that the 'HIV-1 infected cell burdens' fluctuated over time and that one patient 'had a substantially higher viral burden on the initial polymerase chain-reaction positive sample relative to the subsequent samples', although the patient did not receive anti-retroviral treatment⁷⁵.

The fact that when patients are treated with the drug AZT the frequency of HIV isolation is not diminished means that AZT does

not affect the level of proviral DNA. In a paper published in 1994, researchers from the AIDS Research Center, Department of Veterans' Affairs Medical Center, Palo Alto and the Center for AIDS Research, Stanford University, discussing their proviral DNA findings and those of others, wrote: 'Donovan *et al.* found that proviral DNA copy number was constant in six patients who had multiple samples taken during a 5–14 month period while on zidovudine (ZDV) therapy. We have also shown that there was no significant change in provirus level in four patients who were followed for a mean of 13 months'⁷⁶. That AZT does not have any effects on the proviral DNA has been confirmed by other researchers⁷⁷. Since, contrary to its putative action, proviral DNA remains unaffected by AZT treatment, and since AZT does not affect the expression of HIV, one would expect the drug to have no effect on the p24 antigenaemia and 'HIV RNA'.

3. p24 Antigenaemia

If p24 is an HIV protein, and if the cause of ARC and AIDS is HIV, then one would expect at least these patients, if not all HIV seropositive patients, to have high levels of p24 antigenaemia. If AZT is an anti-HIV drug, then the concentration of p24 should decrease in all patients who are treated with AZT. The decrease should be observed only in treated patients.

As mentioned, in their 1987 'double-blind, placebo-controlled trial', Margaret Fischl and her associates had 145 patients who received AZT and 137 who received placebo. 'Thirty-six AZT recipients and 40 placebo recipients were found to have detectable serum p24 antigen. Of these, 28 in each group had both a serum specimen obtained at entry and specimens obtained later in which changes in antigen level could be evaluated. Statistically significant decreases

from the serum level of p24 antigen at entry were found among AZT recipients at weeks 4, 8 and 12 (overall, $P < 0.05$). Similar trends were also noted at weeks 16 and 20, but the numbers of subjects were small for statistical analysis'⁷².

In 1988, several studies were published in which the relationship between AZT treatment and p24 was examined. In the study by Surbone *et al.* mentioned above, 'Serum obtained at periodic intervals from the patients was assayed for HIV p24 antigen using an enzyme-linked immunosorbent assay (Abbott Laboratories...)....Patients 4 and 6 had detectable serum p24 antigen at entry; in each of these patients, p24 could no longer be detected at week 10 of therapy. The other four patients had no detectable HIV p24 antigen either at entry or during treatment', although the patients had either AIDS or ARC⁶².

In a letter to *Lancet*, researchers from the University of Amsterdam wrote: 'An *in vitro* study has lately demonstrated resumption of virus production in HIV-infected T lymphocytes in the continued presence of initially highly inhibitory doses of zidovudine. As indicated by HIV antigen levels in two patients we have treated, a similar resumption of antigen production may occur after prolonged zidovudine treatment. Both were HIV-Ag seropositive (Abbott enzyme immunoassay) AIDS patients and were treated with 200 mg zidovudine 4-hourly. Serum HIV-Ag concentrations fell rapidly below the cut-off level for the assay [50 pg/ml]. However, despite continuation of the same drug regimen and patient compliance with the therapy, HIV-Ag serum levels subsequently rose in both patients. Neither had diarrhoea or clinical evidence of malabsorption'⁷⁸.

In the same year, in yet another study by researchers from the University of California, Burroughs Wellcome and Abbott Laboratories, the authors noted that 'Clinical testing of drugs potentially active against the

human immunodeficiency virus (HIV) has been seriously impeded by the lack of a reproducible quantitative method of estimating viral burden. We have investigated the clinical utility of an antigen capture assay for the HIV gag gene product p24 in patients undergoing treatment with zidovudine. Previous studies have shown that HIV gag or core antigen can be detected with greater frequency in patients with more advanced HIV infection, and presence of antigen is a predictor of disease progression in initially asymptomatic HIV seropositive homosexual men and hemophiliacs. In addition, HIV antigen can be reliably quantitated in picogram amounts allowing the possibility of dose-effect observations. We previously reported the use of a serum HIV core antigen (HIV-Ag) capture assay in a preliminary study of the *in vivo* antiviral effect of zidovudine⁵. We describe results of a larger study of serum HIV-Ag levels in patients enrolled in the multicenter phase II trial of zidovudine for the treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). (Reference 5 cited in this extract is the 1987 paper by Fischl *et al.*) 'Two hundred eighty-two subjects with either severe ARC (weight loss or oral candidiasis and fever, leukoplakia, lymphadenopathy, night sweats or herpes zoster) or a recent episode of *Pneumocystis carinii* pneumonia were recruited for the study... Subjects were randomly assigned to receive either 250 mg of zidovudine or placebo every 4 hours in a double-blind fashion. Dose modifications were made at the investigators' discretion based on toxicity. Median duration of treatment was 16 weeks...One hundred fifty-eight subjects, 83 treated with zidovudine and 75 given placebo, had serum samples available for testing and are the subject of this article. The prevalence of HIV-Ag at any time in subjects from whom baseline samples were available was 43% for zidovudine-treated individuals and 48% for placebo re-

ipients. The prevalence of HIV-Ag varied by diagnosis: 59% of subjects with AIDS have detectable HIV-Ag vs 37% of those with ARC'.

Their findings are presented in a table and also are discussed in the text. In the text, one reads: 'Thirty-one zidovudine and 32 placebo recipients who were HIV-Ag positive had a baseline and at least one additional sample available to evaluate changes in HIV-Ag levels according to treatment. Median HIV-Ag levels in zidovudine patients declined significantly with treatment, falling from 111 pg/mL at entry to 46 pg/mL at four weeks, and stabilizing at that level through 16 weeks. In contrast, HIV-Ag levels in placebo recipients varied little over time with a non-significant increment at 16 weeks....Fifty-nine percent of zidovudine-treated patients who were initially HIV-Ag positive became HIV-Ag negative during therapy compared with only 7% of placebo treated subjects ($P < .0001$)'. (However, it is obvious from the data presented in the table that at least one patient from each group was HIV-Ag negative already at day 0).

Commenting on their findings, the authors wrote: 'The use of HIV-Ag assay to monitor patients treated with zidovudine is limited by the prevalence of antigenemia in patients with AIDS and ARC. As previously reported, a greater proportion of our patients with AIDS, 59%, had HIV-Ag present compared with patients with ARC, where the prevalence was 37%. Approximately half the patients in each treatment group (zidovudine or placebo) were HIV-Ag positive during the course of the trial'⁷⁹.

In a study by researchers from University of Illinois and Abbott Laboratories, 16 patients with AIDS or ARC were enrolled 'using criteria applied in the national placebo-controlled trial. One half of the patients were randomised to receive zidovudine in an initial dose of 250 mg orally every 4 h. Changes in dosage were made by protocol definition based on reduction in leukocytes, hemoglobin,

hematocrit, or platelets...Positive cultures were identified by the presence of HIV antigen (predominantly p24) in the culture supernatant (EIA) (Abbott Laboratories...).... Serum or plasma was tested for HIV antigen by the same EIA....Antigenemia was found in the initial serum specimen from 11 and in serial specimens during the study from 12 of 16 patients with AIDS or severe AIDS-related complex. Three of the four antigen-negative patients had detectable serum anti-p24 antibody. Among patients who had antigenemia on entry, 8 could be characterised as having a high level (greater than 100 pg/mL), 4 had a low level (15 to 65 pg/mL), and 4 had no detectable antigenemia.'

Three out of the 4 patients with no detectable antigenaemia, 3/4 with low level and 2/8 with high level of antigenaemia were treated with AZT; and 1/4 with no detectable antigenemia, 1/4 with low level and 6/8 with high level of antigenaemia were given placebo (can this be said to be a randomised placebo-controlled study for p24?). 'Treatment was always begun with 250 mg or 200 mg every 4 h. The regimen consistently reduced the serum level of HIV antigen. Doses of 100 mg every 4 h or 250 mg every 8 h often permitted an increase in the serum level of HIV antigenemia....Cultures for HIV were nearly always positive in many patients with antigenemia regardless of the level'⁸⁰.

Three-hundred and sixty-five consecutive patients with ARC (80) or AIDS (285) who were eligible for AZT treatment by the Claude Bernard Hospital AZT Committee were followed up for a mean of 31 weeks. 'The full dose of AZT was 200 mg orally every 4 h. Patients with haemoglobin below 9 g/dl and/or PMN count below 1000/ μ l were treated with 200 mg 8-hourly. For patients treated with the full dose the dosage was reduced to 200 mg 8-hourly if PMN count dropped to less than 1000/ μ l, haemoglobin to less than 9 g/dl, platelets to less than 50,000/ μ l, or if non-haematological side-

effects occurred. AZT treatment was temporarily interrupted when PMN count fell below 750/ μ l, haemoglobin below 7 g/dl, or if non-haematological side-effects could not be tolerated'.

Antigenaemia analysis 'was restricted to the 52 patients with detectable p24 antigen (cut-off level 40 U/ml) before treatment who could be maintained on the full-dose or half-dose regimen for at least 12 months. The patients were stratified by pretreatment p24 antigen level (200 or more, or less than 200 U/ml). For patients with p24 antigen at 200 U/ml or above, the relative decrease was similar in the full-dose and the half-dose groups. The 16 full-dose patients were still p24 antigen positive at month 1, and only 1 was negative at month 2; none of the 7 half-dose patients became p24 antigen negative at months 1 or 2. Conversely, for patients with pretreatment p24 antigen level less than 200 U/ml, the relative decrease was significantly greater (ANOVA and *t*-test, $p < 0.02$) in the patients treated at full-dose than in those treated at half-dose. Of the 19 full-dose patients 9 (47%) and 10 (53%) became p24 antigen negative at months 1 and 2; only 2/10 and 3/10 half-dose patients were p24 antigen negative at months 1 and 2'.

Discussing their findings in general and of antigenaemia in particular, the authors wrote: 'Generally, in our series, full-dose AZT for 2 months did not eliminate antigenemia in patients with pretreatment p24 levels of 200 U/ml or higher...in AIDS and ARC patients, the rationale for adhering to high-dose regimens of AZT, which in many instances heads to toxicity and interruption of treatment, seems questionable'⁸¹.

In the 1990 study by Collier *et al.*⁷³ discussed earlier, of 67 patients enrolled, 51 (76%) had antigenaemia before treatment with AZT. 'Forty of the 47 subjects who completed 12 weeks of therapy continued treatment for a median of 29 additional weeks.... Only 13 of 37 of the subjects pos-

itive for HIV antigen (28%) became negative during this period. The proportion in whom HIV antigenemia resolved after therapy was 32% in the 300 mg group, 21% in the 600 mg group, and 33% in the 1500 mg group. The median change in the level of HIV antigen was 82% in the low-dose group, 71% in the medium-dose group, and 74% in the high-dose group (P not significant)...The decrease or increase in the dose of zidovudine had no effect on levels of HIV antigen during the eight-week crossover period....Among the subjects positive for HIV antigen, there was a 50% decrease in the level of antigen during the first 12 weeks in 76% of the 25 treated with zidovudine alone and in 79% of the 24 treated with the combination (*p* not significant)'.

In a study published in 1994, Victor DeGruttola and many of his associates, including Margaret Fischl, Paul Volberding and the Aids Clinical Trials Group Virology Laboratories, from several institutions from the USA pointed out that 'The primary clinical end points for evaluation of antiretroviral therapies in phase II and III studies are the development of AIDS-related complex (ARC) or AIDS or death. As therapy is initiated earlier in the course of human immunodeficiency virus type 1 (HIV-1) infection, there is an increased need for surrogate markers for clinical end points that can be used as early indicators of therapeutic efficacy'. In their study they 'investigated whether changes in serum p24 antigen levels can be used as a surrogate marker for clinical end points in phase II and III studies by examining whether pretreatment and follow-up serum p24 antigen measurements predicted subsequent clinical end points in three completed phase III clinical trials of zidovudine in persons with HIV-1 infection or AIDS'.

