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SECOND ANSWER TO DR. CHERRY

"…everybody will tell you that AZT is not very good. If you prescribe it as a single drug to a patient, it would be deemed to be malpractice".

Dr. Arthur Gottleib[1]

Dr Cherry: In our view, there are two issues at stake: the efficacy of AZT; and whether its benefits outweigh the disadvantages associated with its toxicity.

We agree.

Dr Cherry: With regard to the first, there is very little doubt about the efficacy of AZT.

In our extensive search of the literature regarding the efficacy of AZT, including mother to child transmission, we could find no such proof. We would be grateful to Dr Cherry if he could indicate references where such proof exists.

Dr Cherry: AZT monotherapy was the only therapy available in 1986 (when treatment was started), but since the early nineties AZT has been more commonly used in combination therapy with other drugs. The early AZT monotherapy studies were conducted before the development of the techniques currently available to quantify the virus accurately in humans (in vivo).

(1) The efficacy of AZT given either singly or as combination therapy can be judged only by determining its effects on the end point, that is, the presence of AIDS or death of the patient from AIDS. Not by its effect on the quantity of virus. The only way to obtain such data epidemiologically is to conduct a long term, prospective randomised double-blind, placebo-controlled study using either AZT monotherapy or in combination with other drugs. There is only one such study, the 1994 Concorde study where AZT was shown to have a detrimental effect. This study involved 1749 symptom-free, HIV-infected individuals from centres in the UK, Ireland and France. In the first report from this study it was stated that "there was no statistically significant difference in clinical outcome between the two therapeutic policies".[2] In 1995, extended results of Concorde showed a significant increased risk of death among the patients treated early.[3]

(2) There are several problems with the statement: **"Techniques currently available to quantify the virus accurately in humans (in vivo)"**, including the following:

(a) The techniques used at present to quantify "HIV" do not measure the number of HIV particles. Instead they purport to count molecules, in

this case, the number of "HIV RNA" molecules. Even if it is possible to perform such tests, first it is absolutely necessary, but not sufficient, to start with RNA which is known to be **the** HIV RNA. These tests can be compared to the DNA test which is sometimes used to prove a suspected criminal guilty of a crime. For such a trial to proceed one must have proof that the DNA came from the suspected individual and nobody else. Similarly, to quantify HIV RNA one must have proof that such RNA originates in an HIV particle. Since viruses are very small and it is not possible to take the RNA from one particle, the next best thing is to take the RNA from material which contains nothing else but HIV particles, that is to take the RNA from a mass of isolated, purified particles. However, contrary to the many claims, to date nobody has presented proof for the isolation (purification) of HIV. In fact it appears that "HIV RNA" has been obtained from material which was not constituted even with retrovirus-like particles. In 1997, the French investigative Journalist Djamel Tahi interviewed Professor Luc Montagnier in camera at the Pasteur Institute in Paris regarding our claim that Montagnier and his colleagues did not publish any proof that what they called purified virus actually contained nothing else but isolated retroviral particles. Montagnier was asked, "Why do the EM [electron microscope] photographs published by you [in 1983] come from the culture and not the purification?". His reply was, "There was so little production of virus it was impossible to see what might be in a concentrate of the virus from the gradient ["pure virus"]. There was not enough virus to do that. Of course one looked for it, one looked for it in the tissues at the start, likewise the biopsy. *We saw some particles but they did not have the morphology typical of retroviruses*. They were very different. Relatively different. So with the [unpurified] cultures it took many hours to find the first pictures. It was a Roman effort!…Charles Dauget [an EM expert] looked at the plasma, the concentrate, etc…he saw nothing major" (italics ours). Questioned about the Gallo group he replied, "Gallo? I don't know if he really purified. I don't believe so".[4] Since what Montagnier and Gallo called "purified" virus did not contain even retrovirus-like particles, it could NOT have been a special retrovirus, HIV.

To tell someone he or she is infected with a deadly virus, HIV, based on "techniques currently available to quantify the virus accurately in humans (in vivo)" is no different from condemning a presumed criminal using DNA testing where there is no proof that the DNA used to compare with the DNA present on the victim belongs to the presumed criminal or even a human being.

(b) Once one has the HIV RNA, then, and only then, one can proceed to develop techniques to quantify the HIV RNA in humans. As we have stressed in our AZT paper, at present no such tests exist. Currently three tests are used to determine the "HIV viral load". If there was an HIV RNA, and if all these tests measured the same thing, then no matter what test one uses, the result for a sample which contain the same amount of RNA should be the same. This is not the case. 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 glades A to H…These isolates were expanded in culture. Virus was collected by ultracentrifugation and resuspended in HIV-seronegative plasma. To standardise 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 10 pg of p24 antigen per ml", that is each sample contained the same amount of HIV RNA. The "HIV-1 RNA copies" per ml of plasma obtained were as follows (where <400 is considered zero RNA[5]):

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

If the HIV RNA PCR "viral load" test were a pregnancy test there would be simultaneous cries of joy and woe on the basis of the same blood sample.

(c) If the "viral load" test measures the concentration of HIV in the blood, then given that millions of copies of HIV RNA/mL are reported, there should be no problems detecting and quantifying both the HIV RNA and DNA (without the need for prior PCR amplification) using classic hybridisation techniques (hybridisation requires more than infintesimal amounts of nucleic acids). Yet nobody has reported such data. In fact in 1994 Gallo admitted: "We have never found DNA in the tumour cells of KS…and in fact we've never found HIV DNA in T-cells".[6, 7] If there is no HIV DNA, there can be no HIV RNA.

If the HIV RNA, that is the "viral load" tests measure the concentration of HIV in the blood, then given that millions of copies of HIV RNA/mL are reported, there should be no problems detecting and quantifying HIV by electron microscopy. Yet to date, not one electron microscopy picture has been published showing even one HIV particle in blood.

Dr Cherry: Nonetheless, two recent studies have clearly documented a decrease in HIV-1 RNA *in vivo* in association with the administration of AZT alone.

Let us repeat, as we have stressed in both our AZT paper and in our first answer to Dr Cherry, HIV experts themselves will not accept that a drug possesses an anti-HIV effect unless it induces a sustained decrease in the "HIV RNA", that is, "viral load" of at least 0.5 – 1 log.[8, 9] The data from Dr. Cherry's refs. 1 and 2 show that AZT does not cause such a decrease in the "HIV-1 RNA". (See Appendix 1). In other words, if a decrease in "HIV-1 RNA" is used as a criteria for the efficacy of AZT, as Dr Cherry now advocates, then the data from the references he cites prove AZT is a failure. In ref. 1, one reads: "Known baseline plasma HIV RNA concentration and the presence of non-syncytium inducing phenotype at baseline were significant predictors of a decreased hazard of disease progression…Anti-retroviral treatment before entry into the study was associated with lower CD4 cell counts and higher rate of the presence of syncytium-induced phonotype". In other words, anti-retroviral treatment results in a detrimental effect, rather than benefiting. The data in ref.1 not only proves the failure of AZT as an anti-retroviral drug, it also poses a significant problem in regard to the HIV theory of AIDS. According to the authors of ref.1, "CD4 [T4] cell counts were not significantly associated with the risk of progression" to disease. In other words, a decrease in T4 cells is neither necessary nor sufficient for disease to develop. This finding totally contradicts the HIV theory of AIDS, that is, that HIV infection → decrease in T4 cells (AID) → S (diseases).

