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Treatment of HIV infection with didanosine and foscarnet

Submitted by

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TREATMENT OF HIV INFECTION WITH DIDANOSINE AND FOSCARNET.

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THESIS ABSTRACT

Treatment options for the management of HIV disease are increasing with a range of new antiretovirals as well as drugs for the treatment of and prophylaxis against opportunistic dieases becoming available. This thesis will cover clinical data relating to trials with two antiretroviral agents, didanosine (ddI) and foscarnet, conducted at St Stephen's clinic, London, discussing aspects of their therapetic efficacy, effect on survival, clinical and laboratory tolerability.

Didanosine (ddI) is an orally available purine based nucleoside analogue which, after intracellular triphosphorylation to ddATP, is a potent inhibitor of HIV *in vitro*. Data from phase 2 trials with ddI showed evidence of beneficial effects on surrogate markers such as CD4 cell counts and P24, with the principal dose-limiting toxicity being identified as peripheral neuropathy. This thesis presents data from a phase 3 open-label noncomparative single centre study of ddI in zidovudine-intolerant HIV infected persons with symptomatic disease and provides evidence that ddI is well tolerated in this patient group. Comparison of survival data with an historical database of zidovudine-treated patients suggests that ddI provides at least the same survival as that which would have been provided by continued zidovudine and superior to receiving no further antiretroviral therapy. These conclusions are supported by data from subsequent randomised blinded prospective trials of ddI.

The pyrophosphate analogue Foscarnet (phosphonoformate) is an antiviral agent with *in vitro* activity against both Herpes family viruses and HIV. In clinical practice it has utility as an anti-cytomegalovirus (anti-CMV) agent and in persons with acyclovir-resistant herpes virus. Wider use of Foscarnet has been precluded by the need for intravenous administration and toxicities which include renal dysfunction, disturbance of calcium and magnesium levels and penile ulceration. Data presented in this thesis show that in a randomised open-label comparative study, Foscarnet provides similar efficacy to the estsblished anti-CMV agent ganciclovir both as an initial treatment for CMV retinitis and as a maintainance therapy to prevent further recurrence. Additionally, although Foscarnet was not noted to provide a survival advantage over ganciclovir in this patient group, foscarnet patients were more likely to be able to continue zidovudine, hence gain the survival advantage provided by this therapy. Foscarnet was noted to have a distinctly different toxicity profile to ganciclovir providing the possibilities of both sequencing and combining these agents.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and that, to the best of my knowledge and beleif, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I, Graeme John Moyle, the author of this thesis consent to the thesis being made available for photocopying and loan if accepted for the award of Doctor of Medicine.

Signed Date 15.11.95

Dr Graeme John Moyle

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I would also like to thank and dedicate this thesis to all the patients who participated in the studies and hope that their participation added to their lives and that the information gleaned from their involvement can benefit humanity as a whole.

Graeme Moyle, London UK June 1995

1. PART I: INTRODUCTION: HIV DISEASE

1.1. NEED FOR THE THESIS: EPIDEMIOLOGICAL BACKGROUND

The Acquired Immune Deficiency Syndrome (AIDS) was first recognized in the USA in 1981 (Gottlieb M et al 1981) with cases having been subsequently reported from most countries throughout the world. The aetiological agent, an immunologically destructive retrovirus now called the Human Immunodeficency Virus (HIV-I), was subsequently identified in 1983 (Barre-Sinoussi F et al 1983).

The diagnosis of AIDS is based on certain criteria including the presence of opportunistic infections, neoplasm's, neurological disease or wasting syndrome as defined by the US. Centre for Disease Control (CDC) in 1987 (MMWR 1987). This has been recently revised (MMWR 1992) to include immunological parameters based on CD_4 counts and several new clinical diagnoses. Most available epidemiological data is based on the 1987 definition (Appendix 1).

By June 30, 1992, the World Health Organization (WHO) had received approximately 501'300 reported cases of AIDS although this figure is thought to be a substantial underestimate, with some countries reporting 5% or less of cases. The Harvard School of Public Health Global AIDS Policy Coalition estimate the true figure to be 2.5 million persons with AIDS with a further 13 million persons HIV infected (Mann J, 1992). The highest rate of infection is thought to be in sub-Saharan Africa where as many as 1 in 40 persons are HIV antibody positive. In Australia and the UK the case rate is though to be closer to 1 in 200 males and 1 in 1400 females. Globally women represent 40% of those infected with a further 1 million HIV infected children. Considerable alarm has been expressed regarding the rapid increase in prevalence of HIV infection in Southeast

Asia, South America and the Indian subcontinent. The incidence of new infection in the USA and probably Australia and the UK are thought to be slowing, presumably due to education and behavior modification.

In developed countries, progression from primary HIV infection to first AIDS defining diagnosis takes a mean of 8-10 years. It is as yet unknown whether all those infected with HIV eventually progress to AIDS. Time from infection to first AIDS diagnosis may be increasing due to antiretroviral treatment and prophylactic regimens. However, time from AIDS diagnosis to death may be shortening due to changing patterns of opportunistic infections and neoplasm's.

The major route of HIV transmission is sexual with 71% heterosexual and 15% homosexual transmission on a global basis. In Europe 45% of transmission occur in homosexuals with a further 33% related to drug injecting (Mann,J 1992). The peak age related incidence is between 20 and 35 years, a point which underlines the considerable social and economic impact of AIDS on communities. The number of children orphaned by AIDS is expected to increase from 1.8 million in 1992 to 3.7 million in 1995. Morbidity and mortality amongst persons of working age has considerable impact on economies both at local and national levels and place further demands on social and welfare services, as well as on medical and scientific systems.

With forecasts for year 2000 ranging from 30-120 million persons infected with HIV, the Global costs could be as high as US$ 500 billion per annum, equivalent to 1% of world gross national product (Mann J 1992). Already the cumulative costs, direct and indirect (1981-1991) are estimated at US$ 240.1 billion.

This thesis will examine the impact of two antiretroviral medications on survival, surrogate markers of survival, disease progression and quality of life in patients attending an urban, UK treatment center.

1.2. REPRODUCTIVE CYCLE OF HIV, THEORIES OF PATHOGENESIS OF DISEASE.

Specific drug therapy for HIV infection is based around knowledge of the structure, and replicative cycle of the virus. HIV, as with other animal retroviruses, consists of genetic material in the form of Ribonucleic acid (RNA) coupled with the enzyme reverse transcriptase (viral DNA polymerase). This is surrounded by a core protein 24 (p24), a lipid bilayer and outside coating (envelope) of glycoprotien consisting of the binding glycoprotien Gp120 anchored by a second glycoprotein Gp41 (Appendix 2). The genome consists of at least 8 genes including 3 common to all retroviruses; *gag* (coding for core proteins), *pol* (coding for reverse transcriptase and other enzymes) and *env* (coding for envelope protein) plus several regulator genes (*tat*, *rev*, *nef*).

1.2.1. The infection-replication cycle

The first step in infection of the target cell involves binding to a receptor protein. In this case the surface Gp120 of HIV binds specifically to the CD_4 surface receptor of the helper T cell and other cells carrying this receptor. HIV is then able to enter the target cell, probably by a process of fusion. The virus then "uncoats" releasing RNA into the host cytoplasm, probably in the form of a dimer of two identical genomic RNA sub-units. This RNA is then used as a template by the viral enzyme Reverse transcriptase for the production of a "minus" DNA strand. This DNA is then recopied to produce a double stranded piece of "viral" DNA which may then be integrated into host cell genome or remain circularized near the nucleus. The integration process is catalyzed by a viral integrase protein

which is an endonuclease enzyme capable of joining viral with human DNA.

Transcription of integrated viral DNA to messenger RNA utilizes host cellular machinery. Initiation and continuation of transcription is influenced by both viral and cellular factors. In resting CD_4 + cells no viral RNA may be detected despite presence of viral DNA (Schnittman SM, Greenhouse JJ 1989) and production of new virus may be dependent of T4 stimulation (Folks T,et al 1987). Initiation of full length viral transcripts begins at a long repeater sequence (LTR) and may be influenced by the interplay of viral regulator genes, principally *tat* , *rev* and *nef*. The protein product of the *tat* gene is a transactivator of transcription which regulates the rate of viral RNA transcription and possibly viral protein production (Wright CM, et al 1986). The *rev* gene is a regulator of viral protein expression which influences accumulation of unspliced, singly spliced or multiply spliced viral RNA. (Malim MH, et al, 1989). The *nef* protein is not required for viral replication, however, *nef* negative viruses replicate more rapidly than those producing *nef* suggesting a down regulator role (Cheng-Mayer C, et al 1989). During initial viral replication only viral regulator proteins are produced, however, once sufficient *rev* and *tat* have accumulated full length viral transcripts are produced.

Proteins are then produced in a precursor form and assembled near the plasma membrane. Immature virus particles are then released by a poorly understood budding process. The envelope glycoprotien (GP 120) is deposited on the exterior surface of the plasma membrane at this time, and 2 copies of viral genomic RNA incorporated. The protein precursors are then processed into mature forms by a specific viral proteinase

enzyme which cleaves proteins at specific sites. Mature HIV particles may then go on to infect further CD_4 positive cells.

1.2.2. Immune Dysfunction

The primary abnormality in patients presenting with AIDS is a significant decline in the numbers of circulating CD_4 positive cells. This decline occurs despite the number of circulating CD_4 cells infected with HIV at any one time appearing to be a low percentage of the total and direct T cell death due to rapid viral replication is an infrequent event. Recent data has suggested that HIV replication, particularly in early disease, may be occurring principally in the lymphatic tissue rather than circulating cells accounting for the low levels of plasma viraemia in early disease. (Pantaleo G, et al, 1993).

HIV positive individuals also show functional deficits related to CD_4 positive cell function such as delayed type hypersensitivity and mitogen induced responses, as well as deficits in CD_8 positive cell responses which are influenced by CD_4 cell secreted cytokines such as interleukin 2 (Lane HC,Fauci AS 1985). Defects in CD_4 cell response to certain soluble antigens may in part account for the increased incidence of some infections, e.g. *Cryptococcus neoformans* in persons with HIV. (Hoy JF, et al 1988).

Other divisions of the immune system are also influenced with B cells showing chronic activation (Lane HC, 1983) leading to hypergammaglobulinaemia. However, despite their hyperactivity B cell function is defective in regard to response to mitogens throughout infection. (Edelman AS, et al 1990).

Although monocyte-macrophages may be chronically infected with HIV, most of their functions, specifically antigen presentation are preserved (Rosenberg ZF, Fauci AS 1989) although chemotaxis and monocyte-dependent T cell proliferation may be dysfunctional. Virus production in these cells is predominately intracellular and HIV antigens are not expressed on the surface suggesting a role as reservoir of infection for these cells (Gartner S, et al 1986).

1.2.3. Theories of Pathogenesis

Polymerase chain reaction (PCR) techniques have shown that around 1% of CD_4 cells from persons with AIDS, and in earlier disease as few as 0.01%, are positive for HIV DNA. Viral load directly correlates with T cell decline and disease progression and the proportion of circulating HIV infected T cells rises with disease progression. (Schnittman SM, Greenhouse JJ 1990). *In vitro* CD_4 cell death can occur following HIV infection (Gallo RC, 1984), however if direct killing were the sole mechanism of CD_4 + T cell loss then one would expect replacement of lost cells by new production unless the homeostatic mechanism for balancing the CD_4 /CD_8 ratio did not exist and /or thymic precursors were infected (Schnittman SM, Denning SM 1990). One report has suggested that human thymopoiesis is suppressed by HIV infection and may contribute to limiting the regeneration of the peripheral T-cell compartment (Bonyhadi ML, et al, 1993). However, several studies examing viral and cellular turnover suggest that production of CD_4 is not a central problem, but that both substantial viral and CD_4 cell turnover occur every day (Wei X 1995, Ho 1995), supporting the view that viral replication is the engine which drives CD_4 depletion.

The cytopathic effect of HIV on the CD_4 positive cell may be due to disruption of host cell membrane by large numbers of budding virus particles or interference of cellular metabolic function because of viral requirements for RNA and protein synthesis. (Rozenberg ZF, Fauci AS 1989).

Indirect mechanisms for CD_4 cell death have also been proposed, principally involving the GP 120 and GP 160 envelope glycoprotiens. Binding of CD_4 and GP 160 may lead to blocking nucelo-cytoplasmic transport as GP 160-CD_4 complexes fill pores in the nuclear membrane (Koga Y, et al, 1990). Furthermore, expression of HIV envelope glycoprotien on the surface of infected CD_4 cells leads to attachment of uninfected cells and the formation of syncytia or multinucleated giant cells. (Rozenberg ZF, Fauci AS 1989). A direct relationship between the presence of syncytia and the degree of cytopathic effect of the virus in individual cells has been demonstrated *in vitro* (Pantaleo G, et al 1991). The appearance of syncytia forming virus *in vivo* has been proposed as a marker of disease progression (Koot M, et al, 1993) and may be associated with more rapid CD_4 cell decline, however, most patients with asymptomatic disease and normal CD_4 counts do not exhibit the syncytia forming phenotype but continue to lose CD_4 cells, albeit at a lower rate.

A further factor influencing disease development may be HIV virulence. A recent report from Australia has documented a group of 6 people infected by blood products and the original donor, all of whom remain well but HIV positive after at least 8 years of infection suggesting they may share a virus of low pathogenicity (Learmont J, Tindall B, et al, 1992).

Autoimmune mechanisms may also be involved and some clinical manifestations of HIV disease have been noted to resemble Graft versus Host disease. Structural homology between GP 120, GP41 and the nonpolymorphic determinants of major histocompatibility-complex (MHC) class II molecules HLA-DR and HLA-DQ may lead to production of antibodies to these molecules which prevent interaction between CD_4 and MHC II molecules on antigen presenting cells. This would impair effective antigen presentation and inhibit antigen specific functions of CD_4 T cells. (Golding H, et al, 1988, Golding H, et al, 1989). The binding of free GP 120 to CD_4 receptors on uninfected T cells may make these cells targets for destruction by cytotoxic cells. (Weinhold KJ, et al, 1989). Complexes of GP 120 antibody and antigen bind to CD_4 molecules and cause CD_4 cells *in vitro* to become refractory to stimulation through activation of CD_3 molecules. This may contribute to anergy seen in HIV infected persons (Mittler RS, 1989). Superantigens, viral protiens which can bind to all T cells causing massive stimulation followed by deletion have also been implicated in disease pathogenesis although *in vivo* evidence for this is lacking.

The host immune response may also be critical in influencing the natural history of HIV infection. The host response appears influenced by factors including the size of innoculum and genetic predisposition (Itescu S, et al, 1990). Aspects of the immune response may also enhance HIV infectivity (Robinson WE, et al, 1990). The high rate of mutation in antigenic areas of HIV proteins provides an effective escape mechanism from initial immune responses (Albert J, et al, 1990). Cellular responses are also likely to be important in control of HIV. (Planta F, et al, 1989). Finally, Programmed cell death or apoptosis, a normal regulatory phenomenon in T cells, may

be increased in HIV infection, possibly due to increased immunostimulation by viral antigens.

In summary, infection of CD_4 cells and production of new virus and/or viral antigens appears crucial to the immune dysfunction seen in HIV infected individuals. Although unanswered questions remain it appears rational that therapeutic approaches should focus on slowing replication of HIV and limiting the number of cells infected with HIV. As changes in immune function appear very early in infection therapeutic interventions may also be valuable from this time.

1.3. THE NATURAL HISTORY OF HIV

As knowledge of the replicative cycle of HIV and pathogenesis of disease assists in targeting the sites for therapeutic intervention, knowledge of the natural history of HIV disease enables planning of medical interventions to improve patient survival, quality of life and reduced number of opportunistic diseases.

1.3.1. Seroconversion

Following HIV exposure with an sufficient inoculum of virus, acute HIV infection produces a symptom complex which may resemble infectious mononucleosis or other acute viral illnesses. Prospective studies in Australia (Cooper DA, et al 1985 Tindall B, et al 1988) have suggested some illness may occur in over 90% of cases although only a small proportion of these attend for medical care. The symptoms generally develop within 3 months of initial infection and last for 1 - 2 weeks. Most commonly clinical manifestations include fever, pharyngitis, headache, malaise and diffuse cutaneous erythematous rash. Lymphadenopathy is also present in many cases. Changes in laboratory markers include leucopenia, monocytopenia and raised liver transaminases. The CD_4 count is frequently reduced. Antibodies to HIV are not present until several weeks after inoculation and the infection may readily be missed unless other virus markers such as p24 antigen are assessed (Moyle G, et al 1992). More severe symptoms have been reported including viral meningitis and opportunistic infections such as oral and oesophageal candidosis and *Pneumocystis carinii* pneumonia. Use of antiretroviral medications at this time is uncertain as ZDV has been reported to blunt the possibly protective CD_8 response (Tindall B, et al, 1993).

1.3.2. Asymptomatic Phase

After acute infection and seroconversion to HIV antibody positivity, the infected person enters a phase of variable duration in which they are asymptomatic or only have signs related to lymphadenopathy. However, immunological changes may be present including inversion of the CD_4 /CD_8 ratio and a decline in numbers of CD_4 positive cells in circulation. Thrombocytopenia of idiopathic origin may also be present. Hypergammaglobulinaemia related to non specific B cell activation is also characteristic and levels of ß2 microglobulin and neopterin (see section 1.7) may also be affected. Virological markers such as p24 antigen are routinely negative although viral replication continues, principally in the lymphatic system. (Pantaleo G, et al, 1993) and virus may be detected in plasma by techniques such as polymerase chain reaction (PCR). The mean time from initial infection to development of AIDS is 8-10 years, however, patients may not be asymptomatic throughout that time. As immune function deteriorates and CD_4 cell numbers decline non specific symptoms become frequent. These signs and symptoms may involve most body systems and include skin disorders, fevers and night sweats, headache, fatigue, diarrhoea, and oral infections including candida, hairy leukoplakia (caused by Epstien Barr virus) and accelerated dental caries.

1.3.3. Symptomatic Disease

As the CD_4 count declines further, particularly below 200 cells/mm3 individuals become at risk opportunistic infections which constitute a diagnosis of AIDS (MMWR 1987; MMWR 1993, Appendix I). Neoplasms which are AIDS defining such as B cell lymphoma, Kaposi's sarcoma and

primary cerebral lymphoma may occur any time during the disease process but are also more common with greater immune dysfunction. A variety of other neoplasms including squamous cell carcinoma of the anus and seminoma have also been reported to be more common during HIV infection (Kaplan MH, et al, 1987, Moyle G, et al, 1991). Risk of death in HIV infected persons increases as CD_4 count declines below 50 (Yarchoan R, et al. 1991) and usually results from opportunistic disease rather than directly from HIV.

Natural history and manifestations of HIV disease vary with geographic location, sex, race, age of acquisition of infection and possibly mode of infection. In particular, Kaposi's Sarcoma appears more common amongst male homosexuals, prompting the theory that a transmissible agent other than HIV maybe involved in the pathogenesis of this neoplasm. Further more, the use of treatment and prophylaxis regimens are influencing the natural history of HIV infection (Rosenberg PS, et al, 1991, Moore RD, et al, 1991) and new interventions and earlier treatment of HIV are likely to further influence this natural history.

1.4. THERAPY FOR HIV INFECTION

Since the discovery of HIV, unprecedented resource effort has been directed at finding effective anti-HIV therapies by both governments and pharmaceutical industry. Methods persued in the search include empirical screening of chemical classes for activity and rational or semirational drug design based on knowledge of the structure of viral protiens and receptors. Understanding of the replication cycle of HIV (see section 1.2) permits the identification of numerous steps in this process which are suitable for intervention.

A wide variety of compounds are currently under investigation for the treatment of HIV infection with several sites in the replicative cycle being targeted.(Appendix 3)

BINDING

Monoclonal neutralizing antibodies and soluble CD_4 molecules can bind free virus and free Gp120 to prevent binding to uninfected cells. Problems with this approach currently include viral antigenic drift (specifically in the hypervariable loop of envelope protein) and the observation that disease progression does occur despite some hosts producing high quality neutralizing antibodies (i.e. cell medicated immunity may be more relevant to protection against infection than humoural immunity).

ASSEMBLY AND RELEASE

Interferons (IFN), particularly Interferon Alpha, exert an antiviral effect on retroviruses by inhibiting virus assembly and release (Sen GC, 1982). However, *in vitro* studies have not found IFN Alpha to be fully inhibitory of HIV replication as a single agent (Harlshorn KL, 1987), although *in vitro*

synergy leading to complete inhibition has been reported using Interferon Alpha with a number of nucleoside analogues including ZDV (Harlshorn 1987), ddC (Epstein. JS, et al, 1992) and phosphonoformate (Harlshorn K, et al, 1986). The drug is currently only available via subcutaneous injection and frequently induces side-effects including flu-like symptoms and neutropenia, even in asymptomatic populations (Lane HC, et al, 1990).

Clinical studies have generally involved only small number of patients and have used a variety of dosages and preparations. Interferon Alpha monotherapy has been licensed in a number of countries for the treatment of Kaposi's sarcoma. (Fischl MA, et al, 1991). However, only trends to immunologic response are seen at doses producing a high incidence of side-effects. A number of protocols are currently examining combinations

expression in glial cells of JC virus, the human papovavirus implicated in Progressive Multifocal Leukoencephalopathy (PML), (Tada H, et al, 1990).

One anti-*tat* agent has been based on a benzodiazepine ring but which does not interact with central benzodiazepine receptors. *In vitro* inhibitory activity has been demonstrated in acute and chronically HIV infected cell tissues and against ZDV resistant strains. (Hsu MC, et al, 1991). However, first Phase I/II studies recently presented have not demonstrated clinical activity (Haubrich R, 1993) and development has been stopped.Wong-Staal has recently presented data suggesting *tat*-negative mutants continue to replicate under certain conditions, suggesting that this gene (or its protein product) may not be an ideal therapy target (Wong-Staal F 1993).

VIRAL PROTEINASE

Inhibitors of HIV`s aspartic proteinase have been synthesized from several different base compounds. The HIV proteinase is responsible for the post-translational processing of *gag* and *gag-pol* polyprotein gene products into function proteins. Inhibition leads to release of immature, non-infectious virions from cells. These compounds do not appear active against human cellular proteases, at therapeutic concentrations. Several compounds are currently in phase II/III clinical trials in USA, Europe and Australia with one compound Saquinavir (formerly Ro 31, 8959) showing low toxicity and evidence of activity with CD_4 count rises similar to ZDV in patients with advanced HIV disease. (Data on file F.Hoffmann-La Roche).

REVERSE TRANSCRIPTASE

Reverse transcriptase is an obvious target for specific therapies, being unique to retroviruses, although substrates used by host DNA polymerases are the same. Several reverse transcriptase inhibitors have

not been shown to confer clinical benefit (e.g. Suranim, HPA-23) whereas the dideoxy-nucleoside analogues and phosphonoformates have shown benefit in studies of both surrogate markers and clinical endpoints.

Both agents derived from pyrimidine and purine nucleosides (see below) and non nucleoside agents have been described as inhibitors. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) include products from a variety of chemical families including benzodiazepines, pyridinones, anthraquinones and others which share properties including low toxicity, excellent pharmacokinetics, high potency and synergy with nucleoside analogues. However, high level resistance appears to develop rapidly following mutations in the amino acid residues 100 to 108 and 181 to 190 which form the binding areas for these compounds (Richman DD, 1993). Trials are currently underway investigating a number of these compounds both as single agents and in combination regimes.

NUCLEOSIDE ANALOGUES

The dideoxynucleosides (nucleoside analogues) are a family of compounds based on modifications to the basic components of DNA, namely Thymine, Guanine, Adenine and Cytosine. Their active form is the 5`triphosphate but these forms have poor intracellular penetration. The compounds are therefore given in the absorbable 2`3`dideoxynucleoside form which then undergo intracellular triphosphorylation by cellular kinases (e.g. Thymidine Kinase) to mono-, di- and triphosphate. The resultant compound mimics natural substrates for reverse transcriptase (viral DNA polymerase) and host DNA polymerase. The compounds are preferentially used by reverse transcriptase and are competitive inhibitors of this enzyme. Once incorporated into the growing DNA chain, the compounds are unable to form the next 5` -> 3`phosphodiester link

resulting in chain termination. The dideoxynucleoside analogues are not preferred substrates for host DNA polymerase alpha, the host enzyme responsible for nuclear DNA synthesis or for polymerase beta the host "repair" enzyme, thus limiting toxicity. The host mitochondrial DNA polymerase gamma is, however, sensitive to the nucleoside analogues and this may, in part, be responsible for the toxicity of these compounds (Furman PA, et al, 1986). Steps within the phosphorylation process vary for each nucleoside analogue and rates of phosphorylation vary between each of the target cell lines. Accumulation of mono- and di-phosphate analogues may also be a cause of toxicity and may also alter the degree of *in vitro* protection offered by these compounds against HIV cytopathic effects in different cell lines. Phosphonoformate (see below) does not require anabolic phosphorylation and may therefore be more protective to those cell lines which have low levels of some kinases (e.g. monocyte derived cells).

ZIDOVUDINE

The first nucleoside analogue to reach human clinical trials was 3`azido-2`-3`dideoxythymidine [AZT, Zidovudine (ZDV)]. The incorporation of an (-N3) group at the 3`carbon atom was found to increase the compounds affinity for reverse transcriptase considerably from its base compound 2`3`dideoxythymidine. ZDV had originally been synthesized by Horwitz in 1964 (Horwitz JP, et al, 1964) and unsuccessfully investigated as an anti cancer drug. Subsequently it was shown to be an *in vitro* inhibitor of C type Murine retrovirus (Ostertag W, et al, 1974). After successful *in vitro* testing (Mitsuya H, et al, 1985) ZDV began clinical phase 1 trials in July 1985 which rapidly led to a large multicentre placebo controlled, double blind study.

Bioavailability studies show ZDV is rapidly absorbed from the gastrointestinal tract with peak serum levels occurring within 1 - 5 hours. The serum half life is around one hour but intracellular levels of the bioactive 5`triphosphate form persist for several hours. Oral bioavailability averages around 65% although this may be decreased by high fat meals. Steady state cerebral spinal fluid (CSF) concentrations are around 55% of plasma levels. ZDV is metabolized by both hepatic glucoronidation to an inactive metabolite and by renal tubular excretion (Blum MR, et al, 1988 Cload PA et al, 1989).

Phase 1 studies with ZDV established evidence of clinical and surrogate marker efficacy over a wide range of doses (1 - 90 mg/kg/day) with toxicity occurring readily at high doses. Toxicities included headaches, nausea and myalgia particularly during the first few weeks of therapy and anaemia with megaloblastic changes, and leukopenia subsequently (Yarchoan R, et al, 1986.). The increases in mean red corpuscular volume are thought to be due to accumulation of pools of ZDV - monophosphate (the second phosphorylation being the rate limiting step) which act as a substrate inhibitor for DTMP kinase resulting in a decline in intracellular thymidine triphosphate. This process is also dependent on Vitamin B12 and folic acid whose deficiencies are known to lead to megaloblastic anaemia. Supplementation within these vitamins does not lead to improvement in ZDV induced megaloblastosis or anaemia.

ZDV IN LATE DISEASE

The phase 2 multicentre double blind placebo controlled trial of ZDV (BW 002) began in February 1986 and enrolled 282 patients of whom 160 had first episode *Pneumocystis carinii* pneumonia (pcp) within the last 120 days, and 122 had AIDS related complex (ARC) on the basis of oral

candidiasis. Each patient received capsules, at regular 4 hours intervals, containing either 250 mg of ZDV or placebo. By September 1986, 20 deaths had occurred on the study of which 19 were in the placebo group and only one was receiving ZDV. This reached statistical significance at a level of $p < 0.001$ (Fischl MA, et al, 1987). This trial rapidly led to wide availability of ZDV to AIDS patients and its licensing by the US Food and Drug Administration (FDA) on March 20 1987 for use in patients with confirmed PCP or absolute CD_4 lymphocyte counts below 200/mm^3. Changes in immunological parameters were particularly significant ($p < 0.0001$) with ZDV patients showing an average rise in CD_4 lymphocyte counts of 80/mm^3 against on mean fall of 8 cells /mm^3 in placebo patients at week 4. These differences were no longer significant by week 20 in AIDS patients and week 24 in ARC patients. Patients with circulating p24 antigen showed significant decreases up to week 12 in the ZDV group, as compared with the patients receiving placebo. The trial also demonstrated significant ($p < 0.05$) increases in body weight with ZDV patients averaging a gain of 0.5 kg compared with an average loss of 0.1 kg in placebo patients.