The first trial 'was a randomized, open-label trial evaluating a reduced dose of zidovudine in 524 subjects with AIDS and a first episode of *Pneumocystis carinii* pneumo-

nia (PCP)...Randomized subjects (262 in each group) received zidovudine at 1500 or 600 mg/day....Serum p24 antigen levels were measured before treatment and at weeks 8, 16, 24, 48, 64, 80, 96, 112 and 128 of treatment'. Because only 406 patients had a pretreatment serum p24 antigen measurement, the analysis was restricted to those patients. Only 65% of these patients with AIDS and PCP 'had measureable pretreatment concentration of serum p24 antigen (≥ 10 pg/mL)' ('Estimated concentrations of serum p24 antigen < 10 pg/ml were considered to be negative'). Of the 203 patients on 600 mg/day AZT, 69 had a negative pretreatment p24 antigen. In the follow-up period 5 had no further measurements, 53 remained negative and 11 became positive. Of the 134 who had a pretreatment positive p24 antigen level, 21 had no follow-up measurement, 73 had $> 50\%$ decrease, 25 had $\leq 50\%$ decrease and 15 had an increased p24 antigen level.

Of the 203 patients who received 1500 mg/day AZT, 73 had a negative p24 antigen level. Of those, 14 had no follow-up measurements, 51 remained negative and 8 became positive. Of the 130 who were positive, 23 had no follow-up measurement, 74 had 50% decrease, 17 had $\leq 50\%$ decrease and 16 had increased p24 antigen levels. The survival of the 406 patients 'was unrelated to the pretreatment concentration of p24 antigen in serum, and among those with available pretreatment antigen data there was no difference in survival....Changes during treatment were not associated with reduced mortality'.

The second study was a 'randomized, double-blind, placebo-controlled' study consisting of 713 subjects with 'mildly symptomatic HIV-1 infection and CD4⁺ cell counts of 200–800/mm³...Serum p24 antigen levels were measured before treatment and at weeks 4, 8, 16, 24, 40, 52, 76 and 88 of treatment. In this 'mildly symptomatic' study, 150 (24%) of the 637 patients with a pre-

treatment serum p24 antigen measurement had ≥ 10 pg/ml'.

Of the 238 patients who were given placebo and who had a negative pretreatment p24 antigen, 9 had no follow-up measurement, 206 remained negative and 23 became positive. Of the 71 patients who were positive, 4 had no follow-up measurements, 5 had $> 50\%$ decrease, 25 had $< 50\%$ decrease and 37 an increase.

Of the 249 patients treated with AZT (dose not given) and who had a negative pretreatment p24 antigen, 11 had no follow-up measurement, 220 remained negative and 18 became positive. Of the 79 patients who had a positive pretreatment p24 antigen, 5 had no follow-up measurement, 41 had 50% decrease, 28 had $< 50\%$ decrease and 5 had an increase in their p24 antigen level. In this study, having measurable serum p24 antigen before treatment 'about doubled the risk of developing advanced ARC or AIDS or dying...regardless of treatment'. Changes in the p24 levels 'were marginally associated with increased time until more advanced disease'.

The third study 'was a randomised, double-blind, placebo-controlled trial of two dosages of zidovudine in 1323 asymptomatic HIV-1 infected subjects who had CD4⁺ cell counts of $< 500/\text{mm}^3$Serum p24 antigen levels were measured before treatment and at weeks 8, 16, 32, 48 and 64 of treatment.... Of 683 asymptomatic subjects, 123 (18%) with a pretreatment serum p24 antigen measurement had ≥ 10 pg/mL'. Of 204 individuals who were given placebo and who had negative pretreatment p24 antigen, 34 had no follow-up measurements, 155 remained negative and 15 became positive. Of 35 who had positive p24 antigen, 6 had no follow-up measurement, 7 had 50% decrease, 8 had $\leq 50\%$ decrease and 14 had an increased level of p24 antigen. Of 356 individuals who were treated with AZT there was no difference in the results of the 500 or 1500 mg dosages and

because of this the results were combined, 58 had no follow-up measurement, 287 remained negative and 11 became positive. Of 88 individuals with a positive pretreatment p24 antigen, 19 had no follow-up measurement, 38 had $> 50\%$ decrease, 21 had $\leq 50\%$ and 10 had an increased p24 antigen level.

In this trial, changes in the p24 antigen levels 'were not associated with increased time until progression'. According to the authors of this study, serum p24 antigen is 'a specific marker of HIV-1 replication' but their study shows that 'much of the clinical improvement with zidovudine must be due to some other drug effect not mediated through p24'; that is, virus replication, viral load⁸².

4. Virus Quantitation

If HIV is the cause of AIDS, the appearance of immune deficiency and of the clinical syndrome should be preceded by an increase in the 'viral load' and not vice versa. AZT treatment should lead to a significant decrease, if not to a complete elimination, of the 'viral load'.

Two methods have been used to quantify HIV in plasma, viral load.

1. Plasma culture. Plasma from infected individuals is cultured with normal stimulated PBMC and the p24 in cultures measured. However, according to an article published in 1996 in *Nature Medicine* by some of the best known workers in the fields of HIV research and viral treatment, including Saag, Shaw, Volberging, Coombs, 'fewer than 50% of patients with CD4⁺ counts greater than 200 cell/ul had positive plasma cultures, and inherent biologic variability in virus quantitation required that a 25-fold (approximately 1.4 log) increase was seen before it was likely to be clinically meaningful'⁸³.

2. HIV RNA. To the quantitation of HIV performed by measuring p24 in cultures, a test apparently introduced by David Ho⁸⁴, he and many others have added a test in which 'HIV RNA' in plasma is quantified. The three assays frequently used to quantify the 'viral load' are reverse transcription-polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA) and branched chain DNA (bDNA). To assess the impact of the assays used and of 'genetic variability in HIV-1 RNA quantification', researchers from France 'evaluated three commercial kits by using a panel of HIV-1 isolates representing clades A to H...These isolates were expanded in culture. Virus was collected by ultracentrifugation and re-suspended in HIV-seronegative plasma. To standardize the quantities of virus to similar levels in each preparation, the p24 antigen was determined and the volume adjusted so that each specimen contained approximately 10pg of p24 antigen per ml'. The 'HIV-1 RNA copies' per ml of plasma obtained were as indicated in Table 1.

These results prove that 'quantification of HIV-1 RNA is highly influenced' by the 'HIV-

1 clade' and the test kit used. Indeed, given their data it is virtually impossible to make any sense at all of 'viral load' findings⁸⁵.

There are two practical reasons for measuring plasma RNA levels:

1. *The RNA levels and its changes are said to predict disease progression.* However, in a paper published in 1997 by researchers from the Walter Reed Army Institute of Research and the Henry M. Jackson Foundation, the authors wrote: 'Whereas levels of cell-free viral RNA were shown in cross-sectional studies to vary over 1 to 2 logs with disease progression, four recent longitudinal studies have revealed a more complex view of viral RNA dynamics. Although all these reports have shown approximately 1 log higher levels of initial cell-free RNA from rapid versus slow progressors, in three of these studies cell-free RNA levels showed a < 1 log increase in the majority of rapid progressors. In contrast, Mellors and co-workers showed a > 1 log plasma RNA increase in three patients but a < 1 log RNA change in two of five patients studied intensively in their report'. In Michael and colleagues' study, there were 17 patients who were rapid progressors and 20 slow. They reported

Table 1

| HIV-1 STRAIN | RT-PCR | bDNA | NASBA |
|--------------|---------|---------|---------|
| DJ258 | <400 | 111,500 | 100,000 |
| DJ263 | <400 | 79,800 | 60,000 |
| SF2 | 225,500 | 38,000 | 240,000 |
| III-B | 54,000 | 17,000 | 360,000 |
| ZAM18 | 78,300 | 70,000 | 66,000 |
| ZAM20 | 178,800 | 125,800 | 420,000 |
| UG270 | 179,800 | 29,200 | 170,000 |
| UG274 | 320,000 | 41,400 | 32,300 |
| CM241 | 18,800 | 72,800 | 35,000 |
| CM235 | 4,700 | 52,000 | 15,000 |
| 163.3069 | 36,200 | 94,000 | 57,000 |
| 162.307 | 2,800 | 78,100 | 26,000 |
| G98 | 254,700 | 269,000 | <400 |
| LBV21 | 184,500 | 295,000 | <400 |
| VI557 | 950,000 | 587,000 | 125,000 |

that 'The mean \pm SD for the initial serum RNA (expressed in \log_{10} copies/ml) in the rapid progressor (4.07 ± 0.53) exceeded that for the slow progressors (3.07 ± 1.25) [note the large SD in the latter group]....Dynamics of serum viral burden in rapid progressors reveal two distinct patterns. Serum viral burden changes of < 0.5 log were previously shown to be consistent with biological variation. This level of variance was used to sort the rapid progressors into two groups [contrary to the HIV theory of AIDS]. Seven rapid progressors show a ≤ 0.5 log change in viral burden (static group), and 10 showed a > 0.5 log increase in viral burden (increase group) over time' (one patient 0.6 log; 3 patients 0.7 log, 2 patients 0.8 log; 1 patient 0.9 log; 1 patient 1 log and 2 patients 1.4 log)⁸⁶.

2. *To determine the effects of treatment.* According to the 1997 British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals, 'If the viral load has not fallen by about 1 log 8–12 weeks after treatment initiation consideration should be given to modify therapy'⁸⁷. In their 1996 paper in *Nature Medicine*, Saag, Shaw, Coombs and their associates stated that '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', and that 'Zidovudine monotherapy results in a median 0.7 log decrease in plasma HIV RNA level within two weeks, which returns toward baseline values by 24 weeks'^{19,20}. At least the claim regarding the effects of AZT on the RNA level is not substantiated by the presently available

data, not even in the two studies which they are citing. Reference 19 in the above extract is a 1996 paper by William O'Brien and his associates from the Veteran Affairs Cooperative study group of AIDS. In this 'blinded study' the authors made a 'comparison of immediate with deferred zidovudine therapy...All the patients in the immediate-therapy group received open-label zidovudine for the entire study period, whereas those in the deferred-therapy group received placebo until their CD4+ lymphocyte counts fell below 200 cells per cubic millilitre or an AIDS-defining illness developed, when they were switched to open-label zidovudine'. In the immediate therapy group the maximum decrease, which is reached at two months, was about 0.6 log. By 12 weeks the RNA level returns to the baseline value, and at 24 months it is about 0.25 log above the baseline. In the deferred therapy group the RNA level never dropped below the baseline value⁸⁸. Reference 20 is a 1996 paper by Coombs *et al.* In this study, 'In total, 913 subjects who had received at least 16 weeks of previous zidovudine therapy were enrolled in ACTG protocol 116B/117 and followed a mean of 48 weeks for disease progression defined as a new AIDS event or death. A subset of subjects for whom plasma samples were obtained for HIV-1 RNA quantitation were enrolled throughout the distribution of randomization dates for all subject participants enrolled. These subjects were followed in the study for a median of 304 days (range, 12–736). Plasma samples were available from 100 subjects at baseline; 71 of them had plasma samples at week 4, 72 at week 8, 66 at week 12, and 49 at week 24.'

The only data given on changes of the RNA level with therapy are the following:

'The plasma HIV-1 RNA level declined by a median of $0.2 \log_{10}$ during therapy for subjects who were switched to didanosine (figure 2A) but not for those who continued zidovudine (figure 2B)'. In figure 2B, where the effect of AZT treatment on the RNA level is shown, the level is always above the baseline⁸⁹.

