2-9

IN THE HIGH COURT OF SOUTH AFRICA
(NATAL PROVINCIAL DIVISION)

Case No: 1894/2001

In the matter between:

ANNET HAYMAN	First Plaintiff
ANNET HAYMAN obo DAVID HAYMAN	Second Plaintiff

and

GLAXO WELLCOME SOUTH AFRICA (PTY) LTD t/a GLAXOSMITHKLINE	First Defendant
THABO MBEKI	Second Defendant
DR MANTO TSHABALALA-MSIMANG	Third Defendant
PROFESSOR PETER EAGLES	Fourth Defendant

FIRST DEFENDANT'S NOTICE – RULE 36(9)
PROFESSOR DAVID BACK

BE PLEASED TO TAKE NOTICE that at the trial of the above matter the first defendant intends to call Professor David Back to testify as an expert witness.

FURTHER TAKE NOTICE that a summary of his evidence, containing his opinions and his reasons for them, is attached hereto.

DATED AT JOHANNESBURG ON THIS THE 3 DAY OF JULY 2002

BELL DEWAR & HALL
First Defendant's Attorneys
c/o **Tatham Wilkes & Co**
200 Berg Street
Pietermaritzburg
P O Box 161
Tel: 033 345-3501
Fax: 033 394-9199
Ref: Mr N R Tatham

To: **The Registrar**
High Court
Natal Provincial Division
Pietermaritzburg

And to: **Lister & Lister**
Plaintiffs' Attorneys
11th Floor United Building
194 Longmarket Street
Pietermaritzburg
Ref: Mr R Stretch
Tel: 033 345-4530

Received a copy hereof on

Plaintiffs' Attorneys

REPORT OF PROFESSOR DAVID BACK

1. General

1.1 I have been asked by Simmons & Simmons (the UK solicitors of GlaxoSmithKline) to comment on paragraphs 15.1 - 16.4 of the Particulars of Claim.

1.2 I have also been asked by Simmons & Simmons to prepare an introductory primer, outlining the technical background to the areas covered by this report. This document is presently being completed and will then be attached to this report as Annex 1.

1.3 In this report I refer to a number of scientific papers; these are listed as Annex 2.

1.4 When I was first requested to give evidence in this case, it was explained to me that my overriding duty is to provide expert and impartial assistance to the Court. I fully subscribe to that approach and have sought to comply with it at all times in producing this report.

2. Qualification and Experience

2.1 I am Professor of Pharmacology at Liverpool University, having been appointed to a personal chair in 1994 on the basis of external testimony. I hold the degrees of BSc and PhD.

2.2 I am the author or co-author of more than 300 peer-reviewed papers in the field of pharmacokinetics, drug disposition and drug interactions. I am the author or co-author of more than 100 peer-reviewed papers on the metabolism and pharmacokinetics of anti-HIV drugs. I am currently head of the Liverpool HIV Pharmacology Group which comprises a team of approximately 20 basic and clinical researchers working in the

following areas: intracellular phosphorylation of nucleoside analogues; therapeutic drug monitoring of protease inhibitors; pharmacological mechanisms of failure of therapy and pharmacogenomics.

2.3 I am currently a member of the UK Medical Research Council AIDS Therapeutic Trials Committee and several other trials committees. I was Editor in Chief of the British Journal of Clinical Pharmacology from 1995 – 2000. I am a recent co-founder of the International Society for HIV Pharmacology and a member of the International AIDS Society. I attach a copy of my *curriculum vitae* as Annex 3.

2.4 I have received research grants from the following companies: GlaxoSmithKline, Bristol Myers Squibb, Agouron Pharmaceuticals, Abbott Pharmaceuticals, Triangle Pharmaceuticals, Shire Biochemicals, Boehringer Ingelheim, Merck Sharp & Dohme, Roche Pharmaceuticals. I have acted as consultant and guest lecturer for the above.