ZDV patients also developed fewer new opportunistic infections over the study period and improvement in skin anergy was noted in some of the patients. After unblinding patients were continued to be followed with the 36 week mortality being 6.2% of ZDV-treated and 39.3% for the original placebo group. By 52 weeks the ZDV group still only had a mortality rate of 10.3% suggesting a survival benefit with treatment of at least one year. Some of the improvements in survival may have been due to the use of prophylaxis against *Pneumocystis carinii* pneumonia which was instituted in the extended phase of the study (Fischl MA, et al, 1989).

Other benefits suggested for ZDV from this and other studies include improvement in HIV related neurologic dysfunction (Yarchoan R, et al, 1987) and in HIV related thrombocytopenia (Pottage JC, et al, 1988).

Concern has been expressed regarding the development of lymphoma in several of the ZDV patients (Pluda JM, et al, 1990), however this is thought likely to be due to chance as increased rates of lymphoma are seen in a variety of familial immunodeficiency disorders (e.g. Wiskott-Aldrich syndrome) and other immunosuppressed states. Genesis of this lymphoma has not been seen in animal studies with ZDV. The conclusions of this first large study of ZDV have been further supported by studies from many institutions in most major western countries.

LOWER DOSES OF ZDV

In 1990 a study by the US AIDS Clinical Trials Group (ACTG) suggested that lower doses of ZDV, around 600 mg/day may be at least as efficacious as 1500 mg/day with lower toxicity, particularly anaemia (< 8g/day) and neutropenia (< 130 cells/mm^3) over long periods of follow-up (Fischl MA, Parker CR, et al, 1990) in AIDS patients. After 24 months therapy, survival was significantly greater in the 600 mg/day dose group (34%) than in the 1'500 mg/day dose group (27%) ($p=0.033$). Incidence of opportunistic infections and CD_4 counts were not significantly different.

ZDV IN EARLY DISEASE

The demonstration of efficacy with Zidovudine in advanced HIV disease prompted investigators to assess this drug in earlier disease. One study (ACTG 016) in patients with mildly symptomatic HIV disease (principally patients with CD_4 counts 200-500 cells/mm^3) using 1200 mg Zidovudine

per day showed a significant delay in the development clinical end points (AIDS, advanced ARC or death) in the treated arm as compared to placebo ($p = 0.0002$). (Fischl MA, Richman DD, et al, 1990). A second ACTG study (ACTG 019) compared two doses of ZDV 500 mg/day and 1500 mg/day with placebo in 1338 asymptomatic patients. At 55 weeks follow-up more patients in the placebo group had progressed to AIDS than the low dose group ($p = 0.002$) or the high dose group ($p = 0.05$). The lower dose group also experienced fewer adverse events as compared with the higher dose group (Volberding PA, et al, 1990).

The Veterans Affairs Cooperative study compared immediate (or 'early') with delayed (or 'late') initiation of 1500 mg/day of Zidovudine in 338 patients with CD_4 counts between 200 and 500 cells/mm^3. The delayed therapy patients received placebo, in a double blind manner, until CD_4 counts reached 200 cells/mm^3 or an AIDS defining event occurred. Patients were followed for a mean of 25.6 months. Greater numbers of patients in the late treatment group experienced an AIDS defining event ($p=0.02$). However, no survival advantage was found. Interpretation of survival data was, however, complicated by the large number of non-AIDS-related deaths during the study. Patients receiving early therapy also showed a delay in the fall of their CD_4 counts and a higher proportion of these patients became p24 antigen negative during the study (Hamilton JD, et al, 1992).

The Multicenter AIDS Cohort study (MACS) has also found advantage for early Zidovudine use including showing a survival benefit in patients with CD_4 counts between 200 and 349 cells/mm^3. (Graham NM, et al, 1992) and the Australian European Collaborative Group also showed delay in progression from asymptomatic to CDC group IV disease using

Zidovudine 1000 mg/day in patients with CD_4 > 400 cells/mm^3 with 2 years follow-up. No Survival advantage was seen (Cooper DA, Gattell JM, et al, 1993).

The large Anglo-French Concorde 1 study involving 1749 asymptomatic HIV infected individuals in a multicentre placebo controlled, double blind study compared immediate versus delayed treatment with Zidovudine at a dose of 1000 mg per day. Patients with asymptomatic HIV disease were randomized to receive either ZDV (immediate treatment) or placebo (delayed treatment).

In the delayed treatment arm Zidovudine was commenced at the endpoint of progression to CDC IV disease (AIDS or ARC). The trial was analyzed on an 'intent to treat' basis, however, data may be somewhat skewed in the placebo arm following an October 1989 protocol amendment following data from ACTG trial 019. This amendment led to 282 (32%) of this arm receiving Zidovudine having not clinically progressed but having CD_4 counts below 500 cells/mm^3. However, total time on ZDV in this group was 6 times less than the initial treatment arm prior to progression (14% vs. 82%).

Clear differences in CD_4 counts favoring the Zidovudine group were observed from 3 months until study end at 3 years follow-up. However, there was no difference in clinical outcomes including survival and disease progression seen at 3 years follow-up, although disease progression had been delayed in the immediate treatment group at one year. Haematological events were infrequent but more common with Zidovudine (5% vs. 1% at 1 year, 9% vs. 2% at 3 years). A further 9% Zidovudine patients stopped for other, mainly gastrointestinal side effects compared

with 4% in the delayed treatment arm. (Aboulker JP, 1993). The lack of survival data seen in several of these studies, and the Concorde study in particular has bought into question the value of early therapy with Zidovudine and the usefulness of surrogate markers, in particular CD_4 at predicating the value of treatment response.

The theoretical advantage of early therapy with ZDV include evidence of viral replication in early disease (Pantaleo G, et al, 1993), improved tolerance of ZDV and slower development of ZDV resistance in patients with better preserved immune systems (Richman DD, et al 1990). Long term follow-up from ACTG 019 reflects the view that, as in late disease, the duration of ZDV's benefits in early disease are of limited duration. (Volberding P, et al 1993). Decisions about use of ZDV must be balanced by physicians and patients view on when is the prudent time to intervene with ZDV and this balance lies not simply in the science of the clinical trial data but also in Quality of Life and psycho-social issues.

TOXICITIES WITH ZDV

These studies and others have also further elucidated the toxicities of Zidovudine. The major dose limiting toxicities of ZDV are neutropenia and anaemia. Incidence of both these adverse events is greater with higher doses and in patients with AIDS. From the above described studies BW002, ACTG 016 and 019, rates of anaemia (Hb <7.5) in AIDS patients receiving 1500 mg ZDV/day to be 31.3%, compared with, only 15% of ARC patients experienced anaemia (Hb <8.0) on 1500 mg/day and 1.1% on 500 mg/day. Similar figures for neutropenia (<750 cells/mm^3) were AIDS 31.3%, ARC 11.7%, asymptomatic 6.3 and low dose in asymptomatic 1.8%. Myopathy has been reported with prolonged therapy (Bessen LJ, et al, 1988) although myalgia may be more common. The

underlying process is thought to relate to mitochondrial dysfunction (Dalakas MC, et al, 1990). The process usually begins proximally and creatinine kinase is frequently elevated. Resolution occurs in most patients within 2 months of stopping therapy (Chalmers AC, et al, 1991). Nausea is also a common, usually initial, side effect of ZDV therapy which probably occurs less frequently in asymptomatic patients receiving lower doses. Similarly, insomnia and fatigue may occur with Zidovudine particularly in the weeks following commencement of therapy.

Ongoing trials with ZDV are looking at efficacy of lower doses and combinations with many other compounds including other nucleoside analogues, non-nucleoside reverse transcriptase inhibitors, proteinase inhibitors, acyclovir and interferon alpha.

DDI

2`3`dideoxyinosine (ddI) is a nucleoside analogue of the natural purine inosine and is closely related to dideoxyadenosine (ddA). It was first noted to be an inhibitor of HIV replication in Human T cells in 1985 (Mitsuya H, et al, 1986) and subsequently monocyte/macrophage lines (Perno CF, et al, 1988). Similar to ZDV, ddI can completely inhibit HIV replication *in vitro* at concentrations 10 - 20 fold lower then those required to inhibit growth of T cells (Mitsuya H, 1986) but with considerably less toxicity to haemopoietic progenitor cells (Du DL, et al, 1989). HIV strains resistant to ZDV (see section 1.4) remain sensitive to ddI *in vitro* (Larder BA, 1990).

As with ZDV , ddI diffuses into cellular cytoplasm without active transport and undergoes stepwise phosphorylation to the active form, 5`triphosphate dideoxyadenosine triphosphate (ddATP). This pathway of activation is distinct from the metabolic pathway used for ZDV

triphosphorylation. Although being competitive with the natural substrates for both cellular and retroviral DNA polymerase, the ddATP has a higher affinity for the viral enzyme Reverse transcriptase. Chain termination by preventing formation of further 5`-> 3`phosphodiesterase links again appears an important mechanism of action (Mitsuya H, et al, 1987). ddI has also shown activity against most other retroviruses and the Hepadena virus. In chronically infected ducks ddI has been shown to suppress duck Hepatitis B when given in an intravenous preparation (Martin P, et al, 1989),however, evidence of activity against Hepatitis B was not seen in patients with concommitant advanced HIV infection who received ddI during the St. Stephens study (Catterall A, Moyle G, 1992).

PRECLINICAL DEVELOPMENT

During pre clinical development it was noted that ddI solubility was increased in alkali environments (Anderson BD, et al, 1988) and that a rapid acid catalyzed hydrolysis to hypoxanthine and dideoxyribose occurred in low pH environments (York JL, 1981). This led to oral formulation of ddI with alkaline buffer to prevent gastric acid degradation, enabling oral bioavailability around 35% (Yarchoan R, et al, 1989). A subsequent study confirmed the reduction of bioavailability by the presence of food in the stomach (Shyu WC, et al, 1991) leading to advice to administer ddI under "fasting conditions".

PHASE I/II STUDIES

The active moiety of ddI, ddATP has an intracellular half life around 12 - 24 hours, suggesting that once or twice daily dosing is practical (Ahluwalia G, et al, 1988). Phase 1 studies (Lambert JS, et al, 1990, Cooley TP,et al

1990) with ddI suggested improvements in the surrogate markers of CD_4 count and p24 antigen (see section 1.5) as well as clinical improvement and weight gain. The dose of ddI was limited to a maximum of 12 mg/kg by toxicity, principally painful peripheral neuropathy and pancreatitis. No haematological toxicity was observed. In particular the neuropathy appeared dose related and was noted to resolve in most patients on stopping with ddI. Laboratory changes of elevated uric acid and aminotransferases were also noted in patients. Asymptomatic hyperuricaemia, particularly at higher dosage levels, probable reflects metabolic degradation of ddI via purine catabolic pathways. A longer term phase 1 study (Yarchoan R, Pluda JM, Thomas RV, et al 1990) suggested sustained surrogate marker benefit particularly for patients who had previously received ZDV for less than 4 months, and noted improvement in cognitive function in 5 patients. ddI subsequently entered a compassionate release programme in the USA and several studies, principally uncontrolled, across USA, Europe and Australia leading to licensing in the USA in late 1991.

OTHER NUCLEOSIDES

A number of other nucleoside analogues are in development or licensed including ddC, d4T, and 3TC. All appear to act by the same mechanism as ZDV and ddI. ddC and d4T both have peripheral neuropathy as their dose limiting toxicity but pancreatitis appears less frequent with these medications. Limited published data is available on these drugs but both ddC and d4T have been reported to produce transient changes in surrogate markers similar to those seen with ZDV and ddI (Yarchoan R, Perno CF, et al 1988 Browne MJ, et al, 1993). A review of recent data on ddC is provided in appendix 4 (Moyle G. 1995)

FOSCARNET

Trisodium phosphonoformate (Foscarnet) is a pyrophosphate analogue which was recognized in 1973 to have anti-herpes activity in cell culture (Shipkowitz NL, et al, 1973) through inhibition of herpes virus DNA polymerase. Subsequently it was found to inhibit reverse transcriptase from a wide variety of retroviruses (Sundquist B, 1979) including HIV (Sandstrom EG, et al, 1985, Sarin PS, et al, 1985). Other herpes viruses, notably cytomegalovirus (CMV) are also inhibited by foscarnet at low concentration (Eriksson B, 1982). Inhibition of reverse transcriptase by Foscarnet is non competitive with respect to both substrate and template (Sundquist B, 1979) and chain termination appears to be part of the mode of action(Sundquist B, 1979). Although intracellular penetration of Foscarnet is slow (Oberg B, 1983) further activation is not required and foscarnet is active against Thymidine Kinase negative herpes virus mutants (De Clercq E, et al, 1980). Foscarnet shows low toxicity against host cells (Sternberg K, et al, 1978).

Foscarnet is currently only available in intravenous formulation although several oral preparations have been tried in the past, resulting in osmotic diarrhoea and minimal absorption. Ninety four percent of intravenously administered Foscarnet is rapidly excreted in the urine (Sjorall J, et al, 1988) with some of the remainder being retained in bone and cartilage as calcium complexes (Helgstrand E, et al, 1980). Foscarnet toxicity relates to these two facts, with renal dysfunction and disturbed calcium levels, particularly hypocalcaemia being the most common. Urine containing concentrated Foscarnet may accumulate under the foreskin causing acute irritant reaction leading to ulceration, as is seen with the 3% Foscarnet cream. Renal dysfunction caused by Foscarnet may be prevented by

hyperhydration of patients during Foscarnet infusion (Deray G, et al, 1990).

In vitro data suggests additive or synergistic inhibition of HIV replication occurs when ZDV and Foscarnet are used together (Eriksson B, et al, 1989 Kostida R, et al, 1989). A further theoretical advantage is that for the use of Foscarnet as an anti HIV agent is that suppression of herpes family virus may secondarily reduce the replication of HIV, as a cellular product of herpes virus infection, infected cell protein (ICPO) can potentiate HIV transcription (Mosca JD, et al, 1987).

CLINICAL STUDIES WITH FOSCARNET

Small scale studies in HIV showed reduction of viral isolation in patients receiving Foscarnet (Farthing CF, et al, 1987 Gaub J, et al, 1987). Subsequent studies combining ZDV with Foscarnet have shown significant reductions in p24 antigen in patients persistently p24 antigen positive despite 9 - 27 weeks of ZDV monotherapy(Jacobson MA, van der Horst C et al, 1991). A further trial in patients with CMV retinitis demonstrated a significant survival advantage in patients treated with Foscarnet rather than ganciclovir and that this advantage could not be wholly accounted for by consumption or ZDV of other nucleoside analogues (Studies of Ocular Complications of AIDS Research group in collaboration with the AIDS Clinical trials group, 1992). However a retrospective analysis from the San Francisco General Hospital in a similar group of patients did not show any survival advantage for Foscarnet treatment over Ganciclovir (Harb GE, et al, 1991).

1.5. CMV DISEASE AND RETINITIS

Cytomegalovirus infection (CMV) is widespread in the adult population with seroprevalence in the HIV infected and 'at risk' populations ranging upto 90% (Drew WL, et al, 1981 Quinn TC, et al, 1987). Rates of isolation of CMV from urine and blood increase with immune dysfunction (Collier AC, et al, 1987) but are of poor diagnostic or predictive value of present or future clinical status (Zurlo JJ, et al, 1993). In the near future it is hoped that qualitative and quantitavive PCR techniques may act as both baseline predictors of CMV risk and therapy response.

Although replication of CMV is present, clinical manifestations are rare until immune dysfunction is severe, patients with CD_4 < 100 cells/mm^3 being at greatest risk. (Gallant JE, et al, 1992, Perter P, et al, 1992). Sites of disease related to CMV include retina, gastrointestinal tract, lung and adrenal tissue. Encephalopathy has also been described. Furthermore, CMV and other herpes viruses have been implicated as cofactors in HIV disease by promoting HIV replication through a mechanism involving HIV's *tat* gene. (Gendelman HE, et al, l986, Skolnik PR, 1988).

CMV retinitis is the most common cause of visual loss in persons with HIV and its prevalence has been estimated between 15 and 46% (Palestine AG, et al, 1984 Holland GN, et al, 1983). It generally presents as a painless progressive visual loss in individuals with advanced HIV disease. Some patients describe 'floaters' or flashing lights as their first symptoms. Peripheral lesions may not produce symptoms until considerable retinal area is lost and asymptomatic disease may be seen on routine ophthalmological assessment. Retinal appearances are characteristic with yellow/white exudates and superficial hemorrhages, initially along the

vascular arcades. Multiple sites in a single eye and bilateral disease are both frequent and the disease may rapidly spread resulting in complete loss of vision (Palestine AG, 1988).

Differential diagnosis includes cottonwool spots, *Toxoplasma gondii* retinitis, candidal retintis, syphilis, and varicella zoster and herpes simplex virus infections all of which probably occur more frequently in patients with HIV infection. Although vitreal sampling may be useful in assisting diagnosis this is rarely done in practice and the ophthalmologist generally relies of clinical features. Response to treatment may be measured quantitatively or qualitatively (Palestine AG, et al, 1986, Fanning MM, et al, 1990).

CMV retinitis is generally a localized manifestation of a systemic disease and patients may also experience fevers, malaise, weight loss or have CMV related manifestations at other sites prior to diagnosis. Systemic therapy is therefore preferred in patients with CMV retinitis although intra vitreal treatment has been described. The two agents currently licensed for CMV treatment Ganciclovir and Foscarnet are both available in intravenous form only although oral presentations are under development.

1.5.1. Ganciclovir

Ganciclovir (9-[(1,3-dihydroxy-2-propoxy)methyl] guanine) is a nucleoside analogue with activity against all herpes family viruses but not against HIV (Cheng Y-C, et al, 1983). Like other nucleoside analogues it is active in the triphosphate form, thus requiring activation via cellular kinases, most likely guanylate kinase. (Boehme RE, 1984). Intracellular half life of the active moiety is in excess of 12 hours. Excretion is primarily renal,

however, toxicity is mainly hematological with neutropenia being most frequent. Response of CMV retinitis to a 3 week course of Ganciclovir has been reported in upto 90% of patients (Collaborative DHPG treatment study group, 1986). Relapse is common and rapid if maintenance regimes are not employed and maintenance must therefore by lifelong. However, emergence of Ganciclovir resistant isolates has been reported with long term usage. (Erice A, et al, 1989). Disease caused by Ganciclovir resistant CMV appears to continue to respond to Foscarnet therapy (Parenti DM, et al, 1990).

Patients unable to tolerate Ganciclovir due to toxicity may benefit from intravitreal injections or implants although these techniques do not treat systemic CMV disease and carries a risk of endophthalmitis. (Cantrill HL, et al, 1989).

1.5.2. Foscarnet

Foscarnet has earlier been described in Section 1.4. It appears effective in the treatment of CMV retinitis (Fanning MM 1990) and as maintenance therapy although dosage and regimes of administration remain unclear. A previous uncontrolled study has suggested response of CMV retinitis to Foscarnet may be slower than Ganciclovir, possibly related to its ability to cross the blood brain barrier (Walmsley SL, et al, 1988). Although resistance has been described *in vitro* there have been few *in vivo* reports of resistance (Erikson B, 1979).

1.6. VIRAL RESISTANCE

Patients chronically infected with HIV express virus with a wide degree of biodiversity, so called quasi-species, including variants which may cause altered cell tropism, or resistance to treatment. Nucleotide sequencing has exhibited differences of more than 25% between strains of HIV-I (Haseltine WA, 1988). Mutations may occur at 3 steps in the replicative process. The reverse transcriptase (viral DNA polymerase) lacks the error correcting feature seen with Human DNA polymerases, thus allowing errors to occur when synthesizing the single stranded viral DNA and subsequently the complementary strand. Similarly the Human Cellular mRNA polymerase, used during transcription of viral and Human DNA to messenger RNA also fails to correct errors. Furthermore, the secondary structure of the RNA template of the reverse transcriptase gene, may contribute to base misincorporations during reverse transcription possibly at specific regions of this enzyme's genome (Schinazi RF, et al 1994). Saag and colleagues (Saag MS, et al, 1988) showed that HIV-I variation develops rapidly *in vivo* and that considerable genotypic variations coexist within chronically infected individuals. Many of the mutations leave the HIV unviable and so are eliminated whereas others cause insignificant changes in their gene product which have no effect on viral behavior. Mutations in regulatory genes may result in more rapid viral replication and therefore possibly more rapid disease progression. Mutations of the exposed regions of the virus, such as in the so called hypervariable loop of the envelope protein may enable the virus to evade the immune response. Underlying immunodeficiency may also favor the emergence of variant strains particularly as the number of replicative events increase.

Larder and colleagues (Larder B, et al, 1989) were the first to isolate strains of HIV resistant to ZDV and noted the absence of cross resistance to some other nucleoside analogues (e.g. ddI, ddC) or Foscarnet but resistance to other nucleosides containing the azido group (e.g. AZU), however, a more recent report has suggested that for every 10-fold decrease in ZDV susceptibility ddI and ddC susceptibility may decrease by around 2-fold (Mayers DL, et al 1994). Other studies have suggested that ZDV resistance may develop more rapidly in patients with more advanced disease and that the presence of resistant strains persisted after the withdrawal of ZDV (Rooke R, et al, 1989 Richman D, 1990). Studies of reverse transcriptase gene *pol* using selective polymerase chain reaction technology have identified 4 codons which increase ZDV resistance in a stepwise manner. Boucher found these mutations occur in an ordered manner involving codons 70, 215, 67 and 219 although partial resistance with the codon 215 mutation appeared the most stable variant (Boucher C, et al, 1992) The resultant amino acid substitutions are thought to alter the charge or structure of the catalytic site of reverse transcriptase and may therefore also reduce the enzymes ability to deal with natural substrate.

Subsequently other codons on the reverse transcriptase gene have been identified for resistance to ddI (codon 74) (St Clair M, et al, 1991) and ddC (codon 69) (Fitzgibbon JE et al 1992). Non-nucleoside reverse transcriptase inhibitors such as nevirapine, TIBO and BHap have also had resistant mutants recognized *in vitro*. Rapid appearance of such mutants has been noted *in vivo* studies (Richman D, 1992). HIV strains highly resistant to Foscarnet have not been described although chronically HIV infected monocyte-macrophage cells show a marked reduction in their

strain sensitivities compared with those acutely infected (Crowe S, et al, 1991).

The clinical significance of diminished drug susceptibility is, as yet, not clearly established. Resistant mutants may exhibit less virulence such as occurs with Thymidine Kinase negative Acyclovir resistant mutants of Herpes Simplex virus (Field HJ, Darby G.1980) and individuals may have a mixture of different resistance genotypes circulating simultaneously (Boucher C, et al,1990). Clinical endpoints with HIV are not reached directly as a consequence of viral load or genotype but are a consequence of immunosuppression, which is itself a determinant of likelihood of resistance development. A recent study from a Canadian cohort has suggested that viral resistance may be a marker of subsequent disease progression suggesting that presence of such mutants may be clinically meaningful (Montaner JS, et al 1993). The area remains complex with careful case control studies as well as comparisons with animal models needed. A comprehensive review of resistance and possible therapeutic implications is provided in Appendix 4 (Moyle G 1995).

1.7. SURROGATE MARKERS OF DISEASE PROGRESSION AND SURVIVAL

HIV infection produces disease many years after acquisition and patients frequently experience long periods of symptomatic disease prior to death. Trials in HIV patients which use survival as the primary end point may therefore involve many years of patient follow-up, particularly if commenced during the asymptomatic period. The use of surrogate markers can greatly shorten the time needed to conduct a trial and could reduce the number of persons required in a study and, therefore, have the potential to reduce the time needed for drug development. Desire, and at times political pressure, from both patients and physicians has been for rapid evaluation of new drugs to gain prompt patient access. Many events occurring prior to death have been studied to assess their value as predictors of death and changes in these parameters may reflect therapeutic effect and hence survival benefit. For a marker to be useful the effects on treatment on that marker must predict the impact of therapy on clinical progression or mortality. Use of invalid markers can potentially lead to the licensing of ineffective agents or the dismissal of effective agents as ineffective. Surrogate endpoints are now accepted by regulatory agencies for drug licensing both in HIV and other fields of medicine. These markers include immunological, virological, biological and clinical values some of which exhibit rapidly observable changes, often within weeks of starting therapy. However, the short term effect of a treatment on a marker does not necessarily imply the treatment has an ultimate effect on survival, particularly if significant drug toxicity occurs (Mochado SG, et al, 1990) and marker changes occurring with one drug may not necessarily apply to drugs with different mechanisms of action.

In HIV infection, changes in surrogate markers with therapy have only been validated against actual survival in the case of ZDV (Jacobson MA, Bacchetti P, et al, 1991). This reflects the newness of therapies for HIV rather than the lack of vigor of other investigators. It seems likely that the conclusion regarding surrogate markers drawn from ZDV studies are at least reliable for other nucleoside analogues with the same mechanism of action (e.g. ddI, ddC) if not for other therapeutic agents. Of course, some markers predictive of survival such as Age > 40 years, diagnosis of AIDS (Bacchetti P, et al, 1988) cannot change with treatment and therefore can only be used as baseline values for comparison between treated and untreated groups. In this section I will only discuss markers which may change with therapy.

The ideal surrogate marker should correlate with the risk of death or clinical progression and any effect of treatment on the risk of clinical progression must be explainable and predictable by its effect on the marker (Lagakos SW, 1993).

Surrogate biological markers, when used independently reveal nothing about patients well being and quality of life or about therapy toxicity. Quality of life issues, particularly in patients with short life expectancies may be more important to individuals than short increases in length of life.

IMMUNOLOGICAL SURROGATE MARKERS

The principal immunological markers with predictive value in HIV are CD_4 count, CD_4 /CD8 (or helper/suppresser) ratio, ß2 microglobulin and serum neopterin. CD_4 positive cells are the principal targets of HIV infection and it was recognized early on that substantial declines in CD_4 positive lymphocytes cells occurred prior to the occurrence of AIDS defining

opportunistic infections (Fauci AS, et al, 1984). CD_4 count has been widely studied as the best predictive marker of progression to AIDS and a count below 50 cells/mm^3 is a powerful predictor of risk of death (Yarchoan J, 1991). Changes in CD_4 count (and the logarithm of CD_4 count) with ZDV therapy have been shown to be predictive of survival at 12 weeks of treatment when controlled for pre-treatment prognostic characteristics (Jacobson MA, Bacchetti P, et al, 1991). However, this correlation was not seen in the Concorde 1 study in which patients receiving 'immediate' ZDV therapy had significantly higher CD_4 counts than those not receiving 'delayed' ZDV but had equivalent rates of disease progression and death (Aboulker JP, 1993). This data does not question the value of the CD_4 count as a predictor *per se*, but if changes in CD_4 count *with therapy* are predictive of future outcome. Precise normal ranges for absolute numbers or the percentage of lymphocytes which are CD_4 positive are poorly established. Flow cytometers (so called FACS autoanalysers) which are the standard laboratory techniques for measuring lymphocyte counts commonly show variation of 10 - 15% within the same sample which at higher CD_4 counts may mean fluctuations of over 50 cells/mm^3. CD_4 counts also show diurnal variation, being higher shortly after waking, and the absolute numbers decline due to intercurrent viral illnesses (including HIV seroconversion illness). It has also been suggested that exercise, stress, general and micronutritional status may also influence absolute numbers of circulating CD_4 cells. CD_4 positive lymphocytes as percentage of the total number of circulating lymphocytes may be a better surrogate marker for disease progression than absolute numbers. Circulating numbers of CD_4 cells also give no indication to function with studies of bulk cell populations from HIV infected persons showing decreased response to mitogens (Hoffmann B, et al, 1987) and soluble antigens (Lane HC, et al, 1985) as well as depressed expression

of interleukin 2 receptors (Prince HE, et al, 1984). Functional changes occur in large populations of CD_4 lymphocytes despite only small numbers being infected directly by HIV (Schnittman S,Greenhouse JJ et al, 1990). This may be due to circulating viral proteins (Cunningham-Rundles S, et al, 1983) or loss of sub populations (Giorgi JV, et al, 1987) responsible for certain responses. Rather than being a marker of disease, loss of CD_4 lymphocytes (and some other immune cells) may represent the disease process of HIV infection with the clinical syndrome of AIDS (and ultimately death) being "surrogate markers" of this loss.

CD_4 is validated as a predictor of death or clinical progression with analyses from 2 prospective studies demonstrating that as subjects CD_4 counts change over time their risk of clinical progression changes proportionately (Lagakos SW, 1993 DeGrutolla V, et al 1993). This correlation applies to both treated and untreated subjects. However, changes in CD_4 lymphocyte counts only explain some of the effects of ZDV in delaying clinical progression and hence CD_4 positive lymphocyte count is not an ideal surrogate marker.