In a paper published in 1993 by Ann Collier and her associates, including Coombs and Fischl, the authors conducted 'a clinical trial to characterize the safety and efficiency of a range of doses using combination zidovudine and didanosine therapy compared with zidovudine therapy alone'. From 25 out of the 69 patients in their study they took sequential plasma samples at 0, 12 and 24 weeks and the plasma HIV-1 RNA was determined 'by a semiquantitative assay'. They reported that: 'Seventeen patients had a one log or more decrease in virion-associated HIV-1 RNA copy number during therapy, 7 had no change, and 1 had an increase. Nine patients had a decrease in virion RNA from pretreatment levels at both 3 and 6 months, 5 had a decrease between 3 and 6 months, and 3 had a decrease at 3 months that was not sustained at 6 months. Of 17 patients who had a decrease in plasma RNA titers, 15 were treated with a combination regimen. Overall, 15 (83%) of 18 patients receiving combination regimens had a decrease in plasma HIV-1 RNA titers compared with 2 (29%) of 7 patients receiving zidovudine alone'⁹⁰.

In their well known 1993 study 'High Levels of HIV-1 in Plasma During All Stages of Infection Determined by Competitive PCR', Piatak, Saag, Shaw and their associates reported that 'Sixty-six consecutive enrolled HIV-1-infected subjects representing all stages of infection [Centers for Disease Control (CDC) Stages I to IV] and ten HIV-1 seronegative healthy donors were evaluated for virion-associated HIV-1 RNA by QC-PCR. Infected subjects were also tested for culturable virus and for p24 antigen with both

standard and immune complex dissociation (ICD) test procedures'. They reported that the 'RNA copy numbers ranged from 1.00×10^2 to 2.18×10^7 HIV-1 RNA copies per millilitre of plasma....The average decline in HIV-1 RNA among the ten patients treated with AZT was 11-fold, whereas the average decline associated with resolution of the acute retroviral syndrome in six patients was 72-fold'. They also claimed to have found a correlation between plasma HIV-1 RNA and 'virus titers measured by endpoint dilution culture'. Given their finding that: 'Whereas the QC-PCR method quantified virion-associated HIV-1 RNA in all 66 patients tested, virus culture and standard p24 antigen assays were much less sensitive, with positive results in 4/20 and 5/20 subjects with CD4⁺ T-cell counts >500 per cubic millimeter, 6/18 and 7/18 subjects with CD4⁺ T-cell counts of 200 to 500 per cubic millimeter, and in 22/28 and 24/28 subjects with CD4⁺ cells fewer than 200 per cubic millimeter, respectively', it is difficult to see how such correlation can be determined⁹¹.

In a paper published in 1995, Joseph Eron and his associates for the North American HIV Working Party 'studied two doses of lamivudine in combination with zidovudine in patients with little or no prior antiretroviral therapy who had 200 to 500 CD4⁺ cells per cubic millimeter'. In this study, 'The greatest mean reductions in the plasma concentration of HIV-1 RNA were 0.52 ± 0.04 log in the zidovudine-only group, 1.19 ± 0.07 log in the lamivudine-only group, 1.56 ± 0.10 log in the low-dose combination-therapy group, and 1.55 ± 0.09 log in the high-dose combination-therapy group'⁹².

In a paper published in 1996, David Katzenstein and his associates in the AIDS Clinical Trials Group Study, 175 Virology Study Team, determined the relationship of virological and immunological factors to clinical progression. The virology subgroup comprised 391 subjects. Blood was collected on

'two occasions, at least 72 h apart, during the 14 days preceding treatment, to determine plasma HIV RNA concentrations; the geometric mean of these two measurements was defined as the baseline value. Plasma HIV RNA concentrations were measured at weeks 8, 20 and 56 provided that the subjects continued to receive the assigned treatment'. Eighty-nine subjects were treated with AZT only, 107 with didanosine only, 102 with AZT plus didanosine, and 93 with AZT plus zalcitabine. In this study, the 'mean baseline plasma HIV RNA concentration was 4.20 log (15,791 copies per milliliter), and the values ranged as high as 6.61 log. For 80 percent of the subjects, the difference in the log concentration between the two baseline measurements was less than 0.26 and for 90 percent it was less than 0.41'. No data are given for the other 10%. The presence of symptoms such as oral hairy leukoplakia, candidiasis or herpes zoster was significantly associated with increased HIV RNA concentration. 'Homosexuality was associated with a significantly higher plasma concentration of HIV RNA ($p = 0.002$), and intravenous drug use with a significantly lower concentration ($p = 0.003$). Women had significantly lower plasma HIV RNA concentrations ($p < 0.001$), as did black subjects ($p = 0.013$). ... Antiretroviral treatment before entry into the study was associated with lower CD4 cell counts and a higher rate of the presence of syncytium-inducing phenotype, but not with differences in plasma HIV RNA concentrations....Measurements made eight weeks after the start of treatment revealed significant differences in the response of plasma HIV RNA concentrations to anti-retroviral therapy among the treatment groups. There was a mean decrease of 0.26 ± 0.06 log (45%) in the HIV-RNA concentration in 65 subjects who received zidovudine alone, a decrease of 0.65 ± 0.07 (78%) in 87 subjects who received didanosine alone, a decrease of 0.93 ± 0.10 (88%) in 81

subjects who received zidovudine plus didanosine, and a decrease of 0.89 ± 0.06 (87%) in 76 subjects who received zidovudine plus zalcitabine....Subjects without a history of antiretroviral treatment who took zidovudine alone had a mean reduction at week 8 of 0.47 log; subjects with that history had a mean reduction of 0.02'.

During the follow-up, 48 (12%) of the 391 subjects 'were given a diagnosis of AIDS or died; and 28 (7%) died....A decrease of 1.0 log in the concentration of HIV RNA from baseline to week 8 was associated with a significant lowering to 0.35 in the hazard ratio for AIDS or death (i.e., 65% reduction in the risk of AIDS or death)....There was a 90% reduction in the risk of progression of disease associated with a reduction of 1.0 log in the plasma HIV RNA concentration between baseline and week 56'. (How was it possible to determine such relationships when only a small percentage of patients developed AIDS or died, and even a smaller proportion if any of these patients had a decrease of 1.0 log at week 8 and nobody at week 56?)

Discussing their finding, the authors wrote: 'The presence of lower baseline plasma HIV RNA concentrations among women and among intravenous drug users is an interesting but unexplained observation. However, risk factors for HIV infection, sex, ethnic group, and a history of previous antiretroviral treatment were not independently associated with differences in clinical outcome. Neither are the clinical results of ACTG 175 fully explained by the overall comparison of the changes in HIV RNA concentrations in the different treatment regimens. Therapy with didanosine alone led to clinical results comparable to those with the combination of zidovudine and didanosine, although patients treated with the latter regimen had a clearly larger mean decrease in plasma HIV RNA concentrations. The reduction in plasma HIV RNA concentrations after treatment with zidovudine plus zalcitabine

was similar to that after zidovudine plus didanosine, yet the latter regimen was more effective in the subjects with a history of anti-retroviral therapy, and similar results have been observed in a recently reported study of combination therapies in subjects with more advanced disease, but without a history of antiretroviral therapy... These differences point to the importance of other factors in the treatment of HIV infection⁹³.

In a study published in the same year (1996), researchers from Spain and Belgium conducted a 6-month follow-up study in 46 patients previously treated for at least 6 months with AZT plus zalcitabine (ddC) who were subsequently allocated to receive either ZDV/ddC/3TC (15 patients), ZDV/3TC (15 patients), or to continue with the ZDV/ddC regimen (16 patients). 'Maximum mean decrease in VL [plasma HIV-1 RNA] was achieved at week 4 in the ZDV/ddC/3TC (-0.64 log) and ZDV/3TC (-0.72 log) groups. At week 12 and 24 the decrease in the ZDV/ddC/3TC group was 0.41 log and 0.45 log, respectively. The corresponding values for the ZDV/3TC group were 0.16 log and 0.15 log. In the ZDV/ddC group there was a continuous increase in the plasma HIV-1 RNA level, and at week 24 was 0.36 log above the baseline level⁹⁴.

In 1996 there was also a paper by Ann Collier and her associates for the AIDS Clinical Trials Group. Because, in patients treated with RT inhibitors, 'the disease eventually progresses to the acquired immunodeficiency syndrome (AIDS) despite the use of these agents', the authors 'studied the safety and efficacy of saquinavir, an HIV-protease inhibitor, given with one or two nucleoside antiretroviral agents, as compared with the safety and efficacy of a combination of two nucleosides alone'. The patients were given either saquinavir plus AZT and zalcitabine or AZT plus either saquinavir or zalcitabine. The study lasted 24 weeks, with an option of an additional 12 to 32 weeks. The

plasma HIV-1 RNA was quantified by using two methods, branched chain DNA and the quantitative polymerase-chain-reaction amplification by the RT method. The mean plasma RNA levels were given in separate graphs for the two methods. The average decrease in patients treated with saquinavir plus AZT was 0.1 log; for the zalcitabine plus AZT group, 0.39 log; and for the group which received the three-drug combination, 0.68 log.

In addition to the plasma RNA, Collier and her colleagues also determined the quantity of 'HIV in PBMCs'. 'In the quantitative analysis of HIV in PBMCs, the titer of infectious units per million cells was calculated for each sample²⁸'. In reference 28 they cite Susan Fiscus *et al.*⁹⁵ who used a method, 'Quantitative cell microculture assay (QMC)', where the quantity of HIV in a co-culture is determined by measuring p24. 'Six serial dilutions of each subject's PBMC, starting at a concentration of 10^6 PBMC, were cocultured in duplicate with 10^6 HIV-seronegative donor PBMC that had been prestimulated with PHA for 1-3 days according to standard procedures.... A culture was scored as positive if > 30 pg/mL HIV-1 p24 antigen (Abbott, Abbott Park, IL) was present in the supernatant.... Several dilution schemes, including 2-fold, 5-fold, and 10-fold serial dilutions of the subjects' PBMC, were tested as part of the early development of the QMC method for the quantitation of virus load. However, since all dilution schemes used 10^6 PBMC/well as one of the serial dilutions tested, an algorithm was calculated to express the results as infectious units per million cells (IUPM). The median change in \log_{10} IUPM (hereafter called log IUPM) from study entry with treatment over time was determined'.

They reported: 'At baseline, 107 (98%) of the 109 evaluable subjects had cultivatable HIV-1 from at least 1 PBMC specimen. For 94 subjects, 2 independent baseline blood specimens, drawn a median of 10 days apart, were available for PBMC HIV-1 culture.

Duplicate specimens were both positive in 78 (83%), discordant in 15 (16%), and negative in 1 (1%) case. For the baseline comparison (ignoring the effect of censoring i.e. failing to reach a dilution end point), 56 (60%) of 94 duplicate specimens differed in HIV-1 titer by ≤ 1 log IUPM; 20 (21%) of 94 differed by >1 but <2 log IUPM; and 18 (19%) of 94 differed by ≥ 2 log IUPM. In an analysis that accounted for the effect of censoring, the within-person SD with paired baseline PBMC culture data was 0.72 log IUPM '.

Using Fiscus *et al.*'s methods, Collier and her colleagues found in their patients that 'The mean titer of HIV in PBMCs decreased by 0.8 log in the three-drug group, as compared with no change in the saquinavir-zidovudine group and a change of less than 0.4 log in the zalcitabine-zidovudine group. Zalcitabine and zidovudine lowered titers more than did saquinavir and zidovudine ($P=0.004$). The patients assigned to three-drug therapy had titers that remained below baseline longer than those of the patients assigned to saquinavir and zidovudine, although over time, even in the three-drug group, there was a gradual return toward the baseline titer'.

Regarding the clinical outcome, they reported that 'No statistically significant differences were found among the three regimens with respect to any clinical or laboratory measure during either the first 24 weeks or the overall study....One of the interesting observations was that the suppressive effect of the three-drug combination on viral load, as measured by quantitative microculture of PBMCs, HIV RNA titers, and effects on serum activation markers, appeared to be more durable than the elevation of CD4+ counts. That the antiviral response was sustained longer than the CD4+ cell response raises intriguing questions about the association between quantitative measures of HIV, immune activation, and CD4+ cell counts. Nonetheless, these results suggest that the

combination of saquinavir, zalcitabine, and zidovudine should be further investigated in long-term studies'⁹⁶.

