The renaissance of fixed dose combinations: Combivir

Abstract: Combivir is a fixed dose combination tablet of two antiretroviral drugs; zidovudine and lamivudine, used in the treatment of HIV-1 infection. AZT was the first antiretroviral used in clinical trials and the addition of lamivudine improved its effectiveness. With the introduction of highly active antiretroviral therapy in the form of a combination of three drugs including two nucleoside analogues, Combivir became the gold standard nucleoside ‘backbone’ until very recently. Combivir was the first combination agent and simplified HIV therapy greatly. The introduction of newer fixed dose combinations with the advantage of once daily dosing and improved tolerability and toxicity profiles has made Combivir a less popular choice in treatment naïve individuals needing to start therapy.

Keywords: Combivir, zidovudine, lamivudine, antiretroviral, HAART, HIV

The first fixed dose combination antiretroviral

Combination antiretroviral therapy, sometimes called highly active combination therapy (HAART) has revolutionized the management of HIV infection and rendered it a chronic manageable disease (Palella 1998).

Most combination therapies for HIV use two nucleoside reverse transcriptase inhibitors (NRTI) as a backbone with a third agent either from the non-nucleoside reverse transcriptase inhibitor (NNRTI) class or a protease inhibitor to make up a very potent therapy that will successfully suppress viral replication and allow immune reconstitution (Dronda 2002).

Combivir™ (GlaxoSmithKline Ltd, Brentford Middlesex, UK) is a combination of two NRTI’s, azidothymidine (zidovudine, 3’-Azido-3’-deoxythymidine, AZT) which is a thymidine analogue, and lamivudine (2’-Deoxy-3’-thiacytidine, 3TC, GlaxoSmithKline Ltd, Brentford Middlesex, UK) a cytosine analogue. Each Combivir pill contains 300 mg of AZT and 150 mg of lamivudine and is taken every twelve hours with or without food. It was the first fixed dose combination therapy made available for HIV infected individuals. Combivir became available in 1997 and was licensed by the United States Food and Drug Administration (FDA) in October that year. The European launch followed in March 1998. Combivir has maintained a very important place in HIV management and here we discuss the history and utility of its two component agents. We describe the pivotal studies which for some time maintained Combivir as the preferred NRTI backbone. More recent developments in HIV therapy that have led to Combivir becoming a less chosen first line option and we will discuss the future of Combivir in antiretroviral management.

AZT was developed in the 1960’s as an antitumor agent but was not used clinically for this indication. When a cell line model for HIV infection became available, AZT was amongst the first compounds screened by the then Wellcome pharmaceutical research laboratories (Personal communication GlaxoSmithKline UK Ltd). In 1984 Wellcome started working on an assay that would be used to identify agents to
inhibit HIV. AZT was one of the first 100 compounds that ran through the assay and was found to have in vitro efficacy at reducing reverse transcriptase activity (Furman 1986) and for attenuating the infectivity and cytopathic effects of this newly discovered virus (Mitsuya 1985). AZT was trialed as monotherapy in humans in the early 1980’s with notable improvements in clinical status noted (Yarchoan 1986). The 076 study was a placebo controlled study that enrolled 287 individual who were clinically classified as having Acquired Immune Deficiency Syndrome (AIDS) or AIDS related complex. The results were dramatic and the study was unblinded and terminated early by the data safety monitoring committee. Only one death had occurred in the AZT arm compared to 19 deaths in the placebo arm and there was a significant reduction in disease progression in those on active drug (Fischl 1987). AZT was found to have other benefits such as improvements in AIDS related neurological syndromes (Yarchoan 1987). These benefits however came at a cost with over 24% of those on AZT becoming profoundly anemic, and other toxicities such as myositis, macrocytosis, headaches and neutropenia recognised (Richman 1987). It must be noted that in this trial and in other earlier trials AZT was dosed at 250 mg every four hours compared to the currently licensed dose of 250 mg every twelve hourly or 300 mg twelve hourly as a component of Combivir. At these doses the drug is considerably more tolerable and has less severe toxicity.