Activation of CD_8 + lymphocytes (suppressor and natural killer cells) also occurs early in HIV infections leading to proliferation and an increase in circulating numbers, hence a depression of the normal CD_4 :CD_8 ratio. Again certain subsets are particularly influenced (Giorgi JV, et al, 1987, Gupta S, 1986, Giorgi JV, et al, 1993). These changes may represent cytotoxic responses against HIV due to frequent antigenic stimulation by circulating HIV viral proteins or be an attempt to down regulate other immune responses such as polyclonal activation of B cells. Addition of CD_8 + cells to CD_4 lymphocyte cultures suppresses HIV propagation (Walker CM, et al, 1986). Late in the course of disease, AIDS patients

show a decline in all lymphoid cell lines including the CD_8^+ cells (Lane HC, et al, 1985). The CD_4 : CD_8 ratio has been used as a surrogate marker of HIV infection by a variety of authors although its popularity has waned in recent years in favor of CD_4 counts (Taylor JMG, et al, 1989). CD_8 positive lymphocyte counts do not appear to undergo dramatic changes during treatment with currently available therapies.

Beta 2 Microglobulin is a protein product of mitogen stimulated lymphocytes (Kubo RT, et al, 1976) which binds to class 1 major histocompatibility antigens on most nucleated cells and plays an imprecise role in the immune response. It is renally excreted and levels are elevated in renal dysfunction (Karlsson FA,et al 1980). The elevated levels in HIV infection may be due to either increased production during lymphocyte activation or increased release upon lymphocyte destruction. Levels of β2 microglobulin are negatively correlated with CD_4 lymphocyte numbers (Antonen J, et al, 1987). Studies have shown a correlation between elevations in β2 microglobulin, AIDS and reduced survival. Falls in β2 microglobulin occur with ZDV therapy and remain predictive of survival at 8-12 weeks of therapy (Jacobson MA, Bacchetti P, et al, 1991).

Neopterin, a metabolite of guanosine triphosphate, is produced by macrophages when they are stimulated by interferon gamma from activated T cells (Huber C, et al, 1984). Serum and urinary neopterin levels have been shown in several studies to be elevated in HIV infection and to correlate with prognosis (Fuchs D, et al, 1988, Melmed RN, et al, 1989). It may improve the power of the CD_4 + T cell as an indicator of prognosis when used in combination and may predict the future rate of decrease in CD_4 cells (Melmed RN, et al, 1989).

VIROLOGICAL MARKERS

The principal virological marker of HIV disease until recently has been the p24 core antigen (also called HIV antigen) which has been proposed as both a prognostic marker (Pedersen C, et al, 1987 Paul DA, et al, 1987) and a method of monitoring response to antiretroviral therapy (de Wolf F, et al, 1988 Chaision RE, et al, 1986). The p24 antigen is well correlated with plasma viraemia although virus may be cultured in serum of patients in the absence of p24 antigen (Ho DD, 1989). The p24 antigen tests based on ELISA techniques thus have limitations of false negative results and reduced sensitivity, usually due to the presence of p24 antibodies (Lange JM, 1987) although improved detection and quantitation can be achieved by pretreating serum samples with an acid solution (Nishanian P, et al, 1990). Using standard methods different tests show variable sensitivity and the results may not be reproducible for the same sample (Kruppenbacher JP, 1988). After initial infection, when p24 antigen may be detected, levels rapidly fall following seroconversion and production of anti p24 antibodies (von Sydow M, et al, 1988). p24 antigen is not detectable in the serum of 85 percent or more of asymptomatic HIV seropositive men (Fahey JL, et al, 1990) although levels increase with clinical stage, due to either increasing production of antigen or decreasing production of anti-p24 antibody, only about two thirds of patients ever show detectable levels on conventional assays (Goudsmit J, et al, 1986). P_{24} antigen is therefore a marker of disease stage and may also be a predictor of survival (Fahey JL, et al, 1990). P_{24} antigen levels fall rapidly with nucleoside therapy but this decline may be short lived, particularly if resistant virus develops. Fall in p_{24} antigen may be an indicator of antiviral activity of a compound but fall in p_{24} with therapy has not been shown to correlate with prolonged survival. Absence of antibody to p24 antigen is a better predictor of HIV plasma viraemia (Coombs R, et al,

1989) and is also a prognostic indicator (Allain JP, et al, 1989) but tests for this are not currently widely available, and presence of antibody has not been shown to increase with nucleoside treatment. More recently, quantifiable amplification techniques such as RNA PCR and branch-chain DNA have been proposed as potential baseline markers of disease risk and as dynamic treatment-dependent variables. Although further validation against clinical endpoints is required, it appears likely that these techniques will be used widely as the principal virologic markers in the future.

CLINICAL MARKERS

Body weight and other markers of nutritional status such as body mass index, lean body mass and serum albumin have been observed as prognostic markers in a number of diseases including HIV and AIDS. Studies in advanced cancer patients using intravenous hyperalimentation have shown success in achieving weight gain, but no improvement or a decrease in survival time (Clamen G, et al, 1985, Samuels M, et al, 1981, Nixon DW, et al, 1981) suggesting at best a limited correlation between improvement of these parameters with survival. Low albumin is a better marker of infection than nutritional status in patients with malnutrition and this is also likely to be the case in AIDS. Weight loss principally occurs in AIDS at times of active infection due to many mechanisms including reduced intake, malabsorption and diarrhoea, hypermetabolism and inefficient use of nutrients (so called futile cycling). Stability or rising weight (and albumin) may therefore be a better used as a marker of health and possibly quality of life. Kotler et al (Kotler DP, 1989) showed in AIDS patients that a fall in lean body mass to 66 % of ideal was inevitably associated with death and suggested that death in these circumstances was due to the metabolic disruption of starvation, implying that, in some

circumstances, weight gain may prolong survival. Although weight gain may occur with ZDV therapy, this has not been directly correlated with improved survival.

Skin testing and anergy to common allergens have also be used by some researchers, particularly in the USA, as a marker of disease state and this has been incorporated into the Walter Reed Staging system for HIV disease (Redfield RR, 1986). It is not widely used in clinical trials in Australia or Europe at the present time.

USE OF DISEASE STATUS AS A CLINICAL TRIAL ENDPOINT

Development of clinical disease such as AIDS defining opportunistic infections and so called severe ARC have also been used as endpoints in clinical trials. Changes in practice, most notably the use of primary Pneumocystis carinii prophylaxis and the increased use of imidazole antifungals have resulted in a change in patterns of disease occurring as first AIDS diagnosis. Formerly Pneumocystis carinii pneumonia constituted upto 60% of first AIDS diagnosis in homosexuals males in the USA (Phair J, et al, 1990). This number has now fallen substantially, to around 33%. Systemic antifungal use at the time oral candida first appears has also led to a decline in the diagnosis of oesophageal candida, another AIDS defining opportunistic infection which commonly occurred relatively earlier in the natural history. Data from the San Francisco cohort suggested although survival from initial HIV infection to death may be increasing due to both antiretroviral treatment and improved treatment of individual opportunistic infections; survival from first AIDS diagnosis is declining due to changes in the natural history of HIV. Opportunistic disease is now occurring at lower CD_4 counts and manifesting as poorer prognostic disease such as B cell lymphoma, cytomegalovirus (CMV) infection,

disseminated Mycobacterium avium complex (MAC) and cryptosporidial diarrhoea (Osmond D, 1991, Brebinder S, 1991, Graham N, 1991).

QUALITY OF LIFE

Quality of life (QOL) or functional status (e.g. Karnofsky score) have not been validated as surrogate markers of survival or disease progression but have been used as secondary end points in clinical trials. Quality of life particularly the aspects of health and psychological status decline with time and greater declines in QOL occur in patients with AIDS than in those with symptomatic disease. Information on QOL may be used to complement clinical data in trials and so assist patients and physicians in making informed treatment choices. Improvements in QOL have been noted in studies with ZDV but have not been correlated with improved survival.

SUMMARY

No surrogate markers currently available are ideal. The likely way forward with their use is to combine a battery of partial surrogate markers which collectively explain more of a treatments effect on clinical progression than each alone. Such a battery is likely include markers which look at both immunological and virological markers.

1.8. CLINICAL TRIAL METHODOLOGY AND USE OF HISTORICAL CONTROLS

Choosing the most appropriate design for a clinical trial is determined by multiple perspectives including medical or scientific objectives, statistical considerations, ethical and patient requirements. Furthermore, trials conducted with unlicensed medications have objectives set by the sponsoring pharmaceutical company which relate to both their regulatory and marketing needs. In the area of AIDS care, political and social factors, particularly in the USA, have led to some changes in trial design and drug development processes with the establishment of large compassionate use protocols collecting, largely, safety data as well as wider use of open label studies, the concept of large simple trials and the development of "parallel track" licensing procedures.

To assess both efficacy and safety of a new drug the ideal trial design is a prospective, randomized blinded placebo controlled study. Alternatively, when an established therapy is available (e.g. ZDV in HIV disease) this can be used as the comparator, although a placebo arm specifically enables proof of efficacy against no therapy. The use of randomization enables bias based on physicians pre-perceptions to be limited and provide a sound basis for statistical analysis.

Placebo controlled trials in HIV specific therapy have largely been limited to those with ZDV and are specially problematic with patient groups who generally prefer "treatment" to "no treatment" particularly in the light of *in vitro* and phase I/II evidence of surrogate efficacy. The failure to recruit onto the placebo arm of the Alpha Study in Europe and Australia is strong evidence of this phenomena although the limited efficacy evidence gained

from this study suggest such a study may still be of value (Darbyshire JH, Aboulker JP, 1992). As the scientific merit of a study containing no control group is considerably limited, the possible options for a single arm prospective study are to use case controls or historical controls from a data base.

All clinical trials, whether randomized, controlled or not should be interpreted in the historical light of previous studies in the same sphere of practice. Those results inconsistent with previous data will tend to be viewed with skepticism whereas those yielding results in keeping with expectations will be readily accepted. Information gained in the past is the guide to construction of future trials.

The use of historical controls has both advantages as well as pitfalls. Smaller numbers of patients are required enabling the trial to be completed more rapidly. Such trials are easier to recruit as patients and doctors do not have dilemmas about the use of placebos, the patient is assured the new, presumably active, agent. Reducing the number of patients not entering the study therefore reduces some recruitment bias. However, bias can enter due to changes in diagnostic procedures or other aspects of clinical care thus making comparisons of some endpoints invalid. For example, the advent of primary and secondary prophylaxis against *Pneumocystis carinii* pneumonia into HIV care during 1987 largely invalidates the use of data from prior to this time for survival, opportunistic infection attack rates and progression to AIDS as this infection was a common cause of death and the most common first AIDS defining illness at that time.

Historical control groups also do not have clearly defined entry criteria and are frequently not monitored as closely as those in a clinical trial. The availability of a new potential treatment may also alter physician decision making. Thus the availability of ddI for ZDV-intolerant patients may result in the doctor choosing to define a patient as intolerant, due to, say, nausea or headaches whom, given no therapeutic option, would otherwise have been encouraged to continue therapy.

Green and Byar warn of the fallacy of omnimetrics "the ill-conceived idea that if one identifies the right things to measure and develops the appropriate scales of measurement, then one can determine all that one needs to know about prognosis and can make valid treatment comparisons ... there are many reasons why treatment is chosen in clinical practice ... treatment assignments are tied up very closely with prognosis thus producing inherent bias" (Green SB, 1984). Randomized trials provide a defined "time zero", whereas this may vary between historical controls. (Byar DP, 1991). Specifically, changes in the use of ZDV over late 1980s due to the influence of studies demonstrating the efficacy of lower doses and earlier interventions alters the "time zero" in successive years. Such problems can be limited by conducting trials using the same groups of physicians the same clinic (hence equivalent diagnostic facilities) as were used during the construction of our historical control data base and by choosing that part of the data base most close in time to the trial for comparison. Awareness of known prognostic characteristics, such as disease stage, CD_4 count, hemoglobin when constructing the historical data base enables meaningful comparisons to be made with new treatment group thus limiting the problem of Simpson`s paradox (Green SB 1984). Large databases of historical controls also enable the physician to "fish" for data or go "data dredging" to find a

comparison which reaches statistical significance. The definition of specific hypotheses to be tested prior to commencing the trial helps to avoid this search by creating defined goals of study, such as survival, CD_4 and p24 changes and changes in clinical markers such as weight.

The net result of these problems is generally considered to be exaggeration of the value of a new treatment. Adjusted analyses may go some way in reducing bias although historical data often have only limited details on some co-factors (e.g. Performance Status) and other factors may not be included.

The use of concomitant case controls is generally impractical with new HIV therapies as most patients choose the new option rather than no further therapy. Those refusing the study may do so for particular reasons such as performance status, or lack of desire to participate in the rigors of a trial. Matched historical case controls have many similar disadvantages to historical control data bases. "Perfect matches" are particularly difficult to find even from a large patient group particularly if large numbers of baseline variables are to be matched.

In conclusion, confirmatory studies are essential for all clinical trials. Results will not gain complete acceptance unless they can be repeated by other investigators treating a similar patient group. This is especially relevant for historical control studies where false positive results may be more common. However, a well constructed historical control trial can and do yield valuable information. Pocock suggested that the requirements for a valid historical control group were 1) the historical group received a precisely defined treatment in a recent previous study 2) that the eligibility of patients, diagnostic procedures, techniques of evaluation of treatment,

and other features related to outcome should be known and controlled in both groups 3) that prognostic features should be known and controlled in both groups and 4) that there should be no unexplained indications leading on to different results between treatment groups (Pocock SJ, 1976).

2. PART II: METHODOLOGY & RESULTS

METHODOLOGY, RESULTS AND SHORT DISCUSSIONS

2.1. GENERAL METHODOLOGY

The John Hunter Clinic at St Stephen's Hospital is a long established centre in London SW10 for the treatment of sexually transmitted disease (STD). Together with Outpatient Clinic number 6 (OP6) at Westminster Hospital, London SW1, it provides comprehensive services covering all facets of STD investigation and treatment for the East Riverside district of South West London. In 1988 a further unit, the Kobler Centre opened at the John Hunter Clinic site to provide outpatient and day case investigation and treatment facilities for persons infected with HIV.

Laboratory back-up was provided initially by pathology services based at St Stephen's Hospital London SW10 until its closure in 1990 when staff and equipment were relocated to the Westminster Hospital, London SW1. Similarly, inpatient facilities for care of persons with HIV and AIDS were transferred intact from St Stephen's to Westminster hospital at that time.

Patients recruited to the studies mentioned in this thesis and whose data entered the data base were drawn from three sources:

John Hunter Clinic, London SW10. After the establishment of the Kobler centre some patients with HIV continued to have regular follow-up in this unit, which was also the point of HIV testing for the London SW10 site.

The Kobler Centre, London SW 10.

OP6, London SW1.

These 3 units shared the same clinic guide of treatment recommendations as well as interchanged staff and shared the same inpatient facilities. HIV and AIDS care at each site was under the directorship of the Head of HIV care in East Riverside Dr. Brian Gazzard.

All Clinical trials in East Riverside required approval by the local ethics committee after submission of a final protocol. Any amendments to the protocol also required approval from the ethical committee chairman. Information sheets were used to back up verbal discussions to enable full informed consent from patients.

Laboratory services were provided within East Riverside by facilities based at St Stephen's and Westminster sites. The main departments used were Hematology (Dr. Christine Costello), Chemical Pathology (Dr. John Stevens) and Microbiology (Dr. David Shanson).

The Vacutainer ® system of blood collection was used at all sites with blood generally being draw by clinic nursing staff. Preservatives and anticlotting agents were used with some samples including Lithium Heparin with Chemical Pathology specimens, Potassium oxalate and Sodium fluoride with Glucose and EDTA with full blood counts. No additives were used with samples for HIV antibody or p24 antigen tests.

The CD_4 lymphocyte counts were measured on a Becton-Dickinson autoanalysing flow cytometer. This uses the Simutest®, immune monitoring kit which utilizes lysed whole blood to measure lymphocyte subsets and was checked for accuracy during 1990 by the Medical Research Council and found to be consistent to within 10%.

Collectively patients from these three clinics will be described as the St Stephen's cohort throughout this thesis.

2.2. AZT-TREATED HISTORICAL CONTROLS

INTRODUCTION

The St Stephens' ddI study was planned as an open label single arm study, as a placebo controlled study was not thought ethical in the patient groups of ZDV intolerant patients, following discussion with patients advocacy groups and physicians at the clinic.

As this study was conducted at a single unit, the use of historical control data was thought feasible, particularly given that the personnel, diagnostic procedures, laboratory facilities and patient demography had been consistent for several years prior to this study. Data from a previous similar study was not available and, therefore, data was collected from patient files, pharmacy and laboratory records. A number of possible prognostic markers were collected together with clinical data and dates of death. Details of concomitant medications, specifically for *Pneumocystis carinii* prophylaxis were collected as it was known that wide spread use of these therapies for primary and secondary prophylaxis were not fully instituted until 1988. The dose of ZDV in patients fully tolerating this drug was 1 - 1.2g/day in 1987, 1988 and most of 1989 with 600 mg being increasingly used as the standard dose from late 1989.

Following the establishment of this data base survival data was regularly updated and new patients commencing ZDV added, laboratory and clinical prognostic markers being collected prospectively. Following the publication of data by Yarchoan and colleagues (Yarchoan R, Venzon DJ, et al, 1991) showing the association with CD_4 and risk of death, our, larger, cohort was analyzed to confirm this report.

2.2.1. Objectives

The objective of establishing the historical data base of ZDV-treated patients was to create a legitimate, comparable control group for the ddI study. As comparison of raw data was expected to contain substantial bias (see section 2.8). Analysis of the control group was performed, aimed at finding the group of patients most closely matched to those patients entering the ddI study.

2.2.2. Patient Population

Data for these analyses included baseline characteristics and survival experience of all patients with AIDS or ARC within the East Riverside Health Authority, who received ZDV therapy from the first date of ZDV availability. 598 patients had initiated ZDV therapy from June 1986 until February 1990. This number excludes patients enrolled in the placebo controlled double-blind Concorde study, which was on-going at that time. Of this group of 598 patients, data on baseline characteristics and survival were available for 529 patients. Thirteen patients, documented to be HIV-positive but without symptoms of AIDS or ARC at initiation of ZDV, were excluded from this analysis. The final group of all ZDV patients thus consisted of 516 patients with AIDS or ARC at baseline, as defined by 1987 United States Centers for Disease Control (CDC) criteria. They initiated ZDV therapy during the interval 23 September 1986 to 16 February 1990 (the date of commencement of the ddI trial).This group of patients is identified as Historical Control Analysis I (HCA 1) throughout this report. A further analysis of 1415 patients treated with ZDV up to October 31, 1991 will also be presented as Historical Control Analysis II

(HCA 2). This analysis includes all patients commencing ZDV during that time period regardless of disease status.

Patient characteristics analyzed included age, sex, diagnosis (Asymptomatic, ARC or AIDS), risk factors for HIV acquisition and date of initiation of ZDV therapy. Baseline laboratory data included hemoglobin, white blood cell count, granulocyte count, platelet count, HIV immunologic markers (CD_4 Lymphocyte count) and HIV virologic markers (p24 antigen level).

Laboratory data taken within sixty days prior to the start of ZDV was accepted as baseline.

Survival was assessed from date of initiation of ZDV therapy to the date of death or date last known alive. Ascertainment of date of death was from hospital records, local death records and the national death index. Data available as of 17 September 1990 were included in the first survival analysis (HCA 1) and to December 31, 1991, for the second analysis (HCA 2).

2.2.3. Statistical Methods

All analyses were performed using SAS (Statistical Analysis System, SAS Institute, North Carolina. USA). Proportions were compared using the Fisher's Exact test and continuous variable distributions in different subsets were compared using the Wilcoxon Rank sum test. The survival

durations for various subsets of patients were compared using the log rank test. The proportion of surviving patients in each group was planned as a function of time using the Kaplan-Meier product limit estimates. The 95 percent confidence intervals for the median survival were estimated using the nonparametric method.

The effect of baseline characteristics as prognostic factors for duration of survival was estimated using the Cox proportional hazards model. Using a forward stepwise procedure, a factor entered the model if it was significant at the 0.10 level; it was removed if its significance level was greater than 0.15 upon entry of additional factors. The potential prognostic factors used in the analysis were age at start of ZDV therapy ($\geq$ 35 yrs versus < 35 yrs), AIDS or ARC, baseline CD_4 ($\geq$ 100 cells/mm^3 versus c 100 cells/mm^3), p24 antigen level ($\geq$ 32 pg/ml versus < 32 pg/ml), hemoglobin ($\geq$ 11 g/dl versus < 11 g/dl), WBC ($\geq$ 4000/μl versus < 4000/μl) and granulocyte count ($\geq$ 2000/μl versus < 2000/μl).

Indicator variables for the year of start of ZDV therapy were always retained in the model regardless of significance level. The p-values, regression coefficients beta and standard error of beta are reported in the final model for the year of starting ZDV therapy and for factors with p-value < 0.10.

In HCA 1 survival analyses by baseline characteristics and prognostic factors as defined were performed on the entire group of 516 evaluable patients receiving ZDV. Additionally, analyses were performed by year of initiation of ZDV to detect changes in survival with time. For these analyses, the 516 patients were grouped in the 1987, 1988 or 1989 cohorts according to the date of initiation of ZDV therapy: 1987=

September 12, 1986 - December 31, 1987; 1988 = January 1, 1988, December 31, 1988; 1989 = January 1, 1989 - February 16, 1990.

2.2.4. Results Of Analysis Of Historical Controls Data: HCA 1

HCA 1:

PATIENT CHARACTERISTICS:

The baseline characteristics of the 516 eligible patients were analyzed at start of ZDV therapy for the entire population and by year of ZDV initiation (Table 1). The median age of the population was 35 years (range 19 years to 69 years) with a predominance of homosexual Caucasian males. There was also an increase in the percentage of patients starting ZDV with ARC from 1987 (36 percent) to 1989 (71 percent: p-value <0.001). Overall, 57 percent of the population had ARC at the start of ZDV therapy and 43 percent had AIDS. Eighty-four percent of the patients with AIDS began therapy with ZDV within one year of the diagnosis.

The median CD_4 cell count at start of ZDV therapy was 132 cells/mm^3 (range 1 - 1008 cells/$mm^{3)}$. Patients with AIDS had a median baseline CD_4 count of 71 cells/mm^3 (range 1 - 630 cells/mm^3 whereas patients with ARC had a median CD_4 count of 180 cells/mm^3 (range 5 - 1008 cells/mm^3) at the start of ZDV therapy. There was a trend toward increasing baseline CD_4 counts by year of ZDV initiation from 1987 to 1989 (Table 1; p-value = 0.030).

Baseline levels of p24 viral antigen were obtained in 473 patients. Overall, 26 percent of these patients had detectable levels of p24 antigen (≥ 32 pg/ μl) at the start of ZDV therapy. There was no difference in the proportion of patients with detectable p24 levels by year of ZDV initiation (p-value = 0.518).

The median hemoglobin level at baseline was 13.3 g/dl (range 8.1 g/dl - 17.5 g/μl). There was an increase in baseline hemoglobin values when analyzed by year of ZDV initiation (Table 1; p-value = 0.026).

The median white blood cell count (WBC) was 4400/μl (range 1,500/μl - 14,100/μl) at the start of ZDV therapy. Sixty-two percent of the patients had baseline WBC ≥ 4000/μl. The median baseline WBC varied significantly by year of ZDV initiation (p-value = 0.002).

Table 1 Patient Characteristics At Start Of ZDV

Number of Patients (%) by Year

	1987	%	1988	%	1989	%	All ZDV Patients	%
Patients Analyzed	138		160		218		516	
Age (y)								
Median	33		35		35		35	
Range	19-69		20-66		19-65		19-69	
Sex								
Male	136	99	158	99	214	98	508	98
Female	2	1	2	1	4	2	8	2
Diagnosis								
AIDS	88	64	68	42	64	29	220	43
ARC	60	36	92	58	154	71	296	57
CD_4 cell count (cells/μl)								
Patients evaluable	114		136		207		457	
<50 cells/μl	29	25	24	18	40	19	93	20
50-99 cells/μl	31	27	29	21	31	15	91	20
≥ 100 cells/μl	54	47	83	61	136	66	273	60
P24 Level								
Patients evaluable	116		148		209		473	
< 32 pg/ml	83	72	109	74	157	75	349	74
≥ 32 pg/ml	33	28	39	26	52	25	124	26
Hemoglobin								
Patients evaluable	136		149		212		497	
< 11 g/dl	24	18	13	9	22	10	59	12
≥ 11 g/dl	112	82	136	91	190	90	438	88

HCA 1: SURVIVAL BY YEAR OF ZDV INITIATION

Median survival of the 138 patients who began therapy with ZDV during 1987 was 100 weeks from start of ZDV. Ninety-nine deaths were reported. In the group of 160 patients who began ZDV therapy during 1988, median survival was 121 weeks, with 62 deaths reported. Ninety-two percent of the 218 patients who began ZDV therapy during 1989 were alive 1 year after the start of ZDV therapy. Overall there was a significant prolongation of survival by year of start of ZDV therapy (unadjusted p-value = 0.0001)(Figure 1).

HCA 1: EFFECT OF PATIENT CHARACTERISTICS ON SURVIVAL DURATION

The effects of characteristics and year of initiation of ZDV therapy on survival were assessed for the 516 patients using the Cox proportional hazards model. Year of initiation of ZDV treatment was included in the model regardless of the level of significance. Factors which emerged with significant negative impact on survival duration (final p-value < 0.10) included initiation of treatment during 1987, the diagnosis of AIDS at baseline and hemoglobin < 11 g/dl at start of ZDV therapy (Table 2). Baseline CD_4 count $\geq$ 100 cells/mm^3 had significant positive impact on survival from start of ZDV therapy (adjusted p-value < 0.001). Initiation of ZDV therapy during 1988 was of borderline prognostic significance (adjusted p-value = 0.103). Prognostic factors at baseline which did not emerge from the model with significant impact on survival included age, p24 antigen level, WBC and granulocyte count.