Dr Cherry: AZT is administered as a non-phosphorylated molecule to facilitate uptake by infected cells. It is phosphorylated to its active form (AZT triphosphate) intracellulary[3].

There is no proof of a higher cellular uptake of the non-phosphorylated AZT compared to the phosphorylated moiety. Nobody apart from Dr. Cherry has ever claimed that the reason the drug is given in the non-phosphorylated form is to increase its uptake. The level of triphosphorylate reported in ref. 3 is not significantly different from that reported in the other "recent and more sophisticated studies". (See fig. 2).

Dr Cherry: The issue of whether it is sufficiently phosphorylated to be active in humans has to be answered by documenting an *in vivo* decrease in HIV-1 RNA in patients treated with AZT, which has now been done.[1,2]

"The issue of whether it is sufficiently" triphosphorylated is best documented by performing direct measurements of the triphosphorylated AZT with the extremely sensitive, accurate and sophisticated presently available methods. If for reasons which are not clear, one must answer the triphosphorylation question indirectly and also by an unproven method, that is by "documenting an *in vivo* decrease in HIV-RNA" then, looking in fig. 1 where the data from ref. 1,2 are presented, one has no choice but to conclude that AZT is not "sufficiently" triphosphorylated.

Dr Cherry: With regard to test tube (*in vitro*) studies, they usually involve the stimulation of patient-derived cells in culture followed by radio-labelled nucleoside exposure. Thus, these methods measure the maximal ability of stimulated cells to incorporate exogenous nucleotide triphosphate, which may or may not be related to what the subjects' cells would do if they were

naturally exposed following drug administration. Several studies have documented higher triphosphorylation in vitro[4-8].

(a) The methods do not "measure the maximal ability of stimulated cells to incorporate exogenous nucleotide triphosphate", but the maximal level of triphosphorylation which can be achieved in the cells exposed to the same form of drug administered to patients, that is, non-phosphorylated AZT. In fact, unless AZT is administered in its triphosphorylated form, there can be no "exogenous nucleotide triphosphate".

(b) It is true that some studies *in vitro*, using leukaemic cell lines or other immortal cell lines reported higher levels of triphosphorylation than those found *in vivo*. The highest to date was reported by the authors (Furman and his associates, including Gallo and researchers from Glaxo-Wellcome) of Dr Cherry's reference 5. However, when Furman *et al*, conducted their *in vitro* study to prove HIV inhibition by AZT using the leukaemic cell line H9 (HUT-78), instead of using non-triphosphorylated AZT, they used triphosphorylated AZT which was "prepared from azidothymidine [non-phosphorylated AZT] by published methods".[10] More importantly, we repeat, no matter what the findings are *in vitro*, they cannot be extrapolated *in vivo*. According to the authors of Dr Cherry's ref 9, "However, infected human cell lines or ex vivo studies of lymphocytes are unlikely to be representative of the complex milieu of the HIV-infected patient".

(c) If the triphosphorylation obtained in vitro, under the most ideal conditions is not sufficient to inhibit HIV *in vitro*, again under the most ideal conditions, then the lower triphosphorylation obtained *in vivo*, would be even less sufficient.

Dr Cherry: Several studies have documented higher triphosphorylation *in vitro*[4-8] than cited in her graph and table, but the varying levels reported are being used by Professor Papadopulos-Eleopulos as a red herring, as the *in vivo* studies referred to above[1-3] are clearly of far greater significance.

(a) The level of triphosphorylated AZT reported by us in the graph and table, which he claims we use as a red herring, are not from *in vitro* as Dr Cherry seems to imply but from *in vivo*. Everybody, including the patients and the doctors who treat them, are interested only in what is happening *in vivo*, not *in vitro*. Dr Cherry, himself, in his first reply agreed that "AZT can inhibit HIV replication by acting as a chain terminator only in the triphosphorylated form". What is (are) the reason(s) which now lead him to consider the data on triphosphorylated AZT a red herring and contradict himself? Again, if one agrees with Dr Cherry's new criteria for AZT efficacy, then one would also have to accept that by these criteria the data in ref. 1-3, prove that AZT has no anti-viral effect. That is, the drug is not triphosphorylated and does not decrease the HIV RNA.

Dr Cherry: It has been found that it is of limited clinical relevance to measure levels of phosphorylation, as the triphosphorylated form of AZT has a limited life span and there are a number of confounding factors which make it is

difficult to interpret the results. These factors include difference in exposure, host cell intracellular kinase activity, sample collection intervals and analytical techniques. The relationship between the plasma levels of AZT and intracellular triphosphate concentrations is usually unclear[9-11], due to cell regulatory pathways resulting in a saturable intracellular metabolism. Generally, drugs are used at levels that are higher than the concentration needed to saturate the phosphorylation pathway. We therefore think it is largely irrelevant to continue to discuss the levels of phosphorylation, as the critical issue is that individuals who are treated with AZT monotherapy show a significant, but modest, reduction (between 45% and 72%) in viral RNA levels[1,2]

(a) In our search of the AZT literature we did not come across any evidence or anybody claiming that "it has been found that it is of limited clinical relevance to measure levels of phosphorylates" of AZT. To the contrary. According to the authors of ref. 9, cited by Dr Cherry: "As zidovudine triphosphate is important for both antiviral activity in HIV-infected cells and host toxicity in target tissue such as bone marrow, an improved understanding of the systemic and cellular pharmacokinetics of zidovudine can improve the efficacy and safety of therapy". In ref.11 one reads: "These results would support the hypothesis, based on the mechanism of action of dideoxynucleoside inhibition of HIV reverse transcriptase, that intracellular triphosphate concentration predicts the antiretroviral effect more accurately than the plasma concentration", of non-triphosphorylated AZT.

(b) It is true that the metabolism of AZT depends on many factors, but this is no unusual for any drug and would be expected. The relationship between the plasma levels of AZT and the intracellular triphosphate concentration is not "unclear", but as the authors of ref. 11 state "complex". It depends on the plasma concentration, the proliferative and metabolic state of the cells, and extent of disease. Dr Cherry contradicts himself. In his first answer he expressed the view that AZT "can inhibit HIV replication by acting as a chain terminator only in the triphosphorylated form". Now instead of given UNCLEAR as the "recent and more sophisticated studies showing higher degrees of triphosphorylation", as we have asked, he claims that it is "largely irrelevant to discuss the level of phosphorylation". No matter which criteria one uses to determine the efficacy of AZT, his first, that is, triphosphorylated AZT, or his latest, a reduction in "viral RNA" levels, the evidence allows only one logical conclusion: AZT cannot and does not have an anti-HIV effect.

Like other researchers, the authors of ref. 9 found "low degrees of triphosphorylation" of AZT, which "are at the current limit of detection for available analytical methods". Ref. 10 is an abstract from the 1998 World AIDS Conference which we could not access. From the title it appears that the authors studied the triphosphorylation of lamivudine not AZT.