3. Background to AZT and reverse transcription

3.1 Zidovudine (AZT) is a prodrug that has to be converted inside cells to the active triphosphate anabolite, AZT-5'- triphosphate (AZTTP), before it can have a pharmacological effect. The initial conversion step is by the cellular enzyme, thymidine kinase, to zidovudine-5'-monophosphate (AZTMP) which is then in turn phosphorylated by thymidylate kinase to the 5'-diphosphate (AZTDP) and then by pyrimidine nucleoside diphosphate kinase to the 5'-triphosphate (AZTTP).

3.2 In the triphosphorylated form AZTTP is an analogue of thymidine, one of the four building blocks of DNA. As explained in the Introductory Primer to this report (Annex 1), the replication of HIV requires the conversion of viral RNA into DNA, which then becomes incorporated in the host's own DNA (as proviral DNA). This process is called reverse transcription and is catalysed by the viral enzyme reverse transcriptase. As it is an analogue of thymidine, AZTTP becomes incorporated into a growing DNA

strand by the action of reverse transcriptase. However, because it lacks an essential hydroxyl (OH) group at the 5' position that is necessary for forming a bond with the next deoxynucleoside triphosphate (dNTP – the generic name for the four building blocks of DNA) and hence elongation of the growing DNA chain, the incorporation of AZTTP in place of thymidine results in chain termination. In this way AZTTP blocks the conversion of viral RNA into DNA and so inhibits the replication of HIV.

3.3 The rate-limiting step in the above process is the conversion of AZTMP to AZTDP, hence AZTMP accumulates so that the concentration within the cell of the monophosphate is greater than either the di- or triphosphate.

3.4 Since it is the triphosphate anabolite that interrupts the formation of viral DNA from the host's endogenous dNTP pool it is correct to focus on pivotal *in vitro* and *in vivo* studies which have examined the intracellular pharmacology (concentrations and mechanism of action) of the triphosphate.

4. *In vitro* studies of the IC_{50} for HIV reverse transcriptase

4.1 It is the principal contention of the section of the Particulars of Claim dealing with AZT triphosphorylation (paragraphs 15 and 16) that much higher concentrations of AZTTP are necessary to inhibit reverse transcriptase than have to date been determined in cells *in vivo.* This contention is based on the extrapolation of findings drawn from a single *in vitro* study by Furman and colleagues (1986). However, the Plaintiff's contentions are fundamentally flawed for the reasons set out below.

4.2 In studies with purified enzymes or cells in culture, it is common practice to calculate IC_{50} values. The IC_{50} is the concentration of an inhibitor that either produces 50% inhibition of enzyme activity or, taking another example with whole cells, produces 50% decline in virus production. So the IC_{50} is an indicator of how effective a compound (a

drug) is at inhibiting a particular cellular process i.e. the lower the IC50 is the greater the inhibition. It is important to note that IC_{50} values are typically used not as an absolute number that can be transposed to other experimental set-ups, but for purposes of comparison within a self-contained study, i.e. as a screen of the inhibition of RT by different NRTIs. Provided that such an experiment is designed in such a way that the only variable between different arms of the study is the drug used (all other conditions are held constant), then in such circumstances a comparison of the IC_{50} of the different drugs would be valid. As a result of such an experiment, a rank order of the inhibitory ability of different drugs could be compiled. However, for the reasons set out in the following paragraph, this is not necessarily predictive of how the drugs would compare in patients.

4.3 Data generated *in vitro* must always be extrapolated with caution to the *in vivo* situation (which I describe in detail below in relation to AZTTP). This general point can be illustrated through the following example: Ritonavir (a protease inhibitor approved for treating HIV) is a potent inhibitor of *in vitro* metabolism but in patients *in vivo* can either induce or inhibit enzymes. Hence, when ritonavir was found to inhibit methadone metabolism *in vitro* (Iribane et al 1998), the data were extrapolated to the *in vivo* situation and it was concluded that patients would require a reduction in methadone dosage. However, when a study was performed in subjects receiving methadone (Hsu et al 1998), it was found that ritonavir increased the rate of metabolism so that patients would actually require an increase in dose. This example illustrates that an *in vitro* result is not necessarily indicative of the more complex *in vivo* situation. *In vitro* data can seldom be regarded as the definitive finding when looking at a complex cellular process. *In vitro* experiments help design other *in vitro* experiments and give important direction to the establishing of *in vivo* studies.