Table 2 Survival Duration: Effect of Pretreatment Characteristics

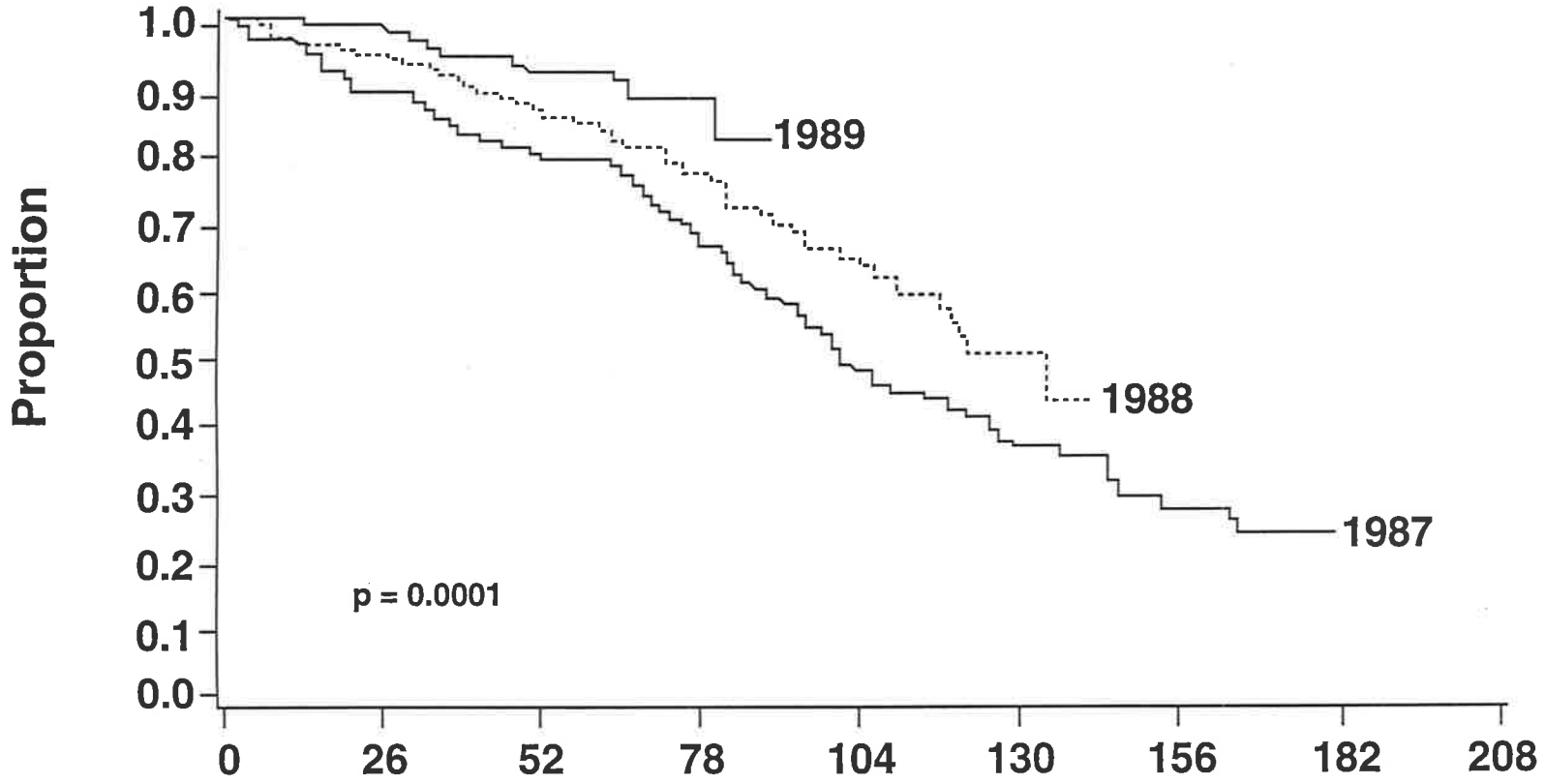
Factor	Final p-value
Start ZDV = 1987	0.026
Start ZDV = 1988	0.103
CD_4 count $\geq$ 100/μl	<0.001
Diagnosis (0=ARC;1=AIDS)	0.001
Hemoglobin <11g/dl	0.002
Age $\geq$ 35y	0.627
P24 $\geq$ 32 pg/ml	0.368
WBC <4000/μl	0.421
Granulocytes <2000/μl	0.377

HCA 1: SUBSEQUENT THERAPY WITH DDI

Overall, 103 of all 516 ZDV patients (20 percent) went on to receive subsequent therapy with ddI. Fifteen percent of patients who initiated ZDV therapy in 1987 received second-line treatment with ddI, compared with 27 percent of patients who began ZDV therapy in 1988 and 18 percent of patients in the 1989 cohort. Kaplan-Meier analysis revealed a significant trend toward earlier initiation of second-line ddI therapy in the later years of ZDV therapy (p-value = 0.001). (Figure 1)

Figure 1

All ZDV patients survival by year



1987 N = 138	Median survival 100 (95% CI 91-119)
1988 N = 160	Median survival 121 (95% CI 111-)
1989 N = 218	Median survival not reached

HCA 1: DISCUSSION

This analysis of all 516 ZDV-treated patients revealed a median survival of 121 weeks from initiation of ZDV. Eighty-seven percent of patients survived one year; 58 percent of patients were alive at two years from start of ZDV. Overall, patients with the diagnosis of AIDS had significantly poorer outcome than patients with ARC at the start of ZDV therapy (adjusted p-value < 0.001)

This survival experience is comparable to the published data from clinical trials of ZDV treatment of AIDS and ARC. (Fischl M, Richman D, Greico M, et al, 1987, Fischl M, Parker CR, et al, 1990, Creagh-Kirk J, et al, 1988). The St Stephen's patients who began therapy with ZDV in 1987 had shorter survival than those who started treatment in 1988 or 1989. The median survival of the St Stephen's patients with AIDS and baseline $CD_4 \geq 100$ cells/mm^3 who initiated ZDV therapy in 1987 was 125 weeks, (88 percent at 1 year, 52 percent at 2 years). These results are at least equivalent to the experience in AIDS patients with baseline $CD_4 > 100$ cells/mm^3 enrolled in the Study BW 002. Patients in the latter study initiated ZDV therapy between December 1986 and November 1987 and had a median survival of 93 weeks (83 percent at 1 year, 38 percent at 2 years) (Fischl MA,Parker CR 1990).

As with all analyses of historical control populations, the prognostic variables already identified and others, such as patient compliance with the treatment regimen and differences in supportive care measures, (e.g. PCP prophylaxis) may not be equivalent between years.

2.2.5. Results of Analysis of Historical Controls Data: HCA 2

Following the establishment of the ZDV historical data base, all new patients commencing ZDV in East Riverside were added to the data base. A further analysis was performed including patients who had commenced ZDV upto October 1991. The demographics of this larger group were similar to those in HCA 1 although asymptomatic patients were included reflecting changing patterns of care at that time.(Table 3)

Table 3: HCA 2: Patient Characteristics

Mode of HIV Acquisition	Number	%
Homo/bisexual	1237	(87.4)
Heterosexual	90	(6.4%)
IVDU	61	(4.4%)
Blood products	9	(0.6%)
Unknown	18	(1.2%)
	1'415	(100%)

Disease Stage		
AIDS	476	(33.6%)
Symptomatic non AIDS	687	(48.6%)
Asymptomatic	194	(13.7%)
Unknown	58	(4.1%)

Median CD_4 by disease stage

AIDS	77 cells/mm^3	
Symptomatic	194 cells/mm^3	
Asymptomatic	269 cells/mm^3	

HCA 2: STATISTICAL METHODS AND RESULTS

For HCA 2 analysis was restricted to examination of survival only. Analysis of survival used data on death upto December 31, 1991 at which time 432 patients had died. Patients alive at that date were censored and a Kaplan-Meier life table analysis used to examine survival distribution according to when CD_4 cell count first fell into the ranges of $\leq$50, 51-100, 101-150, 151-200 cells/mm^3. Survival time was thus calculated from time of first CD_4 count in a defined range to date of death or censoring.

Table 4 shows the survival estimates from when a patients CD_4 count first entered the defined range. The 1 and 2 year survival once patients CD_4 counts fell below 50 cells/mm^3 was substantially shorter than those with CD_4 counts in higher ranges. The $\leq$ 50 cells/mm^3 group was the only group to reach a median survival time after 2 years follow-up. Of note over 25% were still alive after 2 years.

When the < 50 cell patient group is subdivided into 10 cell ranges ($\leq$ 10, 11-20, 21-30, 31-40 and 41-50) further trends are seen towards poorer survival with lower CD_4 counts with the CD_4 < 10 cells/mm^3 group having only a 17.8% survival at 2 years, compared with 36.4% of those patients in the 41-50 cells/mm^3 range surviving to 2 years.

Table 4 Cumulative percent of Zidovudine-treated patients surviving from first CD_4 lymphocyte count within defined ranges

			Cumulative % surviving (SE)			
Range of CD4 lymphocyte count (cells/mm^3)*	No. of patients	No who died	12 months	18 months	24 months	Median survival (months)
151-200	186	29	97.4 (0.01)	94.6 (0.05)	76.5 (0.05)	-
101-150	180	43	93.2 (0.02)	81.7 (0.03)	67.3 (0.05)	-
51-100	252	85	87.0 (0.02)	68.2 (0.04)	51.4 (0.05)	-
≤ 50	552	288	68.5 (0.02)	42.8 (0.03)	25.7 (0.03)	17.3
First CD_4 cell count≤ 50/mm3						
41-50	142	55	83.2 (0.04)	58.4 (0.06)	36.4 (0.06)	20.8
31-40	134	61	76.6 (0.04)	46.7 (0.06)	29.5 (0.06)	19.0
21-30	126	67	63.5 (0.05)	35.7 (0.05)	22.7 (0.05)	16.5
11-20	88	60	59.8 (0.06)	33.9 (0.07)	15.9 (0.05)	16.0
≤ 10	62	45	47.0 (0.07)	31.6 (0.07)	17.8 (0.06)	11.0

** 108 patients who had no CD_4 measurements during follow-up and 431 patients with no CD_4 counts below 200 were excluded from the analysis. Since a patient with a CD_4 count falling into the range 151-200 cells/mm^3 may have a subsequent CD_4 measurement in the range 101-150, 51-100, or ≤ 50 cells/mm^3, a single patient may contribute to the survival estimates of more than one CD_4 category. SE, standard error.*

365 of the 432 patients in this cohort who had died had at least one CD_4 cell count measure in East Riverside in the 2 years prior to their death. Table 5 shows the distribution of CD_4 counts at various intervals during this period. The percentage of patients with CD_4 counts below 50 increased from 33% at 24 months to 93% at 1 week. The corresponding median CD_4 cell counts at these times were 113 at 2 years prior to death to 7.5 in the week prior to death.

Table 5 CD_4 cell counts among Zidovudine-treated patients in the 2 years prior to death

	Months prior to death							
	-24 (n=42)	-18 (n=89)	-12 (n=107)	-9 (n=147)	-6 (n=143)	-3 (n=148)	-1 (n=100)	-1 week (n=14)
CD_4cell count/mm^3								
Median	113	70	35	29	17	13	11	7.5
0-49	14 (33)	36 (40)	62 (58)	106 (72)	119 (83)	119 (80)	86 (86)	13 (93)
50-99	6 (14)	17 (19)	22 (21)	17 (12)	11 (8)	14 (10)	7 (7)	0
100-199	14 (33)	24 (27)	17 (16)	15 (10)	9 (6)	8 (5)	4 (4)	0
$\geq$ 200	8 (19)	12 (14)	6 (6)	9 (6)	4 (3)	7 (5)	3 (3)	1 (7)

Data in parentheses are given as percentages. Percentages may not add up to 100, because of rounding error.

Fourteen patients had CD_4 counts greater than 50 cells/mm^3 in the month prior to death with 7 having $CD_4 \geq 100$ cells/mm^3. The causes of death in these patients were attributed to PCP (4 patients), disseminated Mycobacterium avium complex (1), lymphoma (2), Kaposi's sarcoma (2),

alcoholic liver disease with hepatic encephalopathy (2). The cause of death was not ascertained in 3 patients. Patients dying with CD_4 counts >50 cells/mm^3 were significantly older at time of commencing ZDV (median 49 years) and death (median 50 years) compared with those who died with CD_4 <50 cells/mm^3 (median age at start of ZDV 34 years, at death 36 years), ($p < 0.001$). There were no significant differences between the two groups in either HIV risk category, clinical status at start of ZDV therapy, duration of ZDV therapy or use of PCP prophylaxis.

DISCUSSION

This data suggests a strong association between CD_4 count and risk of death, confirming previously published data. However, this data does not examine change of CD_4 with therapy and, therefore, cannot be used to support the assertion the CD_4 cell count is a valid surrogate marker in assessing therapies for HIV disease.

2.3. THE DDI STUDY

INTRODUCTION

At the time of commencing this study ddI had not been licensed in any country but phase I/II studies had shown, in small numbers of patients, that ddI therapy led to rises in CD_4 cell counts and falls in HIV p24 antigen. The dose limiting toxicity of peripheral neuropathy had been identified, being particularly common a higher doses. From dose ranging studies, doses below 12.5 mg/kg/day had been identified as providing surrogate marker efficacy with a reduced risk of intolerance. Given the differing side effect profiles of ZDV and ddI, ZDV intolerant patients had been identified as the most suitable candidates for ddI therapy.

There were considerable expectations placed on ddI by both physicians and patients; it was hoped that ddI would in some way revolutionize the treatment of AIDS, as it was the first drug of a 'new generation' of 'safer', 'better' anti-HIV therapies. If nothing else it was doubling the options for antiretroviral therapy. Following discussions with physicians at the clinic and with patient advocacy groups it was concluded that a placebo controlled study in ZDV intolerant patients was not feasible.

As the study was to be conducted in a single unit the use of a historical control group was thought feasible, and specifically, this would enable more meaningful conclusions regarding a survival benefit of ddI to be reached. A single arm open label study was designed and a Case Report Form developed with the cooperation of Bristol Myers company (Later Bristol Myers Squibb).

The trial commenced on 16 February 1990 with recruitment of 151 patients (one more than originally planned) being completed by 18 January 1991. As limited safety data was available on ddI at that time, an interim analysis was planned on the first 100 patients. This number of patients having been recruited by 15 May 1990.

2.3.1. Objectives

The primary objective of this study was to evaluate the efficacy and safety of a weight-adjusted dose of ddI, administered orally twice a day, in the treatment of patients with AIDS or ARC who had received prior therapy with Zidovudine (for at least eight weeks) and developed intolerance to that drug.

2.3.2. Study Design

The trial was designed as an open label, single-institution, prospective phase II study of ddI in previously ZDV-treated AIDS and ARC patients with intolerance to ZDV. The dosage of ddI was adjusted according to body weight, and treatment with ddI could continue for up to 18 months.

The protocol was submitted to and approved by the Ethics Committee of the Riverside Health Authority, covering both the Westminster and St Stephen's Hospitals.

A case record form was developed in conjunction with Bristol-Myers Squibb incorporating clinical, laboratory, and quality of life information (Appendix 5).

2.3.3. Material And Methods

2.3.3.1. Patient Selection

The study was opened to adult patients (16 years or older) with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC), as defined by 1987 United States Centers for Disease Control (CDC) criteria. All patients had to have received prior treatment with Zidovudine for at least eight weeks. Prior Zidovudine treatment was administered at the Kobler Unit (outpatient clinic) of the St Stephen's Hospital or at the Westminster Hospital in London. Patients had to have experienced intolerance to Zidovudine therapy, including hematologic, gastrointestinal and other forms of intolerance. The ascertainment of Zidovudine intolerance was left to the referring physician's discretion.

Baseline laboratory tests were required to meet the following criteria: leukocyte count $\geq$ 750/mm^3, platelet count > 50,000/mm^3, hemoglobin concentration $\geq$ 8 g/dl, serum creatinine < 240 μmol/L, uric acid < 0.9 μ mol/L, SGOT < 5 times the upper normal limits.

Signed informed consent was required. Next-of-kin written consent was acceptable for patients with CNS impairment.

A number of exclusion criteria applied: presence of malignant neoplasms other than Kaposi's sarcoma, pregnancy or breast feeding, refusal of adequate contraception, history of acute or chronic pancreatitis. Patients with active acute opportunistic infections or poorly controlled seizure disorder, as well as patients receiving neurotoxic drugs within 3 months from start of ddI, were not eligible. A list of neurotoxic drugs was provided

in the Appendix to the study protocol. A list of drugs with known or potential pancreatic toxicity was included, and their use discouraged.

2.3.3.2.

Therapy

The treatment was administered on an outpatient basis, whenever possible. Patients were administered ddI orally every 12 hours, and the dosage was assigned according to pretreatment body weight (Table 6). The dosage could be modified for body weight variations subsequent to the starting of treatment.

Table 6 Dosage

Body weight	ddI dose
> 60 kg	375 mg b.i.d.
40 - 60 kg	250 mg b.i.d.
< 40 kg	167 mg b.i.d.

ddI for oral administration was provided by Bristol-Myers Squibb as a buffer powder blend packaged in sealed foil sachets in several strengths (100 mg, 167 mg, 250 mg and 375 mg of ddI per sachet). Each sachet contained 5.2g of citrate/phosphate buffer. Sucrose was included in the formulation to counteract the salty taste of the buffering ingredients, with the amount of sucrose adjusted to yield a final net weight of 20g per sachet. Sachets were stored at room temperature in the pharmacy of St Stephen's Hospital and the central pharmacy at Westminster Hospital.

Before administration, the content of each sachet was dissolved in approximately 120 ml of drinking water. The drug was administered on an empty stomach (at least 2 hours after and half an hour before taking other food or drink). Temporary interruption due to adverse effects or to receive other therapies was allowed. Prophylaxis for *Pneumocystis carinii* pneumonia followed the standard practice of the unit. Suppressive therapy for other previously diagnosed infections was also allowed. Use of erythropoietin was not permitted.

2.3.3.3. Study Parameters

Prior to enrollment into this study, patients received a complete history and physical examination. Assessment included ECG, neurological assessment, temperature and body weight. Information on prior Zidovudine therapy was documented at this time. A quality of life assessment, using a Spitzer Index questionnaire was carried out at baseline. Hematology tests included hemoglobin, hematocrit, white blood cell (WBC) count, differential, mean corpuscular volume, and platelet counts. Chemistry studies included serum glucose, urea, creatinine, total bilirubin, SGOT, calcium, phosphorus, sodium, potassium, amylase, triglycerides, uric acid and creatinine phosphokinase (CPK). Immunologic evaluation including CD_4 and CD_8 cell counts was performed two weeks prior to starting therapy and at baseline. Virologic evaluation included serum p24 antigen determination. Serum p24 antigen levels were assayed by the same laboratory using the Abbott Laboratories quantitation panel for HIV antigen. Female patients received a pregnancy test.

Medical history and physical examination (including careful assessment of neurological status and completion of the quality of life questionnaire) were repeated on day 14 and monthly thereafter. All blood evaluations were also repeated on day 14 and monthly thereafter.

2.3.4. Definitions And Criteria For Data Analysis

2.3.4.1.

Pretreatment Characteristics

Baseline values for all pretreatment characteristics were those on the first day (day 1) of ddI therapy.

Time from diagnosis was calculated as elapsed time from the first day of HIV positive test until the first day (day 1) of ddI therapy. When exact dates were not available, they were approximated to the 15th of the month.

The history of prior antiretroviral therapy was recorded from the patient's charts. Duration of prior zidovudine therapy was computed as the elapsed time from the first day to the last date of Zidovudine therapy.

2.3.4.2.

Efficacy

Efficacy was assessed based on improvement of (1) immunological status (CD_4 cell counts), (2) virologic status (serum p24 antigen levels), and (3) clinical observations (weight, changes in hemoglobin level and/or signs

and symptoms related to the disease). Quality of life was evaluated using the Spitzer index questionnaire. Efficacy analyses were performed in the entire patient population as well as in subsets of prognostic relevance (AIDS vs. ARC, baseline CD_4 groups, etc.)

IMMUNOLOGIC, VIROLOGIC AND WEIGHT STATUS

Changes in the CD_4 cell count, p_{24} antigen and weight over time while on ddI therapy were each analyzed by examining the mean change from baseline.

In addition the change from baseline, expressed as the natural logarithm of the ratio of the value in the interval (and pre) to the baseline value, was examined to determine if the log-ratio differed significantly from 0. Multiple log-ratios for a patient in the same interval were averaged.

Response: Response rates for CD_4 were calculated by defining the response as a 50% increase over baseline and a minimum increase of 50 cells observed for two consecutive observations at least four weeks apart. To be assessable for response by this definition, a patient had to have a minimum of two observations after the baseline value, at least seven days apart. The response rates were tabulated by dose groups and other prognostic factors. For CD_4 response rates were also calculated using less stringent criteria of CD_4 increase, i.e., 25% increase and 25 cells, or 10%/10 cell increase. For p24 response: the sensitivity of the p24 serum assays is such that values <31 pg/ml are not considered detectable or reliable. In patients with baseline p24 level above 64 pg/ml, the p24 marker response rates in the study population were calculated by defining the response as a 50% decrease from baseline observed for two consecutive observations at least four weeks apart. In patients with

baseline value between 32 to 63, the on-study p24 had to be below 31 for 4 weeks. To be assessable for response by these definitions, a patient has to have a minimum of two p24 observations after the baseline value, at least seven days apart. The response rates among assessable patients were tabulated by dose groups and other prognostic factors.

Weight response rates in the study population were calculated by defining the response as 2.5 kg increase over baseline observed for two consecutive observations at least four weeks apart. To be assessable for response by this definition, a patient had to have a minimum of two observations after the baseline value, at least seven days apart. The response rates among assessable patients were tabulated by dose groups and other prognostic factors.

Response duration: time to achieve a response was computed as the first day the "response" started. Among the responders, the duration of response was counted as the calendar interval from the day 1 of response to the first time the marker (CD_4, p_{24}, weight) returned to a value below the subjects' baseline value. For those responders in whom the value did not return below baseline the response duration was censored on the last date on which the marker was observed. By definition of the response criteria, the response duration is guaranteed to be at least four weeks for all responders.

The time on study spent at marker values equal to baseline or better is defined as the time on ddI therapy during which the marker value was at or above the baseline value. This is computed by subtracting from duration of therapy (a censored observation for those on therapy at the last contact) the length of time the marker value was below the baseline

value. Since the markers are sampled at 2 to 4 week intervals, a simple method of carrying the last observed value forward in time is used to estimate the period below baseline.

SIGNS AND SYMPTOMS

Changes in clinical signs and symptoms were independently assessed by the investigator as well as by the patient during the conduct of the study. A response was defined as a marked improvement of the most significant signs and symptoms noted at a given study visit with respect to the evaluation performed during the prior study visit.

ANEMIA

The change in hemoglobin value for prior to baseline (at least one week before day 1 of ddI) was examined to determine the direction and magnitude of the marker value prior to initiating ddI therapy.

Changes in the hemoglobin over time while on ddI therapy were analyzed by examining the mean change from baseline.

In addition, the change from baseline, expressed as the natural logarithm of the ratio of the interval (or pre) value to the baseline value was examined to determine if the log-ratio differed significantly from 0 at each interval. Multiple log-ratios for a patient in the same interval were averaged.

OPPORTUNISTIC INFECTIONS

AIDS-defining opportunistic infections (OI's) were classified as recurrent or new. The rates were tabulated by dose groups and other prognostic

factors. The time to the first OI was calculated from day 1 of ddI. The patients who remained OI-free were censored at the last day on ddI therapy.

QUALITY OF LIFE

Quality of life evaluation was based upon the Spitzer index score. Each of the five elements of evaluation (activity, daily living, heath, support and outlook) were analyzed separately, as well as a total score resulting from the sum of

individual scores. Since, for most of the parameters, most of the patients had an optimal (2 points) or near-optimal (1 point) baseline score, subsequent improvements of the index were less likely to be noted. Therefore, time-to-first-deterioration in the Spitzer index by one point or more in patients who started at or reached the higher scores of 2 or 1 were examined. In addition, patients who died within 30 days from the last administration of ddI were considered to have shown deterioration, even if a Spitzer index score was not available. The time was computed from day 1 of ddI therapy and censored on the last day the Spitzer index was noted for those patients whose index did not decrease by one point or more. For the total score, a similar approach was adopted, and considered time-to-first deterioration a decrease of the total score below 8 points in patients who started at or reached a high score of 8, 9 or 10. Similar to the individual parameters, death within 30 days from the last dose of ddI was considered equaling a score below 8.

SURVIVAL

Survival time on ddI study was calculated from day 1 of ddI until the death date. Those who were alive at the last follow-up were censored at the

time. The follow-up for survival status information extended beyond the date off ddI therapy, as necessary.

OVERALL TIME ON STUDY SPENT AT BASELINE OR BETTER VALUES

The analysis combining the CD_4 and p24 markers, weight and opportunistic infections was computed by subtracting from duration of therapy the time any one of the 3 markers was worse than the baseline value a 4 week interval period following an opportunistic infection. The criteria for determining the marker value at any given time was the same as before, i.e., the last observed value until a new one is noted.

ON-STUDY THERAPY

Duration of ddI therapy was computed as the calendar interval between day 1 of ddI till the last recorded day of dosing. For those who were still on therapy at the last follow-up, the duration was a censored observation on the last known day of dosing.

On-study therapy was also evaluated by tabulating cumulative dose expressed as mg/kg/day by entry dose levels. The average daily dose, also in mg/kg/day, was computed as the total cumulative dose divided by the duration of therapy.

SAFETY

Safety analyses were performed in the entire patient population, as well as in subsets of prognostic relevance, i.e., AIDS vs. ARC, baseline CD_4.

HEMATOLOGY

Hematologic toxicities were evaluated according to WHO criteria. Nadirs were defined as the lowest recorded values during the entire duration of

the study with the exclusion of baseline (day one or pretreatment). Whenever possible, the observation period was extended up to 30 days after the patient went off-study. Leukopenia was defined as a WBC count below 4,000/mm^3, granulocytopenia a granulocyte count below 2,000/mm^3 and thrombocytopenia a platelet count below 100,000/mm^3. Anemia was defined as a hemoglobin level below 11 g/dl.

SERUM CHEMISTRIES

For serum chemistries, the worst value was defined as the highest recorded value during the entire duration of the study with the exclusion of baseline. The observation, period was extended by 30 days after the off-treatment date,

or by longer if values were still rising at that time. All evaluations were assigned to WHO grades.

DOSE-LIMITING TOXICITIES

Dose-limiting toxicity was defined as a toxic effect which required dose reduction and/or discontinuation of therapy. Time to occurrence of dose-limiting toxicities was calculated from day 1 of therapy until the day of dose modification or treatment discontinuation. Cumulative dose until the day of toxicity was also used for analysis.

2.3.5. Statistical Methods

No unplanned analyses were performed. All statistical analyses were performed using the SAS (Statistical Analysis Systems, SAS Institute, North Carolina, USA) software package. All proportions were compared using the Fisher's exact test. Appropriate continuous variables were compared by the Wilcoxon rank sum test. The log-ratios were tested using

the two-sided t-test to reject the null hypothesis that the log-ratio was zero. Kaplan-Meier methods were used to estimate the proportion of events in analyzing the censored time-to-event data (time on ddI therapy, time to first OI, response duration, survival, time to change in the Spitzer Index, time at baseline or better values, etc.). Comparison of failure-time curves was generally made using the log rank test or its appropriate stratified version. All tests were conducted at two-sided alpha level of 0.05.

Response rates for markers, on-study therapy, efficacy and toxicity were tabulated by subgroups of prognostic relevance, e.g. CD_4, diagnosis.

2.3.6. Results of the ddI Study

All patients in East Riverside who met the criteria of ZDV intolerance between 16 February 1990 and 18 January 1991 were offered ddI therapy. Five patients were excluded due to hepatic dysfunction, history of pancreatitis or raised serum amylase. A further eight patients chose to decline further anti retroviral therapy for personal reasons. 151 patients entered the study.

Some safety data included here is limited to the first 105 patients who had been entered on study only up to May 15, 1990. In order to allow for a sufficient follow-up of the patients included in this interim analysis full collection of data was carried out for these patients up to August 1, 1990, thus allowing a minimum follow-up of three months for each patient still on study. This first analysis was a planned interim analysis focusing primarily on safety data and was conducted with Bristol Myers Squibb's assistance and cooperation. The subsequent final analysis of the 151 patient group is more survival and efficacy oriented and also conducted with Bristol Myers

Squibb's assistance. This analysis included data up to August 1, 1991. Further detailed update of the safety analysis was not considered necessary for the final analysis as no unexpected events had occurred after the interim analysis. However, data for the two most serious adverse events, peripheral neuropathy and pancreatic dysfunction were further examined.

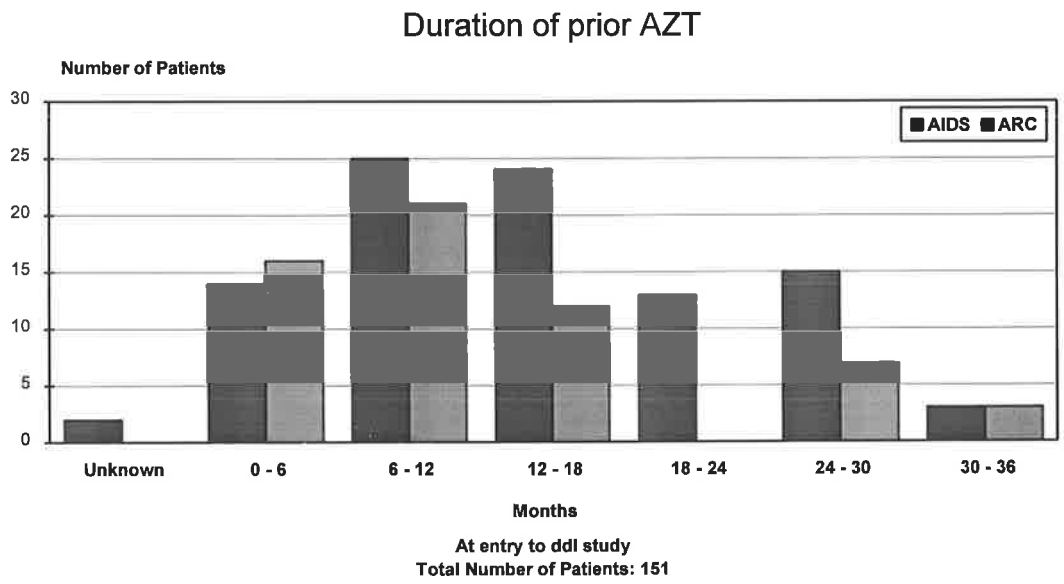
2.3.6.1. Demographics.

