REPLY TO DR CHERRY

Dr Cherry: They "selectively review the literature".

It is true we did not mention all the thousands of articles, which have been written, on AZT. Our aim was not to review the AZT literature but assuming that a retrovirus, HIV exists, to answer the following questions:

1. Is it possible for AZT, in the form administered to humans, to have an anti-HIV effect?
2. From human studies is there proof that AZT has an inhibitory effect on the parameters which are assumed to prove HIV infection?

The answer is, that in no study published to date is there proof for an affirmative answer to either question.

Dr Cherry: "AZT can inhibit HIV replication by acting as a chain terminator only in the triphosphate form".

The AZT given to patients is not triphosphorylated, it is given as a non-active drug, a pro-drug. Thus it goes without saying that before one starts to use AZT to treat patients one must have proof that:

a.) in humans the non-active form of AZT is metabolised to an active form, that is, it is triphosphorylated.
b.) the level of triphosphorylation achieved is sufficient to inhibit HIV replication, that is, to significantly decrease the HIV DNA and thus HIV RNA in humans.
c.) the therapeutic effects are proven and outweigh the toxic effects (all drugs have toxicities to greater or lesser degrees and these must be substantially less than the benefits).

Yet AZT was introduced into clinical practice in 1986 without proof that humans are capable of triphosphorylating the parent compound which is given to patients. In fact the first studies which examined this aspect of AZT pharmacology in humans only appeared in 1991.

Dr Cherry: "The authors appear to have ignored a large number of studies in the scientific literature which provide evidence that AZT is adequately triphosphorylated in human cells".

Even if we assume that there is proof (no such proof exists) that "human cells" in the test tube can triphosphorylate AZT "adequately", test tubes and "human cells" in the test tube are not the same as humans and the cells from which they are constituted. If by "adequately triphosphorylated" Dr Cherry means levels of triphosphorylated AZT in humans sufficient to induce a significant decrease in HIV, that is, in HIV DNA and HIV RNA, we could find no such proof neither when we wrote the paper nor since. According to researchers from Glaxo-Wellcome, the manufacturers of AZT, in the test tube, under the most ideal conditions, the minimum concentration of triphosphorylated AZT required to decrease by half the synthesis of DNA using an RNA template is 0.7 μM.[1] In vivo the conditions are more complex which means that higher concentrations would be necessary. For example, unlike in the *in vitro* conditions used by these researchers, in humans triphosphorylated AZT has to compete with the natural nucleotides for binding to HIV DNA. Furthermore, although these researchers were trying to prove that AZT inhibits HIV DNA synthesis:

a.) instead of using AZT as given to patients they used an already triphosphorylated AZT;

b.) instead of using HIV RNA as a template they used an artificial (synthetic) RNA which does not occur in Nature and bears no relationship whatsoever to HIV RNA.

However, using the best available data to date, the concentrations of triphosphorylated AZT are about ten times lower than the concentration necessary in the test tube to decrease by half the synthesis of DNA using a synthetic RNA as a template (see table and figure).

If, despite our extensive search of the literature, we have missed the "large number of studies in the scientific literature which provides evidence that AZT is adequately triphosphorylated in humans", we would be grateful to Dr Cherry for providing us with the relevant references.

Dr Cherry: "This [adequate triphosphorylation of AZT] allows it to work well as a blocker of HIV replication in vitro, and in vivo when tested on mammalian cells in sensible concentration. It's routinely used in academic research laboratories in experiments where inhibiting HIV replication is part of the experimental protocol".

Again, even if one assumes that in "vitro" and "in academic research laboratories", one can obtain a "sensible concentration" of triphosphorylated AZT, which will block HIV replication, the data cannot be extrapolated to humans. As researchers from the Department of Clinical Pharmacology University of California point out, in vitro "experiments are often performed under extreme conditions and do not necessarily reflect the in vivo situation".[2] The patients and their carers are not interested in what happens in the test tube and in academic research institutions. All they want to know is whether there is a “sensible" AZT concentration which will "inhibit HIV replication" without killing them or make them worse.

Dr Cherry: "The original research reports cited by Papadopulos-Eleopulos *et al.* do not, to our knowledge, come to the conclusion which Papadopulos-Eleopulos *et al.* do, viz. that AZT is inadequately triphosphorylated in human cells to be effective."