AZT was considered a potential therapy for HIV infection and a larger joint US and European study named ‘Concorde’ study was designed. Participants were enrolled in the Concorde study if they had not had a history of an AIDS diagnosis and were randomized to receive either 1000 mg a day of AZT (‘immediate treatment’) or placebo. Placebo recipients were switched to receive active drug on disease progression (‘delayed treatment’). Over 1700 participants were enrolled in the Concorde study with a view to compare the clinical outcomes of either disease progression or death in the two observed groups. After about one year into Concorde a further study of similar size and design, ACTG 019, had shown a slowing of CD4 decline but no clinical advantages in AZT use (Volberding 1990). The Concorde study ultimately demonstrated that immediate or deferred AZT had no clinical advantage (Concorde Coordinating committee 1994) and evidence was mounting that at the doses used had unwanted effects that may have had a negative overall effect on quality of life.

From what is now understood about HIV viral dynamics and the rapid emergence of resistant virus with incompletely suppressed virus, it is clear that monotherapy with an NRTI will have only short term benefits. AZT had been shown to have limited clinical effectiveness and it was clear that newer drugs were needed and then perhaps used in combination together. Subsequently three new NRTI’s became available including the cytosine analogue lamivudine (3TC) (van Leeuwen 1992). In-vitro work suggested a combination of AZT with 3TC delayed the emergence of AZT resistant virus (Soudelayens 1991) and AZT resistant virus retained some susceptibility to 3TC. AZT continued to be used in monotherapy but further clinical trials of dual therapy with two NRTI’s including AZT showed a more dramatic slowing of clinical disease progression (Staszewski 1996). It was also noted that 3TC was less cytotoxic than AZT and that with repeated passage of virus through culture there was a rapid emergence of viral resistance to 3TC with a mutation in the YMDD catalytic portion of reverse transcriptase with a substitution of valine for methionine at position 184 (Gao 1993; Tisdale 1993). This mutation, known as the M184V, is the signature mutation for 3TC and is known to develop very rapidly with 3TC monotherapy or with virological failure of a 3TC containing combination (Pluda 1995). It was noted that 3TC was very well tolerated with headache and insomnia being the only major side effects. Serious toxicity was strikingly absent (Ingrand 1995). In clinical trials, dual therapy adding 3TC to AZT monotherapy resulted in significant virological suppression (Katlama 1996; Katzenstein 2000) with the resulting clinical benefits of delayed disease progression (Staszewski 1997).

It was established that dual therapy was better than monotherapy, however the effects were short term and resistance still developed albeit more slowly, leading to disease progression (Delta Coordinating committee 1996). In 1996 trials of combination therapy with two NRTI’s and a protease inhibitor showed dramatic reductions in short term mortality in clinical trials (Steigbigel 1996). Subsequently another class of antiretroviral agents, the non-nucleoside reverse transcriptase inhibitors (NNRTI) were found to be effective in combination with two NRTI’s and thus the era of triple combination HAART was heralded. This triple combination therapy suppressed circulating virus profoundly to levels below the limit of detection. With such limited viral replication a realistic goal of complete viral suppression and the prevention of resistant virus emerging was possible (Pollard 1999).

Other NRTI’s that became available had disadvantages over AZT and 3TC. The adenosine analogue, didanosine (ddI), was poorly absorbed and had to be chewed or dissolved and taken on an empty stomach with a bulky antacid buffer. Stavudine (d4T), another thymidine analogue was relative
well tolerated but carried a high risk of the development of peripheral neuropathy. Zalcitabine (ddC) was poorly tolerated and caused unpleasant mouth ulceration and peripheral neuropathy.

Throughout the late 1990’s and early 2000’s the choice of nucleoside backbone became a matter of fashion with stavudine and lamivudine being the most frequently prescribed due to relatively good tolerability and the belief that stavudine had a better barrier to drug resistance than AZT. A combination of stavudine and didanosine was also frequently used although the emergence of cases of lactic acidosis and hepatic steatosis associated with this combination moved it out of vogue (Carr 2000). The arrival of Combivir in 1997 lead to a new dawn for AZT and many clinical trials have supported this NRTI backbone in terms of efficacy and a relatively good tolerability and toxicity profile. The ACTG 384 study demonstrated the advantages of this combination over the prescription of stavudine and didanosine (Robbins 2003). Stavudine has been associated with the development of peripheral and facial lipoatrophy (Dube 2002) and this has almost eliminated its use in current practice.