Pregnant women are treated with AZT to prevent vertical transmission of HIV⁹⁷. However, in a paper published in 1997, researchers from the University of Washington summarised the results of a study in which they treated pregnant women with AZT as follows: 'In summary, HIV-1 levels in asymptomatic women, most with low viral loads, reveal stable level of HIV-1 RNA in plasma and infectivity of PBMC during pregnancy. The use of ZDV in pregnancy did not lead to a significant decrease in the viral load at delivery when controlled for the effect of pregnancy'⁹⁸.

The results for the CAESAR trial, a trial conducted in Canada, Australia, Europe and South Africa were published in *Lancet* in 1997. The trial evaluated the impact of adding lamivudine or lamivudine plus loviride to the current anti-HIV regimens consisting of either AZT monotherapy or AZT plus didanosine or zalcitabine combination therapy. 'CD4 counts and plasma HIV-1 viral load were measured prospectively over the first 28 weeks of treatment in the 326 patients recruited in France and Belgium. There was a minimal response in the CD4 count and viral load [<0.1 log at 2 weeks], followed by a similar increase thereafter of patients continuing current treatment alone....The maximum change in viral load for patients in the lamivudine arm was a median reduction of 0.67 log₁₀ HIV-1 RNA copies at week 2, returning to 0.1 log₁₀ below baseline by week 28. The corresponding reductions at weeks 2 and 28 for patients in the lamivudine plus loviride arm were 0.79 log₁₀ and 0.25 log₁₀ HIV-1 RNA copies below baseline, respectively'⁹⁹.

In 1993 Saag, Shaw and their colleagues reported that, in patients with signs and symptoms 'of primary infection' with HIV, 'Virion-associated HIV-1 RNA levels peaked between 8 and 23 days after the onset of symptoms, reaching values between 3.55 x

10^5 and 2.18×10^7 copies per milliliter (corresponding to 1.78×10^5 to 1.09×10^7 virions per milliliter)...Within the first 100 days after onset of symptoms, plasma RNA levels fell by between 20 and 235-fold', even without anti-retroviral therapy⁹¹.

Because annual influenza vaccination 'was and still is recommended for all HIV-infected individuals', William O'Brien and his associates from several institutions in the USA studied the effect of an influenza vaccine on the 'HIV DNA and RNA...Study subjects were self-assigned to the vaccinated ($n = 20$) or nonvaccinated control group ($n = 14$)...Subjects were to have CD4+ lymphocyte counts of 200 to 500/mm³, although those having 100 to 200/mm³ or 500 to 550/mm³ at study entry were not excluded if willing to participate...Patients were excluded if there was clinical or laboratory evidence of acute viral hepatitis, active herpes simplex virus infection, pneumonia, or other acute respiratory infection, psychosis, or transfusion within the last 2 months. Patients receiving immunization did not have an allergy to eggs, because vaccine antigens were derived from influenza virus preparations grown in eggs. At each point, patients were specifically questioned about the presence of fever, cough, rash, cutaneous or respiratory infection, and diarrhea... Patient histories and examinations at 1- to 2-week intervals during the 2-month study period did not show any side effects from vaccination, nor were there any symptoms of acute infections in the study population. Therefore, we do not believe overt infections with bacteria or with heterologous viruses were important confounders during the course of observation. This analysis is critical, because infection may be another potential source of stimulation, and hence, viral induction. In addition, none of the study patients reported blood transfusions or symptoms of allergy or hay fever during the study period'.

Although all but two of the study patients

were receiving AZT, 'Over the study period, there was little change in levels of proviral DNA in peripheral blood mononuclear cells... In contrast with what was observed for viral DNA, there was a significant relative increase in postvaccination HIV-1 RNA levels in PBMC from the 20 patients receiving influenza vaccination (11.6 ± 5.0 -fold increase, median 2.7, $p < 0.002$)...The peak HIV-1 RNA levels typically occurred at 1 or 2 weeks postvaccination (in 9/10 patients showing greater than fourfold increase), and returned to baseline at later time points. Thus, in most patients, HIV-1 RNA induction was transient. Equivalent increases in peak PBMC RNA levels during the same time frame were not seen in the 14 nonvaccinated controls (2.4 ± 1.6 -fold increase; median, 0.0; $P=.24$), and only 2/14 control patients (14%) had increases greater than fourfold'. To determine the relationship between virological responses to vaccination and clinical outcome, the study patients were followed for a mean of 3 years. 'Of the 10 vaccinated patients who exhibited a fourfold increase in HIV-1 RNA, 5 had a fall in CD4+ lymphocyte number at 6 months of 20% or more, and all 5 of these subjects developed AIDS. Moreover, 3 of 5 vaccinated subjects with HIV-1 RNA increases who did not have a decrease in CD4 cell count have not developed AIDS. Finally, 3 of the 10 study subjects who did not show an HIV-1 RNA increase developed AIDS over 6 months, and 2 of these patients had a 20% decrease in CD4 cell count. Therefore, the pattern of virologic response to influenza vaccination does not entirely predict outcome. Other factors appear to be involved in determining the rate of clinical progression'. Discussing their results, the authors wrote: 'Our results suggest that continued immunologic (antigenic) stimulation may result in increased virus load *in vivo*... In addition, our assay would not reliably detect increases in HIV-1 DNA of twofold or less, which may still be relevant for clinically

important increases in proviral burden. It is also possible that vaccination increased the number of infected cells in compartments not assayed here, such as lymphoid tissues. Although in our study we did not detect increases in HIV-1 DNA over 2 months, it seems likely that the progressive increases in viral load during the course of HIV disease are a consequence of many such small inductions of HIV-1 replication which occur intermittently over several years. Our study involved only a single immune stimulation event which may be inconsequential to a chronic disease such as AIDS where an infected individual is expected to be exposed to numerous antigenic stimuli....Co-expression of HIV and either herpes simplex virus or cytomegalovirus genes in T-cells, or stimulation of HIV-infected cells by HTLV particles, can result in increases in HIV-1 replication....In addition, a recent study suggests that recurrent herpes simplex virus infection can also lead to marked increases in HIV-1 expression. Furthermore, actual influenza virus infection may lead to a greater level of HIV-1 expression than the transient nature of the increase in viral expression observed here, because of the prolonged nature of the infection. This relationship may hold true for other vaccine-versus-disease combinations'⁷⁷.

Researchers from the Gladstone Institute of Virology and Immunology, and a number of other institutions from the USA, noted that 'It is generally recommended that HIV-1 infected individuals be vaccinated against several important pathogens, including influenza viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and hepatitis B. In addition, it is recommended that HIV-1 infected infants be vaccinated against diphtheria, tetanus, measles, mumps, rubella, polio, and pertussis. Although the efficacy of these vaccines in immunocompetent individuals has been established, the protective value of vaccination in the context of HIV-1 infection has

not been demonstrated. It has been reported that many HIV-1 infected individuals do not make a significant antibody response to vaccine antigens, suggesting that routine vaccination of HIV-1 seropositive patients may be of little benefit'. To clarify some of these problems, they vaccinated 32 adults 'with HIV-1 infection' and 10 seronegative controls with 'a standard dose (0.5 ml) of the 1993-1994 formulation of trivalent influenza vaccine....The majority of HIV-1 infected participants were receiving antiretroviral therapy (with the nucleoside analogues zidovudine, zalcitabine, didanosine, or stavudine monotherapy or various combinations thereof) before and during the study period. None of the study participants had evidence of active opportunistic infections at the time of study entry. There were no adverse clinical reactions to the influenza vaccine and there were no reports of an influenza-like illness'.

Considering a 3-fold [0.5 log] change in the level of plasma HIV-RNA to be significant, they found that 'the majority (83%) of vaccinated individuals experienced a significant increase in plasma HIV-1 RNA levels within 1-2 wk of immunization and returned to their prevaccination levels within 4 weeks after immunization ($p = 0.0009$). The mean fold increase in HIV-1 RNA copy number was substantially greater in those individuals with higher CD4⁺ T-cell counts. At baseline, before immunization, the individual plasma HIV-1 RNA copy number measures covered a wide range (50-402,500 copies/ml). After immunization, the peak titers observed had a significantly more narrow distribution (range 5,800-1,600,000 copies/ml; $p = 0.0009$). Among all HIV-1 infected participants, peak plasma HIV-1 RNA levels seen after vaccination ranged from 1- to 369-fold above baseline values (median 7.3)....Patients on antiretroviral therapy were not noticeably different from those not on therapy with regard to increases in plasma viremia. In a few patients, plasma viremia did not return to

baseline or showed a second increase during the study. None of these study participants were given a second vaccination during the study period, but these few subjects did have evidence of an intercurrent infection, such as the development of CMV retinitis or *Pneumocystis carinii* pneumonia, which may have caused the second wave of viremia'.

Commenting on their findings, the authors wrote: 'given the significant activation of virus production that follows a discrete vaccine-induced antigenic exposure, it is likely that the immune activation associated with an actual opportunistic infection may cause even more dramatic stimulation of virus production. These issues must be considered in determining the advisability of particular vaccines. Our additional anecdotal experience suggests that acute *M. tuberculosis* and *P. carinii* infections can cause increased viral load (Staprans, S., and M.B. Feinberg, unpublished observations). Indeed, intercurrent infections occurred in a few of the influenza-vaccinated patients whose plasma viremia levels either did not return to baseline values or who manifested a second, later peak in plasma viremia....It is hoped that the recently developed methods to monitor HIV-1 RNA levels in plasma, including the techniques used in this study [branched DNA], will provide valuable tools to assess the risk of disease progression and the efficacy of antiviral drugs in infected individuals. However, little information is available concerning the factors that influence the biological variation of these new assays. The observed changes in plasma viremia after vaccination or infection by pathogens are important to consider in determining the clinical utility of HIV-1 RNA assays, as such perturbations may significantly influence the interpretation of viral load measures. Further studies will be required to elucidate the possible pathogenic consequences of immune stimulation in HIV-1 infection'¹⁰⁰.

Comments

The presently available data on HIV isolation do not prove that AZT alone or in combination with other nucleoside analogues has any effect on the frequency of HIV isolation. Nor is there proof that these drugs have any effect on the level of proviral DNA. However:

1. According to the HIV experts, the anti-HIV effects of AZT are due to its inhibition of the reverse transcription of HIV-RNA into proviral DNA;
2. Since 1995, with the publication of the well known papers by Ho *et al.*²⁰ and Wei *et al.*²¹ the concept of prolonged virological latency has been 'replaced by a new paradigm of ongoing, high-level viral replication from the time of initial infection until death. Indeed, as many as 10 billion new HIV virions are produced per day, with a half-life in plasma of 6 h. CD4⁺ lymphocytes, one of the principal cell targets responsible for viral replication *in vivo*, are also produced in high numbers and, once productively infected, have a half-life of about 1.6 days'.

The combination of the 'extraordinarily high level...of cell destruction and cell replacement', and of treatment with RT inhibitors – that is, of drugs which inhibit the formation of new proviral DNA – should rapidly result in a state in which cells from such treated patients have no detectable HIV-DNA. The fact that both the frequency of isolation of HIV and the level of HIV-DNA are not affected by treating patients with AZT means that:

1. AZT has no anti-HIV effect; or
2. 'HIV-DNA' and 'HIV isolation' are not specific to HIV; or
3. both (1) and (2).

Furthermore, since AZT and the other RT

inhibitors have no effect on the level of 'HIV DNA', and since these drugs do not inhibit viral activation, then it is not possible for these drugs to have an effect on levels of 'HIV RNA' and 'HIV p24 antigenaemia'.