This is true. However, it is also true that the authors of these papers did not conclude that AZT is adequately triphosphorylated. They could not reach such a conclusion from their data because their experiments were designed to determine the degree to which AZT is triphosphorylated in humans, not to determine the effective concentration required to block HIV replication in humans. However, the effect of AZT on HIV replication, that is, on HIV DNA and RNA, has been studied by many other researchers. The analysis of these studies form the majority of our paper. Yet for some unknown reason Dr Cherry failed to comment on this part of our analysis and its conclusion, that is, AZT as given to patients, whatever its level of triphosphorylation, has no effect on HIV replication.

As was stated in our paper, 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".[3] According to American HIV experts: "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".[4] Thus, according to the most eminent experts on anti-HIV drugs, any drug which does not produce a sustained decrease of HIV RNA (the "viral load") by at least 0.5 log

cannot be considered to be an anti-HIV drug. Neither when we wrote the paper nor since, have there been any publications proving that AZT causes even a peak decrease in the HIV RNA of more than 0.5 log, much less of a sustained decrease of more than 1 log or 0.5 log. In other words, consistent with its putative action and its insignificant level of triphosphorylation, the presently available data shows that AZT has no effect on HIV replication.

Dr Cherry: "These reports [on AZT triphosphorylation] appear, however, mostly to date from the period 1991 to 1994, when assays for determining phosphorylation were not nearly as sophisticated as they are now. This, combined with the fact that different assays were used by different workers in these experiments, may explain why these results indicate varying and low degrees of triphosphorylation."

As researchers from the Max Plank Institute in Germany point out, the low level of triphosphorylated AZT achieved in humans is not a matter of measurement but of biological facts.[5] An assay can be improved either in regard to its specificity (the earlier methods may not be as specific as they are now) or its sensitivity, which means that with the assays used more recently it is possible to measure a much lower level of triphosphorylated AZT. If with the assays used in "the period 1991 to 1994" one could detect "low degrees of triphosphorylation" than with the more specific and sensitive assays used now, the levels will be either the same or lower.

Dr Cherry: "The article is not comprehensive and not up-to-date, as it omits to refer to many important recent studies which are relevant to the field under review. Both recent and more sophisticated studies showing higher degrees of triphosphorylation, as well as other studies reporting on the efficacy of drugs-based trials on mother-to-child transmission, appear to have been ignored by the authors."

As can be seen from the table and figure, where the peak levels of triphosphorylated AZT are shown, the most "recent and more sophisticated studies" do not show "higher degrees of triphosphorylation". In fact the highest levels of triphosphorylated AZT were reported in the initial studies, which were later shown not to have been properly validated. (The Toyoshima *et al* 1991 study of 5.6pmol/10^6 cells was omitted from the graph because it is too high relative to all other studies and out of scale). It is also important to note that the triphosphorylated AZT rapidly decays (in hours) to non-active forms. Because of this, to sustain the peak level of triphosphorylated AZT, the drug would have to be administered a least a few times every 24 hours.

The aim of our analysis was to evaluate the effect on HIV replication, not the clinical effect of AZT. However given that:

a.) Dr Cherry admits that "AZT can inhibit replication", "only in its triphosphorylated form" and that even the most recent results indicate "low degrees of triphosphorylation";
b.) For AZT to be accepted as an anti-HIV drug it must induce a sustained decrease in the HIV DNA of 0.5 log but to date there is not one single study proving such an effect;
c.) "HIV RNA" is a strong predictor" for transmission[6, 7] but AZT produces "only a minimal (ie, 0.24 $\log_{10}$) reduction in maternal and antenatal plasma HIV/RNA copy number".[8] According to David Ho and his associates there is "a significant rise in viral

load and a decline in the $CD4^+$ lymphocytes in the six months after delivery...These changes can not be attributed to the cessation of zidovudine [AZT] since most women continued this therapy post partum";[9]

one has no choice but to conclude that AZT has no effect on HIV transmission from mother to child.

As far as the clinical results are concerned, it suffices to mention that according to the 1998 Public Health Task Force Recommendations for the Use of Antiretroviral Drugs in Pregnant Women Infected with HIV-1 for Maternal Health and for Reducing Perinatal HIV-1 Transmission in the United States, "when considering treatment of pregnant women with HIV infection, antiretroviral monotherapy [AZT] is now considered suboptimal for treatment; combination drug therapy is the current standard of care".[8]

Dr Cherry: "Like many medical interventions AZT is widely acknowledged to have toxic effects, which should be weighed up against its potential benefits."