Demographically, the patients entering the study were a representative sample of patients attending for HIV care in East Riverside, being predominately young Caucasian homosexual or bisexual males. Only 2 female patients participated in the study. Ninety patients had an AIDS defining diagnosis (CDC 1987) with only 10 patients being in this category due to Kaposi`s Sarcoma alone. The most common prior AIDS defining diseases were *Pneumocystis carinii* (30%), oesophageal candida and cytomegalovirus infection. The remaining 61 patients had symptomatic HIV infection (called ARC in tables) mostly due to either oropharyngeal candida or oral hairy leukoplakia (Table 7). 48% of the patients had been knowingly HIV positive for four years or more prior to entering the study.

Table 7 - Demographics of Patients receiving Dideoxyinosine

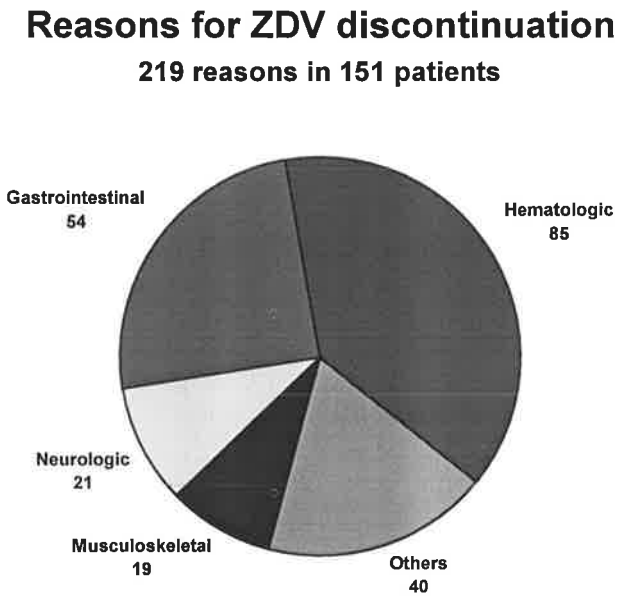
	AIDS	ARC	Total
Diagnosis at time of recruitment (No.)	90	61	151
Age/years - mean	36.9	37.4	37.1
Risk factor for HIV positive status			
Homosexual/Bisexual	86	58	144
Intravenous Drug User (IVDU)	1	0	1
IVDU + Homosexual	1	0	1
Blood transfusion	1	1	2
African Heterosexual contact	1	0	1
Haemophiliac	0	1	1
Heterosexual	0	1	1

All the patients had received a minimum of 8 weeks prior ZDV but most patients had continued ZDV for considerable lengths of time (mean 62 weeks, range 10 - 153 weeks) prior to developing intolerance (Figure 2).



The commonest cause for ZDV intolerance was bone marrow suppression (Figure 3).

Figure 3



For most patients the typical prior ZDV dose had been 200 mg orally 4 hourly in waking hours (i.e. 1000 mg/day) although from late 1989 many patients received 200 mg three times per day (600 mg/day). Myelosuppression was principally manifested as anaemia although 6 patients had neutropenia as their sole reason for ZDV discontinuation. Gastrointestinal toxicity consisted mostly of nausea and headaches were the principal neurological cause for stopping ZDV. Myalgia and/or myositis were also a common cause for cessation. Lack of efficacy was never given as the sole reason for stopping ZDV.

2.3.7. Treatment

2.3.7.1. Assigned Dose Level

Three initial dosage levels were assigned, according to baseline body weight (Table 8). Three patients with baseline weight of exactly 60 kg received the highest dose (375 mg b.i.d.).

When daily dosage was adjusted for the actual patient weight, all the patients received a dose between 8.3 and 12.5 mg/kg/day except one (a patient weighing 107 kg who received 375 mg b.i.d., or 7 mg/kg/day).

Table 8 Initial dosage

Weight group	b.i.d. dosage	Actual weight ranges	Daily range
> 60 kg	375 mg	107 - 60 kg	7.0 - 12.5 mg/kg
60 - 40 kg	250 mg	60 - 43 kg	8.3 - 11.6 mg/kg
< 40 kg	167 mg	38 kg	8.8 mg/kg

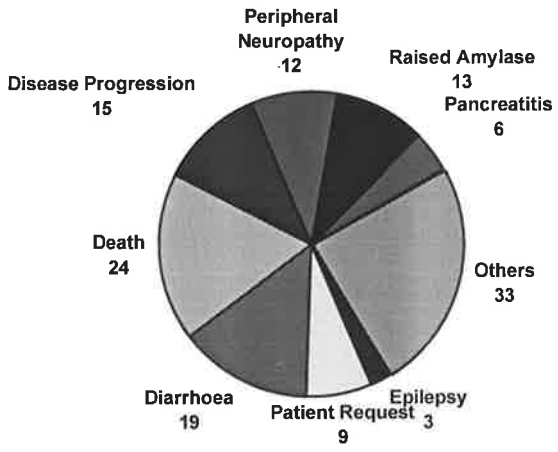
2.3.7.2. Dosage Modifications

Dosage modifications occurred rarely during treatment. Four patients had a dose escalation (three from 250 mg b.i.d. to 375 mg b.i.d., one from 167 mg b.i.d. to 250 mg b.i.d.) due to weight increase. Four patients had a dose de-escalation (two from 375 mg b.i.d. to 250 mg b.i.d., one from 375 mg b.i.d. to 167 mg b.i.d., and one from 250 mg b.i.d. to 167 mg b.i.d.). These dose reductions were prompted by diarrhea in three patients (two eventually interrupted treatment due to the same reason, despite

reduction to 167 mg b.i.d., one continued therapy at 250 mg b.i.d.) and by weight decrease below 60 kg in one patient, who continued therapy at 250 mg b.i.d. Temporary, brief (median duration 10 days, mean 10 days, range 1 to 19 days) interruption of treatment occurred in 11 patients (twice in two patients). In the majority of the cases this was prompted by diarrhea (8 patients) or headache (2 cases). Definitive discontinuation of ddI therapy occurred in 102 (65%) of the patients (Figure 4). More than one reason for discontinuation was given in some cases.

Figure 4

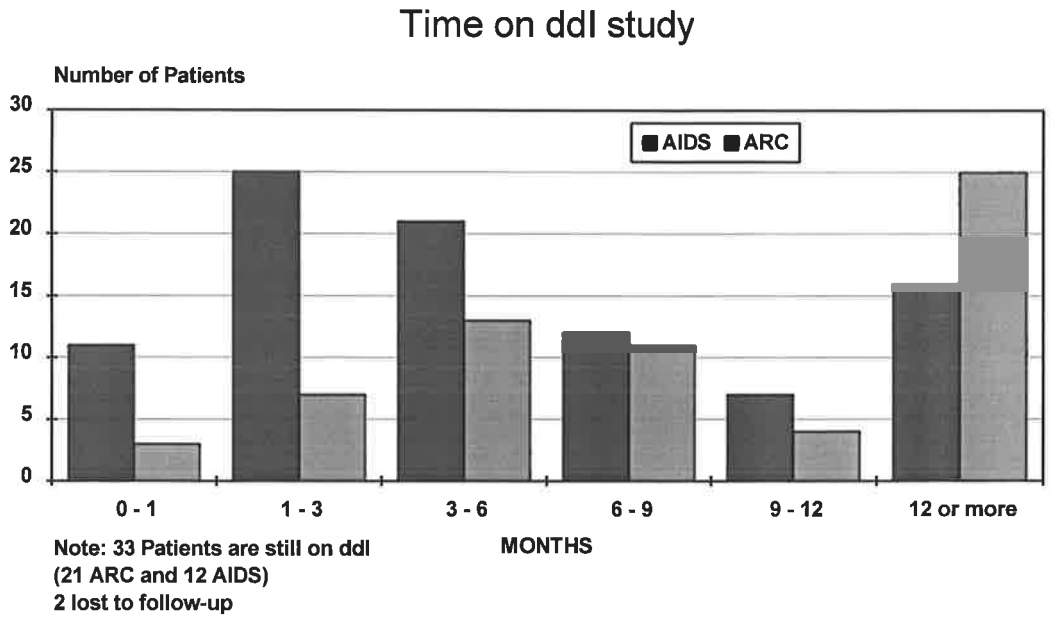
Reasons for discontinuation of ddI



2.3.7.3. Treatment duration

At the time of the final analysis (March 1992) only 12 of the 90 AIDS patients and 21 of the 61 patients with symptomatic disease remained on therapy. More of the AIDS patients, 57 (64%) stopped therapy before 6 months than those with symptomatic disease, 23 patients (38%).(Figure 5).

Figure 5



2.3.7.4. Average Daily Dosage

The average daily dose was calculated by dividing the cumulative dose administered to each patient by the duration of treatment.

The median average daily dose dispensed during the trial was 741 mg/day (mean 679, range 333 to 750 mg/day).

When these values were expressed according to the patient body weight, the median average daily dose was 10.4 mg/kg/day (mean 10.2, range 5.7 to 12.5 mg/kg/day).

2.3.7.5. Concomitant Medications

The administration of prophylactic treatment for *Pneumocystis carinii* pneumonia was documented 92 % of patients. A large number of

concomitant medications were administered during the trial. The most common medications used were acyclovir, cotrimoxazole, ciprofloxacin, erethromycin, ampicillin, Rifabutin, a variety of non steroidal analgesics and antipyretics, Fluconazole, itraconazole and ketoconazole, vitamin preparations, loperamide and megestrol acetate. Of note, 10 patients received Ganciclovir concomitantly with ddI.

2.3.8. Clinical Toxicities

DIARRHOEA

The commonest side effect of ddI was diarrhoea which resulted in cessation of therapy in 23 patients. Diarrhoea was defined as greater than 3 liquid motions a day persisting for more than one week, or worsening of preexistent diarrhoea, which occurred in 90 patients. In just under half the patients (39), the diarrhoea regressed over the first month of therapy. Patients with an AIDS diagnosis (62 out of 90) were more likely to develop diarrhoea than ARC patients (28 out of 61) ($p < 0.01$), and this was more likely to persist in AIDS patients with continued ddI therapy for more than 4 weeks (37 AIDS patients compared with 14 ARC patients) ($p < 0.025$). Other Gastrointestinal tract problems include pancreatic disturbance (see below), raised liver function tests (see below), nausea and vomiting, abdominal pain and bloating.

PERIPHERAL NEUROPATHY

Peripheral neuropathy (WHO grade III-IV), occurred in 12 patients (5 ARC, 7 AIDS) after a mean period of 27.3 weeks (range 13 to 42) which was similar for both ARC and AIDS patients. This affected only the lower limbs and was always painful. In 6 patients (4 ARC, 2 AIDS) the

symptoms resolved partially or completely when therapy was stopped. (see below)

PANCREATIC DAMAGE

Pancreatitis only occurred in AIDS patients, and was responsible for cessation of therapy in 6 patients, including 2 deaths. A further 13 patients stopped ddI due to raised amylase. 7 patients developed diabetes mellitus whilst on therapy leading to cessation of therapy in 3 patients (see below).

OTHER SIDE EFFECTS

Elevated levels of serum aminotransferase were noted in 12 patients, but in 5 of these cessation of other drug therapy produced a resolution of the liver function test abnormality. Two patients were re challenged with ddI and neither has developed further abnormalities of liver function.

Five patients underwent a marked personality change that was noticed by hospital staff, the patient, and their relatives. This was reversed in 4 on stopping therapy.

Three patients developed epilepsy whilst on ddI but in 2 another potential cause was found. In the third patient recent complex partial seizures ceased on stopping ddI.

2.3.8.1. Peripheral Neuropathy

INTRODUCTION

As peripheral neuropathy had previously been identified on the major dose limiting toxicity of ddI, assessment of the peripheral nervous system at each attendance was an important part of patient follow-up. Symptoms

of PN were discussed with patients at the start of therapy and early attendance encouraged if PN was suspected.

PN was assessed on the WHO criteria based on duration of symptoms, level of discomfort and presence or absence of deep tendon reflexes (principally ankle jerk). Furthermore, nerve conduction studies were prospectively offered to all patients commencing ddI. Patients with known preexisting PN were excluded from entry into the study.

OBJECTIVE

To prospectively assess change in peripheral nerve function during ddI therapy.

METHODOLOGY

Direct questions regarding PN symptoms were included in case report forms and asked at each attendance. Patients were examined and ankle jerks repeated at each attendance.

Nerve Conduction Studies (NCS) using a Dantec N2000 nerve conduction machine (Dantec Medical Supplies, UK) were offered to all patients with a view to repeating studies at 6 months follow-up or at the time of appearance of symptomatology as suggestive of peripheral neuropathy. The nerves identified for study were 2 motor nerves, median (upper limb) and common peroneal (lower limb) and 2 sensory nerves, ulnar (upper limb) and sural (lower limb).

Simulatory impulses were sent via a movable probe adjusted until the point of maximal response was attained. Receptors were fixed at standardized sites and the interface between skin surface and receptor

maximized by the use of saline soaked pads. Only the right limbs were studied. All studies were conducted by the same operator (me) to further standardize the procedure.

RESULTS

No patients entered the study with bilateral symptoms suggestive of PN. 7 patients were considered to have reduced ankle jerks at baseline.

Complete baseline values for nerve conduction were obtained on 107 trial participants. The remaining patients refused NCS or withdrew consent once testing had begun due to the discomfort caused by the test impulses.

Mean baseline common peroneal conduction speed was 24.3 m/sec (R 18.6 - 34.4 m/s) and for sural nerve amplitudes 10.9 μv (R 0-22.9 μv). Nine patients had undetectable Sural nerve amplitudes at baseline but had no symptoms of PN on direct questioning.

Twelve patients developed symptoms suggestive of PN during the study. Only the lower limb was affected. Both patients with AIDS (7) and ARC (5) developed PN with the mean time on study being 27.3 weeks (R 13-42 weeks), a mean cumulative dose of 2.03 g/kg of ddI. 4 patients with symptoms suggestive of PN had loss of their deep tendon reflex, equivalent to WHO grade 3 PN. No other patients lost their ankle jerk during the study. 6 patients with PN declined NCS, principally due to pain.

A case control group of 14 patients who had received ddI for approximately 6 months (mean 29.2 weeks R 24 - 33 weeks) and who were matched to the patients with symptoms of PN for baseline CDC

group, CD_4 count and age were used as comparators for nerve conduction.

Statistical comparison was made using a 2 tail T-test. Results are shown below (Table 9).

Table 9: Nerve conduction in patients with and without PN

	Neuropathy N = 6		No Neuropathy N = 14	
	Baseline	at Symptoms	Baseline	at 6 months
Peroneal m/s (Range)	24.9 (20.4-34.4)	19.5 (15.6-22.8)	24.3 (18.6-28.9)	23.3 (19.2-28)
Sural μv (Range)	8.25 (5.9-11.4)	6.1 (0-12.2)	10.6 (0-20.6)	11.6 (0-17.6)

Peroneal nerve conduction speeds were significantly reduced in patients developing PN compared with those who did not developed PN (p=0.035). Changes in sural nerve amplitudes showed a trend towards lower microvoltages in symptomatic patients although this did not reach statistical significance.

DISCUSSION

Nerve conduction speed and amplitudes appear reduced in patients presenting with symptoms suggestive of ddI related PN. Baseline Nerve conduction values do not appear to predict the future development of PN.

2.3.8.2. Pancreatic Damage

Pancreatitis and raised amylase had previously be identified as probable toxicities of ddI during phase I/II studies. Symptoms of pancreatitis were discussed with all patients prior to commencing ddI and patients were encouraged to attend promptly if any suggestive symptoms developed.

Patients with a history of pancreatitis were excluded from the study. All patients were asked directly about abdominal pain at each attendance and the abdomen routinely examined. Serum amylase was performed at each attendance but fractionation facilities were not available.

OBJECTIVE

To assess the frequency of pancreatic disease in patients treated with ddI

METHODOLOGY

As well as direct questioning, abdominal examination and routine serum amylase at each attendance, a random blood glucose was performed at each visit and patients with a result above 9 mg/ml were requested to attend for a standard 75 g glucose tolerance test to more adequately assess their glucose tolerance.

RESULTS

Patients with a history of pancreatitis were excluded from the study. Random blood glucoses performed at baseline were below 8 mg/ml in all patients and no patient gave a history of diabetes mellitus or glucose intolerance.

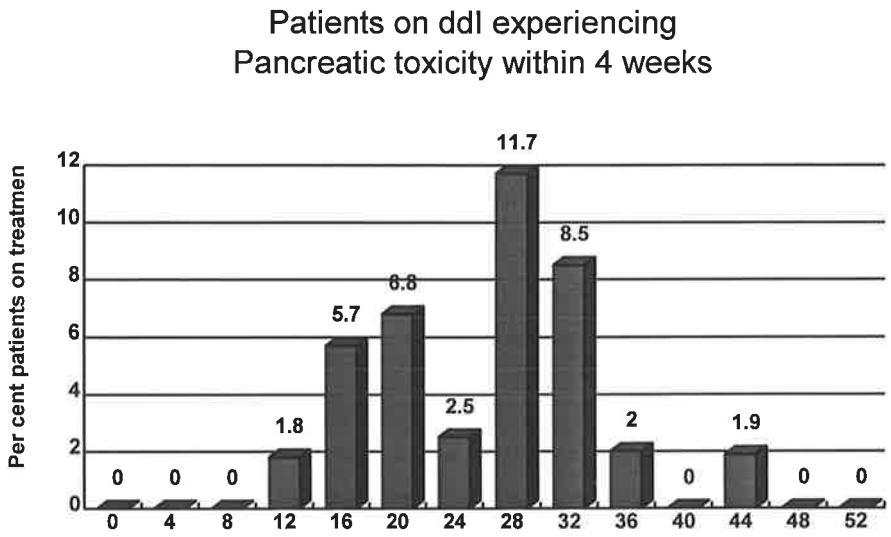
Pancreatitis, defined as upper or central abdominal pain with an amylase greater than twice the upper laboratory range of normal was significantly more common in AIDS patients (6) than patients with a baseline ARC diagnosis (0) ($p<0.05$) and was responsible for death in 2 individuals. The mean duration of therapy prior to the diagnosis of pancreatitis was 17.8 weeks (range 11-26). A further 13 patients, 9 with AIDS developed raised serum amylase after a mean of 21 weeks therapy (range 12-32), but had no other symptoms or abdominal pain. All 13 patients discontinued ddI

therapy with a return of their amylase to normal levels. One patient has been re-challenged and showed no increase in serum amylase after 3 months further therapy. A raised amylase was associated with Xerostomia in 6 patients, 2 of whom had parotitis.

Seven patients developed a markedly abnormal random serum glucose, all of whom had had random glucoses prior to inclusion in the study below the upper limit of normal for fasting glucose. Six of these 7 patients had diabetes mellitus as measured by a standard 75g glucose intolerance test, and the seventh patient had a random glucose of > 11 mg/ml. Three of these patients ceased ddI therapy and their glucose tolerance tests returned to normal. In the other 4 patients the abnormality of glucose tolerance persisted, but was mild and did not require pharmaceutical intervention. None of these patients were receiving concommitant treatment with pentamidine.

The chances of developing possible pancreatic damage (diabetes mellitus, pancreatitis or a raised amylase) during ddI therapy were slight prior to 12 weeks therapy. However, by 28 weeks the chances of pancreatic damage occurring within the next 4 weeks was 11.1%, although at 40 weeks the risk had declined to 1.9% within the succeeding 16 weeks (Figure 6).

Figure 6



DISCUSSION

Clinical pancreatitis developed in 4% of patients with a further 8.6% showing raised amylase although at least some of these elevations may have related to salivary gland disturbance being associated with Xerostomia. Diabetes mellitus had not previously been reported as complication of ddI therapy but developed in 7 (4.6%) of our patients and may be suggestive of chronic pancreatic damage. Development of pancreatic problems (Pancreatitis, raised amylase or diabetes mellitus) was rare in the first 12 weeks and after 40 weeks of therapy suggesting an idiosyncratic dose related response.

2.3.9. Haematology and Biochemistry

As safety data on these parameters was only examined at the interim analysis, values on the first 105 patients included in that analysis are shown here.

2.3.9.1. Baseline Values

2.3.9.1.1. Baseline Leukocytes

The majority of patients presented to the study with mild leukopenia. Median white blood cell (WBC) count was 3,300 cells/mm^3 (mean 3,704, range 1,000 to 9,100). The respective median values were 4,050 cells/mm^3 in ARC patients and 3,100 cells/mm^3 in AIDS patients (Table 10).

Table 10 Baseline leukocytes

	No.	%
Total	105	100
Leukocytes/cells/mm^3		
$\geq$ 4,000	40	38
3,999 - 3,000	25	24
2,999 - 2,000	31	30
1,999 - 1,000	9	9

2.3.9.1.2. Baseline Granulocytes

Median granulocyte count was 1,900 cells/mm^3 (mean 2,110, range 400 to 6,367). respective median values were 2,000 cells/mm^3 in patients diagnosed with ARC and 1,700 cells/mm^3 in patients with AIDS.

More than one-half of the patients presented granulocytopenia at the time ddI was started, including seven patients with severe granulocytopenia (Table 11).

Table 11 Baseline granulocytes

	No.	%
Total	105	100
Analyzed	104	100
Granulocytes/cells/mm^3		
≥ 2,000	50	48
1,999 - 1,500	27	26
1,499 - 1,000	20	19
999 - 500	6	6
< 500	1	1

2.3.9.1.3. Baseline Hemoglobin

All patients met the protocol eligibility criterion requiring values of ≥ 8 g/dl. Median hemoglobin concentration was 12.4 g/dl (mean 12.3, range 8.3 to 15.7 g/dl). Corresponding median values were 13 g/dl in ARC and 11.9 g/dl in AIDS patients, respectively (Table 12).

Table 12 Baseline hemoglobin

	No.	%
Total %	105	100
Hemoglobin, g/dL		
≥ 11	82	78
10.9 - 9.5	20	19
9.4 - 8.0	3	3

Hematocrit values at baseline ranged between 24.9 and 46.2 percent (median 37.3, mean 37.3). Respective median values were 39.7 percent in ARC and 36.8 percent in AIDS patients.

2.3.9.1.4. Baseline Platelets

Median platelet count was 156,500/mm^3 (mean 161'692, range 49'000 to 393'000). The corresponding median values were 157'500/mm^3 and 150,000/mm^3 in ARC and AIDS patients, respectively. About one-fourth of the study population presented with thrombocytopenia before starting ddI (Table 13).

Table 13 Baseline platelets

	No.	%
Total	105	
Analyzed	104	100
Platelets/cells/mm^3		
≥ 100,000	79	76
99,000 - 75,000	14	13
74,999 - 50,000	10	10
49,999 - 25,000	1	1

2.3.9.1.5. Baseline Hepatic Function Tests

More than two-thirds of the patients had minor transaminase elevations whereas abnormalities of bilirubin were less frequent. The origin of these liver function alterations is unclear, although it is most likely related to the very advanced stage of disease and/or, possibly, to the prior or concomitant therapies(Table 14).

Table 14 Baseline liver function

	SGOT*		Bilirubin*	
	No	**%**	**No**	**%**
Evaluable (%)	103	100	103	100
Normal (N)	31	30	92	89
1.01 - 1.25 x N	22	21	5	5
1.26 - 2.50 x N	43	42	5	5
2.51 - 5.00 x N	5	5	1	1
5.01 - 10.0 x N	1	1	-	
> 10.0 x N	1	1	-	

** 2 values missing.*

2.3.9.1.6. Baseline Renal Function Tests

Renal function was generally normal. A few patients showed minimal elevations in these parameters at baseline(Table 15).

Table 15 Baseline kidney function

	UREA		S.creatinine	
	No	**%**	**No**	**%**
Evaluable (%)	105	100	105	100
Normal (N)	98	93	102	97
1.01 - 1.25 x N	6	6	3	3
1.26 - 2.50 x N	1	1	-	

2.3.9.1.7. Other Laboratory Values At Baseline

Minimal elevations of uric acid, serum amylase levels and Creatinine phosphokinase (CPK) were present at baseline (Table 16).

Table 16 Other baseline laboratory tests

	Uric acid		Amylase		CPK	
Evaluable (%)	101	100	105	100	101	100
Normal (N)	79	78	89	85	98	97
1.01 - 1.25 x N	17	17	12	11	-	
1.26 - 2.50 x N	5	5	3	3	2	2
2.51 - 5.00 x N	-		-		1	1
5.01 - 10.0 x N	-		1	1	-	

2.3.9.2. *Results*

2.3.9.2.1. *Leukocytes*

The majority of the patient population with presented leukopenia before starting ddI treatment. Two patients had no on-study leukocyte counts. Of the 103 evaluable patients, 39 (38%) experienced deterioration their WHO grade during therapy (Table 17). No life-threatening leukopenia (WBC 1,000/mm^3, WHO grade IV) occurred during treatment. Fourteen (22%) of the 63 patients with abnormal baseline values actually improved their WHO grades during therapy.

Median WBC nadir on study was 3,000/mm^3 (mean 3, 196, range 1,200 to 7,000).

Table 17 Leukopenia

		Worst WHO grade on study			
	Eval.	**0**	**I**	**II**	**III**
Total	103	29	27	26	21
By baseline grade (HBC/cells/mm^3)					
WHO 0 (≥ 400)	40	24	11	3	2
WHO I (3,999 - 3,000)	24	4	9	8	3
WHO II (2,999 - 2,000)	31	-	6	13	12
WHO III (1,999 - 1,000)	8	1	1	2	4
By diagnosis					
ARC	40	16	8	10	6
AIDS	63	13	19	16	15

2.3.9.2.2. Granulocytes

Granulocytopenia, present in more than one-half of the patients at baseline, was also frequently observed during ddI treatment (Table 18). Two patients had no on-study granulocyte counts. Low nadir counts were observed in 28 of the 50 patients (56%) with normal baseline counts. However, granulocytopenia was never life-threatening (PMN < 500/mm^3) and was severe (PMN 999 - 500/mm^3) in only three patients. Sixteen (31 %) of the 52 patients with baseline granulocytopenia worsened their WHO grade during treatment, whereas 12 (23%) improved it, including 4 out of 7 patients with baseline PMN counts < 1,000/mm^3.

Table 18 Granulocytopenia

		Worst WHO grade on study				
	Eval.	**0**	**I**	**II**	**III**	**IV**
Total	103	30	29	29	14	1
By baseline grade (PMN/cells/mm^3)						
Not available	1	1	-	-	-	-
WHO 0 (≥ 2,000)	50	22	15	10	3	-
WHO I (1,999-1,500)	19	2	2	11	3	1
WHO II (999 - 500)	6	1	2	-	3	-
WHO IV (< 500)	1	-	-	1	-	-
By diagnosis						
ARC	40	12	11	11	6	-
AIDS	63	18	18	18	8	1

The median granulocyte nadir was 1,600 cells/mm^3 (mean 1,730, range 400 to 4,200). The corresponding figures were 1,600 cells/mm^3 (mean

1,780, range 600 to 3,900) for ARC patients and 1,600 cells/mm^3 (mean 1,698, range 400 to 4,200) for AIDS patients.

2.3.9.2.3. Hemoglobin

Two patients had no on study blood counts available and were not evaluable. The majority of patients who started ddI treatment with normal hemoglobin concentrations (Hgb ≥ 11 g/Dl) did not develop anemia during treatment (Table 19). Only four patients developed severe (Hgb 7.9 to 6.5 g/dl) or life-threatening (Hgb < 6.5 g/dl) anemia. All of them had a diagnosis of AIDS and two had abnormal baseline values.

Table 19 Anaemia

		WHO grade				
	Eval.	**0**	**I**	**II**	**III**	**IV**
Total	103	54	32	13	2	2
By baseline grade (Hgb g/dL)						
WHO (0 ≥ 11)	80	53	19	6	-	2
WHO I (10.9 - 9.5)	20	1	12	5	2	-
WHO II (9.4 - 8)	3	-	1	2	-	-
By diagnosis						
ARC	40 (33)	28 (27)	10 (5)	2 (1)	-	-
AIDS	63 (47)	26 (26)	22 (14)	11 (5)	2	2 (2)

** In parenthesis: patients with normal baseline*

Hemoglobin concentrations were analyzed at specific times during the study (Table 20). Differences in hemoglobin values (log Hgb ratio) were compared between baseline and given observation times and were statistically significantly higher at week 4 (p = 0.026) and 16 (p = 0.047).