Combivir became the gold standard and most frequently prescribed nucleoside backbone in initial HIV therapy. This has subsequently been supported by the results of several large randomized studies (Robbins 2003; Gulick 2004). However more recently, AZT has been associated with development of lipoatrophy in some of those using the drug in the long-term (Martin 2004). AZT also has the disadvantage over newer NRTI backbones of requiring twice daily dosing. Tenofovir (TDF) containing and abacavir (ABC) containing regimens may have better short term tolerability and compared to tenofovir containing regimens, AZT containing regimens have been shown to lead to more discontinuations due to anemia (Pozniak 2006).

Contemporary HIV treatment guidelines reflect the uncertainty as to which NRTI backbone is most suitable for therapy naïve individuals. Combivir has the most experience and clinical trial data behind it, yet concerns about short term tolerability and toxicity and the emergence of lipoatrophy has limited its use. The competing fixed dose NRTI/Nucleotide backbone of Truvada® (tenofovir disoproxil fumarate and emtricitabine(FTC)-Gilead sciences) and Kivexa®/Epzicom® (abacavir and lamivudine- GlaxoSmithKline) have some supporting data but are not without disadvantages themselves. Concerns have been raised about renal toxicity from tenofovir (Gallant et al 2005) and 5% of individuals on abacavir develop a potentially life threatening hypersensitivity reaction (Hernandez 2003).

Vertical transmission of HIV from an HIV infected mother to child is a major cause of infection particularly in the developing world where there is limited access to antiretroviral medication. Multiple factors influence the risk of mother to child transmission (MTCT) including amongst others stage of disease, CD4 count, maternal viral load and method of delivery. The risk for MTCT whilst pregnant has been calculated in studies to be 12–25% in the developed world if a mother does not take antiretroviral therapy, reducing to as low as 2% if antiretrovirals are commenced and HIV viral load becomes undetectable (Cooper 2002). The risks are higher in resource limited settings. Antiretroviral therapy consisting of maternal oral AZT monotherapy during pregnancy, intravenous AZT during labor and oral AZT given to the baby was shown to reduce the risk of MTCT by two thirds in the ACTG 076 trial (Connor 1994). AZT was the first and is the most studied of all licensed antiretrovirals. Both AZT and 3TC are classified as Category C drugs in pregnancy by the FDA and there has yet been no evidence of teratogenicity with their usage (Covington 2004). This classification means that these drugs can be used in pregnancy if the potential benefits outweigh the risks. AZT and 3TC are the preferred NRTIs during pregnancy (Mofenson et al 2002). As there is more data to support the use of AZT/3TC in pregnancy, Combivir therefore has a lead over other fixed dose combinations in particular scenarios during pregnancy though there have been no formal studies of the use of Combivir in pregnancy to date.

Antiretroviral drugs are used as post exposure prophylaxis (PEP) agents against HIV infection for both occupational and sexual exposure. Their use is widespread in many countries and in different situations. Although not a licensed indication for its use, retrospective case controlled studies in healthcare workers have shown that AZT monotherapy in occupational exposure was protective against infection (Cardo 1997). AZT is the only antiretroviral currently to have been studied which has shown evidence of the reduction in transmission of HIV. For this reason many clinicians choose to incorporate this drug as part of a combination in PEP. As previously mentioned, triple combination therapy is more effective than one or two drugs for virological suppression so it is biologically plausible that three drugs are preferential than one or two drugs in PEP for preventing HIV transmission following exposure. Zidovudine is favored over the use of abacavir in PEP due to the risks of abacavir hypersensitivity (Hernandez 2003). The low pill burden and simple dosing schedule facilitates is usefulness for this purpose.
So what is the future of Combivir? GlaxoSmithKline’s patent on AZT expired in September 2005 and so the possibility of cheaper, generic versions of AZT may affect Combivir sales. 3TC may be dosed once daily but due to the short intra-cellular half life of AZT means it must be dosed twice daily. The simplicity of once daily therapy, which may have advantages for treatment adherence (Portsmouth 2004) and the improved tolerability of the fixed dose combinations of Kivexa and Truvada has lead to these becoming the most frequently used backbones. Despite Combivir having no food restrictions many patients find taking AZT on an empty stomach difficult and experience nausea.