4.4 It is totally inappropriate to consider a calculation of an IC_{50} determined under *in vitro* conditions to be the defining concentration of drug (in this case AZTTP) that is required *in vivo*, or even to use this as a guideline. This is acknowledged in several of the papers referred to in the Plaintiffs' Particulars of Claim. For example:

(A) Rodman et al (1996) state at p491, column 1, para 1. "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";

(B) Kuster et al (1991) state at p773, column 1, para 2 "A detailed knowledge about *in vivo* phosphorylation is important for several reasons. First, there is documented variability of AZT phosphorylation in various cell systems, and data from *in vitro* experiments cannot necessarily be extrapolated to the *in vivo* situation; and

(C) Robbins et al (1994) state at p115, column 1, para 2 "Because *in vitro* results from human cell lines in culture cannot necessarily be extrapolated to the *in vivo* situation ....."

4.5 Any IC_{50} determination is absolutely dependent on the conditions of the experiment in which it was calculated, such that the 'number' generated can alter by an order of magnitude (10-fold, 100-fold) if the constituent components of the assay are altered. For example, if the concentration of the enzyme or of any of the substrates used in the experiment is altered then the IC_{50} will be altered. The Plaintiffs correctly point out that an IC_{50} value of 0.7 μM for the viral reverse transcriptase was calculated under the conditions used by Furman et al (1986). The essential components of the reaction were: purified HIV reverse transcriptase (the enzyme) radiolabelled [^{3}H] dTTP (substrate), template primer (the growing DNA chain), and AZTTP (inhibitor). It is important to note that the dTTP concentration was 5.6 μM – i.e. in

excess compared to AZTTP. If the dTTP had been lower, then the IC_{50} for AZTTP would have also been reduced. An IC_{50} value is dependent on the substrate concentration as well as the potency of the inhibitor.

4.6 The concentration of AZTTP and dTTP *in vivo* is difficult to measure and in addition may vary between individuals. IC_{50} values have been measured at a range of drug and substrate concentrations with varying calculations of IC_{50} – see paragraph 4.8 and 4.11 below.

4.7 Paragraph 16.2 of the Particulars of Claim describes Furman's *in vitro* conditions as being 'ideal'. The only ideal conditions will be those that reflect as closely as possible the *in vivo* situation. This would require all dNTPs to be present at physiological concentrations and a template primer that reflected the make up of the viral DNA template. The primary purpose of the Furman study was to look at the selectivity of the inhibitory effect of AZT for the viral enzyme reverse transcriptase rather than the human enzyme DNA polymerase α. It was not designed to model the *in vivo* situation and I note that no attempt was made in the Furman paper to assign any *in vivo* significance to the measured IC_{50} value. The experimental conditions cited by Furman can be considered appropriate for generating enzyme kinetic data. However it is worth noting that *in vivo* dTTP concentrations are in the range of 0.5 – 2.5 μM (Hoggard et al 2002;) and therefore the dTTP concentrations used by Furman are up to 10-fold higher than those seen *in vivo*. Also, the CHARM study highlighted the variability of dTTP levels both between and within patients.

4.8 It is important to note that other researchers (White et al 1989;) have given an IC_{50} of 0.01 μM to HIV reverse transcriptase under only slightly different conditions from those of Furman et al (dTTP = 6 μM). The IC_{50} value of White et al is 70 times less than that of Furman et al, demonstrating that even where the conditions used are similar, the IC_{50} calculated will depend on exactly how the experiment was performed.

This 70-fold difference highlights the inappropriateness of considering one *in vitro* value of IC_{50} and extrapolating this to the *in vivo* environment. The table below shows how the IC_{50} depends on the different experimental conditions used. Of particular importance are the data of Heidenreich et al (1990;). In this paper the authors demonstrate the inhibition of HIV RT by AZTTP as a function of dTTP concentration. Using dTTP concentrations between 1.7 and 22 μM the IC_{50} values ranged between 0.08 and 0.32 μM.