Table 20 Hemoglobin concentration variations (g/dL)

	Eval.	Median	Mean	Range
Screening	39	12.4	12.5	9.2 - 15.7
Baseline	105	12.4	12.3	8.3 - 17.7
Week 4	100	11.9	12.0	3.5 - 15.9
Week 8	66	12.6	12.5	9.2 - 16.5
Week 12	61	12.8	12.6	8.9 - 16.8
Week 16	42	12.0	12.1	6.3 - 16.0
Week 20	13	12.7	12.4	10.3 - 14.5

Median hemoglobin nadir during ddI treatment was 11.2 g/dl (mean 11.1, range 3.5 to 14.4). The corresponding nadirs for ARC patients were 12.4 g/dl (mean 12.1, range 9 to 14.4) and for AIDS patients were 10.6 g/dl (mean 10.5, range 3.5 to 13.7). The median hematocrit nadir for all the evaluable patients was 32.9% (mean 33.4, range 12.2 to 44). The corresponding nadirs for ARC patients were 37.4% (mean 36.1, range 12.2 to 44) and for AIDS patients were 31.7% (mean 31.6, range 12.8 to 42.3).

Of note, only three (5%) of the 58 patients who were forced to cease prior Zidovudine treatment due to anemia achieved hemoglobin concentrations below 8 g/dl (WHO grade III-IV) during ddI treatment (Table 21).

Table 21 Anemia in patients who discontinued Zidovudine due to anemia

		WHO grade				
	Eval.	**0**	**I**	**II**	**III**	**IV**
Total	58	18	24	13	2	1
By baseline grade (Hgb g/dL)						
WHO 0 (≥ 11)	37	16	14	6	1	1
WHO I (10.9 - 9.5)	18	2	9	5	1	-
WHO II (9.4 - 8)	3	-	1	2	-	-

Transfusions were administered during ddI treatment to 29/105 (28%) patients. This occurred in 14/36 (39%) of the cases with a history of multiple transfusions before starting ddI, in 13/23 (57%) of the cases with a history of severe anemia on Zidovudine, without documented transfusion-dependency, and in 2/46 (4%) of the patients with no record of prior anemia.

Mean corpuscular volume (MCV) during ddI treatment ranged from 85 to 126 f/L (median 103, mean 102.8) in the 103 patients analyzed.

2.3.9.2.4. Platelets

Two patients had no platelet counts on study (Table 22). Only 10 of the 77 patients with normal baseline values (13%) developed thrombocytopenia.

Of the 25 patients with low baseline thrombocyte counts, 5 (20%) presented a higher WHO grade on study, and 10 (40%) actually improved their WHO grade during treatment.

Only one patient developed a platelet nadir below 50,000/mm^3 while on study.

Table 22 Thrombocytopenia

	Worst WHO grade on study				
	Eval.	0	I	II	III
Total	103	76	14	12	1
By baseline grade (cells/mm^3)					
Unavailable	1	1	-	-	-
WHO 0 (2 100,000)	77	67	8	2	-
WHO I (99,999 - 75,000)	14	6	4	4	-
WHO II (74,999 - 50,000)	10	2	1	6	1
WHO III (49,999 25,000)	1	-	1	-	-
By diagnosis					
ARC	40	31	2	7	-
AIDS	63	45	12	5	1

The median platelet nadir was 140,000 cells/mm^3 (mean 140,282, range 45,000 to 346,000). Corresponding values in ARC patients were 142,000 cells/mm^3 median (mean 138,550, range 51,000 to 233,000) and in AIDS patients were 133,000 cells/mm^3 median (mean 141,381, range 45,000 to 346,000).

2.3.9.2.5. Hepatic Function Tests

Total bilirubin was evaluated in all patients but four, and serum aspartate aminotransferase in all patients but three during ddI treatment.

Elevations of bilirubin never exceeded > 5 times the upper normal laboratory limits, whatever the baseline (Table 23).

Table 23 Bilirubin

	Worst value on study				
	Eval.	Normal	1.01-1.25	1.26-2.50xN	2.51-5.00xN
Total	101	78	6	14	3
By baseline grade					
Unavailable	2	2	-	-	-
Normal (N)	88	75	5	7	1
1.01-1.25xN	5	-	1	4	-
1.26-2.50xN	5	1	-	3	1
2.51-5.00xN	5	1	-	3	1
By diagnosis					
ARC	40	33	1	4	2
AIDS	61	45	5	10	1

Thirteen of the 88 patients with normal baseline values (15%) developed elevations of bilirubin during treatment.

Of the 11 patients with abnormal baseline results, only two had bilirubin values between 2.5 and 5 times the upper normal limit.

Elevations of SGOT levels occurred more frequently. They were observed in 18 (58%) of the 31 patients with normal baseline values, but were never severe or life-threatening (> 5 times the upper normal laboratory limits). In the 69 patients who presented with baseline abnormalities, 27 (39%) developed a higher toxicity grade on study, and 7 (10%) improved. Six patients had severe elevations of SGOT, with actual values ranging between 155 and 247 U/L. (Table 24).

Table 24 SGOT

		Worst value on study					
	Eval.	Normal	1.01-1.5xN	1.26-2.50xN	2.51-5.00xN	5.01-10xN	>10xN
Total	102	15		44	20	7	1
By baseline value							
Unavailable	2	-	-	1	-	1	-
Normal (N)	1	13	5	10	3	-	-
1.01-1.25xN	21	2	6	9	3	1	
1.26-2.50xN	41	-	-	24	9	4	-
2.51-5.00xN	5	-	-	-	4	1	-
5.01-10.0xN	1	-	-	-	1	-	-
>10.0xN	1	-	-	-	-	-	1
By diagnosis							
ARC	40	8	6	13	11	2	-
AIDS	62	7	9	31	9	5	1

Five patients discontinued ddI treatment due to liver function test alterations (concomitant to hyperamylasemia in two cases).

2.3.9.2.6. Renal Function Tests

Serum creatinine and urea were assessed during ddI treatment in all patients but two.

Only one patient in the study population presented a minimal elevation of serum creatinine (Table 25). That patient had a baseline value of 1.12 mg/dl which increased to 1.88 mg/dl. A few other patients had serum creatinine abnormalities which did not exceed 1.25 times the upper normal laboratory values.

Table 25 Serum creatinine

	Worst value on study			
	Eval.	Normal	1.01-1.25xN	1.26-2.50xN
Total	103	96	6	1
By baseline value				
Normal (N)	100	94	5	1
1.01 - 1.25 x N	3	2	1	-
By diagnosis				
ARC	40	37	2	1
AIDS	63	59	4	1

Elevations of urea were even less frequent than those of serum creatinine (Table 26). The only patient with values on study higher than 1.26 times the upper normal limit actually had a lower value on study (62.4 mg/dl) than at baseline (108 mg/dl).

Table 26 Urea

	Worst value on study			
	Eval.	Normal	1.01-1.25xN	1.26-2.50xN
Total	103	96	4	1
By baseline value				
Normal (N)	96	95	1	-
1.01 - 1.25 x N	6	3	3	-
By diagnosis				
ARC	40	40	-	-
AIDS	63	58	4	1

2.3.9.2.7. Other Laboratory Test Alterations

Increase of serum uric acid has been related to the catabolic pathway of ddI. However, in the 101 patients evaluated in this study, elevations were infrequent (Table 27).

Table 27 Serum uric acid

	Worst value on study			
	Eval.	Normal	1.01-1.25xN	1.26-2.50xN
Total	101	56	30	15
By baseline value				
Unavailable	4	3	1	-
Normal (N)	76	51	18	7
1.01 -1.25 x N	16	1	11	4
1.26 - 2.50 x N	5	1	-	4
By diagnosis				
ARC	40	21	10	9
AIDS	61	35	20	6

Median uric acid value on study (worst value) was 7.4 mg/dl (mean 7.5, range 4.4 to 16.2).

Creatine phosphokinase (CPK) elevations were minimal (Table 28), and only ten percent (10/101) of the patients experienced them.

Table 28 Creatine phosphokinase

	Worst value on study			
	Eval	Normal	1.01-1.25xN	1.26-2.50xN
Total	101	91	2	8
By baseline value				
Unavailable	4	3	-	1
Normal (N)	95	87	2	6
1.26-2.50 x N	2	1	-	1
By diagnosis				
ARC	40	37	2	1
AIDS	61	54	-	7

Alterations of serum amylase levels and triglycerides have been discussed in the context of pancreatitis.

DISCUSSION:

Induction of laboratory abnormalities for either hematology or chemical pathology values during ddI therapy were rare. Patients with a history of anaemia related to ZDV therapy rarely had recurrence of this problem during ddI therapy. It is unclear if elevations in liver function tests were related to ddI, to other concomitant medications or disease state.

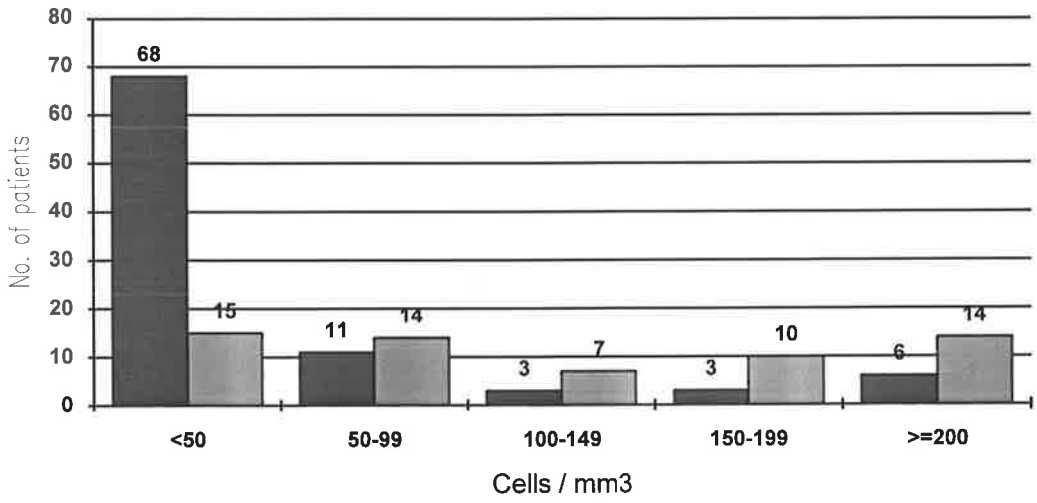
2.3.10. Surrogate Markers

2.3.10.1. Baseline

BASELINE IMMUNOLOGIC FUNCTION

The immunologic status before the beginning of ddI treatment showed severe immune compromise. Over half of the study population had CD_4 lymphocyte counts at baseline below 50 cells/mm^3 (Figure 7). Overall, the median baseline CD_4 count was 32 cells/mm^3 (mean 73, range 1 to 480 cells/mm^3). Median baseline CD_4 count was 96 cells/mm^3 (mean 129, range 7 to 480 cells/mm^3) in ARC patients and 19 cells/mm^3 (mean 38, range 1 to 243 cells/mm^3) in AIDS patients. The median CD_4 count during the screening period was 35 cells/mm^3 (mean 76, range 0 to 546 cells/mm^3).

Fig 7



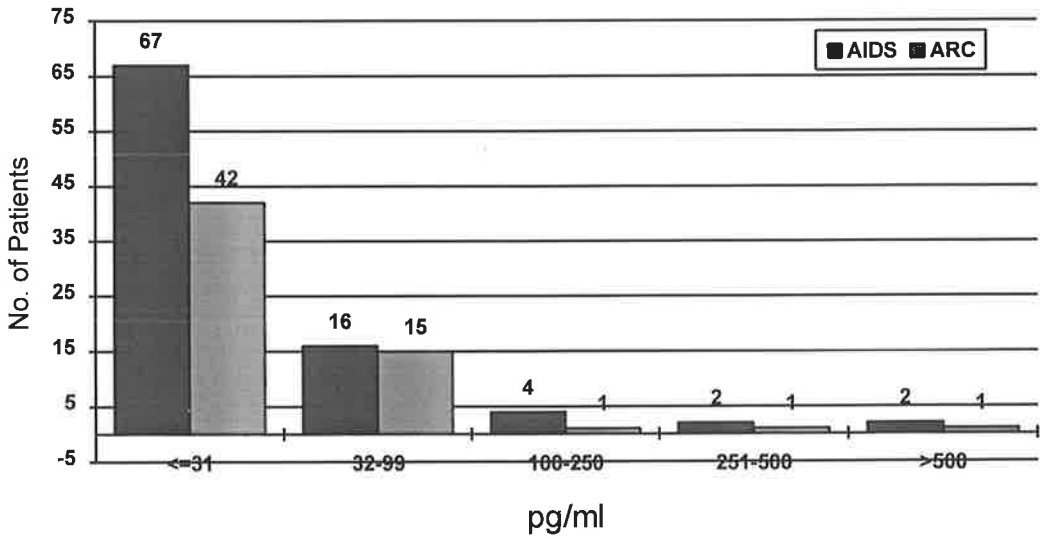
When CD_4 counts taken during the screening period were compared with CD_4 values taken at baseline, there was a statistically significant decrease in this cells population (p = 0.033, log CD_4 ratio).

Median counts at baseline for the total lymphocyte populations were 816/mm^3 (mean 1,035/mm^3, range 100 to 5,900/mm^3). The respective median values were 1,100/mm^3 for ARC patients and 700/mm^3 for AIDS patients.

2.3.10.1.1. Baseline Virologic Status

Only 42 (28%) patients had assessable serum p24 antigen levels (values above 31 pg/ml) before starting ddI (Figure 8). Of the other 109 patients, 60 had undetectable levels and 49 had baseline values of 31 pg/ml or less (range 9 to 31). All of these patients were considered as having unassessible levels of p24 antigen.

Fig.8:p24 by baseline diagnosis



When screening versus baseline p24 levels were compared, there was no statistically significant difference (p = 0.209, log p24 ratio).

2.3.10.1.2. Baseline Weight

All patients had their body weight assessed before starting ddI. Median baseline weight was 66 kg (mean 66.6, range 38 to 107 kg). Only one patient had a baseline weight below 40 kg, 38 had a baseline weight of 40 to 60 kg, and the remainder (112 patients) weighed more than 60 kg.

2.3.10.2. Results

2.3.10.2.1. CD4 Lymphocyte Subset Count

In AIDS patients, only minor rises in CD_4 count were noticed, but the initial CD_4 count was above 50 per mm3 in only 23 of these patients. A more marked rise occurred in those at an earlier stage of disease (Table 29) which remained at baseline or better for up to 6 months (Figure 9).

Table 29 CD_4^+ cell responses in patients taking didanosine

	50% response [a]	25% response [a]	10% response [a]
Total	12/131 (9)	23/131 (18)	37/131 (28)
By diagnosis			
ARC	10/55 (18)	19/55 (35)	25/55 (45)
AIDS	2/76 (3)	4/76 (5)	12/76 (16)
By baseline CD_4 count/mm^3			
< 50	0/71 (0)	3/71 (4)	10/71 (14)
50-99	2/24 (8)	4/24 (17)	6/24 (25)
100-149	1/8 (12)	2/8 (25)	3/8 (38)
150-199	4/12 (33)	5/12 (42)	9/12(75)
≥ 200	5/16 (31)	9/16 (56)	9/16 (56)

[a] *No. responding/no. evaluated. Figures in parentheses are percentages*

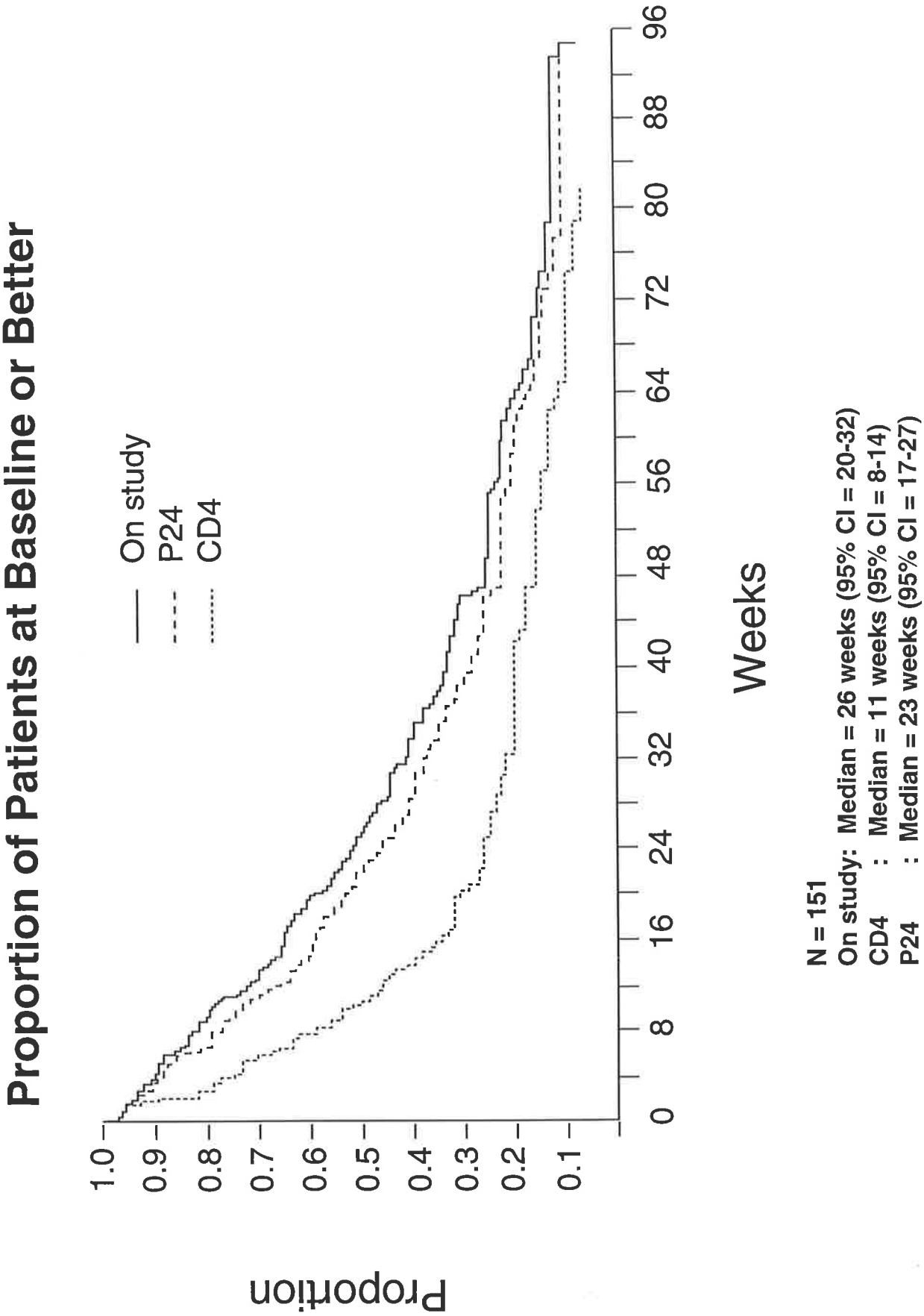
2.3.10.2.2. HIV p24 Antigen

A significant fall in HIV p24 antigen was observed in a third of the 42 patients who had a positive level prior to ddI commencement. A fall in p24 antigen was more likely in those patients with higher CD_4 counts (Table 30). This response in HIV p24 antigenaemia persisted in some patients for greater than 6 months (Figure 9).

Table 30 P24 antigen response to didanosine therapy

	No.	Responders (%)
All patients	42	28 (67)
Diagnosis		
AIDS	23	16 (70)
ARC	19	12 (61)
Baseline CD_4 + lymphocyte count		
< 100	36	22 (63)
≥ 100	6	6 (100)

Figure 9. Proportion of patients whose baseline CD_4 or p24 remained at baseline or better.



2.3.10.2.3. Opportunistic Infections

Both AIDS-defining opportunistic infections and other infections occurred during ddI treatment. Sixty patients (40%) had further AIDS defining infections leading to a first AIDS diagnosis in 16 (27%) of patients with no AIDS diagnosis at baseline.

Analysis of 50 AIDS defining events (CDC 1987)is availably only on the first 105 patients by baseline diagnosis and CD_4 count (Table 31).

Table 31 Opportunistic infections (by patient)

	No. affected	Eval. %
Total	50/105	48
By diagnosis		
ARC	13/40	33
AIDS	37/65	57
By baseline CD_4 count (cells/mm^3)		
< 50	36/63	57
50 - 99	8/16	50
≥ 100	6/26	23
		p = 0.006

In this analysis, there was no statistically significant difference (p = 0.145) in the time to first opportunistic infection between ARC and AIDS patients

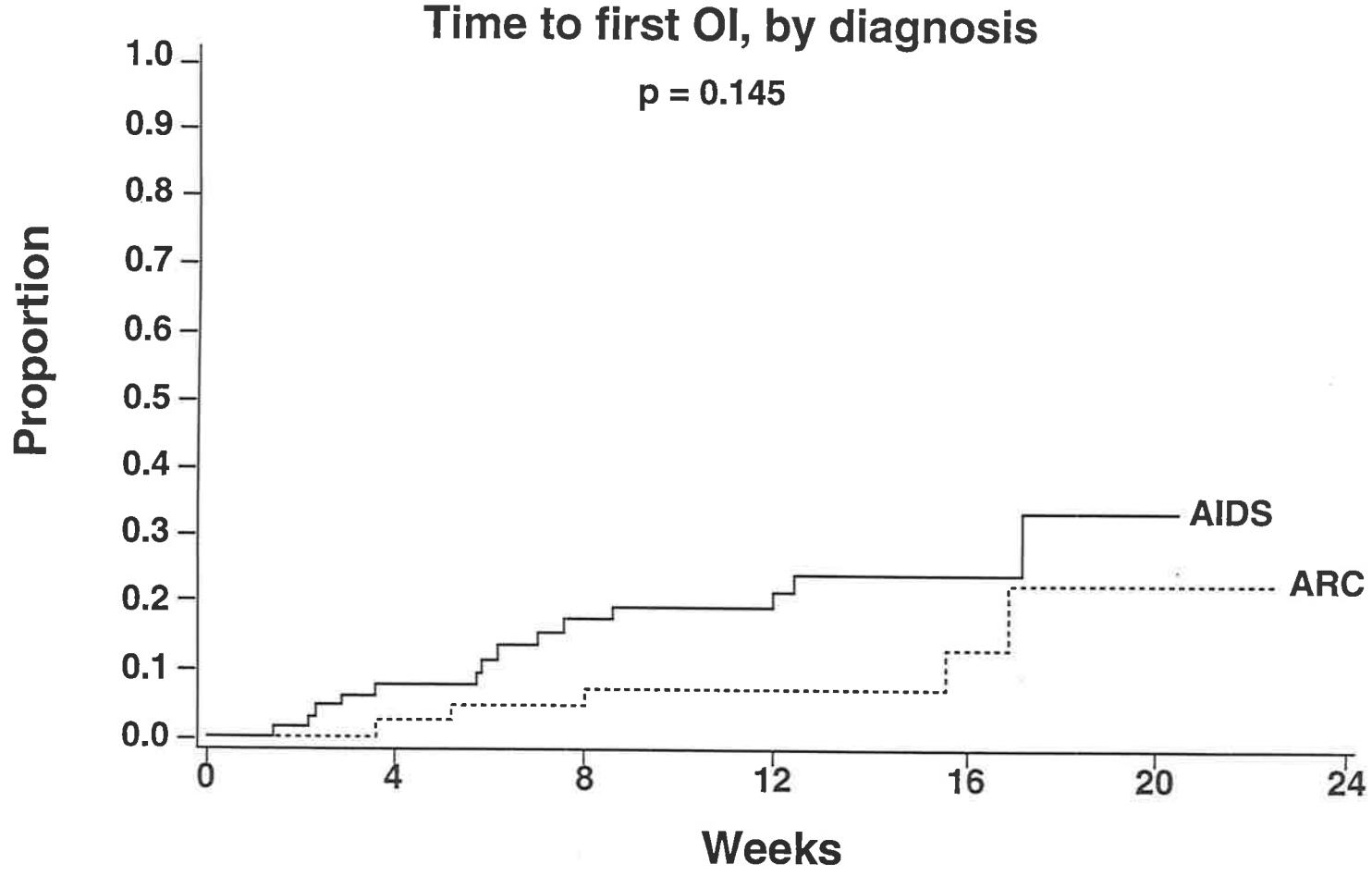

Time to first OI, by diagnosis
p = 0.145
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
4
8
12
16
20
24
Weeks
AIDS
ARC
ARC N = 40
AIDS N = 65

Figure 10

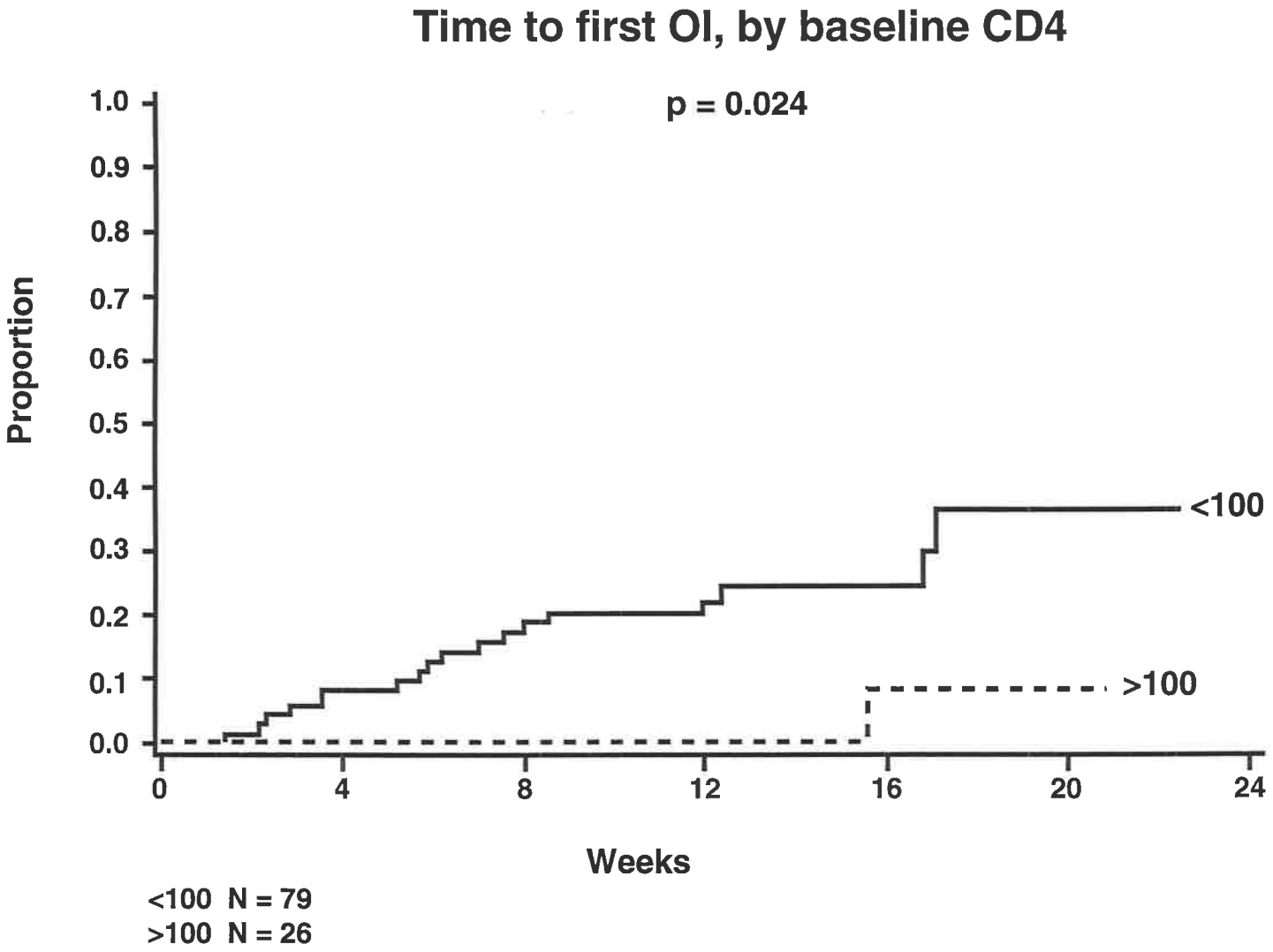

Time to first OI, by baseline CD4
p = 0.024
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
<100
>100
0
4
8
12
16
20
24
Weeks
<100 N = 79
>100 N = 26

Figure 11

However, when baseline CD_4 counts are examined, patients with lower values (CD_4 at baseline < 100 cells/mm^3) developed AIDS-defining infections significantly (p = 0.024) sooner than patients with higher CD_4 counts (Figures 10 and 11).