According to the HIV/AIDS experts:

1. The cause of AIDS is HIV replication;
2. The p24 antigen is 'a specific marker of HIV-1 replication'.

If these are the case, then all ARC and AIDS patients must have high levels of p24. Furthermore, since, according to the 'new paradigm', HIV continuously replicates from the moment of infection, all patients infected with HIV, even if asymptomatic, should have high levels of p24. In all patients who are not treated, the p24 should increase or at least should remain constant; but over time, in all treated patients, the p24 level should decrease. The progression to AIDS should be directly related to p24. However, none of these predictions has been proven by the evidence available to date. It is sufficient to mention that the vast majority of HIV infected individuals have no p24 antigen, and even a significant proportion (~35%) of patients with ARC and AIDS have no p24 detectable in their serum. There is no relationship between p24 and the development of ARC or AIDS, and 'much of the clinical improvement with zidovudine must be due to some other drug effect not mediated through p24'. This means that p24 is not specific to HIV; or HIV is not the cause of AIDS; or both.

The first test introduced to measure viral load was the detection of p24 in cultures of mitogenically stimulated PBMC from healthy individuals containing plasma from patients with AIDS or at risk of AIDS. That AZT has no effect on plasma viraemia is even accepted by Coombs and Collier: 'Unfortunately, zidovudine had no effect on the prevalence of HIV plasma viremia in this

group of subjects with advanced HIV disease. For example, between weeks 4–24 of therapy, 83–91% of subjects on oral zidovudine demonstrated plasma viremia. In addition, zidovudine did not reduce the titers of plasma viremia; in 19 plasma viremic subjects who also had HIV plasma titers determined, no significant differences were noted in either the geometric or arithmetic mean plasma HIV titers over the course of zidovudine therapy'¹⁰¹. In fact, since 'fewer than 50% of patients with CD4⁺ counts greater than 200 cell/ml had positive plasma cultures', the test cannot be used for any purpose in the majority of patients. This fact also raises two questions.

- How is it possible for the CD4⁺ counts to decrease to 200 cell/ml, when no active virus can be detected?
- How is it possible to claim high viral activity from the moment of infection (that is, in asymptomatic patients) till death when viral activity cannot be detected in the majority of them?

If 'p24 antigenemia', 'HIV viremia' and 'HIV plasma RNA' are proof of active HIV infection, and HIV is the cause of AIDS, then:

- (a) a perfect and direct correlation should exist between these three parameters;
- (b) a perfect and direct correlation should exist between the three parameters and the development of immune deficiency and the clinical syndrome;
- (c) anti-HIV treatment should lead to a simultaneous decrease in all three parameters.

HIV experts' own data confirm that this is not the case. In an article published in 1996, Lawrence Deyton from the HIV Research Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, wrote: 'A per-

fect surrogate marker for use in the study of a new therapeutic agent must be biologically plausible, measurable in all patients with the disease, predictive of disease progression (ie, worsening with advancing disease and improving with clinical remission), subject to standardization, and reproducible. The effect of a treatment on a perfect surrogate marker should translate into an effect on the true end point. Valid arguments can be made that no surrogate marker used in testing of HIV therapies yet meets these criteria¹⁰².

In an article published in 1997 in the *British Medical Journal*, Jonathan Cohn from Wayne State University writes: 'However, the experience of these assays has been brief ...inconsistencies between virological, immunological, and clinical responses have been noted; and changes in CD4 cell counts and plasma HIV RNA value still do not account for all of the clinical benefit of antiretroviral treatment. Therefore, it has been suggested that plasma HIV RNA assays need to be validated as predictors of a clinical response for each class of antiretroviral drug and for patients in different stages of HIV infection'¹⁰³.

Be this as it may, it is accepted even by some of the best known HIV experts that 'A three-fold or greater sustained reduction (> 0.5 log) of plasma HIV RNA levels is the minimal response indicative of an antiviral effect'⁸³. However, the presently available data do not prove that AZT, when given alone or in combination, can induce a sustained decrease in the 'plasma HIV level' of > 0.5 log and even less of 'about 1 log', as required by the British HIV Association guidelines for antiretroviral treatment. On the other hand the changes associated with vaccination and appearance and resolution of such signs and symptoms as those said to prove 'primary HIV infection' and AIDS (mycobacterial infections, PCP) are many fold greater than those associated with anti-retroviral therapy.

The inevitable questions which arise are:

- (i) If the appearance of symptoms and signs of the 'primary HIV infection and of such AIDS defining diseases as mycobacterial infections and PCP' leads to increases of 'plasma HIV RNA' of up to 350-fold, why should one claim that 'plasma HIV RNA' is the cause of AIDS and not vice versa, especially when 'plasma HIV RNA' is not significantly affected by anti-retroviral drugs?
- (ii) If the resolution of the signs, symptoms and infections which constitute AIDS leads to a decrease of up to 350-fold in 'plasma HIV RNA', why should one use very expensive and toxic drugs to decrease this RNA by no more than 10-fold instead of treating the infectious diseases which cause these symptoms and signs?

Is the decrease in 'plasma HIV RNA' induced by anti-retroviral drugs due to their effect on HIV or a result of some effect they may have on the aetiological agents of the infectious diseases? Especially if one considers the evidence that AZT can 'inhibit or prevent bacterial infection in immunodepressed hosts' and that 'Opportunistic bacterial infections frequently occur in acquired immunodeficiency syndrome AIDS). Non-typhoid *Salmonella* infections especially are detected at an early stage in HIV patients. These subjects generally develop septicemia that is not of epidemic origin. Since the frequency of *Salmonella* septicemia recurrences without maintenance therapy is about 45%, prophylaxis seems to be recommended'. Furthermore, 'Bacterial infections are known to be very frequent in AIDS patients and generally result in a high fatality rate...The fact that zidovudine is active against Enterobacteria plays an important role in the prophylaxis of opportunistic infections'¹⁰⁴. Indeed, there is ample evidence that AZT has 'potent bacterial activity against many members of the Enterobacteriaceae, including strains of

Escherichia coli, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Enterobacter aerogenes*'. AZT also had activity against *Vibrio cholerae* and the fish pathogen *Vibrio anguillarum*¹⁰⁵. 'The antibacterial effect of zidovudine (AZT) has been demonstrated both *in vitro* and *in vivo* with experimental models of gram-negative bacterial infections...it has been associated with the absence or low occurrence of nontyphoid *Salmonella typhimurium* infections in aids patients treated with AZT...in this study, using an intracellular model, we show that AZT is able to inhibit the intracellular multiplication of *S. typhimurium* at a minimal effective concentration lower than the MIC [minimal inhibitory concentration], indicating its potential for antibacterial accumulation in the macrophages¹⁰⁶. In addition, there is *in vitro* evidence that AZT, as well as another nucleoside analogue (ddG), 'can exert a potent antiviral activity against HBV [hepatitis B virus]', as judged by suppression of the replication of hepatitis B virus in 'hep g2-derived hepatoblastoma cells'¹⁰⁷. According to its manufacturers, AZT had 'an ID₅₀ of 1.4 to 2.7 microgram/ml against the Epstein-Barr virus, the clinical significance of which is not known at this time'²⁵.

- (iii) Is the decline in AIDS deaths due to the use of anti-HIV drugs, as some claim, or
- (a) 'may [it] instead be linked more closely to an increase in federal funding in 1994 for AIDS patients, which led to better prevention and treatment of opportunistic infections', as Mary Ann Chiasson, assistant commissioner of the New York City Department of Health, claims¹⁰⁸;
 - (b) due to a decrease in antigenic stimulation (infectious agents, drugs, semen, blood) as a consequence of the effective education of both patients and physicians?

D. Future Prospects of HIV/AIDS Therapy

That antiviral therapy is not efficacious is best indicated by the fact that, after a decade of experience, HIV/AIDS experts do not agree as to which drugs should be administered, when they should be initiated, and if benefits are conferred in the absence of other therapies. It is also significant that, in 1996, the 'Safety and effectiveness [of AZT] in children have not been established'²⁵. In 1995, David Ho was urging 'to hit HIV, early and hard' with a combination of nucleoside analogues and protease inhibitors¹⁰⁹. One year later, 'A 13-member panel representing international expertise in anti-retroviral research and HIV patient care was selected by the International AIDS Society-USA....To provide clinical recommendations for anti-retroviral therapy' for HIV. Unlike Ho's recommendation, it was the view of this panel that 'Available clinical trial results do not define the optimal treatment strategy for asymptomatic patients with CD4⁺ cell counts above 0.500 x 10⁹/L. In such patients, treatment is recommended for those with more than 30 000 to 50 000 HIV RNA copies/mL or with rapidly declining CD4⁺ cell counts (ie, a greater than 0.300 x 10⁹/L loss over 12 to 18 months), based on the very high progression risk. Treatment should be considered for patients with HIV RNA levels higher than 5000 to 10 000 copies/mL based on the high progression risk. However, any decision to initiate therapy at CD4⁺ cell counts above 0.500 x 10⁹/L must be tempered by the fact that there are no available data to support treatment at this stage of HIV disease, and that such earlier therapy carries with it potential problems related to long-term toxicity, tolerance, acceptance, expense, and the possible induction of drug-resistant virus. Antiretroviral therapy should be initiated in all patients with symptomatic HIV disease (e.g. recurrent mucosal candidiasis; oral

hairy leukoplakia; chronic or otherwise unexplained fever, night sweats, or weight loss'. According to this panel, 'Until longer-term clinical trial data from initial regimens with protease inhibitors are available, most patients in whom therapy is indicated should probably begin with one of the nucleoside analogue-containing regimens described below...The nucleoside analogue combinations with the most demonstrated clinical benefit are zidovudine/didanosine and zidovudine/zalcitabine. Zidovudine/lamivudine may be better tolerated and appears to have comparable antiretroviral potency, but supporting clinical endpoint data are not now available....Although emerging data support combination therapy, didanosine monotherapy is also a reasonable option, particularly for patients who cannot tolerate or who refuse zidovudine. This approach may allow the possibility of adding zidovudine at a later time or switching to zidovudine/zalcitabine or zidovudine/lamivudine, although there are no published data regarding the efficacy of these regimens in patients previously treated with didanosine monotherapy. Initial therapy with other non-zidovudine-containing combinations are less well supported by clinical trial data'. The recommended treatment regimen is summarised in Table 2 overleaf.

One year later (1997), the Panel on Clinical Practices for Treatment of HIV Infection, convened by the Department of Health and Human Services and the Henry J. Kaiser Family Foundation, recommended the following regimen:

Recommended Antiretroviral Agents for Treatment of Established HIV Infection

Preferred (A1)...1 highly active protease inhibitor* + 2 NRTIs (one drug from column A and two from column B. Drugs are listed in random, not priority, order):

| <i>Column A</i> | <i>Column B</i> |
|-----------------|-----------------|
| Indinavir | ZDV + ddl |
| Nelfinavir | d4T + ddl |
| Ritonavir | ZDV + ddC |
| | ZDV + 3TC# |
| | d4T + 3TC# |

Alternative (B11): Less likely to provide sustained virus suppression; clinical benefit is undetermined (30)

- 1 NNRTI (Nevirapine)**+2 NRTIs (Column B, above)
- Saquinavir + 2 NRTIs (Column B, above)'¹¹¹

In the same year the guidelines for anti-retroviral treatment of HIV seropositive individuals of the British HIV Association (BHNA) were:

Initial therapy: evidence from randomised trials

- There is no evidence to indicate the optimum time to start therapy
- Initial treatment should be based on a combination of zidovudine+didanosine, zalcitabine or lamivudine
- ...

Aim of initial treatment

- To reduce plasma viral load as low as possible for as long as possible, preferably to below the assay detection limit, and hence improve clinical outcome...[combinations to achieve this are listed in Table 3 overleaf].

The authors of these guidelines added: 'Antiretroviral therapy has not yet been shown to improve the poor prognosis of patients with a high viral load; nor do we know that clinical benefit is lost if the virus load returns to baseline during therapy'⁸⁷.