Study	**Furman** **PNAS 1986**	**White** **BBRC 1989**	**St Clair** **AAC 1987**	**Heidenreich** **EJB 1990**
Substrate concentration (dTTP)	Variable but 5.6μM used for IC_{50}	6μM	Variable	Variable but 4.4μM used for IC_{50}
Enzyme	HIV RT	HIV RT	HIV RT	HIV RT
Template primer	Poly(rA)-oligo(dT)	Poly(rA)-oligo(dT)	Native template (endogenous HIV RT)	Poly(rA)-oligo(dT)
IC_{50}	0.7μM	0.01μM	-	0.097 μM
Ki	0.04 μM	-	0.01 μM	0.04 μM

4.9 So IC_{50} values are not fixed or absolute and will differ from laboratory to laboratory because they are totally dependent on the experimental protocol and conditions (such as enzyme and substrate concentration or the cell line used and the stage of the cell cycle). Considering an experiment with isolated reverse transcriptase, the competition involves substrate and inhibitor. Therefore the extent of competition will be related to the concentration of substrate (i.e. it is the ratio of the concentration of substrate (dTTP) to the concentration of inhibitor (AZTTP) that is important). In addition, the presence of more enzyme will mean that there are more active sites for binding substrate and this,

too, will alter the rate and extent of the reaction. Furthermore, different enzymes isolated from different viruses are likely to have different affinities for substrate and inhibitor. All of these factors are capable of markedly altering the IC_{50} measured in a particular experiment.

4.10 On the other hand, determination of another value that can be calculated through *in vitro* analysis, a Ki value (inhibition constant of a drug for enzyme), is less prone to variability between experiments because one uses a range of concentrations of substrate (dTTP) and inhibitor (AZTTP) in the assay. In the study referred to above, Furman et al (1986) calculated the K_i to be 0.04 μM. A similar value was shown by Heidenreich et al (1990). Using a different template, St Clair et al (1987) reported the K_i for AZTTP to be 0.01 μM. Note that the K_i is a measure of the association between the enzyme and the inhibitor. It is used to determine the potency of enzyme inhibition and can only be used when you are dealing with an enzyme. It cannot be used for whole cell studies (unlike the IC_{50}). So K_I, not IC_{50} , is the best parameter to generate in enzyme studies. IC_{50} values are the best marker for inhibition of cell growth and/or toxicity.

4.11 That AZT has antiviral activity *in vitro* is undeniable. My research group has recently demonstrated that in persistently infected U-937 cells (a human T-cell derived immortal cell line) the IC_{50} for AZT (that is the concentration of AZT required to give a 50% drop in virus production) was 0.05 μM (Hoggard et al 2000). In a parallel study, intracellular AZTTP and dTTP concentrations and the ratio of AZTTP:dTTP were determined. At a concentration of 0.02 μM AZT, the intracellular level of AZTTP was 0.04 pmol/10^6 cells, which is comparable to values seen *in vivo* (see below). This experiment demonstrates that: a) AZT has antiviral activity; and b) that AZTTP is produced intracellularly.

5. ***In vivo* phosphorylation of AZT**

5.1 As indicated above, AZT is only effective in inhibiting reverse transcriptase (and hence viral replication) in its triphosphorylated form. The Plaintiffs allege (in paragraph 16.2 of the Particulars of Claim) that "AZT is triphosphorylated insignificantly *in vivo*" and that the best designed and executed studies indicate that AZT is "triphosphorylated *in vivo* to levels one or more orders of magnitude below the drug's IC_{50} value, as determined by Furman et al in ideal *in vitro* conditions". I have already dealt with the IC_{50} data of Furman *et al* above and demonstrated that: 1) IC_{50} values depend on the experimental conditions in which they were determined and accordingly vary greatly from laboratory to laboratory; 2) there is no basis for the statement that Furman's conditions were 'ideal'; and 3) it is wholly inappropriate to extrapolate from an *in vitro* measurement of IC_{50} to the *in vivo* situation. I will now address the *in vivo* data on AZT phosphorylation.