Studies comparing ABC + 3TC or TDF + FTC to Combivir have not shown superiority to Combivir as a nucleoside backbone. The CNA30024 (DeJesus 2004) study indicated that in treatment naïve individuals starting ABC + 3TC + efavirenz was as effective over 48 weeks as AZT + 3TC + efavirenz. This was not a head to head trial using the fixed dose combinations of Kivexa or Combivir as the components were given individually. The GS934 Study (Pozniak 2006) shows non-inferiority using Truvada + efavirenz compared to Combivir + efavirenz at 96 weeks.

Patients experiencing treatment failure on Combivir develop resistance patterns which differ from those acquired by individuals using other fixed dose combinations. Patients experiencing treatment failure on Combivir as a first line combination commonly acquire the M184V mutation, reducing susceptibility to lamivudine. This helps increase the sensitivity to AZT and can protect against acquiring further thymidine analogue associated mutations. Combivir may have some uses in second line and in selected treatment experienced patients as guided by genotypic resistance assays. Virological failure on a tenofovir based regime and sometimes on abacavir based regimens leads to the development of the K65R mutation in reverse transcriptase (Winston 2002). Abacavir failure may lead to the development of the L74V mutation and both of these mutations result in a virus that retains sensitivity to AZT (Miranda 2005). Thus if an individual has initiated therapy with either Kivexa or Truvada and then experiences virological failure it is likely that the resistance profile of their virus will retain sensitivity to AZT (Parikh 2006). It would also be very likely that they would also have developed virus with the M184V mutation. Treating physicians may wish to retain the presence of lamivudine in a patient’s regimen especially if the individual has hepatitis B co-infection. 3TC has activity against this virus and stopping 3TC may lead to a hepatitis flare associated with resurgence in hepatitis B virus load. HIV harboring the M184V mutation often has a reduced replicative capacity and may lead to slower disease progression in some circumstances (Castagna 2006). In later lines of therapy some physicians may wish to leave 3TC in a regimen to drive this mutation production and so keep this mutation present. Zidovudine does have well established efficacy at penetrating the blood brain barrier and so confidence remains in its ability to prevent and treat HIV related neurological disease (Enting 1998).

**Conclusion**

In summary, Combivir was the first fixed dose combination antiretroviral agent and for some time has maintained a place as the gold standard in HIV therapy for treatment naïve individuals. It is a highly potent combination but recent concerns over the potential to cause lipodystrophy, and the introduction of better tolerated once daily fixed dose combinations means that Combivir has lost favor as the nucleoside containing backbone of choice.

**References**

Antiretroviral pregnancy register steering committee. Antiretroviral pregnancy registry international interim report 1 January 1989–31 July 2004. Wilmington, NC: Registry coordinating centre; 2004.

Cardo DM, et al. 1997. A case-control study of HIV seroconversion in health care workers after percutaneous exposure. Centers for Disease Control and Prevention Needle stick Surveillance Group. *N Engl J Med*, 337:1485–90.

Carr A, Miller J, Law M, et al. 1997. A case-control study of HIV seroconversion in health care workers after percutaneous exposure. Centres for Disease Control and Prevention Needle stick Surveillance Group. *N Engl J Med*, 337:1485–90.

Delta coordinating committee. 1996. Delta: a randomised double-blind controlled trial of immediate and deferred zidovudine in symptom-free HIV infection. *Lancet*, 343(8902):871–81.

Connor EM, et al. 1994. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Paediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med*, 331(18):1173–80.

Cooper ER, et al. 2002. Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. *J Acquir Immune Defic Syndr*, 29:484–94.