Table 2. Some selected options for changing therapy owing to treatment failure or drug intolerance*¹¹⁰

| Initial regimen | Subsequent regimen options |
|--|---|
| | <i>Treatment failure</i> |
| Zidovudine [†] | Zidovudine/didanosine ± protease inhibitor Zidovudine/lamivudine ± protease inhibitor Didanosine ± protease inhibitor |
| Didanosine inhibitor | Didanosine/stavudine ± protease inhibitor Zidovudine/lamivudine ± protease inhibitor |
| Zidovudine/didanosine | Zidovudine/didanosine/protease inhibitor Stavudine/protease inhibitor |
| Zidovudine/zalcitabine | Zidovudine/lamivudine ± protease inhibitor Stavudine/protease inhibitor |
| Zidovudine/lamivudine | Didanosine/protease inhibitor Stavudine/protease inhibitor Didanosine/stavudine Lamivudine/stavudine |
| | <i>Drug intolerance[‡]</i> |
| Zidovudine [†] | Didanosine Didanosine/stavudine Lamivudine/stavudine Stavudine |
| Didanosine | Zidovudine/lamivudine Lamivudine/stavudine Stavudine/protease inhibitor |
| Zidovudine/zalcitabine intolerance to zidovudine | Didanosine |
| Intolerance to zalcitabine | Didanosine/stavudine Stavudine/protease inhibitor |
| Zidovudine/lamivudine | Zidovudine/lamivudine ± protease inhibitor Didanosine/protease inhibitor Stavudine/protease inhibitor Didanosine/stavudine |

*For patients whose initial regimen includes a protease inhibitor, subsequent regimens should include at least 2 new drugs chosen from among nucleoside analogues, nonnucleoside reverse transcriptase inhibitors (if available), and protease inhibitors (one should be selected for which there is likely to be little or no cross-resistance to the initial protease inhibitor).

[†]Considered a suboptimal regimen; all patients on zidovudine monotherapy should be re-evaluated.

[‡]A protease inhibitor could be added to the nucleoside analogue regimens listed.

Table 3. Possible combinations to reduce plasma viral load to below the limit of detection⁸⁷

| Combination type | Examples |
|--|--|
| Two (or possibly three) nucleoside analogues | Zidovudine+didanosine or zalcitabine or lamivudine |
| Two nucleoside analogues plus a non-nucleoside reverse transcriptase inhibitor | Zidovudine+didanosine+nevirapine |
| Two protease inhibitors | Saquinavir+ritonavir |
| Two nucleoside analogues plus a protease inhibitor | Zidovudine+didanosine+indinavir. |

From these data it is obvious that:

1. AZT is the most often recommended anti-HIV drug.
2. The HIV/AIDS experts profoundly disagree as to what is the best treatment regimen and when treatment should commence.

In a letter published in the *New England Journal of Medicine*, 27th March 1997, Andrew Phillips from the Royal Free Hospital School of Medicine, London, and George Smith from the University of Bristol, wrote: 'in their editorial, Corey and Holmes (Oct. 10 issue) state: "All persons with HIV [human immunodeficiency virus] infection with CD4 cell counts below 500 cells per cubic millimetre should be encouraged to begin anti-retroviral therapy." Such an early instigation of therapy is in line with the current understanding of HIV infection and experience with other infectious diseases, but we do not yet know whether we have good enough therapies – those that have negligible long-term risks and do not jeopardize the efficacy of future therapies the patient may be given – to say that this statement is now true in practice. No randomised trials in asymptomatic patient have established that those treated early survive any longer than those for whom treatment is deferred. Extended follow-up of patients in one trial, the Concorde study, has shown a significantly increased risk of death among the patients treated early. The trials mainly involve monotherapy with zidovudine. The suggestion is that the situation is different for combination therapy. But where is the evidence that for a patient with a CD4 count of 450 cells per cubic millimetre and a low plasma virus level, it would not be better to wait before initiating therapy'¹¹². According to the *Wall Street Journal*, "The new AIDS drugs won Food and Drug Administration approval so rapidly that researchers still don't have a clear understanding...protease patients are,

in effect, guinea pigs in one of the largest and most expensive medical experiments of our time'¹¹³. Dr Andrew Carr of the Centre for Immunology at St. Vincent's Hospital, Sydney, Australia, was reported as saying 'It is therapeutic chaos. Doctors are prescribing what patients ask for, or they're guessing, adding different drugs when they feel like it. I've never seen anything in Medicine quite like it'¹¹⁴.

The latest antiretroviral drugs considered are based on a link that Gallo and his associates claim to have discovered between HIV and chemokines. According to Jon Cohen: 'A pack of academic teams, biotechnology companies and big pharmaceutical houses are now racing to develop treatment that exploit this HIV/chemokine nexus....Researchers caution, however, that even if some of those potential treatments lower HIV level and are well tolerated, they could be tripped up by the same factor that has sent many anti-HIV drugs to an early grave: resistance'. He also reports that John Moore of the Aaron Diamond AIDS Research Center 'worries that companies are going to exaggerate their early findings in HIV trials with chemokine receptor blockers. "I think there's going to be a lot of hot air and smoke" says Moore. "Exploitation clinically? Come back in a couple of years".'. Cohen also says that Anthony Fauci 'notes that the efforts to apply all this new knowledge are running into plenty of complications'. This is not surprising if one considers the theoretical basis for these drugs. According to Cohen, the discovery of the link between HIV and the chemokines 'have answered one of the big mysteries of AIDS research: how HIV infects cells...CD4 receptor binds to gp120 on HIV's surface forming a complex that binds to CCR5 [a chemokine receptor]. When a chemokine – or a drug – occupies CCR5, HIV is shut out'¹¹⁵. However, to date, nobody has presented any evidence – not to mention proof – that the cell-free HIV particles have on their surface knobs, spikes; that is, gp120.

No less an authority on HIV electron microscopy than Hans Gelderblom admits that there is no proof for the existence of such particles¹¹⁶. In fact, to date, nobody has presented any electron microscopy evidence for the existence in plasma of particles with or without gp120.

Surprising as it may seem, Jay Levy appears to be one of the strongest critics of antiviral therapy: 'In virology and in other sciences in which biology plays an important role, statistical significance (even with a P value of .001) and mathematical formulas may not provide the desired insights into a problem unless the correct parameters are being measured....With HIV infection, the basic features of virus multiplication and pathogenesis, particularly, the importance of the virus-infected cell, must be appreciated. Medicine suffers when one is misled by numbers that are not relevant to the clinical problem....Any significant effort to control HIV must provide an extended period of greatly suppressed virus production. Most reports indicate, however, that viruses become resistant to current antiviral therapies within a few months after their initiation. Recent evidence even suggests that resistance to the protease inhibitors is already present in viruses recovered from untreated individuals....Thus, treatment directed at the virus alone is not sufficient. The key issue is the major feature of HIV pathogenesis: Unless the infected cell (the viral reservoir) is eliminated or its production of viruses is stopped, the virus will eventually prevail....A large reservoir of virus-infected cells (up to 250 billion cells) exists in the infected host. Each cell can be a source of continual production of infectious particles or viral products toxic to the host. Most studies indicate no effect of antiretroviral drugs on the level of these virus-infected cells in the blood or lymph nodes. [This appears to be the case not only for the nucleoside analogues but also for protease inhibitors as well, despite the fact that

for both types of drugs the target is HIV-DNA not HIV-RNA¹¹⁷.] Certainly, a reduction in the number of these cells circulating in the blood (and in the lymph nodes) would be a more desirable indication of therapeutic potential than a decrease in the number of viral particles, most of which are noninfectious.... Approaches to control the virus-infected cell need to be developed, particularly cell-mediated immune responses. I envision anti-retroviral drugs being used as adjuncts to immune-modulating therapies that would play the major role in AIDS defence by arming the host to combat HIV infection.... Possible immune-based therapies that merit attention include the use of type 1 cytokines (such as interleukin-2 and interleukin-12), inducers of cytokine production, or activators of CD8⁺ cell antiviral responses'¹¹⁸.

According to Giuseppe Pantaleo, one of the best known HIV experts, antiretroviral therapy, including triple therapy, may not be sufficient to treat HIV infection. One of the reasons given is that 'discontinuation of antiviral therapy [triple therapy, which includes protease inhibitors] after prolonged treatment (up to 1 year) also results in a rapid (10 to 14 day) return of viremia to basal levels, despite the fact that during the period of antiviral therapy plasma viremia was persistently (up to 1 year) found below detectable levels, that is, 200 to 500 HIV RNA copies per millilitre of plasma'. According to Pantaleo, 'The development of immune-based therapeutic intervention may be essential to achieve long-term control of HIV infection.... Therapy with IL-2 or with IL-12 is the ideal strategy for achieving these goals....Administration of immunosuppressive agents, such as cyclosporin A in conjunction with antiviral therapy, may represent, at least in certain stages of disease, a valid strategy for suppressing virus spreading and replication in CD4⁺ T lymphocytes in maintenance therapeutic regimens'¹¹⁹.

However, given the fact that:

- (i) AIDS patients and those at risk are already immunosuppressed (thus the 'AID' in the AIDS acronym).
- (ii) The immunosuppression is associated with abnormally high levels of CD8⁺ ¹⁴.
- (iii) Activation of cells leads to HIV activation. Even at the beginning of the HIV era, both Montagnier's and Gallo's groups accepted that none of the phenomena which collectively are known as 'HIV' appear in the absence of cell activation (IL-2 is almost universally used in 'HIV' cultures);

one can only speculate how additional CD8⁺, immunosuppression and cellular activation would lead to 'long-term control of HIV infection'.

Perhaps now is the time for scientists and physicians to examine treatments predicted by non-HIV theories of AIDS, even if the theories themselves are not accepted, especially when supportive evidence exists. One such theory put forward at the beginning of the AIDS era proposes that oxidative mechanisms are of critical significance in the genesis of AIDS⁸. The discovery of HIV resulted in the broadening of this hypothesis, in that it considered oxidative stress a principal mechanism in both the development of AIDS and the phenomena collectively inferred as proof of the existence of HIV. The theory predicted that the mechanism responsible for HIV and AIDS could be prevented and treated by limiting or ceasing exposure to agents capable of inducing cellular oxidation and administering reducing agents, especially those containing sulphhydryl groups (-SH) or modalities which lead to their increase, in conjunction with diet and general health care^{8,10}.

Although the theory has been ignored, many researchers, for reasons they have not stated, have determined the -SH levels in AIDS patients and those at risk. As the theory predicted, it was found that:

- (i) The tissues of AIDS patients and those at risk, including T4 lymphocytes, are oxidised^{61,120}.
- (ii) Oxidising agents lead to the development of the phenomena which are said to prove HIV infection^{10,11,121}.
- (iii) Reducing agents cause the opposite effect; that is, they inhibit these same phenomena^{10,11,121}.
- (iv) This year, researchers from Stanford University showed that 'GSH [reduced glutathione] levels are lower in subjects with CD4 T-cell counts below 200/ μ l (CD4 < 200) than in subjects at earlier stages of HIV disease; that among subjects with CD4 < 200, lower levels of GSB [glutathione-S-bimane] (an FACS [fluorescence-activated cell sorter] measure of GSH in CD4 T-cells) predict decreased survival; and that the probability of surviving 2-3 years increases dramatically as GSB level approach normal range. In addition, we have presented preliminary evidence suggesting that oral administration of NAC, [*N*-acetylcysteine], which supplies the cysteine required to replenish GSH, may be associated with improved survival of subjects with very low GSH levels'¹²⁰. In other words, and as these data prove, unlike a declining CD4 cell count, there is a direct relationship between decreased cellular -SH levels and patient survival even at CD4 cell counts < 200/uL. These data support our theory^{8,10} that oxidation is of pivotal importance in the development of AIDS. Is there not, then, a scientific justification to begin trials with -SH containing compounds for the prevention and treatment of AIDS - especially when one considers that some of these agents are relatively non-toxic, cheap and readily available?