5.2 In this context, the use of the word 'insignificantly' by the Plaintiffs is not appropriate since numerous studies by different investigators (please see Annex 2) have clearly demonstrated that intracellular concentrations of AZTTP greater than the *in vitro* Ki value can be determined in peripheral blood mononuclear cells (PBMCs) from HIV positive patients receiving zidovudine-containing therapy. Furthermore it is important to remember that the 'significance' of the antiviral effect of the drug (which occurs only via triphosphorylation) has been established in the numerous clinical trials that have shown that AZT alone or as part of a combination of other drugs is extremely beneficial to HIV patients

5.3 My research group has been at the forefront of developing methodologies for intracellular anabolite determinations *in vivo* (including Barry *et al*, 1994; Barry *et al*, 1996; Phiboonbanakit *et al*, 1998; Wattanagoon *et al*, 2000; Moore *et al*, 2000; Hoggard *et al*,

2001; Kewn *et al*, 2002). It is important to observe that the different analytical methodologies used both by my group and others (see Annex 2) give comparable measurements of intracellular AZTTP. Examples of the different methodologies used are 1) high performance liquid chromatography – radioimmunoassay; 2) solid phase extraction – tandem mass spectrometry; 3) cartridge – radioimmunoassay; 4) enzymatic – primer extension. As in all areas of clinical science, methods have been refined and levels of assay sensitivity improved as technology and know-how has advanced. However, *in vivo* studies annexed to the Particulars of Claim are some of the pivotal clinical studies in this area. More recently there have been advances in methodologies such that we now have assays with greater sensitivity with a consequent lowering of the limit of quantitation.

5.4 In clinical studies, the majority of values of AZTTP concentration as calculated by the various methods listed above, lie between 0.04 and 0.15 pmoles/10^6 cells (values will depend amongst other things on the timing of the sample). Based on the volume of a single PBMC these values can be expressed as 'micromolar' concentrations. The average PBMC volume is 0.4 picolitres (data based on FACS analysis or Coulter counter analysis). Therefore the intracellular AZTTP concentration is in the range 0.1 – 0.36 μM, i.e. about ten-fold greater than the computed K_i values, listed above, of 0.01 – 0.04 μM (see paragraph 4.10). So if we are to attempt any *in vitro – in vivo* correlation (and we have to bear in mind all the caveats previously listed), it points to the presence of inhibitory concentrations of AZTTP *in vivo* and not to 'insignificant' phosphorylation.

5.5 Two recent seminal papers should be highlighted.

(A) Firstly, Fletcher *et al* 2000 reported on zidovudine triphosphate and lamivudine triphosphate (3TC) concentration – response relationships in HIV-infected persons. They concluded that two

commonly used markers of HIV infection, the percent change in CD4 cells during therapy (CD4 count) and the rate of decline in HIV RNA (viral load) in plasma were related to the intracellular concentrations of zidovudine and lamivudine triphosphates, i.e. at higher levels of AZTTP and lamivudine triphosphate the increase in CD4 count and decrease in viral load is greater than when the concentration of triphosphorylated AZT and 3TC is lower. This study is important because it shows a direct correlation between AZT triphosphorylation and the immune response in HIV positive patients.

(B) Secondly, Hoggard *et al* (2002) in the CHARM study have examined the intracellular phosphorylation of zidovudine, lamivudine and abacavir over 48 weeks in 22 HIV patients recruited in the Department of Medicine, Somerset Hospital, Cape Town. The novel feature of this study was that all drug triphosphates and endogenous deoxynucleoside triphosphates were assayed. This enabled calculation of the ratio of drug triphosphate to endogenous triphosphate. Since AZTTP and dTTP are 'competing' for incorporation into the growing DNA strand it is the ratio between the two that is important rather than simply the absolute concentration of AZTTP. Importantly in the Hoggard *et al* study the level of AZTTP was found to be in the range 0.02 – 0.2 pmoles/10^6 cells and the ratio of AZTTP:dTTP was shown not to change over 48 weeks, indicating that there were no potentially adverse time dependent changes in the phosphorylation profiles i.e. the ratio of drug triphosphate to endogenous triphosphate did not decrease over the course of the study. This study represents the most comprehensive data-set available for the study of AZTTP levels (i.e. 22 patients with data at week 0, 2, 6, 12, 24 and 48). It represents a total of more than 250 drug triphosphate

determinations with an equivalent number of dTTP determinations.