We agree with Dr Cherry that all drugs have toxic effects. However toxic effects should be weighed not against "potential benefits" but against proven benefits. It makes no sense, indeed it is counter to the Hippocratic oath, to administer a drug with no therapeutic effects. This is the crux of the problem with AZT. While there is no proof that it is anti-viral there is ample evidence that it is toxic to adults as well as to the children whose mothers have been taking it during pregnancy. Two examples will suffice to illustrate this point.

In a study published last year by Italian researchers, the authors followed up, for the first 3 years of life, HIV seropositive children born to mothers who either did or did not receive AZT treatment. The two groups of children were similar with regard to all variables taken into account (year of birth, maternal clinical condition, birth weight and treatments) apart from age at the beginning of PCP chemoprophylaxis, which was undertaken earlier in these children who were born to mothers give AZT. They found that the children born to the mothers given AZT "had a higher probability of developing severe disease" (57.3% versus 37.2%) or severe immune suppression (53.9% versus 37.5%) and a lower survival (72.2% versus 81%).[10]

PROBABILITIES WITHIN THREE YEARS OF:

MOTHERS:	SEVERE DISEASE	SEVERE IMMUNE DEPRESSION	DEATH
TOOK AZT	57%	54%	28%
DID NOT TAKE AZT	37%	38%	19%

In the French National Epidemiological Network for studying mother-to-child transmission between 1986-1998, 1754 mother-child pairs were exposed to AZT. In this study HIV negative children were followed up for 18 months. The diagnosis of two children with serious diseases (visual impairment, refractory epilepsy and deterioration of cognitive and psychomotor abilities), suggestive of mitochondrial dysfunction, led the authors to "investigate mitochondrial toxic effects in children exposed to zidovudine" (AZT). Eight children, all HIV negative born to HIV positive mothers (5 of which were of African origin), "had mitochondrial dysfunction. Five, of whom two died, presented with delayed neurological symptoms and three were symptom-free but had severe biological or neurological

abnormalities". Discussing their findings they wrote: "A continuing study of the incidence of neurological mitochondrial diseases in the UK that correspond to those seen in patients one, two, four and five, has identified only 21 cases in 20 months in about 12 million children younger than 16 years...Even if this UK study had underestimated the number, the observation of several cases in a population of about 1700 exposed children in our network strongly suggests an acquired mitochondrial dysfunction in these non-HIV-1-infected children born to infected mothers".[11]

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Measurement of Triphosphorylation of AZT as a Function of Year Measured