In ref. 11, the patients were treated with two regimes of AZT, "the standard adult zidovudine dose of 100 mg five times daily", and a complicated, not very practical regime where the "dosing strategy was an individualised regime developed to maintain a targeted concentration in the plasma" of non

triphosphorylated AZT. The maximum triphosphorylation obtained with the first regime was 92 fmol/10^6 cells (0.092 pmol/10^6 cells) and with the second 160 fmol/10^6 cells (0.16 pmol/10^6 cells). (Recall (i) 1 pmole/10^6 cells =1 μM; (ii) *in vitro*, under the most ideal conditions, the minimum concentration of triphosphorylated AZT required to decrease by half the synthesis of DNA using a synthetic RNA template is 0.7 μM[10]).

The decrease in "HIV RNA" which these authors detected, like those of ref. 1 and 2 was insignificant "a decrease [maximum decrease] of 0.28 $\log_{10}$ copies/ml with concentration-controlled therapy, and a decrease of 0.05 $\log_{10}$ copies/ml with standard therapy".

Dr Cherry: Treatment with AZT alone is somewhat outdated, as in the long term resistance will develop, and it has only moderate effects, the average duration of efficacy being approximately six months.

To determine that "the average duration of efficacy" of a drug is "six months", one must first show that the drug has an effect. This is not the case for AZT. In fact, since AZT has never been shown to be triphosphorylated to levels sufficient to inhibit reverse transcription or to decrease the viral load for any duration of time, AZT should never have been introduced into clinical practice.

Dr Cherry: Currently, the most appropriate use of monotherapy is for the prevention of transmission, either from mother to child, or after occupational exposure of a health care worker to the virus.

Currently, AZT monotherapy is recommended ONLY for the prevention of mother to child transmission in the developing countries, such as South Africa. AZT monotherapy is considered "suboptimal for treatment; combination drug therapy is the current standard of care" for preventing mother to child transmission in the developed countries, such as the United States.[11] We would be grateful if Dr Cherry could explain the difference between a South African mother-infant pair and a USA mother-infant pair.

Dr Cherry: As more successful combination therapies have been employed, it has been demonstrated that substantial and sustained reductions of virus levels in the blood are associated with substantial and sustained reductions in HIV-1 associated morbidity and mortality.[12]

(a) It is true that there are hundreds of "combination therapies". (Dr. Michael Saag, who supervises research and the care of more than one thousand AIDS patients in Birmingham, admits that in one year 157 of his patients collectively took 189 different drug formulas, with only three patients taking the same mix of drugs[12]). The questions are:

(i) Is there any proof that the drugs used in the combination therapies either above or in combination, decrease the viral "burden", that is, HIV DNA and thus viral "load", that is, HIV RNA? (The action of both AZT and protease inhibitors is to prevent the synthesis of new HIV particles capable of infecting uninfected cells, that is to decrease the formation of more HIV DNA. Hence, after the already infected cells die, (a matter of

days), the HIV DNA should decrease to zero and this should be followed by a similar decrease in HIV RNA).

(ii) Is there any relationship between the "viral load", that is, between HIV RNA and clinical status? If so, which is the cause and which is the effect?

Like many other viruses, the genomes of retroviruses and thus HIV are constituted from RNA and not DNA. According to a theory, WHAT IS UNIQUE ABOUT RETROVIRUSES (known as the proviral theory), when a retrovirus infects a cell, its RNA is reverse transcribed into the complementary DNA, cDNA, by the enzyme reverse transcriptase. This cDNA, known as the provirus, is incorporated into the cellular DNA. When the cell divides its DNA is transcribed as cellular RNA and the retrovirus DNA is transcribed as retroviral RNA. The retroviral RNA may lead to the synthesis of the retroviral proteins and subsequently to the assembly and release of new viral particles. With no exception, all the anti-HIV drugs presently used, by design can only prevent the synthesis of new HIV DNA. Once the DNA is formed these drugs cannot prevent the synthesis of new HIV RNA. In other words the drugs decrease HIV RNA indirectly, by first decreasing the HIV DNA. Yet, as we have stated in our paper, and as confirmed again lately,[13, 14] there is ample evidence that no drug, and no drug combination, including Highly Active Antiretroviral Therapy (HAART) has any effect on the "viral burden", that is HIV DNA.

This means that either:

(i) "HIV DNA" or "HIV RNA" or both are non-HIV;
(ii) The drugs do not decrease the HIV RNA, they only interfere with its measurement;
(iii) The proviral theory of retroviruses is wrong.

In ref. 12 researchers from many institutions in both the USA and United Kingdom, retrospectively analysed selected data from seven clinical trials using Kaplan-Meier statistics and reported:

(a) That baseline HIV RNA levels, that is the level of RNA before treatment was started, were predictors of clinical disease progression;
(b) The decrease, from baseline, at week 24 after treatment, was also a predictor of disease progression;
(c) The baseline HIV RNA level remained a predictor no matter what the level of HIV RNA was at week 24.

Three of the co-authors of reference 12 are also co-authors of ref. 1, where they wrote that the presence of signs and symptoms such as "(oral hairy leukoplakia, candidiasis, or herpes zoster) was significantly associated with increased HIV RNA concentration". In 1993, the senior author of ref. 12, Michael Saag, 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 3.55×10^5 and 2.18×10^7 copies per millilitre (corresponding to 1.78×10^5 to 1.09×10^7 virions per millilitre)…Within the first 100 days after onset of symptoms,

plasma RNA levels fell by between 20 and 235-fold", even without anti-retroviral therapy.[15]

At present there is ample evidence that antigenic stimulation and infectious diseases, including diseases caused by agents other than HIV led to an increase of HIV RNA, and conversely, cessation of antigenic stimulation and resolution of infectious diseases to a decrease, without the necessity of antiviral treatment.[16-18] Since antigenic stimulation and clinical disease led to an increase in viral load, it means that a relationship would exist between the two, but the increase in HIV RNA may be the result not the cause of the disease. That this may be the case, is indicated by the latest data from the Multicenter AIDS Cohort Study (MACS), the longest (began in 1984), largest (over 5,000 gay men), best designed and executed prospective study. Researchers from the MACS reported (in patients who were never treated with antiretroviral drugs) a relatively high level of HIV RNA about the time of seroconversion (at about the time that their antibody tests became positive). This was followed by a decrease, after which the HIV RNA level remained stable. The "slope during the 3 years immediately preceding progression to AIDS was 0.14 – 0.2 $\log_{10}$ copies/mL per year [non significant], and the estimated level at the time of progression to AIDS was 5.2 – 5.3 $\log_{10}$ copies/mL". In addition to showing that the significant increase in HIV RNA did not precede the development of AIDS, as one would expect if HIV was the cause of AIDS, but coincided with it, they have also reported that: "HIV RNA was detected at the last seronegative visit (on average of 3 months before seroconversion) in 15% of seroconverters". Since it is accepted that the maximum time (the window period) between infection and the development of a positive antibody test is 3 months, then at least some of the 15% of patients had a positive HIV RNA test before infection with HIV.[19]

If one assumes that the increase in HIV RNA is the cause of the disease and conversely decrease in the level by the anti-HIV drugs leads to a decrease in clinical disease as Dr Cherry claims then since:

(i) According to the authors of ref. 12 a finding at 24 weeks of an RNA level which is no lower than 0.5 $\log_{10}$ than that at baseline cannot be considered as a significant decrease.