5.6 The Plaintiffs have placed too much emphasis on the absolute concentration of AZTTP without considering also the concentration of dTTP with which it is competing. Since it is the ratio of AZTTP:dTTP that will ultimately determine antiviral response, it is important that data are generated for both anabolites; this gives important additional information. If, for example, AZTTP levels were reduced in a patient but the dTTP levels were also low, then the competing ratio would not necessarily be different from a patient with higher AZTTP and, also, higher dTTP. Enzyme inhibition would likely be comparable. So, if dTTP is low in cells, then less AZTTP will be required to inhibit reverse transcriptase. The recent advances in methodologies to measure both components should enable dose-response (pharmacokinetic-pharmacodynamic) relationships between phosphorylation and clinical effect to emerge.

5.7 There is only one published study (Barry *et al*, 1996) on the relationship between drug dose and intracellular zidovudine triphosphate concentration. This is referred to in paragraph 16.3 of the Particulars of Claim. Although there was no significant difference in the cellular concentration of AZTTP (area under the curve) between the 100 mg (0.42 ± 0.42 pmoles/10^6 cells x h) and 300 mg (0.61 ± 0.81 pmoles/10^6 cells x h) doses, variability was large in this small study of 10 patients. The study was 'under-powered' to detect statistically significant differences (i.e. too few patients) and there was no determination of dTTP concentration (which as indicated above is important to gain the overall picture of antiviral activity). It is also worth pointing out that the 300mg dose was taken twice per day, whereas the 100mg dose was taken 3 times per day – although it is not clear how the different timing would impact on intracellular kinetics. It is not surprising that a linear correlation between dose and intracellular concentration of AZTTP is not

seen, given that there are many steps and variables that could affect the rate of phosphorylation at a particular dose, i.e. drug absorption, uptake into cell and then three phosphorylation steps catalysed by different enzymes. The rate limiting step is the conversion of AZTMP to AZTTP.

6. Conclusion

Based on the above findings, it is evident that zidovudine is phosphorylated *in vivo* and that at the concentrations of active metabolite produced within the cell, the drug has anti-HIV activity.

Professor David Back
1 July 2002
173499v1

Annex 2 - References

Barry M et al **zidovudine phosphorylation in HIV-infected patients and seronegative volunteers.** AIDS 1994 8: F1-F5

Barry M et al. **The effect of zidovudine dose on the formation of intracellular phosphorylated metabolites** AIDS 1996 10: 1361-1367.

Fletcher CV, Kawle SP, Kakuda TN, Anderson PL, Weller D, Bushman LR, Brundage RC, and Remmel RP. **Zidovudine Triphosphate and Lamivudine Triphosphate Concentration-Response Relationships in HIV-Infected Persons.** AIDS 2000, **14**: 2137-2144.

Furman PA et al. **Phosporylation of 3 'azido – 3' deoxythymidine and selective interaction of the 5' - triphosphate with human immunodeficiency virus reverse transcriptase,** Proc Nat Acad Sci USA Vol 83 pp 8333-8337 1986

Heindenreich O, Krufhoffer M, Grosse F, and Eckstein F. **Inhibition of Human Immunodeficiency virus 1 Reverse transcriptase by 3-azidothymidine Triphosphate.** Eur. J. Biochem 1990, **192**: 621-625.

Hoggard PG, Kewn S, Maherbe A, Wood R, Almond LM, Sales SD, Gould J, Lou Y, Vries CD, Back DJ, Khoo SH for the CHARM Study Group. **Time-dependent Changes in HIV Nucleoside Analogue Phosphorylation and the Effect of Hydroxyurea.** Antimicrob. Agents Chemother 2002, in press.