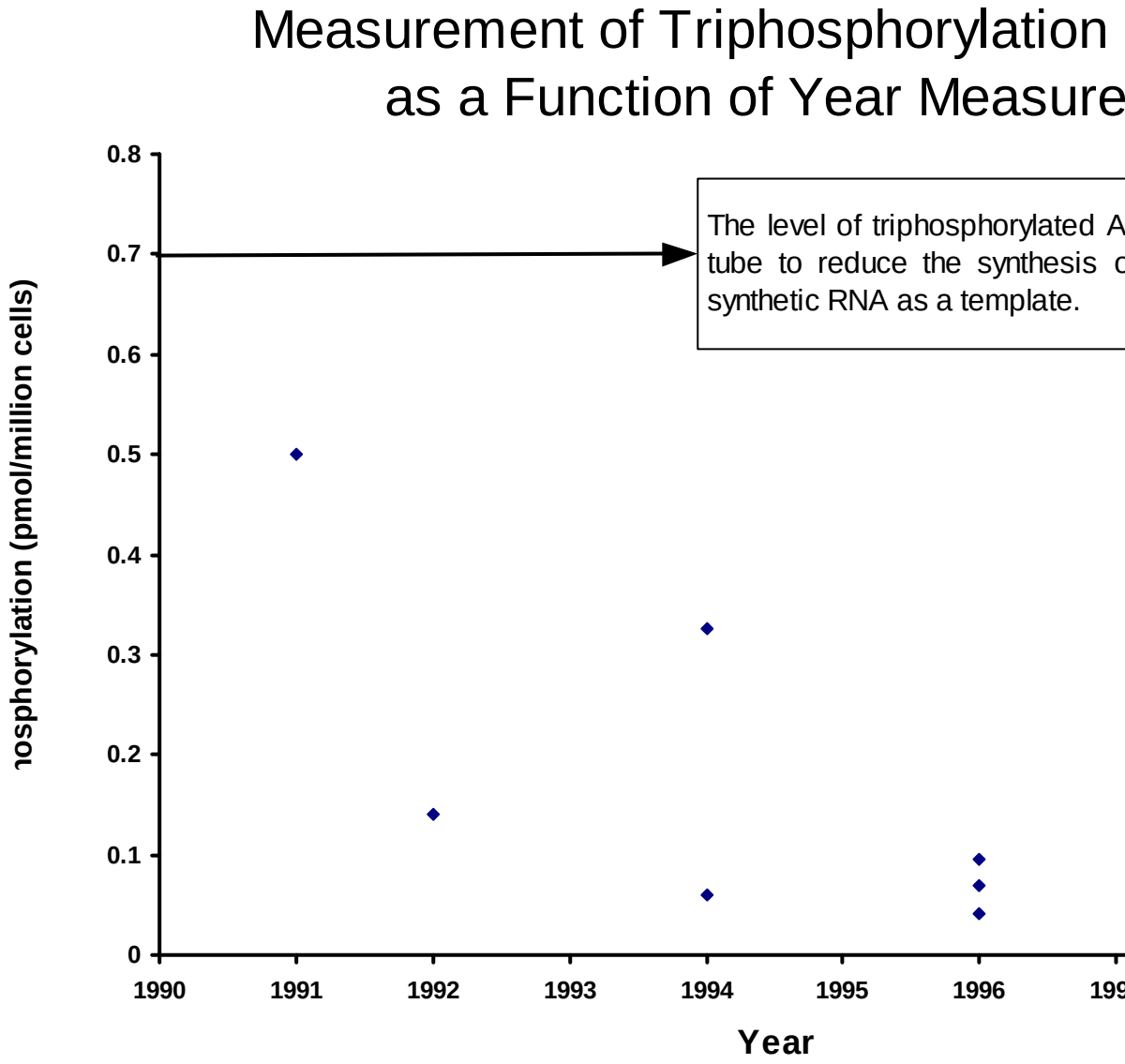
Year	Peak Concentration of Triphosphorylate AZT Reported	Reference
1991	0.5 pmol/10^6 cells	Kuster H, et al. J Infect Dis; 164: 773 – 776
1991	56 pmol/10^7 cells (5.6 pmol/10^6 cells)	Toyoshima t, et al. Analytical Bioch; 196: 302 – 307
1992	0.14 pmol/10^6 cells	Slusher JT, et al. Antimic Agents & Chemoth: 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

$1\mu mol = 10^{-6}$ moles

1 pmol = 10^{-12} moles

1 fmol = 10^{-15} moles

1 pmol/10^6 cells=1 μM


Measurement of Triphosphorylation
as a Function of Year Measure
The level of triphosphorylated A
tube to reduce the synthesis c
synthetic RNA as a template.
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1990
1991
1992
1993
1994
1995
1996
Year

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ADDENDUM

Font E, Rosario O, Santana J, Garcia H, Sommadossi JP, Rodriguez JF. Determination of zidovudine triphosphate intracellular concentrations in peripheral blood mononuclear cells from human immunodeficiency virus-infected individuals by tandem mass spectrometry. Antimicrobial Agents and Chemotherapy 1999;**43**:2964-8

ABSTRACT

Nucleoside reverse transcriptase inhibitors (NRTIs) used against the human immunodeficiency
virus (HIV) need to be activated intracellularly to their triphosphate moiety to inhibit HIV replication. Intracellular concentrations of these NRTI triphosphates, especially zidovudine triphosphate (ZDV-TP), are relatively low (low numbers of femtomoles per 10(6) cells) in HIV-infected patient peripheral blood mononuclear cells. Recently, several methods have used
either high-performance liquid chromatography (HPLC) or solid-phase extraction (SPE) coupled with radioimmunoassay to obtain in vivo measurements of ZDV-TP. The limit of detection (LOD) by these methods ranged from 20 to 200 fmol/10(6) cells. In this report, we describe the development of a method to determine intracellular ZDV-TP concentrations in

HIV-infected patients using SPE and HPLC with tandem mass spectrometry for analysis. The LOD by this method is 4.0 fmol/10(6) cells with a linear concentration range of at least 4 orders of magnitude from 4. 0 to 10,000 fmol/10(6) cells. In hispanic HIV-infected patients, ZDV-TP was detectable even when the sampling time after drug administration was 15 h. Intracellular ZDV-TP concentrations in these patients ranged from 41 to 193 fmol/10(6) cells. The low LOD obtained with this method will provide the opportunity for further in vivo pharmacokinetic studies of intracellular ZDV-TP in different HIV-infected populations. Furthermore, this methodology could be used to perform simultaneous detection of two or more NRTIs, such as ZDV-TP and lamivudine triphosphate.