(ii) All the presently available evidence, including that in ref. 1 and 2, shows that AZT does not decrease HIV RNA by more than 0.5 log, then, by Dr Cherry's criteria, the only possible conclusion is that AZT therapy cannot lead to "sustained reductions in HIV-1 associated morbidity and mortality".

Dr Cherry: **Reduction in HIV-1 replication in patients consistently reduces mortality across all populations studied, regardless of socio-economic status. Consequently, in countries where antiretroviral therapy has been introduced, there has been a progressive reduction in mortality that has paralleled closely the temporal introduction of antiviral therapy.**

The first definition of AIDS was put forward by the CDC in 1982. Between 1982-85, the syndrome (S) consisted mainly of two diseases, Kaposi's sarcoma (KS) and *Pneumocystis carinii* pneumonia. None of the diseases which signified the definition of AIDS was new but, with one exception, they were

previously rare. The exception was KS in Africa. This disease was present in high frequency in an aggressive form in eastern equatorial Africa long before the AIDS era.[20]

By the middle of the 1980s the number of HIV infections as well as the number of AIDS cases especially those with KS, was in decline.[21, 22] However, during the last quarter of 1984, and at the time that HIV was accepted as the cause of AIDS, AIDS was redefined by the addition of "mild and moderate diseases". These included non-Hodgkin's lymphoma and lymphoma limited to the brain. In 1987 the CDC redefined AIDS a second time introducing twelve new diseases including extrapulmonary (but not pulmonary) tuberculosis (TB). The 1987 definition, which came into effect on September 1st, permitted so many degrees of freedom that nearly any sick individual, especially one belonging to a "risk group", could be reported as an AIDS case. The 1987 definition led to an increase of AIDS cases which continued over a number of years. For example, four months after its introduction one institution reported a 19% increase in cases but "Because of the potential lag between publication of the new definition and its widespread use", 19%, "may be an underestimate" of the true impact of the new definition.[23]

By 1990 the reported AIDS cases had levelled out and begun to decline[24] but in 1993 AIDS was redefined yet again. The new definition retained the twenty three AIDS indicator diseases belonging to the 1987 definition and added three more: "pulmonary tuberculosis, recurrent pneumonia, and invasive cervical cancer". In addition, for the first time, any individual was regarded as a case of AIDS without a disease, that is, solely with a laboratory abnormalities, that is, seropositive with a T4 cell count less than 200/μL.[25] According to a CDC report, February 3, 1995 in the USA in 1994 there were a total of 80,691 cases reported to the CDC, "...which followed the 106,618 cases reported in 1993. The number of cases reported in each of these years was greater than that reported in 1992 (47,572) and followed the expansion of the AIDS surveillance case definition for adolescents and adults implemented on January 1, 1993...". In other words by 1994 AIDS cases began another decrease. Furthermore of 79,674 cases reported among adolescents and adults in that year "...43,226 (54% were reported based on the reporting criteria added to the definition in 1993. Of these, 39,513 (91%) persons had severe human immunodeficiency virus (HIV)-related immunodeficiency only (ie., less than 200 CD4+ T-lymphocytes per μL or a CD4+ T-lymphocyte percentage of total lymphocytes less than 14), 2357 (5%) had pulmonary tuberculosis (TB), 1239 (3%) had recurrent pneumonia, and 164 (less than 1%) had invasive cervical cancer; 47 persons were reported with greater than or equal to 2 of these clinical diseases. Of the 3713 persons reported with one of the three opportunistic illnesses (ie., pulmonary TB, recurrent pneumonia, and invasive cervical cancer), 1097 (30%) were women, 2237 (60%) were black, and 1785 (48%) were injection drug users...of the 1017 children, 50% were female, most were black (62%) or Hispanic (23%)".[26]

Although AIDS cases start to decrease again by 1994, most HIV experts, like Dr. Cherry, claim AIDS cases started to decline in 1996, which coincides with the introduction of a new treatment called Highly Active Anti-Retroviral Therapy (HAART) and thus is the result of HAART. Such claim can be proved

only by prospective, blinded, controlled studies. Not one such study exists. The claim that the decrease started in 1996 and is due to HAART is not accepted by all HIV/AIDS experts. According to George Lemp and his colleagues from the AIDS Office, San Francisco Department of Public Health, the annual number of infections peaked in 1982 and the annual number of AIDS cases in 1992. "The decline in the incidence of AIDS in San Francisco reflects the dramatic reductions in new HIV infections that occurred a decade previously and that were achieved as a result of significant changes in high-risk behaviours, primarily among homosexual and bisexual men. Changes in HIV seroincidence must be factored in before attributing the decrease in AIDS incidence to more effective combination antiretoviral treatment".[27] In fact at present evidence exists which shows that despite the name, HAART, the drug combination has no anti-retroviral effect. In fact, Italian researchers reported an increase of HIV DNA following HAART.[28] Although the possibility cannot be excluded that HAART may have a clinical effect independent of HIV, this does not seem to be the case. In a study conducted by Michael Gottlieb and his associates it was shown that after 18 months on HAART, 26% of patients developed AIDS which is a much higher rate of progression than those on no anti-retroviral treatment, less than 2%.[29-31]

Because no simple infectious agent can cause 30 totally different disease, it is claimed that HIV causes immunodeficiency which, in its turn, causes the 30 diseases. If this is the case, and if HAART indeed has an anti-HIV effect, then patients on HAART should have an improved immune response compared to patients who never had received anti-HIV treatment. The improvement should be directly related to the decrease of HIV RNA. This is not the case, to the contrary. In one of the most recent studies from Italy one reads: “It is thus generally accepted that low plasma viraemia [low HIV RNA] and the presence of strong cell-mediated immunity correlates with lack of HIV diseases progression…Drugs designed as a therapeutic tool against HIV infection should consequently be aimed at reproducing these correlates; reduction of HIV viral load and stimulation of a quantitative and qualitative adequate immune response”. Instead they, and others, found “that antigen-stimulated proliferation was defective in most HAART-treated individuals but was strong in the majority of the patients in whom control of HIV replication is achieved in the absence of therapy…In this regard, recent data obtained in HIV-infected and HAART-treated patients show that a more severe immune impairment was detected in those individuals in whom the best suppression of plasma viraemia was obtained”.[32]

Dr Cherry: Multiple clinical trials regarding the role of AZT in preventing transmission of HIV-1 from infected mothers to their exposed infants indicate that AZT clearly reduces HIV-1 transmission of neonates. This has been documented on four continents in multiple blinded trials, of multiple designs, by a large number of investigators.