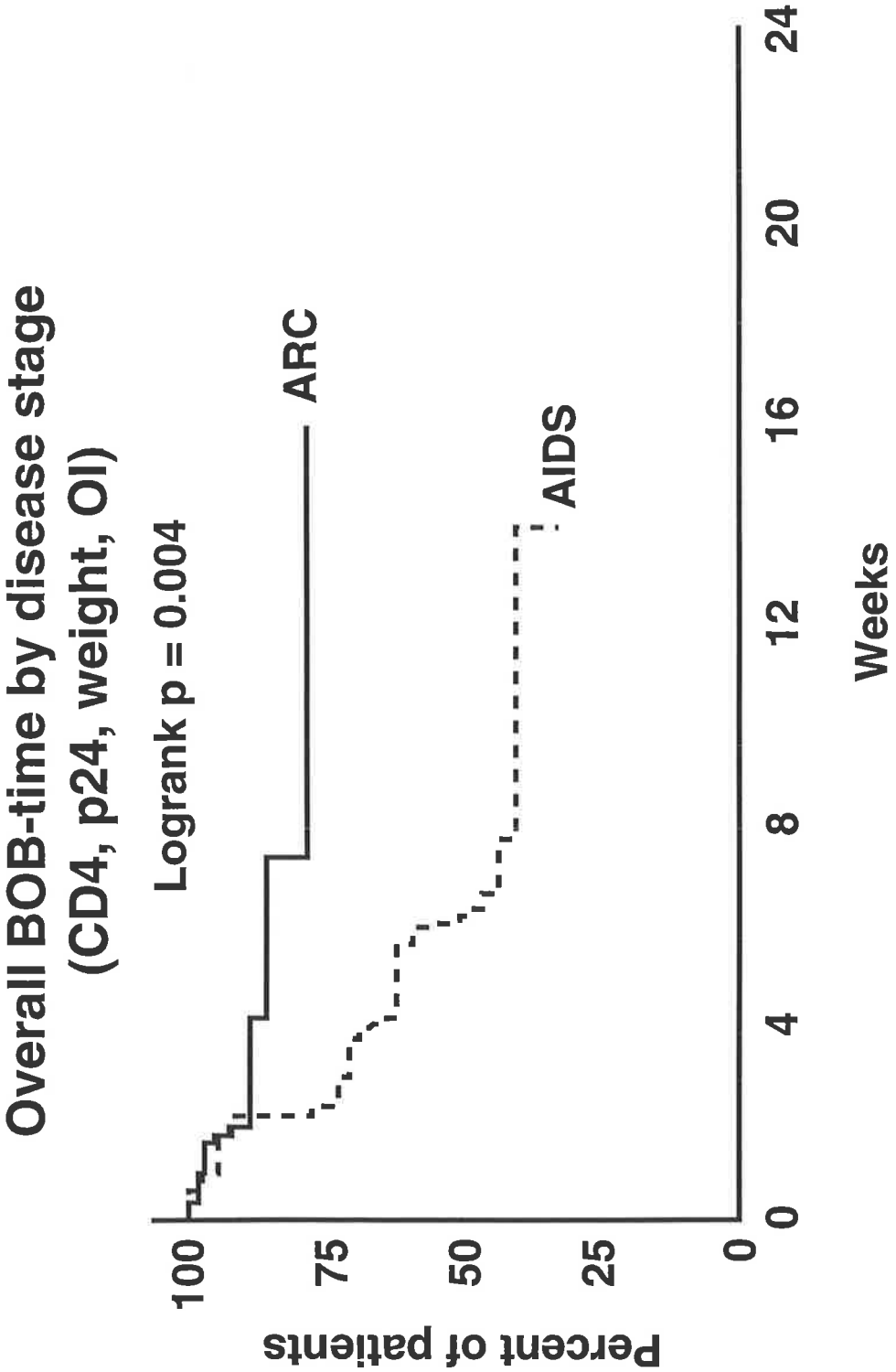
2.3.10.2.4. Time On Study Spent At Baseline Or Better Values

Individual times spent at values equal or superior to the baseline value for CD_4 , p24 have been presented earlier. An overall evaluation which includes CD_4, P24, weight and the occurrence of opportunistic infections are presented in Figure 12

Median time to deterioration was 11 weeks for CD_4 (median not reached for ARC patients, 8 weeks for AIDS patients), 23 weeks for p24 (26 weeks for ARC, 19 weeks for AIDS) and 16 weeks for body weight (19 weeks for ARC, 14 weeks for AIDS).

When the overall time on study at baseline or better values is considered, including the infections, median time to deterioration is 9 weeks. The contribution of AIDS patients (median 6 weeks) to the rapid deterioration of baseline levels as compared to ARC patients (median not reached) is evident and is statistical significant (Log rank p = 0.004)

Figure 12 Overall baseline or better-time by baseline disease status



2.3.11. Quality of Life

2.3.11.1. Baseline Values

The Spitzer Index was assessed at baseline by the trial physician in all patients. As only one therapy data is available on the first 105 patients only this data is included here. (Table 32). The majority of the patients presented with optimal activity, daily living, support and outlook levels. The exception to this observation was represented by the health parameter, which was felt to be less than optimal in more than one-half of the patients and poor in about one-quarter. The relatively good baseline quality of life index was reflected by the high cumulative score presented by the majority of the patients.

ON THERAPY

All patients except two had at least one assessment of their quality of life performed during the study; utilizing the Spitzer Index. Results up to 16 weeks of therapy are presented on the first 105 patients.

The best score achieved during ddI treatment was compared with the baseline score (table 33)

The most frequent variations of score occurred in the health parameter, which was of the parameter with the most patients at a less than optimal level at baseline

Improvements over baseline occurred in 14% of the patients for activity, in 5% for daily living, in 36% for health, in 18% for support, and in 23% for outlook. Conversely, deteriorations of the score occurred in 3% of the

patients for activity, in 2% for daily living, in 14% for health, in 1 % for support, and in 6% for outlook.

Table 32 - Baseline quality of life assessment (Spitzer Index)

	Total	No.	(%)
		105	(100)
	ACTIVITY		
	During the last week, the patient:	83	(79)
2	has been working or studying full-time, or nearly so, in occupation; or managing own household; or participating in unpaid or voluntary activities, whether retired or not.		
1	has been working or studying in usual occupation or managing own household or participating in unpaid or voluntary activities; but requiring major assistance or a significant reduction in hours worked or a sheltered situation or was on sick leave.	13	(12)
0	has not been working or studying in any capacity and not managing own household	9	(9)
	DAILY LIVING		
	During the last week, the patient:		
2	has been self-reliant in eating, washing, toileting and dressing; using public transport or driving own car.	97	(92)
1	has been requiring assistance (another person or special equipment) for daily activities and transport but performing light tasks.	8	(8)
0	has not been managing personal care nor light tasks and/or not leaving own home or institution at all.	-	
	HEALTH		
	During the past week, the patient:		
2	has been appearing to feel well or reporting feeling "great" most of the time.	25	(24)
1	has been lacking energy or not feeling entirely "up to par" more than just occasionally.	58	(55)
0	has been feeling very ill or "lousy", seeming weak and washed out most of the time or was unconscious	22	(21)
	SUPPORT		
	During the last week:		
2	the patient has been having good relationships with others and receiving strong support from at least one family member and/or friend.	81	(77)
1	support received or perceived has been limited from family and friends and friends and/or by the patient's condition	15	(14)
0	support from family and friends occurred infrequently or only when absolutely necessary or patient was unconscious	9	(9)
	OUTLOOK		
	During the past week, the patient:		
2	has usually been appearing calm and positive in outlook, accepting and in control of personal circumstances, including surroundings.	63	(60)
1	has sometimes been troubled because not fully in control of personal circumstances or has been having periods of anxiety or depression.	35	(33)
0	has been seriously confused or very frightened or consistently anxious or depressed or unconscious.	7	(7)
	CUMULATIVE SCORE		
10-8		73	(70)
7-5		25	/24)
≤ 4		7	(7)

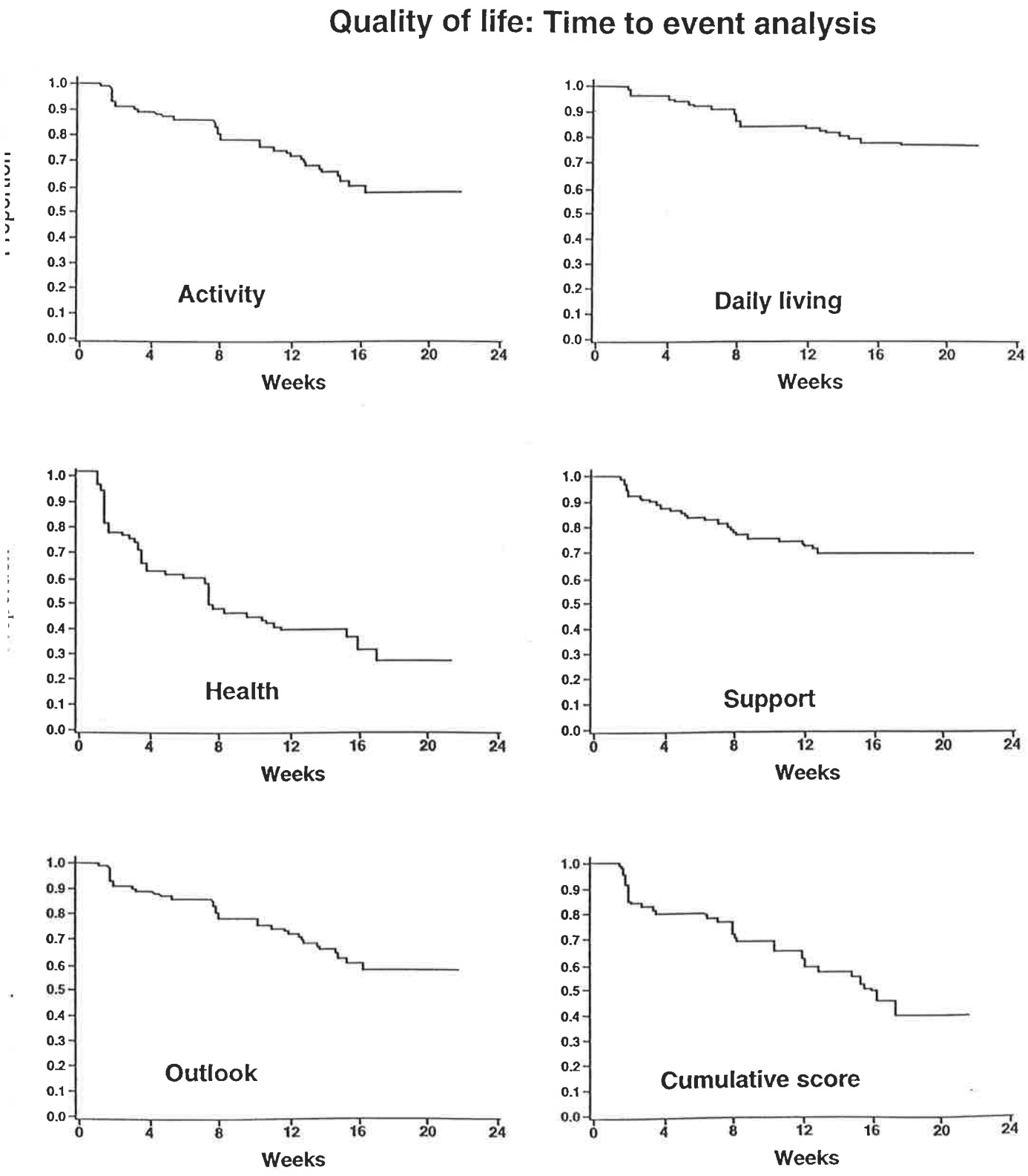
Table 33 Best Spitzer score on study

Baseline score	**Eval.**	**2**	**1**	**0**
Activity				
2	82	80	-	2
1	13	8	4	1
0	8	4	2	2
Daily living				
2	96	94	2	-
1	7	5	2	-
0	-	-	-	-
Health				
2	24	16	7	1
1	58	21	31	6
0	21	5	11	5
Support				
2	80	79	1	-
1	15	12	3	-
0	8	4	3	1
Outlook				
2	63	57	6	-
1	34	18	16	-
0	6	2	4	-
Cumulative score	**Eval.**	**10-8**	**7-5**	**≤ 4**
10 - 8	72	67	4	1
7 - 5	25	15	10	-
≤ 4	6	2	2	2

When the cumulative score was analyzed, a responder was defined as any patient who maintained, for at least four weeks, a total score higher than baseline. A group of 16 patients with a baseline score of 10, who could not have improved, were excluded from the analysis. In the other 89, a total of 27 patients (30%) presented a sustained improvement of the cumulative score. Improvements were observed in 7/29 (24%) of the ARC and 20/60 (33%) of the AIDS patients. When analyzed by baseline CD_4 counts, improvements occurred in 14/57 patients (25%) with CD_4 counts below 50 cells/mm^3, in 7/13 (54%) with CD_4 counts of 50 to 99 cells/mm^3, and in 6/19 (32%) with CD_4 counts > 100 cells/mm^3.

An additional approach to these data took into consideration variations of the quality of life over time. A time to event analysis was performed, considering as a negative event any deterioration by one point of each individual parameter in patients who ever achieved level 1 or 2, at baseline or on study, or a death within 30 days from the last assessment. For the cumulative score, a drop below 8 points was considered, in patients who ever achieved a level 8, 9 or 10, at baseline or on study, as well as a death within 30 days from the last assessment (Figure 13).

Figure13



All patients: N = 105

Discussion

More patients receiving ddI noted improvements in parameters of quality of life than experienced deterioration during the first 16 weeks of therapy. The health parameter was the measure which showed greatest change, improving in 30% of patients and deteriorating in only 14%. Overall improvements occurred in similar proportions of AIDS and ARC patients. Patients with baseline CD_4 < 50 cells/mm^3 were least likely to improve, although a quarter of these patients showed an overall improvement in QOL score.

2.3.12. Signs and Symptoms

A comprehensive evaluation of the clinical progress during the study was independently carried out by both the physician and the patient. They both utilized the same scale in order to assess the overall status in comparison to the prior visit

(1 = deterioration, 2 = no change, 3 = slight improvement, 4 = moderate improvement, 5 = marked improvement). Up to six consecutive evaluations on the first 105 patients are included in this analysis. Twice during the first month of treatment and then once monthly for four months (Table 34).

Table 34 Evaluation of clinical progress

	No.	%
Total	105	100
Missing data	2	2
Evaluated up to:		
week 2	7	7
week 4	12	11
week 8	10	10
week 12	26	25
week 16	32	30
week 20	16	15

When each of the 420 individual assessments were compared for consistency (Table 35), there was an agreement in more than two-thirds of the instances (296/420 or 70%). The physician had a better assessment of the symptomatic status than the patient in 57/420 instances (14%), whereas the patient reported a better symptomatic status than assessed by the physician in 67/420 instances (16%).

Table 35 Comparison of physician vs. patient assessment (all visits)

Assessed by patient

Assessed by physician	Deterio-ration	No change	Slight Improv	Moderate Improve	Marked Improve.	Total	%
Deterioration	63	9	3	1	-	76	18
No change	20	149	22	4	-	195	46
Slight imp.	6	20	43	13	9	91	22
Moderate imp.	1	4	3	29	6	43	10
Marked imp.	-	-	-	3	12	15	4
Total (%)	90 (21)	182 (43)	71 (17)	50 (12)	27 (6)	420	100

When the best score on therapy, as assessed by the physician, was considered, 7% of the patients (79/103) presented a symptomatic improvement(Table 36).

Table 36 Physician evaluation*

Time	No. Eval.	Deterioration	No Change	Slight Improvement	Moderate Improvement	Marked Improvement
Week 2	103	12 (12)	48 (47)	28 (27)	9 (9)	6 (6)
Week 4	96	16 (17)	49 (51)	19 (20)	8 (8)	4 (4)
Week 8	84	19 (23)	36 (43)	18 (21)	9 (11)	2 (2)
Week 12	74	14 (19)	36 (49)	13(18)	9 (12)	2 (3)
Week 16	48	13 (27)	20 (42)	10 (21)	5 (10)	
Week 20	16	2 (13)	7 (44)	3 (19)	3 (19)	1 (6)
Best score	103	6 (6)	18 (17)	38 (37)	27 (26)	14 (14)
Worst score	103	56 (54)	44 (43)	2 (2)	1 (1)	
Last score	103	34 (33)	42 (41)	15 (15)	10 (10)	2 (2)

** In parenthesis: percent*

In the patient's own evaluation, an improvement of the symptomatology occurred in 72% (74/103) of the cases (Table 37).

Table 37 Patient's evaluation*

Time	No. Eval.	Deterioration	No Change	Slight Improvement	Moderate Improvement	Marked Improvement
Week 2	102	18 (18)	40 (39)	20 (20)	17 (17)	7 (7)
Week 4	96	16 (17)	48 (50)	15 (16)	9 (9)	8 (8)
Week 8	84	22 (26)	33 (39)	16 (19)	9 (11)	4 (5)
Week 12	74	14 (19)	36 (49)	13(18)	9 (12)	2 (3)
Week 16	48	13 (27)	20 (42)	10 (21)	5 (10)	
Week 20	16	4 (25)	4 (25)	4 (25)	2 (13)	2 (13)
Best score	103	8 (8)	21 (20)	28 (27)	25 (24)	21 (20)
Worst score	103	65 (53)	34 (33)	3 (3)	1 (1)	
Last score	103	36 (35)	38 (37)	15 (15)	10 (10)	4 (4)

** In parenthesis: percent*

When the incidence of marked symptomatic improvements was analyzed by diagnosis, dose and baseline immunologic status, there was no significant difference among patient subsets (Table 38).

Table 38 Marked symptomatic Improvement

	Assessed by physician		Assessed by patient	
	Resp/Eval.	%	Resp/Eval.	%
All patients	14/103	14	21/103	20
By diagnosis				
ARC	2/40	5	5/40	13
AIDS	12/63	19	16/63	25
By baseline CD_4 count/mm^3				
< 50	9/62	15	11/62	18
50 - 99	2/16	13	4/16	25
≥100	3/25	12	6/25	24
	p = 0.742		p = 0.464	

Since limiting the definition of responder in symptomatic status to those patients with marked improvement might underscore the duration of response or even lead to neglecting cases with slower but constant progress, a different evaluation took into consideration the mean score recorded during the study. Patients with a mean value above 2 (better than no change) were assessed as responders. In the physician evaluation, 60 out of the 103 evaluable patients (58%) were symptomatic responders. These responses occurred in 26/40 (65%) of the ARC patients and in 34/63 (54%) of the AIDS patients. When evaluated according to baseline CD_4 counts, 29/62 (47%) of the patients with counts 50 cells/mm^3 responded, as compared to 12/16 (75%) of those with

counts between 50 to 99 cells/mm^3 and to 19/25 (76%) of those with higher counts.

When the same analysis was applied to the patient evaluation, a total of 51/103 (50%) of the patients responded, or 21/40 (53%) with ARC and 30/63 (48%) with AIDS. Responses according to baseline CD_4 counts were 26/62 (42%) in those with low counts, 10/16 (63%) in those with intermediate counts, and 15/25 (60%) in those with high counts.

DISCUSSION

Physicians and patients showed a high level of congruence between their assessment of overall charge from visit to visit. Similar proportions of patients by diagnosis and by CD_4 strata showed marked improvement. By this measure up to 50% of patients felt they had experienced some improvement during the study.

2.3.13. Survival with DDI

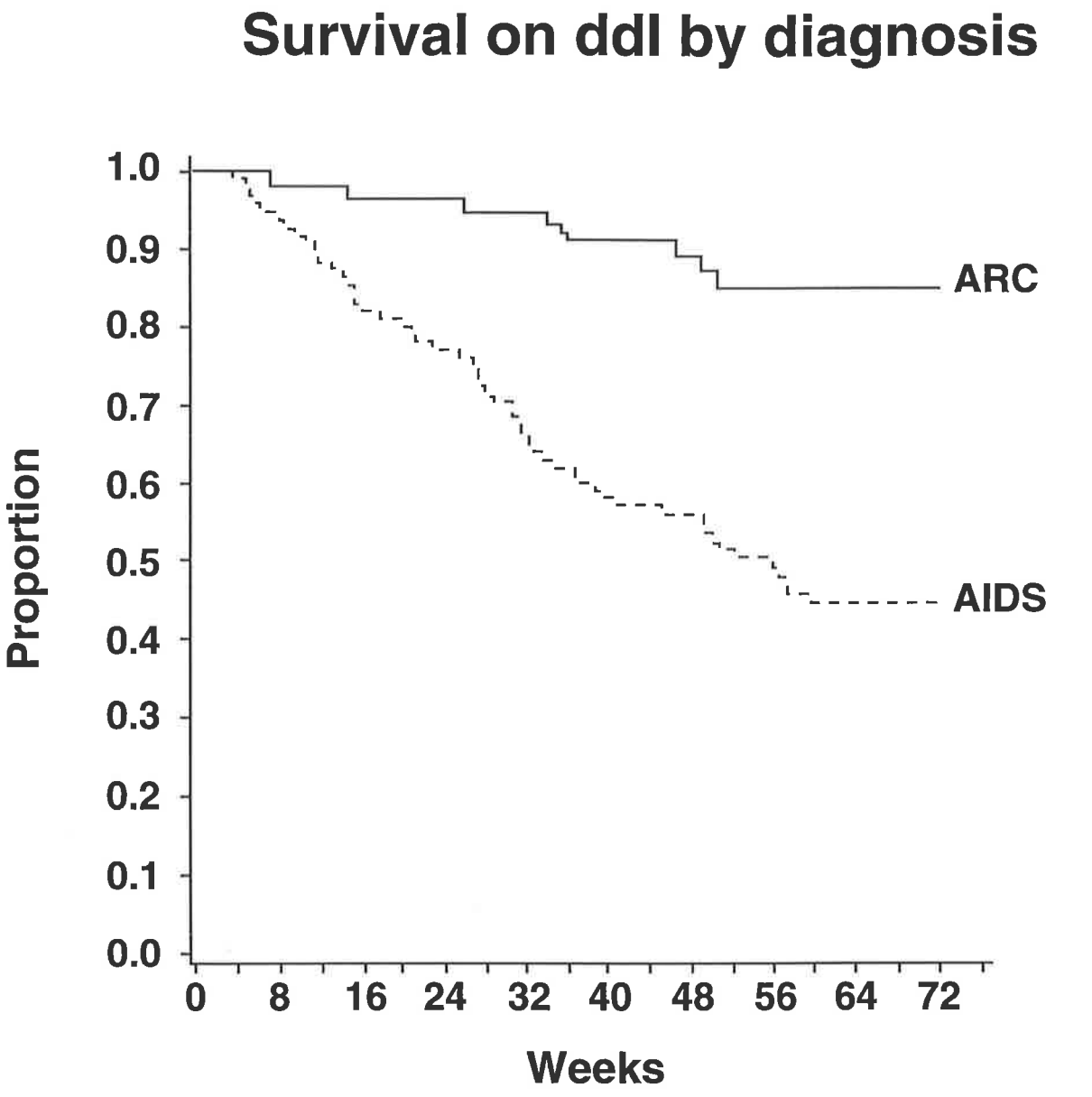
At the time of the final analysis fifty seven deaths had occurred in patients who received ddI in this study. Only 6 patients who died were originally in the symptomatic disease group. 3 AIDS patients, who were thought to be improving by their careers, died unexpectedly whilst on therapy with ddI. All had been seen by the trial physician within one week of death and had normal electrolytes, amylase and electrocardiographs. All cases had Mycobacterium avium (MAI) infection and were concomitantly receiving Rifabutin, Clofazamine and Ciprofloxacin. Post mortem examination was refused in all cases.

Patients with a baseline AIDS diagnosis had a much poorer survival (median 52 weeks) than those with baseline symptomatic HIV disease (median not reached) (Figure 14). The median survival for the whole group was not reached (Figure 15).

When prior duration of ZDV is examined patients with less than 6 months prior ZDV show better survival than those with greater periods of prior ZDV. However, this effect appears to decline after around 24 weeks when the Kaplan-Meier plots become closer (Figure 16).

Further details of survival are provided in the next section.

Figure 14



ARC N = 61 Median survival not reached
AIDS N = 90 Median survival 52 weeks (95% CI 34 -)

Figure 15

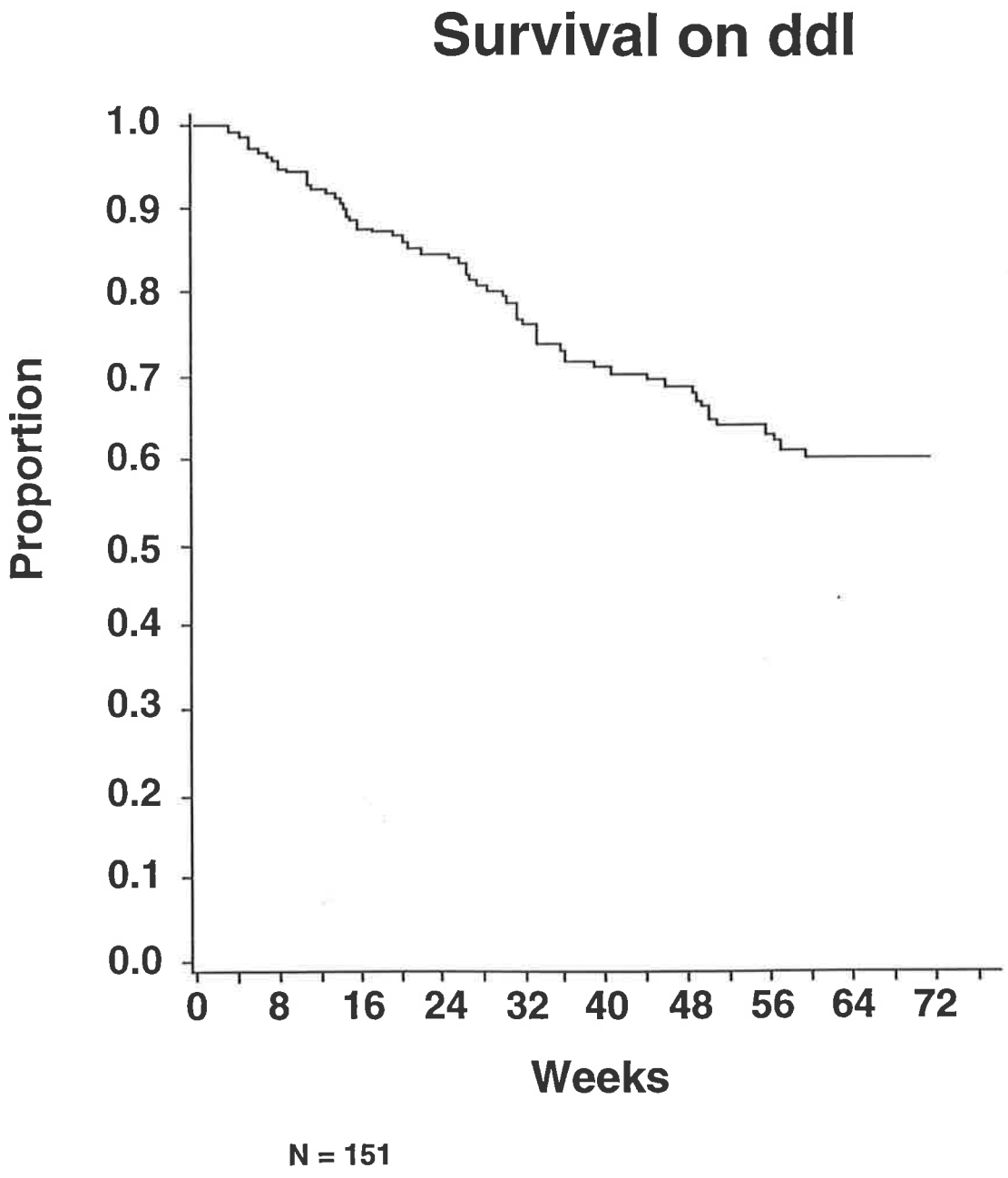
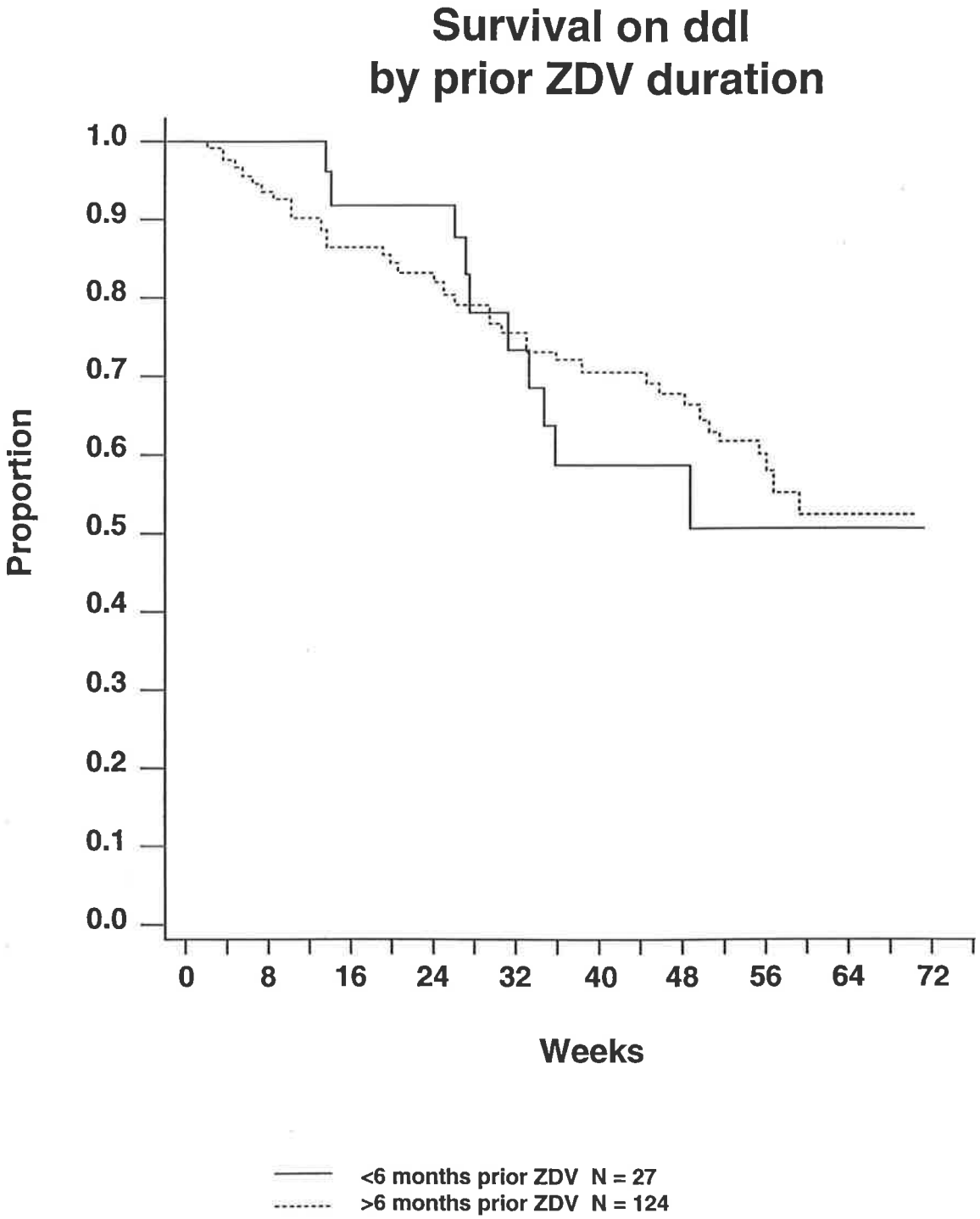

Survival on ddI
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
8
16
24
32
40
48
56
64
72
Weeks
N = 151

Figure 16


Survival on ddI
by prior ZDV duration
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
8
16
24
32
40
48
56
64
72
Weeks
<6 months prior ZDV N = 27
>6 months prior ZDV N = 124

2.4. SURVIVAL WITH DDI COMPARED TO HISTORICAL COMPARISONS

INTRODUCTION

The retrospectively collected ZDV-treated cohort was developed specifically as a historical control group for "the ddI study" with a number of known variables collected to assist matching of groups. The ZDV-treated cohorts were divided into groups based on year of commencement of ZDV, 1987, 1988, and 1989. The 1987 cohort was largely not used for comparison due to the variable use of pcp prophylaxis during that year and the significantly poorer survival in this group. From 1988 on both primary and secondary pcp prophylaxis was routine at all East Riverside HIV clinics (see section 2.1).