Covington DL et al. 2004. Assessing teratogenicity of antiretroviral drugs: monitoring and analysis plan of the Antiretroviral Pregnancy Registry. *Pharmaceutical Drug Saf*, 13(8):537–45.

DeJesus E, Herrera G, Teofilo E, et al. 2004. CNA30024 Study Team. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naïve HIV-infected adults. *Clin Infect Dis*, 39(7):1038–46.

Delta coordinating committee. 1996. Delta: a randomised double-blind controlled trial comparing combinations of zidovudine plus didanosine with zidovudine alone in HIV-infected individuals. *Lancet*, 348:283–91.

Dronda F, Moreno S, Moreno A, et al. 2002. Long-term outcomes among antiretroviral naïve human immunodeficiency virus-infected patients with small increases in CD4+ cell counts after successful virologic suppression. *Clin Infect Dis*, 35(8):1005–9.
Dube MP, Zackin R, Tebas P, et al. 2002. Prospective study of regional body composition in antiretroviral-naive subjects randomised to receive zidovudine + lamivudine or didanosine + stavudine combined with nefinavir, efavirenz or both: A5005S, a substudy of ACTG 384. *Antiviral Ther*, 7(1):8.

Enting RH, Foudraune NA, Lange JM, et al. 1998. Cerebrospinal fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. *Lancet*, 351:1547–51.

Fischl MA, Richman DD, Greico MH, et al. 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med*, 317(4):185–91.

Furman PA, Fyfe JA, St. Clair MH, et al. 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc Natl Acad Sci USA*, 83:8333–7.

Gao Q, Gu Z, Parma MA, et al. 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxynosine and 2',3'-dideoxythidine confers high-level resistance to the (International) anti-2',3'-dideoxy-3'-thiacytidine. *Antimicrob Agents Chemother*, 37(6):1390–2.

Gallant JE, Parish MA, Keruly JC, et al. 2005. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor therapy. *Clin Infect Dis*, 40(8):1194–8.

Gulick RM, Ribaudo HJ, Shikuma CM, et al. 2004. Triple-nucleoside regimens versus efavirenz containing regimens for initial treatment of HIV-1 infection. *N Engl J Med*, 350(18):1850–61

Hernandez JE, et al. 2003. Clinical risk factors for hypersensitivity reactions to abacavir: retrospective analysis of over 8,000 subjects receiving abacavir in 34 clinical trials. Abstract H-2013, 43rd ICAAC, Chicago, September 14–17.

Ingrand D, Weber J, Boucher CA, et al. 1995. Phase II/III study of 3TC (lamivudine) HIV-positive, asymptomatic or mild AIDS-related complex patients: sustained reduction in viral markers. *AIDS*, 9:1323–9.

Katlama C, Ingrand D, Lovejoy C, et al. 1996. Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomised controlled comparison with zidovudine monotherapy. *JAMA*, 276:118–25.

Katzenstein DA, Hughes M, Albrecht M, et al. 2000. Virologic and CD4+ cell responses to new nucleoside regimens: switching to stavudine or adding lamivudine after prolonged zidovudine treatment of human immunodeficiency virus infection. *AIDS Res Hum Retroviruses*, 16:1031–7.

Martin A, Smith DE, Carr A, et al. 2004. Mitochondrial Toxicity Study Group. Reversibility of lipatrophy in HIV-infected patients 2 years after switching from a thymidine analogue to abacavir: the MITOX Extension Study. *AIDS*, 18(7):1029–36.

Miranda LR, Gotte M, Liang F, Kuritzkes DR. 2005. The L74V mutation in human immunodeficiency virus type 1 reverse transcriptase counteracts enhanced excision of zidovudine monophosphate associated with thymidine analogue resistance mutations. *Antimicrob Agents Chemother*, 49:2648–56.

Mitsuya H, Weinhold KJ, Furman PA, et al. 1985. 3’azido-3’-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc Natl Acad Sci USA*, 82(20):7096–100.