The only way to prove that AZT prevents mother to child transmission is to conduct multiple randomised, double-blind, placebo-controlled studies. To date, the authors of only one study, the Paediatric AIDS Clinical Trials Group, protocol 076, have claimed that their study satisfied the above requirements. However, the authors did not give any details, as is required for any epidemiological study, regarding randomisation, how they kept the study blind, and what placebo they used.[33]

Furthermore, in the first part of the study the children were considered "infected" by finding one positive "isolation" result and not two such cultures as it is required by the AIDS Clinical Trial Group.

In the second part of the study, a baby with "a condition considered to define the presence of AIDS", (such conditions are totally nonspecific), was considered HIV infected by virtue of transmission from its mother.

We would be most grateful if Dr Cherry could provide us with the references which prove "that AZT clearly reduces HIV-1 transmission to neonates".

Dr Cherry: The likely event is not through its effect on the level of HIV replication in the mother, but rather through its ability to pre-empt primary infection in the baby.

All through this debate Dr Cherry has insisted that the efficacy of AZT is due to its ability to decrease the HIV RNA. Now he says its effect on transmission is not due to its effect on the HIV RNA but some unidentified "ability to pre-empt primary infection in the baby". According to the presently available evidence, it appears that the unidentified factor cannot be triphosphorylated AZT in the foetus.

In 1997 in a study published by researchers from the National Centre for Toxicological Research/Food and Drug Administration, USA, one reads: "The degree to which AZT and its metabolites accumulate in foetal tissues may be important in the evaluation of the risk-to-benefit ratio of AZT treatment during pregnancy…it is possible that the presence of AZT-MP [AZT monophosphate], even in the absence of significant triphosphate, may put the developing conceptus at risk". Using rhesus monkeys as a model for humans ('The rhesus monkey's placenta structure and function as well as pharmacokinetic similarities to humans, make this an appropriate model for examining AZT distribution during pregnancy"), they reported that: "Only AZT-MP was detected in the foetal tissues…the putative active antiviral metabolite, AZT-TP [AZT triphosphate], was not detected in any monkey foetal tissue". [34]

Unlike Dr Cherry who claims that "The likely effect is not through its effect on the level of HIV replication", that is, the HIV RNA level, the researchers who conduct the mother to child transmission studies, claim that "In multi-variant analysis, the only independent risk factor [for transmission] was the maternal HIV-1 level at baseline".[35] In two of the most recent studies by researchers from the CDC and the Bangkok Collaborative Perinatal HIV Transmission Study Group "Clear evidence was found that elevated maternal viral load is a strong risk factor for both in utero and intrapartum transmission", and "intervention to reduce maternal viral load [according to them this should be by at least 0.5-1 log] should be effective in reducing both in utero and intrapartum transmission".[36, 37]

Dr Cherry: With regard to toxicity, the situation is more complicated. The toxicity of AZT is not disputed; the question related to the relative benefits and disadvantages associated with treatment for particular indications.

Any drug which is shown to have no "relative benefits", even if not toxic should not be prescribed to patients. Since the "toxicity of AZT is not disputed" and since by both Dr Cherry's criteria, AZT has been shown to have no "relative benefits" should he not advise against its use?

Dr Cherry: **Professor Papadopulos-Eleopulos cites two recent studies, one conducted in France[13] and the other in Italy[14], indicating toxicity associated with AZT treatment of mothers during pregnancy. Both are very interesting, but I would like to point out to you that their findings have been placed out of context…The authors [of the French study] recommended specifically that these results do not mitigate against further use of AZT therapy…The last sentence [in the Italian study] reads: "Our findings should not be misinterpreted as a reason not to use ZDV (AZT) prophylaxis, which is effected in preventing perinatal HIV infection. Needless to say, this is precisely what Professor Papadopulos-Eleopulos has done!**

Since the findings of these two studies are "negative", that is, not what is expected, and are not what is claimed in most of the other studies, the authors, if they wanted their finding to be published, may have had no choice but to add the above qualifications. As we know from our experience and as the presently available data shows, it is almost impossible to publish papers with "negative" findings. "Negative studies suffer a substantial time lag. With some exceptions, most of this lag is generated after a trial has been completed...Typical examples in HIV disease include the use of early zidovudine monotherapy in asymptomatic patients, acyclovir, ditiocarb (Imuthiol), and oral granciclovir prophylaxis, where positive and negative trials started at about the same time, but negative studies appeared later or are still unpublished".[38]

We cited the French and Italian studies in the context of Dr Cherry's statement: "Like many medical interventions AZT is widely acknowledged to have toxic effects which should be weighed up against its potential benefits". Since we gave only the data without any comments or conclusion, it is impossible for us to have placed them "out of context". However, Dr Cherry may have done so.

The authors of the Italian study, from which Dr Cherry's quote originated, wrote that children whose mothers received AZT "are more likely to have a rapid course of HIV-1 infection compared with children born to untreated mothers, as disease progression and immunological deterioration are significantly more rapid and the risk of death is actually increased during the first 3 years of life". Furthermore, unlike Dr Cherry, the authors of the Italian study are of the opinion that "A strong association exists between high maternal viral load and an increased risk of transmission. Inability to reduce maternal viral load might explain both treatment failure and rapid disease progression in infected children...Infants with rapid disease progression are likely to be those who have been infected by an intra-uterine infection, which is marginally affected by ZDV [AZT] prophylaxis".

The authors of the French study wrote: "Antiretroviral nucleoside analogues are toxic to mitochondria in HIV-1-infected adults and children…The symptoms in the children in our study were not specific, and may therefore

have not been identified as toxic effects of treatment. In symptom-free children who had only persistent biological abnormalities, the persistent lactic acidosis and the anomalies of myelinisation and electroretinographic findings– the long term progression of which is unknown–were detected by specific diagnostic procedures. Prospective studies designed to investigate this effect are essential. The first data from the long-term follow-up of children exposed to zidovudine (protocol ACTG219) have been published. Three of the 107 children had unexplained symptoms of the heart and eye, which could be related to mitochondrial dysfunction and reinforces the need for specific enzymatic investigation. We are aware that the suggestion that antiretroviral drugs are toxic raises delicate issues…We believe our observations are however sufficiently significant to be shared. It is too early to do a risk/benefit analysis. Our view is that the current recommendations for zidovudine monotherapy prophylaxis should be maintained. We believe that combinations of molecules that could have cumulative toxic effects on the same cellular target should be avoided. Other nucleoside or combinations of nucleosides may have similar toxic effects. Pregnant women should be informed of the potential effects associated with these treatments during pregnancy".

We agree, let the women be fully informed and before they decide.

Dr Cherry: All doses [in the two studies] of AZT were way in excess of those proposed for preventing vertical transmission in South Africa. It is therefore not possible to predict that the same toxicity would occur with very much reduced levels of exposure to the drug.

It is true that if AZT is used in lower doses may result in lower toxicity. However, as we have repeatedly mentioned, our aim has never been to analyse the toxicity of AZT, but its benefits. Since AZT has no benefits, it should not be given to patients even in reduced doses. WANT TO MENTION THAT REDUCED DOSES MIGHT MEAN EVEN LESS AZT-TPP?

Dr Cherry: A very large study in the US of 15,229 children, with and without antiretroviral exposure, born to HIV-infected women, found that no deaths in uninfected children could be attributed to mitochondrial dysfunction[15].