Conclusion

A critical analysis of the presently available data which claim that AZT has anti-HIV effects shows there is neither theoretical nor experimental evidence which proves that AZT, used either alone or in combination with other drugs, has any such effect. The recommendation that AZT, either alone or in combination, is administered to HIV seropositive or AIDS patients warrants urgent revision.

References

1. Duesberg PH. (1992). AIDS acquired by drug consumption and other noncontagious risk factors. *Pharmac. Ther.*, **55**, 201–277.
2. Duesberg PD. (1996). *Inventing the AIDS Virus*. Washington, USA: Regnery Publishing, Inc., 1996.
3. Hodgkinson N. (1996). *AIDS The failure of contemporary science*. London: Fourth Estate, 1996.
4. Duesberg P, Rasnick D. (1997). The drugs–AIDS hypothesis. *Continuum*, **4**, 1s–24s.
5. Chirimuuta RC, Chirimuuta RJ. (1987). *Aids, Africa and Racism*. 1st edn. Bretby House Stanhope Bretby, Burton-on-Trent, United Kingdom: R. Chirimuuta, 1987.
6. De Marchi L, Franchi F. (1996). *AIDS la grande truffa*. ROME: Edizioni SEAM, 1996.
7. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. (1996). The Isolation of HIV: Has it really been achieved? *Continuum*, **4**, 1s–24s.
8. Papadopulos-Eleopulos E. (1988). Reappraisal of AIDS: Is the oxidation caused by the risk factors the primary cause? *Med. Hypotheses*, **25**, 151–162.
9. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. (1992). Kaposi's sarcoma and HIV. *Med. Hypotheses*, **39**, 22–29.
10. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. (1992). Oxidative stress, HIV and AIDS. *Res. Immunol.*, **143**, 145–148.
11. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. (1993). Is a positive Western blot proof of HIV infection? *Bio/Technology*, **11**, 696–707.
12. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. (1993). Has Gallo proven the role of HIV in AIDS? *Emerg. Med. [Australia]*, **5**, 113–123.
13. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. (1995). Factor VIII, HIV and AIDS in haemophiliacs: an analysis of their relationship. *Genetica*, **95**, 25–50.
14. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D, Hedland-Thomas B, Page BA. (1995). A critical analysis of the HIV-T4-cell-AIDS hypothesis. *Genetica*, **95**, 5–24.
15. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Bialy H. (1995). AIDS in Africa: Distinguishing fact and fiction. *World J. Microbiol. Biotechnol.*, **11**, 135–143.
16. Papadopulos-Eleopulos E, Turner VF, Causer DS, Papadimitriou JM. (1996). HIV transmission by donor semen. *Lancet*, **347**, 190–191.
17. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. (1996). Virus Challenge. *Continuum*, **4**, 24–27.
18. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. (1997). HIV antibodies: Further questions and a plea for clarification. *Curr. Med. Res. Opinion*, **13**, 627–634.
19. Papadopulos-Eleopulos E. (1982). A Mitotic Theory. *J. Theor. Biol.*, **96**, 741–758.
20. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*, **373**, 123–126.
21. Wei X, Ghosh SK, Taylor M, et al. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*, **373**, 117–122.
22. Lauritsen J. (1990). *Poison by prescription – The AZT story*. New York: Asklepeios Press, 1990.
23. Zaretsky MD. (1996). The Delta trial. *Lancet*, **348**, 1237.
24. Scott WF. (1996). The Delta trial. *Lancet*, **348**, 1238.
25. Glaxo-Wellcome Ltd. (1996). Product Information. *MIMS Annual* **8**, 591.
26. Mitsuya H, Weinhold KJ, Furman PA, et al. (1985). 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus

- type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. U S A*, **82**, 7096–7100.
27. Furman PA, Fyfe JA, St Clair MH, *et al.* (1986). Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. U S A*, **83**, 8333–8337.
 28. Yarchoan R, Klecker RW, Weinhold KJ, *et al.* (1986). Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet*, **1**, 575–580.
 29. Nakashima H, Matsui T, Harada S, *et al.* (1986). Inhibition of replication and cytopathic effect of human T cell lymphotropic virus type III/lymphadenopathy-associated virus by 3'-azido-3'-deoxythymidine *in vitro*. *Antimicrob. Agents Chemother.*, **30**, 933–937.
 30. Smith MS, Brian EL, Pagano JS. (1987). Resumption of virus production after human immunodeficiency virus infection of T lymphocytes in the presence of azidothymidine. *J. Virol.*, **61**, 3769–3773.
 31. Hoffman AD, Banapour B, Levy JA. (1985). Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. *Virol.*, **147**, 326–335.
 32. Sarngadharan MG, Robert-Guroff M, Gallo RC. (1978). DNA polymerases of normal and neoplastic mammalian cells. *Biochim. Biophys. Acta.*, **516**, 419–487.
 33. Weissbach A, Baltimore D, Bollum F. (1975). Nomenclature of eukaryotic DNA polymerases. *Science*, **190**, 401–402.
 34. Robert-Guroff M, Schrecker AW, Brinkman BJ, Gallo RC. (1977). DNA polymerase gamma of human lymphoblasts. *Biochem.*, **16**, 2866–2873.
 35. Lewis BJ, Abrell JW, Smith RG, Gallo RC. (1974). Human DNA polymerase III (R-DNA): Distinction from DNA polymerase I and reverse transcriptase. *Science*, **183**, 867–869.
 36. Rey MA, Spire B, Dormont D, Barre-Sinoussi F, Montagnier L, Chermann JC. (1984). Characterization of the RNA dependent DNA polymerase of a new human T-lymphotropic retrovirus (lymphadenopathy associated virus). *Biochem. Biophys. Res. Commun.*, **121**, 126–133.
 37. Mitsuya H, Yarchoan R, Broder S. (1990). Molecular targets for AIDS therapy. *Science*, **249**, 1533–1544.
 38. Balzarini J, Pauwels R, Baba M, *et al.* (1988). The *in vitro* and *in vivo* anti-retrovirus activity, and intracellular metabolism, of 3'-azido-2',3'-dideoxythymidine and 2',3'-dideoxycytidine are highly dependent on the cell species. *Biochem. Pharmacol.*, **37**, 897–903.
 39. Tornevik Y, Jacobsson B, Britton S, Eriksson S. (1991). Intracellular metabolism of 3'-azidothymidine in isolated human peripheral blood mononuclear cells. *AIDS Res. Hum. Retrovir.*, **7**, 751–759.
 40. Stretcher BN, Pesce AJ, Murray JA, Hurtubise PE, Vine WH, Frame PT. (1991). Concentrations of phosphorylated zidovudine (ZDV) in patient leukocytes do not correlate with ZDV dose or plasma concentrations. *Ther. Drug Monitor.*, **13**, 325–331.
 41. Stretcher BN, Pesce AJ, Hurtubise PE, Frame PT. (1992). Pharmacokinetics of zidovudine phosphorylation in patients infected with the human immunodeficiency virus. *Ther. Drug Monitor.*, **14**, 281–285.
 42. Stretcher BN, Pesce AJ, Frame PT, Stein DS. (1994). Pharmacokinetics of zidovudine phosphorylation in peripheral blood mononuclear cells from patients infected with human immunodeficiency virus. *Antimicrob. Agents Chemother.*, **38**, 1541–1547.
 43. Toyoshima T, Kimura S, Muramatsu S, Takahagi H, Shimada K. (1991). A sensitive nonisotopic method for the determination of intracellular azidothymidine 5'-mono-, 5'-di-, and 5'-triphosphate. *Analytic. Biochem.*, **196**, 302–307.
 44. Kuster H, Vogt M, Joos B, Nadai V, Luthy R. (1991). A method for the quantification of intracellular zidovudine nucleotides. *J. Infect. Dis.*, **164**, 773–776.
 45. Slusher JT, Kuwahara SK, Hamzeh FM, Lewis LD, Kornhauser DM, Lietman PS. (1992). Intracellular zidovudine (ZDV) and ZDV phosphates as measured by a validated combined high-pressure liquid chromatography-radioimmunoassay procedure. *Antimicrob. Agents Chemother.*, **36**, 2473–2477.
 46. Robbins BL, Rodman J, McDonald C, Srinivas RV, Flynn PM, Fridland A. (1994). Enzymatic assay for measurement of zidovudine triphosphate in peripheral blood mononuclear cells. *Antimicrob. Agents Chemother.*, **38**, 115–21.
 47. Barry M, Wild M, Veal G, *et al.* (1994).

- Zidovudine phosphorylation in HIV-infected patients and seronegative volunteers. *AIDS*, **8**, F1-5.
48. Barry MG, Khoo SH, Veal GJ, et al. (1996). The effect of zidovudine dose on the formation of intracellular phosphorylated metabolites. *AIDS*, **10**, 1361-1367.
 49. Hazuda D, Kuo L. (1997). Failure of AZT: A molecular perspective. *Nat. Med.*, **3**, 836-837.
 50. Lavie A, Schlichting I, Vetter IR, Konrad M, Reinstein J, Goody RS. (1997). The bottleneck in AZT activation. *Nat. Med.*, **3**, 922-924.
 51. Jackobsen B, Britton S, He Q, Karlsson A, Ericksson S. (1995). Decreased thymidine kinase levels in peripheral blood cells from HIV-seropositive individuals: implications for zidovudine metabolism. *AIDS Res. Hum. Retroviruses*, **11**, 805-811.
 52. Bourdais J, Biondi R, Sarfati S, et al. (1996). Cellular phosphorylation of anti-HIV nucleosides. Role of nucleoside diphosphate kinase. *J. Biol. Chem.*, **271**, 7887-7890.
 53. Guettari L, Loubiere L, Brisson E, Klatzmann D. (1997). Use of herpes simplex virus thymidine kinase to improve the antiviral activity of zidovudine. *Virology*, **235**, 398-405.
 54. Fauci AS, Lane HC. Human Immunodeficiency Virus (HIV) Disease: AIDS and Related Disorders. (1994). In: Harrison's Principles of Internal Medicine, Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, eds, 13th edn, McGraw-Hill Inc., New York: pp 1566-1618.
 55. Babior BM, Bunn HF. Megaloblastic anemias. (1994). In: Harrison's Principles of Internal Medicine, Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, eds, 13th edn, McGraw-Hill Inc., New York: pp 1726-1732.
 56. Hayakawa M, Ogawa T, Sugiyama S, Tanaka M, Ozawa T. (1991). Massive conversion of guanosine to 8-hydroxyguanosine in mouse liver mitochondrial DNA by administration of azidothymidine. *Biochem. Biophys. Res. Commun.*, **176**, 87-93.
 57. Hobbs GA, Keilbaugh SA, Rief PM, Simpson MV. (1995). Cellular targets of 3'-azido-3'-deoxythymidine: an early (non-delayed) effect on oxidative phosphorylation. *Biochem. Pharmacol.*, **50**, 381-390.
 58. Benbrik E, Chariot P, Bonavaud S, et al. (1997). Cellular and mitochondrial toxicity of zidovudine (AZT), didanosine (ddI), and zalcitabine, (ddC) on cultured human muscle cells. *J. Neurol. Sci.*, **149**, 19-25.
 59. Handlon AL, Oppenheimer NJ. (1988). Thiol reduction of 3'-azidothymidine to 3'-aminothymidine: kinetics and biomedical implications. *Pharm. Res.*, **5**, 297-299.
 60. Buhl R, Jaffe HA, Holroyd KJ, et al. (1989). Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet*, **2**, 1294-1298.
 61. Eck HP, Gmunder H, Hartmann M, Petzoldt D, Daniel V, Droge W. (1989). Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. *Biol. Chem. Hoppe-Seyler*, **370**, 101-108.
 62. Surbone A, Yarchoan R, McAtee N, et al. (1988). Treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex with a regimen of 3'-azido-2',3'-dideoxythymidine (azidothymidine or zidovudine) and acyclovir. A pilot study. *Ann. Int. Med.*, **108**, 534-540.
 63. Yuzhakov AA, Chidgeavadze ZG, Beabealashvili SS. (1992). 3'-mercapto-2',3'-dideoxynucleotides are high effective terminators of DNA synthesis catalyzed by HIV reverse transcriptase. *FEMS*, **306**, 185-188.
 64. Barré-Sinoussi F, Chermann JC, Rey F. (1983). Isolation of a T-Lymphotropic Retrovirus from a patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). *Science*, **220**, 868-871.
 65. Toplin I. (1973). Tumor Virus Purification using Zonal Rotors. *Spectra*, **4**, 225-235.
 66. Genesca J, Shih JW, Jett BW, Hewlett IK, Epstein JS, Alter HJ. (1989). What do western blot indeterminate patterns for human immunodeficiency virus mean in EIA-negative blood donors? *Lancet*, **2**, 1023-1025.
 67. Belshe RB, Clements ML, Keefer MC, et al. (1994). Interpreting HIV serodiagnostic test results in the 1990s: social risks of HIV vaccine studies in uninfected volunteers. *Ann. Int. Med.*, **121**, 584-589.
 68. Schupbach J, Jendis JB, Bron C, Boni J, Tomasik Z. (1992). False-positive HIV-1 virus cultures using whole blood. *AIDS*, **6**, 1545-1546.
 69. Vincent F, Belec L, Glotz D, Menoyo-Calonge V, Dubost A, Bariety J. (1993). False-positive neutralizable HIV antigens detected in organ transplant recipients. *AIDS*, **7**, 741-742.