Mofenson LM, et al. 2002. Centres for Disease Control and Prevention, U.S. Public Health Service Task Force. U.S. Public Health Service Task Force recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. *MMWR Recomm Rep*, 51(RR-18):1–38

Palella FJ Jr, Delaney KM, Moorman AC, et al. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med*, 338(13):853–60.

Parikh UM, Bacherl L, Koontz D, Mellors JW. 2006. The K65R mutation in human immunodeficiency type 1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analog mutations. *J Virol*, 80:4971–7.

Poziak AL, Galfanti JE, DeJesus E, et al. 2006. For the Study 934 Group. Tenofovir Disoproxil Fumarate, Efavirenz, and Efavirenz Versus Fixed-Dose Zidovudine/Lamivudine and Efavirenz in Antiretroviral-Naïve Patients: Virologic, Immunologic, and Morphologic Changes: A 96-Week Analysis. *J Acquir Immune Defic Syndr*, 43:535–40.

Pluda JM, Coley TP, Montaner JS, et al. 1995. A phase I/II study of 2’deoxy-3’-thiacytidine (lamivudine) in patients with advanced human immunodeficiency virus infection. *J Infect Dis*, 171:1438–47.

Pollard RB, Hall D, Cassuro J, et al. 1999. Durable suppression of HIV with NVP/ZDV/3TC in treatment-naïve patients with advanced and high baseline viral loads. Abstract no 1218, 7th ECCATH, Lisbon.

Portsmouth SD, Osorio J, McCormick K, et al. 2005. Better maintained adherence on switching from twice-daily to once-daily therapy for HIV: a 24-week randomized trial of treatment simplification using stavudine prolonged-release capsules. *HIV Med*, 6(3):185–90.

Richman DD, Fischl MA, Greico MH, et al. 1987. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med*, 317(4):192–7.

Robbins GK, De Gruttola V, Shafer RW, et al. 2003. Comparisons of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med*, 349:2291–301.

Staszewski S, Hill AM, Bartlett J, et al. 1997. Reductions in HIV-1 disease progression for zidovudine/lamivudine relative to control treatment: A meta-analysis of controlled trials. *AIDS*, 11:477–83.

Staszewski S, Lovejoy C, Picazo JJ, et al. 1996. Safety and efficacy of lamivudine-zidovudine combination therapy in zidovudine experienced patients. A randomised controlled comparison with zidovudine monotherapy. *JAMA*, 276:111–17.

Steigbigel R, Berry P, Teplitz H, et al. 1996. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. Abstract no Mo.B.412, 11th World Aids Conference, Vancouver Canada.

Soudeyens H, Yao X, Gao Q, et al. 1991. Anti-Human Immunodeficiency Virus Type 1 activity and in vitro toxicity of 2’deoxy-3’-thiacytidine (BCH-189), a novel heterocyclic nucleoside analogue. *Antimicrob agents Chemother*, 35:1386–90.

Tisdale M, Kemp SD, Parry NR, et al. 1993. Rapid in-vitro selection of human immunodeficiency virus type 1 resistant to 3’ thia-thymidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci USA*, 90(12):5653–6.

van Leeuwen R, Lange J, Hussey EK, et al. 1992. The safety and pharmacokinetics of a reverse transcriptase inhibitor, 3TC, in patients with HIV infection: a phase 1 study. *AIDS*, 6(12):1471–5.

Volberding PA, Lagakos SW, Koch MA, et al. 1990. Zidovudine in asymptomatic HIV infection. A controlled trial in persons with fewer than 500 CD4-positive cells per mm3. *N Engl J Med*, 322:941–9.

Winston A, Mandala, Pillay D, et al. 2002. The prevalence and determinants of the K65R mutation in HIV-1 reverse transcriptase in tenofovir-naive patients. *AIDS*, 16:2087–9

Yarchao R, Berg B, Brouwers P, et al. 1987. Response of human-immunodeficiency-virus-associated neurological disease to 3’-azido-3’-deoxythymidine. *Lancet*, 1(8528):132–5.

Yarchao R, Klecker RW, Weingold KJ, et al. 1986. Administration of 3’-azido-3’-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS hor AIDS related complex. *Lancet*, 1(8481):575–80