Ref. 15 is a presentation at the Second Conference on Global Strategies for Prevention of HIV Transmission from Mothers to Infants, Montreal, 1999. Unfortunately we could not find this reference. Suffice to say here that:

(a) It is only an abstract, and that unless one has details of the study, no conclusions can be drawn regarding its validity, especially if one considers, as the authors of the French study point out, the symptoms of mitochondrial dysfunction are not specific and therefore may not be identified as toxic effects of treatment, unless enzymatic tests are performed.

(b) The abstract cannot be from a single study since to date no study of 15,229 children or even one thousand children has been reported. This means that ref. 15 must consist of a meta-analysis, which makes it even more difficult to draw meaningful conclusions, especially if one considers the evidence that: "Early systematic reviews of the accumulating evidence may give misleading results. An investigation in HIV-related

trials, in particular, has shown that meta-analyses including only the published evidence would have found sizeable treatment benefits for several controversial or even abandoned treatments, while in the case of zidovudine, a meta-analysis of the early published, short-term trials would suggest markedly more favourable results compared with the pooled treatment effect of the trials with longer follow-up that appeared later".

Dr Cherry: In the Italian study, the authors conclude that the babies may have failed therapy as a consequence of infection in the uterus, or transmission of resistant virus. The former has been shown to result in more rapid disease progression irrespective of drug therapy; and the latter would not result from the limited regimens proposed for use in South Africa.

True, WHAT IS TRUE? but they give a mechanism totally different that that of Dr Cherry. Unlike Dr Cherry, the authors of the Italian Study are of the opinion that the "failed therapy" may be due to the inability of AZT to reduce HIV RNA (viral load). "Inability to reduce maternal viral load might explain both treatment failure and rapid disease progression in infected children. The mothers with a high viral load may transmit more virus to their children". As far as failure due to transmission of resistant virus is concerned, suffice to mention that in the first report from the PACTG 076 study, (a study using the highest doses of AZT and the longest duration of treatment), the authors were of the opinion that treatment failure was unlikely to be due to transmission of resistant virus "considering the relatively short duration of the maternal treatment".[33] In a 1999 publication from PACTG, "The development of genotypic zidovudine resistance was assesed among infected infants in the zidovudine treatment group", and it was found that: "When the zidovudine treatment regimen failed to prevent maternal-infant transmission of HIV-1, resistance to zidovudine did not develop". It is also of interest that in the small subset of infants from the PACTG 076 analysed in the 1999 publication, "The rates of rapid disease progression for the Zidovudine group were" 6/14 (43%) and for placebo group 16/43 (37%).[39]

Dr Cherry: Several other studies have shown no untoward effects of AZT on the neonate[15-19].

Ref 16 is an abstract from the same conference as ref. 15 and thus unavailable to us.

In ref. 17 children from the Paediatric AIDS Clinical Trial Group 076, who received either AZT or placebo, were enrolled in "a long-term observational protocol", Protocol 219. The aim was, "To evaluate the long-term effects of in utero exposure to zidovudine [AZT] vs placebo" of uninfected children.

The authors concluded:

(a) "No significant short-term toxic effects were observed in PACTG 076 for those mothers and infants who received zidovudine".
(b) No adverse effects were observed in HIV-uninfected children with in-utero and neonated exposure to zidovudine followed up for as long as 5-6 years".

However, neither the authors of the PACTG 076 protocol[33] nor those of the PACTG 219 mention what placebo was used. In the original 1994 publication from the PACTG 076 study the authors wrote that 22 children stopped therapy "because of toxic effects (11 in each group)". Most importantly the authors of the 219 study, instead of following blindly all the children from the 076 study, as one would expect, chose only 234 (122 in the AZT group and 112 in the placebo), without giving any reason(s). At the end of the study, when the analysis was performed, only "86% of the uninfected children enrolled in PACTG 219 were still participating in the study; 26 children were lost to follow-up or their caregivers refused further contact". No mention is made to what group the children who were enrolled in the 219, but who were lost to follow-up, belonged.

Although "echocardiograms and ophthalmology examinations (including visual acuity assessment and funduscopic examination) were required for all children by 36 months of age", only:

(a) "One hundred and eighty-six uninfected children (80%) had at least one echocardiogram result recorded in the database";
(b) "One hundred and thirty-seven uninfected children (59%) had at least one ophthalmologic examination (including funduscopic results) recorded in the database".

By designing, executing and analysing data, using methods as those used by the authors of the PACTG 219 study, one certainly may find that AZT has no long term toxic effects, even though the drug is very toxic. Equally as certain is the fact that such findings cannot be considered scientific proof. The authors of ref. 17 themselves wrote: "There are caveats to the data presented. Only two thirds of the children enrolled in the original PACTG 076 protocol are currently being followed up in this late-effects protocol…With a sample size of 120 per arm, there is limited power to detect very rare adverse events". Although the authors of the PACTG 219 study concluded that AZT had no toxic effects, the authors of the French study are of the opinion that in the PACTG 219 three "children had unexplained symptoms of the heart and eye, which could be related to mitochondrial dysfunction and reinforces the need for specific enzymatic investigation".

In ref. 18, the records of "HIV-exposed infants with known ZDV exposure (in utero and/or neonatal) were reviewed for reports of the development of tumours. Participants in this review were HIV-exposed infants with known antiretroviral exposure (in utero or neonatal ZDV) and participating in one of two national, multicentre studies: PACTG 076/219 or the Women and Infants Transmission Study (WITS). Of the 188 PACTG 076 participants who met the first two criteria [were taking part in PACTG 076 or WITS and were exposed to AZT either "in utero and/or neonatal"], 115 (61%) co-enrolled in PACTG 219 and could be included in this analysis. WITS is an ongoing observational natural history study of factors that produce an impact on perinatal HIV transmission and disease progression in adult women and their HIV-exposed children. Prospective enrolment has occurred at 6 U.S. sites since 1989. In total, 612 infants in the WITS with in utero or neonatal ZDV exposure were assessed. Infants co-enrolled into both PACTG 076/219 and WITS were reported and analysed in the WITS cohort alone".