Hoggard PG, Lloyd J, Khoo SH, Barry MG, Dann L, Gibbons SE, Wilkins EG, Loveday C, and Back DJ. **Zidovudine Phosphorylation Determined Sequentially over 12 Months in Human Immunodeficiency Virus-infected Patients with or without Exposure to Antiretroviral Agents.** Antimicrob. Agents Chemother 2001, **45**: 976-980.

Hoggard PG, Sales SD, Kewn S, Sunderland D, Khoo SH, Hart CA, and Back DJ. **Correlation between Intracellular Pharmacological Activation of Nucleoside Analogues and HIV Suppression in vitro.** Antiviral Chem. Chemother 2000, **11**: 353-358.

Hsu A, Granneman GR, Carothers L et al. **Ritonavir does not increase methadone exposure in healthy volunteers.** 5th Conference on Retroviruses and Opportunistic Infections, 1998, abstract 342.

Iribarne C, Berthou F, Carlhant D, Dreano Y, Picart D, Lohezic F, Riche C. **Inhibition of methadone and buprenorphine N-dealkylations by three HIV-1 protease inhibitors.** Drug Metab Dispos. 1998, 26: 257-260.

Kewn S, Hoggard PG, Sales SD, Jones K, Maher B, Khoo SH, and Back DJ. **Development of Enzymatic Assays for Quantification of Intracellular Lamivudine and Carbovir Triphosphate Levels in Peripheral Blood Mononuclear Cells from Human Immunodeficiency Virus-Infected Patients.** Antimicrob. Agents Chemother 2002, **46**: 135-143.

Kuster H, Vogt M, Joos B, Nadai V, Lunthy R. A method for the quantification of intracellular zidovudine nucleotides. Journal Infect Dis 1991 164 773-6.

Moore KH, Barrett JE, Shaw S, Pakes GE, Churchus R, Kapoor A, Lloyd J, Barry MG, and Back DJ. **The Pharmacokinetics of Lamivudine Phosphorylation in Peripheral Blood Mononuclear Cells from Patients with HIV-1.** AIDS 1999, **13**: 2239-2250.

Phiboonbanakit D, Barry M G and Back DJ. **Quantification of intracellular zidovudine triphosphate by enzymatic assary.** Br J Clin Pharmacol 46 77P-305P.

Robbins BL, Rodman J, McDonald C, Srinivas RV, Flynn PM and Fridland A. **Enzymatic assay for measurement of zidovudine triphosphate in peripheral blood mononuclear cells.** Antimicrobial Agents and chemotherapy 1994 115-121 Vol 38(1)

Rodman JH, Robbins B, Flynn PM and Fridland. **A systemic and cellular model for zidovudine plasma concentrations and intracellular phosphorylation in patients.** Journal Infect Dis 1996 174 p490.

St Clair MH, Richards CA, Spector T, Weinhold KJ, Miller WH, Langlois AJ, and Furman PA. **3-Azido-3-deoxythymine Triphosphate as an Inhibitor and Substrate of Purified Human Immunodeficiency Virus Reverse Transcriptase.** Antimicrob. Agents Chemother 1987, **31**: 1972-1977.

Wattanagoon Y, Bangchang KNA, Hoggard PG, Khoo SH, Gibbons SE, Phiboonbhanakit D, Karbwang J, and Back DJ. **Pharmacokinetics of Zidovudine Phosphorylation in Human Immunodeficiency Virus-Positive Thai Patients and Healthy Volunteers.** Antimicrob. Agents Chemother 2000, **44**: 1986-1989.

White LE, Parker WB, Macy LJ, Shaddix SC, McCaleb G, Secrist JA, Vince R, and Shannon WM. **Comparison of the Effect of Carbovir, AZT and Dideoxynucleoside Triphosphates on the Activity of Human Immunodeficiency Virus Reverse Transciptase and Selected Human Polymerases.** Biochem. Biophys. Res Commun 1989, **161**: 393-398.