70. Agbalika F, Ferchal F, Garnier JP, Eugene M, Bedrossian J, Lagrange PH. (1992). False-positive HIV antigens related to emergence of a 25–30kD proteins detected in organ recipients. *AIDS*, **6**, 959–962.
71. Mortimer P, Codd A, Connolly J, *et al.* (1992). Towards error free HIV diagnosis: notes on laboratory practice. *Pub. Health Lab. Service Microbiol. Digest*, **9**, 61–64.
72. Fischl MA, Richman DD, Grieco MH, *et al.* (1987). The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *NEJM*, **317**, 185–191.
73. Collier A, Bozzette S, Coombs RW, *et al.* (1990). A pilot study of low-dose zidovudine in human immunodeficiency virus infection. *NEJM*, **323**, 1015–1020.
74. Paxton WB, Coombs RW, McElrath MJ, *et al.* (1997). Longitudinal analysis of quantitative virologic measures in human immunodeficiency virus-infected subjects with > 400 CD4 lymphocytes: Implications for applying measurements in individual patients. *J. Infect. Dis.*, **175**, 247–254.
75. Lee TH, Sheppard HW, Reis M, Dondero D, Osmond D, Busch MP. (1994). Circulating HIV-1-infected cell burden from seroconversion to AIDS: importance of postseroconversion viral load on disease course. *J. Acquir. Immun. Defic. Syndr.*, **7**, 381–388.
76. Holodniy M, Mole L, Winters M, Merigan TC. (1994). Diurnal and short-term stability of HIV virus load as measured by gene amplification. *J. Acquir. Immun. Defic. Syndr.*, **7**, 363–368.
77. O. Brien W, Grovit-Ferbas K, Namazi A, *et al.* (1995). Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood*, **86**, 1082–1089.
78. Reiss P, M LJ, Boucher CA, Danner SA, Goudsmit J. (1988). Resumption of HIV antigen production during continuous zidovudine treatment. *Lancet*, **i**, 421.
79. Chaisson RE, Leuther MD, Allain JP, *et al.* (1988). Effect of zidovudine on serum human immunodeficiency virus core antigen levels. Results from a placebo-controlled trial. *Arch. Int. Med.*, **148**, 2151–2153.
80. Jackson GG, Paul DA, Falk LA, *et al.* (1988). Human immunodeficiency virus (HIV) antigenemia (p24) in the acquired immunodeficiency syndrome (AIDS) and the effect of treatment with zidovudine (AZT). *Ann. Int. Med.*, **108**, 175–180.
81. Dournon E, Matheron S, Rozenbaum W, *et al.* (1988). Effects of zidovudine in 365 consecutive patients with AIDS or AIDS-related complex. *Lancet*, **2**, 1297–1302.
82. DeGruttola V, Beckett LA, Coombs RW, *et al.* (1994). Serum p24 antigen level as an intermediate end point in clinical trials of zidovudine in people infected with human immunodeficiency virus type 1. Aids Clinical Trials Group Virology Laboratories. *J. Infect. Dis.*, **169**, 713–721.
83. Saag MS, Holodniy M, Kuritzkes DR, *et al.* (1996). HIV viral load markers in clinical practice. *Nat. Med.*, **2**, 625–629.
84. Ho DD, Moudgil T, Alam M. (1989). Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *NEJM*, **321**, 1621–1625.
85. Coste J, Montes B, Reynes J, *et al.* (1997). Effect of HIV-1 genetic diversity on HIV-1 RNA quantification in plasma: comparative evaluation of three commercial assays. *J. Acquir. Immun. Def. Syndr. Hum. Retrovirol.*, **15**, 174.
86. Michael NL, Chang M, Kim JH, Birx DL. (1997). Dynamics of cell-free viral burden in HIV-1 infected patients. *J. Acquir. Immun. Def. Syndr. Hum. Retrovirol.*, **14**, 237–242.
87. British HIV Association guidelines for anti-retroviral treatment of HIV seropositive individuals. (1997) *Lancet*, **349**, 1086–1092.
88. O. Brien W, Hartigan PM, Martin D, *et al.* (1996). Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. *NEJM*, **334**, 426–431.
89. Coombs RW, Welles SL, Hooper C, *et al.* (1996). Association of plasma human immunodeficiency virus type 1 RNA level with risk of clinical progression in patients with advanced infection. AIDS Clinical Trials Group (ACTG) 116B/117 Study Team. ACTG Virology Committee Resistance and HIV-1 RNA Working Groups. *J. Infect. Dis.*, **174**, 704–712.
90. Collier AC, Coombs RW, Fischl MA, *et al.* (1993). Combination therapy with zidovudine and didanosine compared with zidovudine alone in HIV-1 infection. *Ann. Int. Med.*, **119**, 786–793.
91. Piatak M, Jr., Saag MS, Yang LC, *et al.* (1993). High levels of HIV-1 in plasma

- during all stages of infection determined by competitive PCR. *Science*, **259**, 1749–1754.
92. Eron JJ, Benoit SL, Jemsek J, *et al.* (1995). Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. *NEJM*, **333**, 1662–1669.
 93. Katzenstein DA, Hammer SM, Hughes MD, *et al.* (1996). 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. *NEJM*, **335**, 1091–1098.
 94. Ruiz L, Romeu J, Martinez-Picado J, *et al.* (1996). Efficacy of triple combination therapy with zidovudine (ZDV) plus zalcitabine (ddC) plus lamivudine (3TC) versus double (ZDV+3TC) combination therapy in patients previously treated with ZDV+ddC. *AIDS*, **10**, F61–66.
 95. Fiscus SA, DeGruttola V, Gupta P, *et al.* (1995). Human immunodeficiency virus type 1 quantitative cell microculture as a measure of antiviral efficacy in a multicenter clinical trial. *J. Infect. Dis.*, **171**, 305–311.
 96. Collier AC, Coombs RW, Schoenfeld DA, *et al.* (1996). Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *NEJM*, **334**, 1011–1017.
 97. Cohen J. (1997). Ethics of AZT studies in poorer countries attacked. *Science*, **276**, 1022.
 98. Melvin AJ, Burchett SK, Watts DH, *et al.* (1997). Effect of pregnancy and zidovudine therapy on viral load in HIV-1 infected women. *J. Acquir. Immun. Def. Syndr. Hum. Retrovirol.*, **14**, 232–236.
 99. Cooper D. (1997). Randomised trial of addition of lamivudine or lamivudine plus loviride to zidovudine-containing regimens for patients with HIV-1 infection: the CAESAR trial. *Lancet*, **349**, 1413–1421.
 100. Staprans SI, Hamilton BL, Follansbee SE, *et al.* (1995). Activation of virus replication after vaccination of HIV-1-infected individuals. *J. Exp. Med.*, **182**, 1727–1737.
 101. Coombs RW, Collier AC, Corey L. Plasma viraemia as an endpoint in evaluating the effectiveness of drugs against human immunodeficiency virus type-1 (HIV) infection: natural history of plasma viraemia and monitoring of antiretroviral therapy. (1991). p. 9–19 *In: Viral Quantitation in HIV Infection*, Andrieu JM, ed., John Libbey Eurotext, Paris.
 102. Deyton L. (1996). Importance of surrogate markers in evaluation of antiviral therapy for HIV infection. *JAMA*, **276**, 159–160.
 103. Cohn JA. (1997). Recent advances HIV infection-I. *Brit. Med. J.*, **314**, 487–491.
 104. Mascellino MT, Iona E, Iegri F, De Gregoris P, Farinelli S. (1993). *In vitro* activity of zidovudine alone and in combination with ciprofloxacin against *Salmonella* and *Escherichia coli*. *FEMS*, **7**, 23–28.
 105. Elwell LP, Ferone R, Freeman GA, *et al.* (1987). Antibacterial activity and mechanism of action of 3'-azido-3'-deoxythymidine (BW A509U). *Antimicrob. Agents Chemother.*, **31**, 274–280.
 106. Herrmann JL, Lagrange PH. (1992). Intracellular activity of zidovudine (3'-azido-3'-deoxythymidine, AZT) against *Salmonella typhimurium* in the macrophage cell line J774-2. *Antimicrob. Agents Chemother.*, **36**, 1081–1085.
 107. Aoki-Sei S, MC OB, Ford H, *et al.* (1991). *In vitro* inhibition of hepatitis B virus replication by 2',3'-dideoxyguanosine, 2',3'-dideoxyinosine, and 3'-azido-2',3'-dideoxythymidine in 2.2.15 (PR) cells. *J. Infect. Dis.*, **164**, 843–851.
 108. Cohen J. (1997). Advances painted in shades of gray at a D.C. conference. *Science*, **275**, 615–616.
 109. Ho DD. (1995). Time to hit HIV, early and hard. *NEJM*, **333**, 450–451.
 110. Carpenter CC, Fischl MA, Hammer SM, *et al.* (1996). Antiretroviral therapy for HIV infection in 1996. Recommendations of an international panel. International AIDS Society-USA. *JAMA*, **276**, 146–154.
 111. Henry J. Kaiser Family Foundation and HIV/AIDS Treatment Information Service (1997) Web site: <http://www.cdcnac.org>.
 112. Phillips AN, Smith GD. (1997). Viral load and combination therapy for human immunodeficiency virus. *NEJM*, **336**, 958–959; discussion 960.
 113. Waldholz M. Some AIDS cases defy new drug 'Cocktails'. *Wall Street Journal*, 1996.
 114. Christie H. (1997). From Hype to Hesitation. *Continuum*, **4**, 11–12.
 115. Cohen J. (1997). Exploiting the HIV-chemokine nexus. *Science*, **276**, 1261–1264.

116. Layne SP, Merges MJ, Dembo M, *et al.* (1992). Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. *Virology*, **189**, 695–714.
117. Schapiro JM, Winters MA, Stewart F, *et al.* (1996). The effect of high-dose saquinavir on viral load and CD4+ T-cell counts in HIV-infected patients [see comments]. *Ann. Int. Med.*, **124**, 1039–1050.
118. Levy JA. (1996). Surrogate markers in AIDS research. Is there truth in numbers? *JAMA*, **276**, 161–162.
119. Pantaleo G. (1997). How immune-based interventions can change HIV therapy. *Nat. Med.*, **3**, 483–486.
120. Herzenberg LA, De Rosa SC, Dubs JG, *et al.* (1997). Glutathione deficiency is associated with impaired survival in HIV disease. *Proc. Natl. Acad. Sci. U S A*, **94**, 1967–1972.
121. Turner VF. (1990). Reducing agents and AIDS – Why are we waiting? *Med. J. Aust.*, **153**, 502.