The authors of ref. 18 reported that: "Race/ethnicity distribution in these cohorts is representative of national demographic patterns of HIV infection exposure in infants with black and Hispanic infants disproportionately represented. Mean infant follow-up was longer for PACTG 076/219 participants at 38.3 months (366.9 person-years follow-up) and reflects closure of the PACTG 076 study in 1994. The WITS study has had continued prospective enrolment to date and has a shorter mean follow-up of 14.5 months with 743.7 person years follow-up. The range of infant follow-up was as short as the first month of life and as long as 6 years" and that: "no tumour of any nature were reported in these 727 HIV and ZDV-exposed infants…These data are reassuring regarding the short-term lack of tumours for ZDV-exposed infants observed to date". Their "reassuring" can be questioned on several grounds, suffice to mention those acknowledged by the authors themselves: "Limitations of this analysis are acknowledged. First, tumour surveillance in the clinical studies PACTG 076, 219, and WITS is relatively passive and underreporting is possible. Assessment of the reproductive tract is included only in PACTG 219 at age appropriate intervals, as instanced, adolescence and young adulthood, but not performed in the young cohort reported in this paper. Second, follow-up for our patient cohort is relatively short, which leads to a wide confidence interval for the relative risk. The longest reported follow-up for infants was just over 6 years with median follow-up by cohort at almost 1 year for WITS and just over 3 years for PACTG 076/219. In the cited rodent study, mice with in utero ZDV exposure were serially sacrificed and examined histologically at the human equivalent of early childhood and again at the second to third decade. Tumours were documented only in mice sacrificed at or after the human equivalent of the second decade…This analysis clearly does not completely define the potential for carcinogenicity of ZDV in the child with foetal/infant exposure for HIV-1 transmission risk reduction". As far as exposure to AZT for "HIV-1 transmission risk reduction" is concerned, it is of interest to note that the summary of ref. 18 starts with the sentence: "Zidovudine (ZDV) therapy during pregnancy and to the neonate reduced perinatal HIV transmission by nearly 70% in Paediatric AID Clinical Trials Group (PACTG) protocol 076". However, in a paper published in 1996 with data from WITS, with two of the co-authors of ref. 18, as co-authors, one reads: "ZDV was not associated with a significant decrease in transmission in this overall cohort (18 versus 20% transmission in ZDV users versus non-users".[40]

In ref. 19 the infants from the PACTG 076 "were followed through 18 months and the clinical and laboratory data of the uninfected children who were exposed to AZT during pregnancy and for 6 weeks after delivery were compared with children who were similarly exposed to placebo. Reading their conclusion: "There were no identified problems that would alter current recommendation for the routine use of ZDV for prevention of mother-to-child HIV-1 transmission", and the title of the paper, "Safety of the Maternal-Infant Zidovudine regimen utilisation in the Paediatric AIDS Clinical Trial Group 076 Study", one may get the impression that in ref. 19, the authors have proven that no toxicity is associated with AZT treatment.

It is true, that by looking at the two tables where they present their data, one gets the impression that the outcome in both the AZT and the placebo groups

are similar. However, this does not mean that AZT is not toxic. From the tables it is obvious that the uninfected children in the placebo group had high levels of clinical and laboratory abnormalities. For example:

	n (%)	
	Zidovudine	**Placebo**
Severe anaemia†	1 (0.5)	3 (2)
Severe neutropenia†	45 (21)	55 (27)
Other severe haematologic toxicity†	2 (1)	2 (1)
Severe chemistry toxicity†	27 (13)	32 (16)

" †Includes protocol-defined severe or life-threatening toxicities"

This means that either both AZT and the placebo used were toxic, or there was an unknown underlying abnormality in either the mothers or the children in the PACTG 076 study. Anyhow, from this study one cannot conclude that AZT is non toxic.

It is significant that HIV infection is said to be the reason why a neonate or child born to an HIV positive mother develops *Pneumocystis carinni* pneumonia (PCP). However in 1997 a report appeared where two HIV positive mothers were given AZT during pregancy and each gave birth to a full term, baby girl ultimately diagnosed non-HIV-infected. Each baby girl received AZT for six weeks and within ten days of ceasing this medication developed PCP (from which they recovered). "Both children had a transient decrease in their CD4 cell counts that was concomitant with the acute PCP episode. However, when CD4 cells were expressed as percentage of total lymphocytes, the percentage never fell below 25; by this criterion, there was no immunosuppression…predisposing conditions known to be related to PCP (e.g. malnutrition and primary immunodeficiency) were excluded in our two cases. Indeed, continued observation of the first infant for 20 months and the second for 16 months revealed that they had normal immunological parameters and normal growth. Thus, there is no signficant or protracted immunodeficiency in these infants". The authors concluded that, contrary to the requirements of the "1994 revised classification for HIV infection in children <18 months of age…the occurrence of PCP in an infant who is perinatally exposed to HIV is not sufficient to diagnose HIV infection".[41]

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APPENDIX 1

Measurement of Triphosphorylation of AZT as a Function of Year Measured

Year	Peak Concentration of Triphosphorylate AZT Reported	Reference
1991	0.5 pmol/10^6 cells	Kuster H, et al. J Infect Dis; 164: 773 – 776
1991	56 pmol/10^7 cells (5.6 pmol/10^6 cells)	Toyoshima t, et al. Analytical Bioch; 196: 302 – 307
1992	0.14 pmol/10^6 cells	Slusher JT, et al. Antimic Agents & Chemoth: 2473 – 2477
1994	326 fmol/10^6 cells (0.326 pmol/10^6 cells)	Robbins BL, et al. Antimicrob Agents Chemother: 115 –121
1994	0.06 pmol/10^6 cells	Barry MG, et al. AIDS; 8: F1 – F5
1996	95 fmol/10^6 cells (0.095 pmol/10^6 cells)	Rodman JH, et al. J Infec Dis; 174: 490-499
1996	0.069 pmol/10^6 cells	Peter K, et al. J Pharm & Biomed Anal; (14): 491 – 499
1996	0.042 pmol/10^6 cells (average)	Peter K and Gambertoglio JC. Clin Pharmacol Ther; 60: 168 – 176
1996	0.07 pmol/10^6 cells	Barry MG, et al. AIDS: 1361 – 1367
1998	0.046 pmol/10^6 cells, in mononuclear cells from lymph nodes. 0.085 pmol/10^6 cells, in PBMC	Peter K et al. AIDS: 1729 –1731
1998	160 fmol/10^6 cells (average) (0.16 pmol/10^6 cells)	Fletcher CV, et al. Clin Pharmacol Ther 64: 331 – 338
1998	0.07 pmol/10^6 cells	Robbins BL, et al. Antimicrob Agents Chemother: 2656 – 2660
1999	193 fmol/10^6 cells (0.193 pmol/10^6 cells)	Font E, et al. Antimicrob Agents Chemother: 2964-8

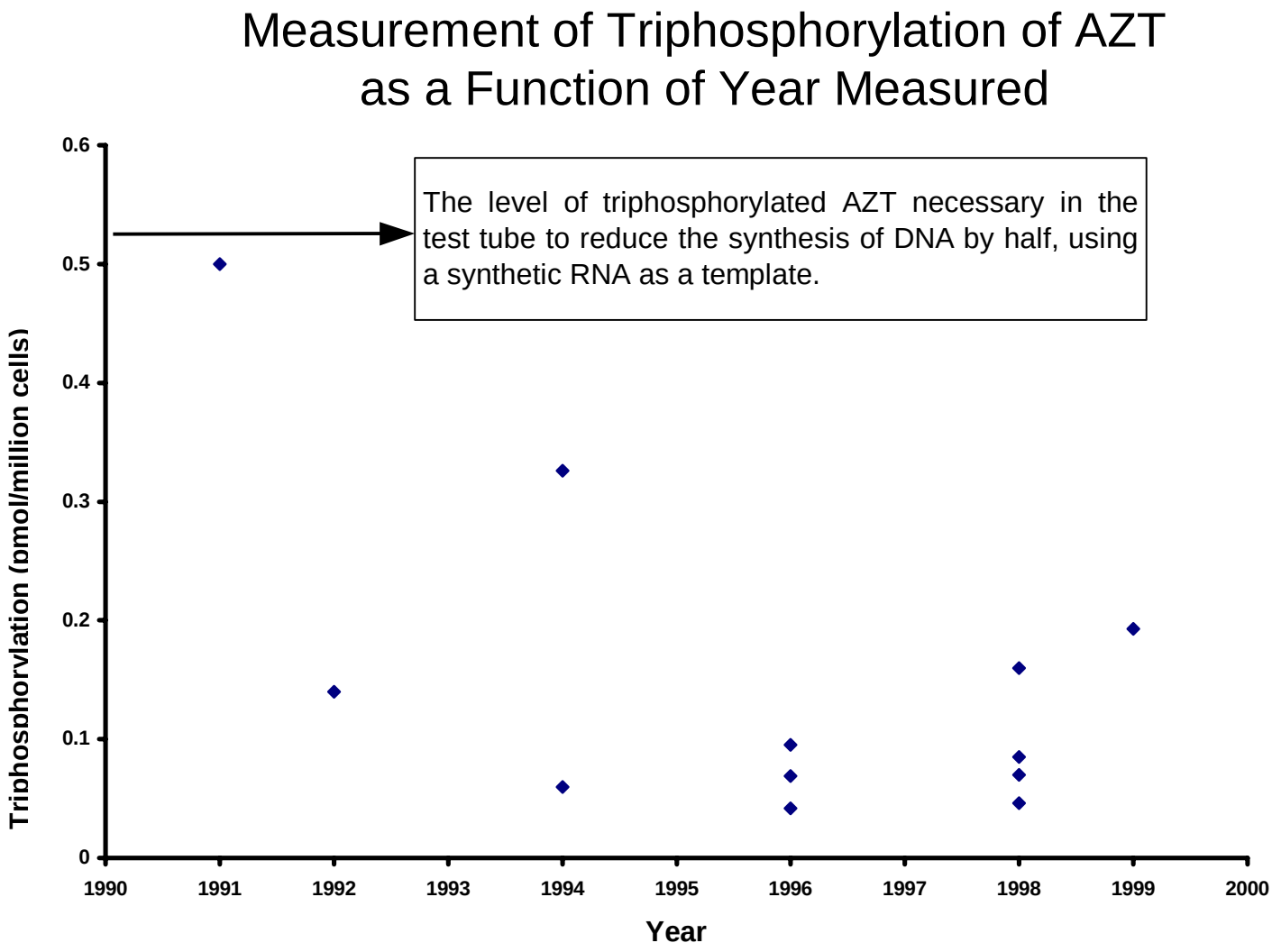
1μmol = 10^{-6} moles

1 pmol = 10^{-12} moles

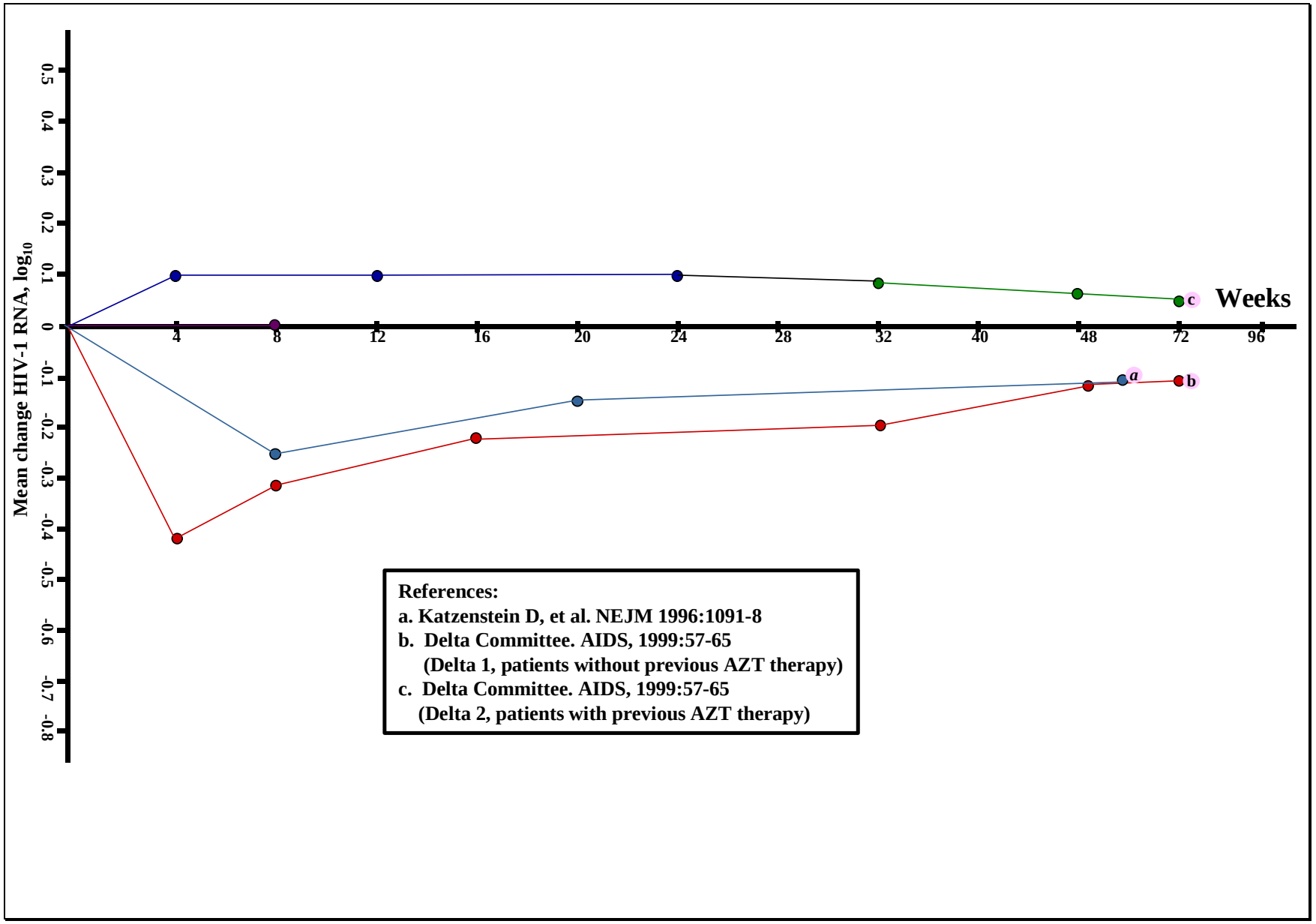
1 fmol = 10^{-15} moles

1 pmol/10^6 cells=1 μM

APPENDIX 1


Measurement of Triphosphorylation of AZT as a Function of Year Measured
The level of triphosphorylated AZT necessary in the test tube to reduce the synthesis of DNA by half, using a synthetic RNA as a template.
Triphosphorylation (pmol/million cells)
0.6
0.5
0.4
0.3
0.2
0.1
0
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
Year

The Effect of AZT on HIV RNA



APPENDIX 2

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