The objective of these analyses was to examine the value in ddI as compared with ZDV in prolonging survival. In assessing the raw data it must be remembered that ddI was given as a second line therapy whereas ZDV had been a first line therapy. Furthermore, patients entering the ddI study had a lower mean CD_4 count and were more likely to have an AIDS diagnosis, particularly when compared to the 1989 ZDV cohort. It was, therefore, planned that comparisons should not only be made on overall data or data comparing similar diagnosis or CD_4 count groups but that a proportional hazard model be used to enable comparison of like against like. Furthermore, as several other ddI studies were commencing or in advanced planning at that time in the USA and we endeavored to design subgroups analyses which could match some of that future data, to provide further construct validity.

In these analyses, survival data were censored on June 30 1991 for the ZDV patients (maximum survival time 182 weeks) and on March 15 1992 for the ddI patients (maximum survival time 108 weeks).

STATISTICAL CONSIDERATIONS

The baseline variables of diagnosis, CD_4 count and hemoglobin were considered as prognostic factors. These factors have been compared between treatment arms using Fisher's exact test for categorical variables or the Willcoxon two sample test for continuous variables. The survival curves have been produced using the Kaplan Meier Method, without any adjustment for baseline variables. A comparison between treatment arms was assessed by means of a log rank test.

The Cox regression method (Cox DR, 1984) has been used to estimate the treatment effect on survival adjusted for prognostic factors. Covariates were selected by means of a forward stepwise regression procedure. A covariate was allowed to enter the model if the significance level was less than 0.10 and, once added, could be removed from the model if the significance level was greater than 0.15. The selected regression model was that including all significant covariates and treatment arm. A comparison between treatment groups was assessed by means of a log likelihood ratio test. A estimate of relative risk with 95% confidence interval has also been included.

The SAS Software package was used to perform all calculations.

2.4.1. Results

Overall, patients receiving ddI showed poorer survival compared with patients who received ZDV in any on the cohort years ($p < 0.01$) [Figure 17]. The ddI group also showed poorer outcome when patients with AIDS, or ARC, or CD_4 count <100 were examined (Figures 18, 19, 20). However, for patients with $CD_4 \geq 100$ cells/mm^3 no differences were seen [Figure 21].

These comparisons were not felt to compare like against like, patients commenced ddI at lower CD_4 counts than when they commenced ZDV and received ddI as their second nucleoside therapy, a factor which may influence response to that therapy (i.e. nuceleoside analogue naive patients may experience responses of greater magnitude and duration than patients who have previously received nucleoside therapy). Three further analyses of subgroups were performed, drawing patients for the 1988 and 1989 ZDV cohorts for comparison with ddI and using a Cox proportional hazards model to adjust for baseline differences. The baseline variables used as prognostic factors were those identified from the historical control analysis namely the categorical variables of diagnosis (ARC or AIDS), CD_4 (> or < 100 cells/mm^3) and haemoglobin (> or < 11 g/dl) and the continuous variables of CD_4 and haemoglobin.

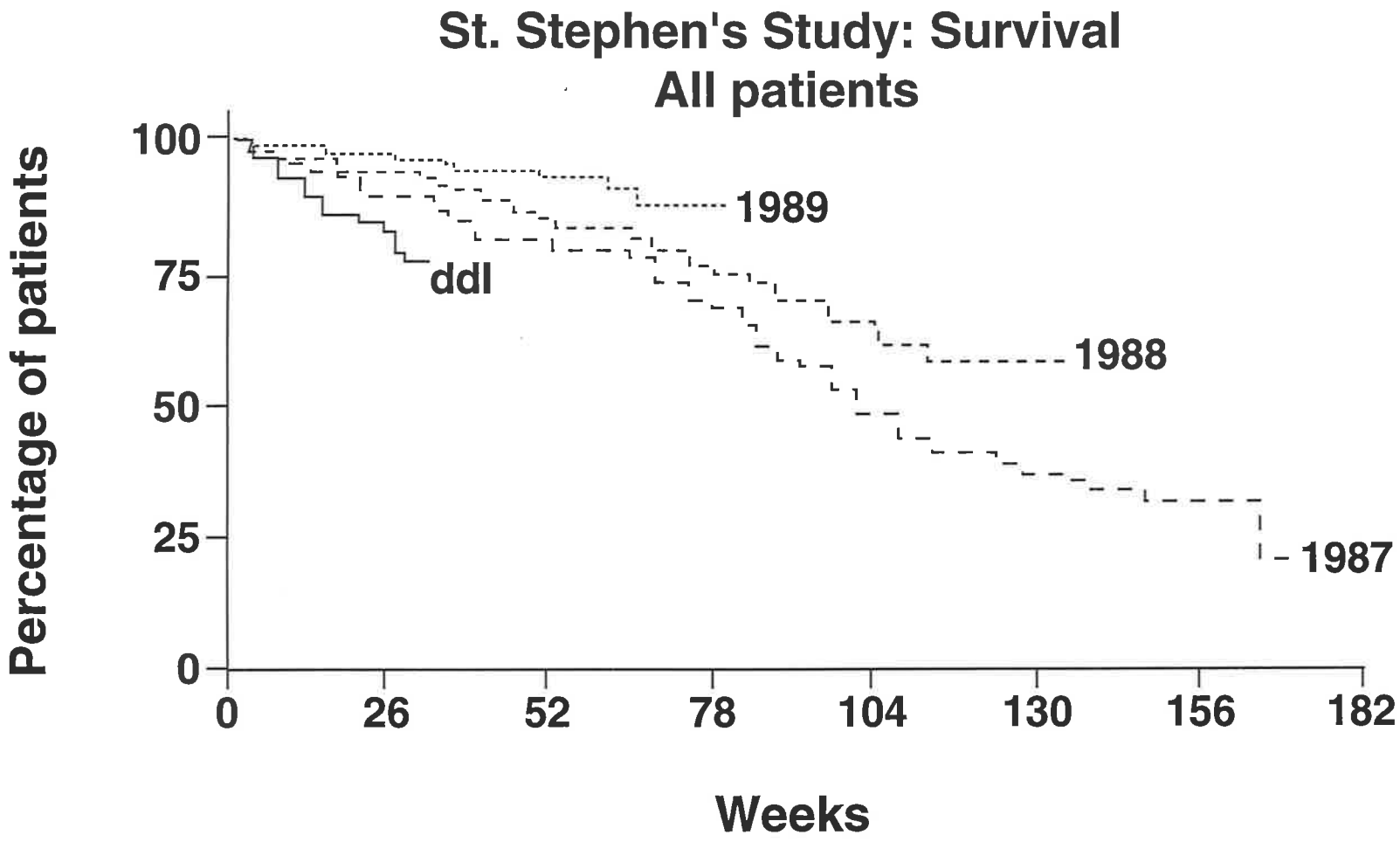

St. Stephen's Study: Survival
All patients
Percentage of patients
100
75
50
25
0
0
26
52
78
104
130
156
182
Weeks
1989
ddl
1988
1987

Figure 17

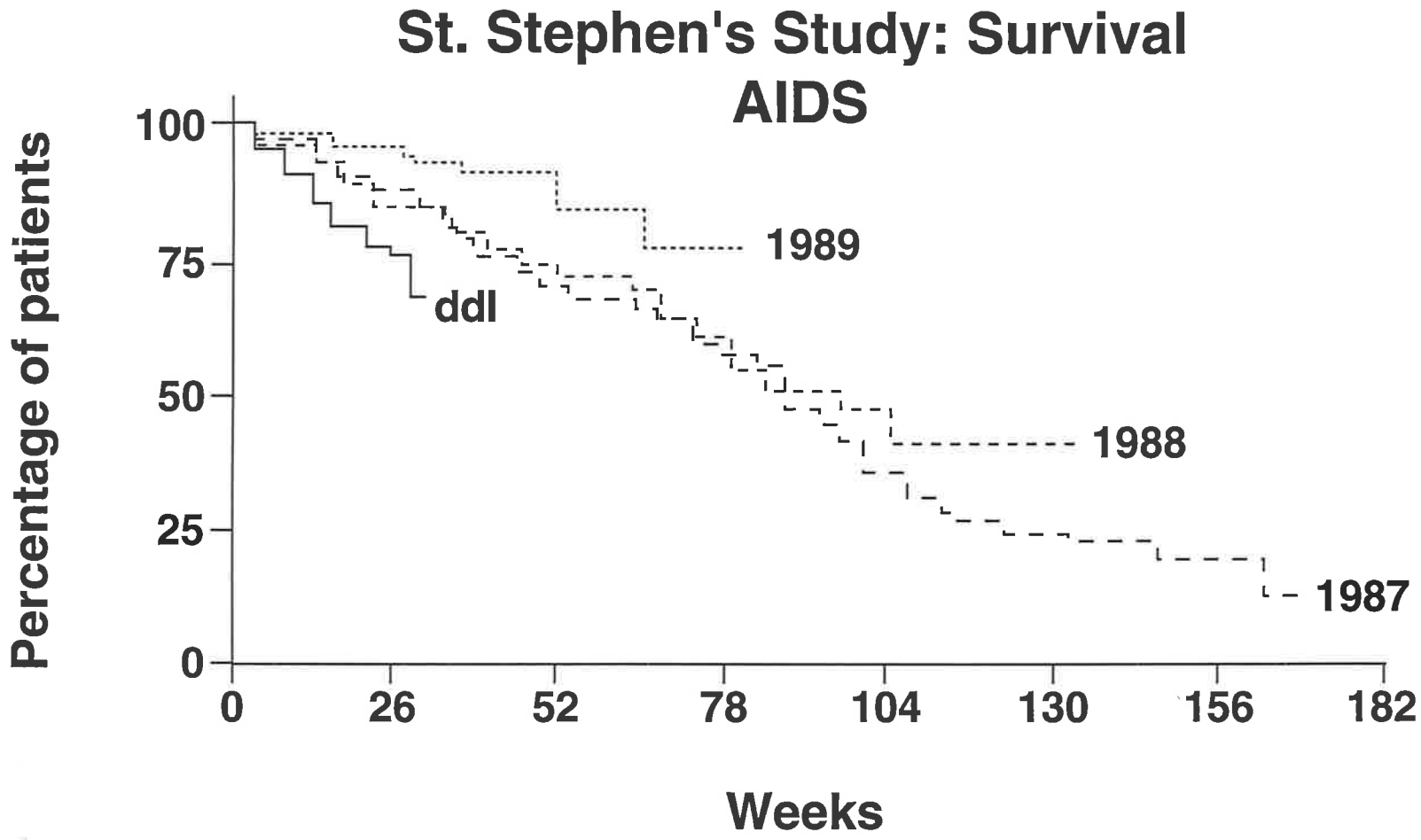

St. Stephen's Study: Survival
AIDS
Percentage of patients
100
75
50
25
0
0
26
52
78
104
130
156
182
Weeks
1989
ddI
1988
1987

Figure 18

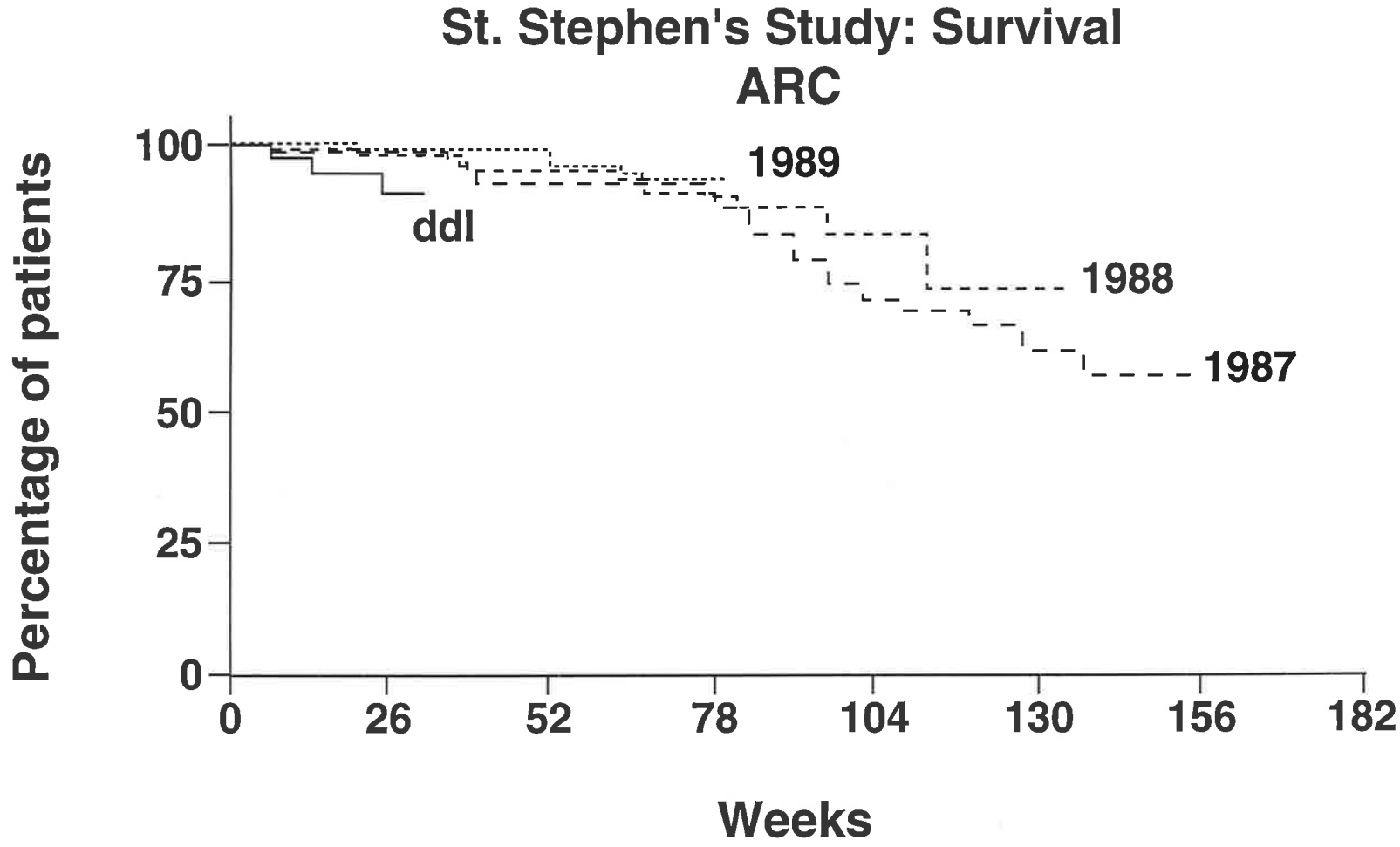
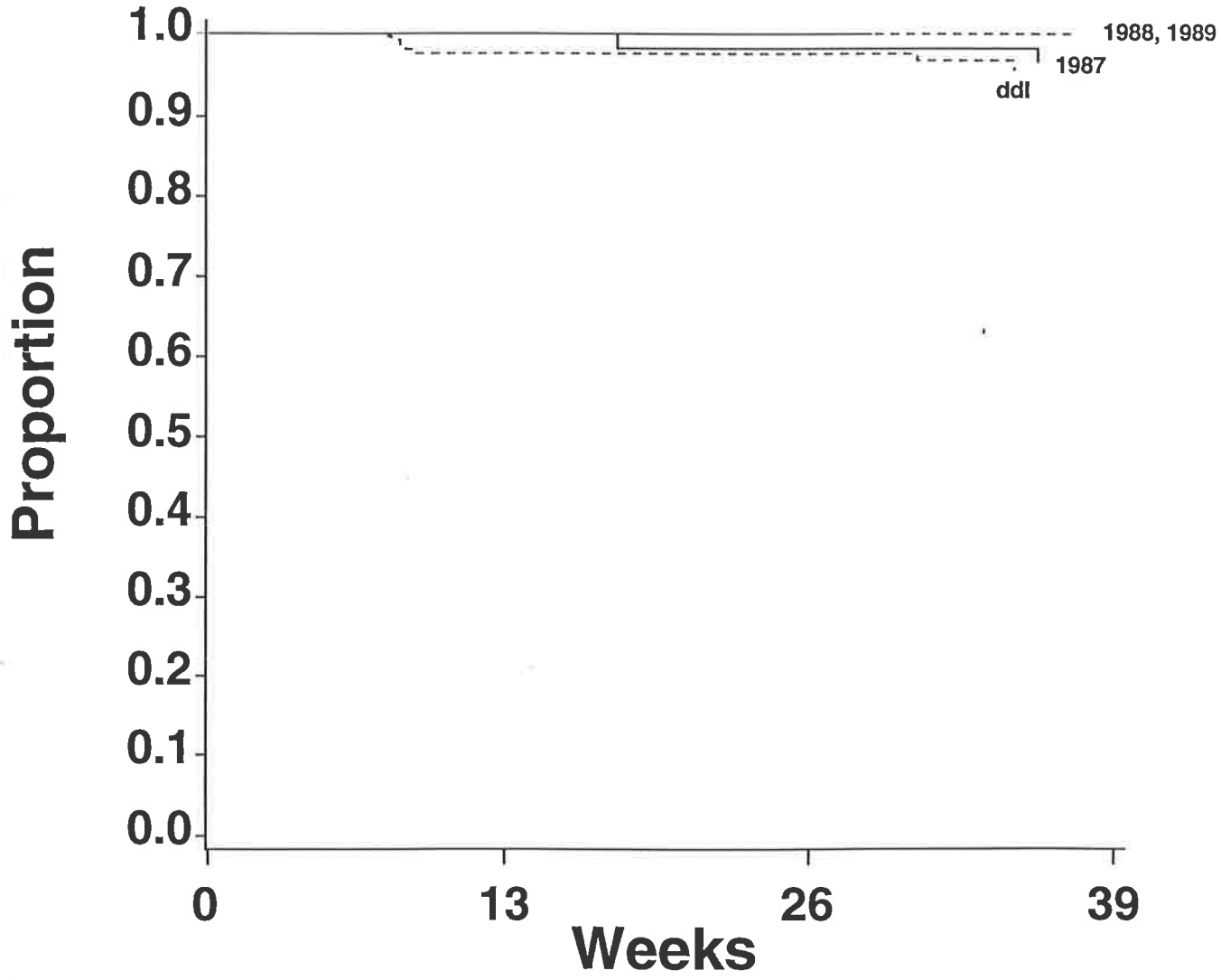

St. Stephen's Study: Survival
ARC
Percentage of patients
100
75
50
25
0
0
26
52
78
104
130
156
182
Weeks
1989
ddl
1988
1987

Figure 19

Figure 20

Overall survival for baseline CD4 count 100 cells/mm^3: AZT by year cohort vs ddI



1987 N = 54
1988 N = 83
1989 N = 138
ddI N = 26

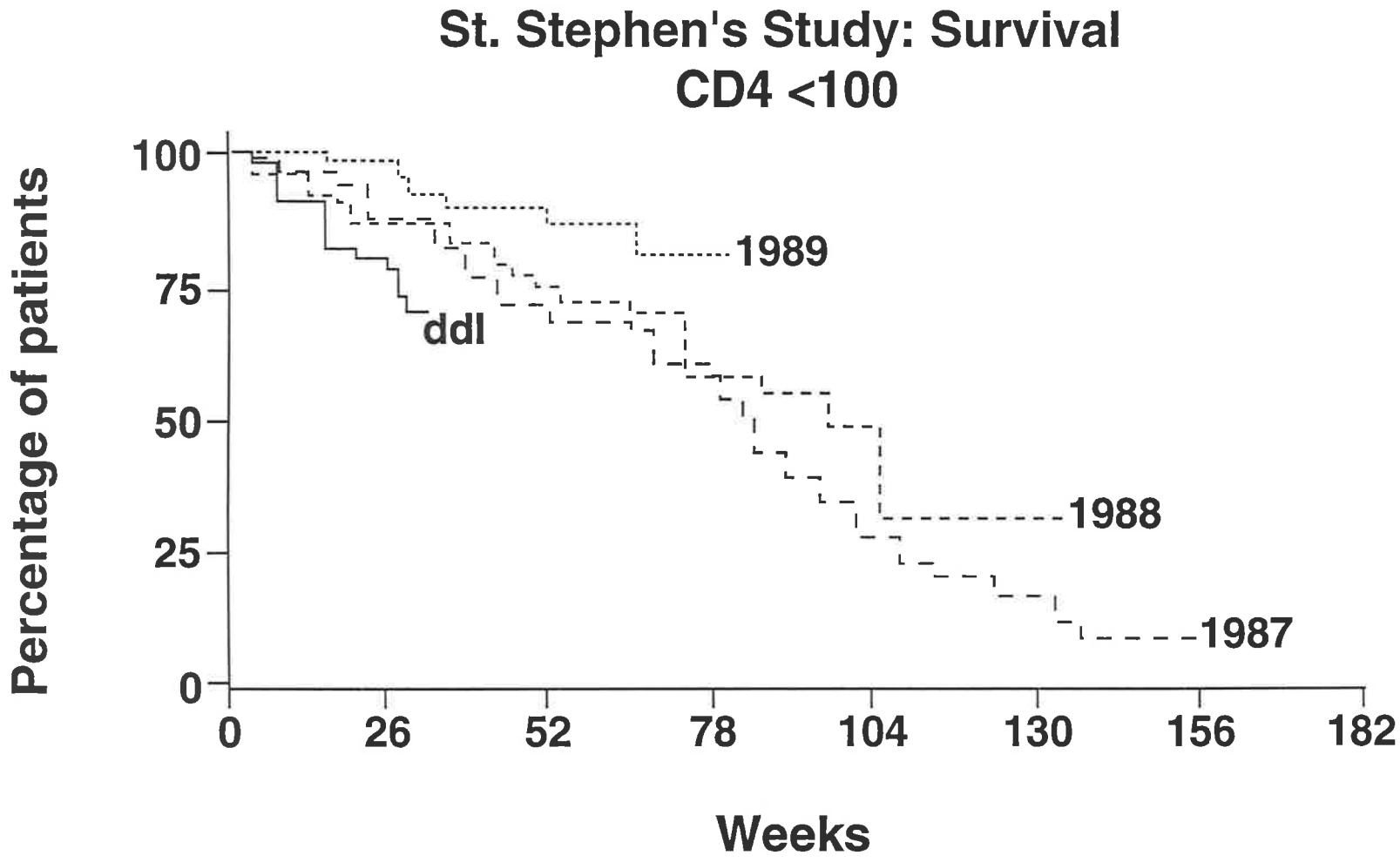

St. Stephen's Study: Survival
CD4 <100
Percentage of patients
100
75
50
25
0
0
26
52
78
104
130
156
182
Weeks
1989
ddl
1988
1987

Figure 21

Analysis I

The first analysis compared the group of patients who has received ZDV for less than 6/12 prior to receiving ddI with those patients who received ZDV "long term" (i.e. greater than 6 months, never switching to ddI) from the 1988 and 1989 cohorts. Based on the analysis presented earlier (HCA 1) the 3 factors in our cohort shown to influence survival were hemoglobin, CD_4 count and disease stage at baseline, these were therefore used to correct the comparison between groups by covariate analysis.

The AZT arm was constituted of patients who started AZT during the year 1988 or 1989 and who were not identified to have switched from AZT to ddI as second line therapy. 35 patients with missing baseline diagnosis, CD_4 count or haemoglobin were excluded, leaving a group of 276 patients.

The ddI arm was constituted of patients from the ddI study and who had received less than 6 months of prior AZT therapy. This resulted in a set of 30 patients.

COMPARISON BETWEEN TREATMENT ARMS

There were significantly more patients with baseline CD_4 count > 100 in the AZT arm compared to the ddI arm (p=0.01). Diagnosis (ARC or AIDS) and hemoglobin (< 11 or > 11 g/dl) categories did not differ significantly between the treatment arms (Table 38).

The mean baseline CD_4 count was significantly higher in the AZT arm compared to the ddI arm (p=0.002). The difference between the two means was 70 cells/mm3 (Table 38).

Table 38 Baseline Variables by Treatment Arm

	AZT + no ddI arm N-276 (100%)	AZT + ddI arm N-30 (100%)
Diagnosis		
ARC	183 (66%)	16 (53%)
AIDS	93 (34%)	14 (47%)
CD_4 count (mm^3)		
Mean	187	117
Median	152	46
Range	1-1008	5-480
<100	98 (36%)	18 (60)
≥ 100	178 (64%)	12 (40%)
Haemoglobin (g/dl)		
Mean	13.3	12.7
Median	13.5	13.1
Range	8.1 - 17.4	8.1 - 16.3
< 11	28 (10%)	6 (20%)
≥ 11	248 (90)	24 (80%)

SURVIVAL ANALYSIS

The survival time was based on the start date of therapy with either AZT or ddI depending on the treatment arm.

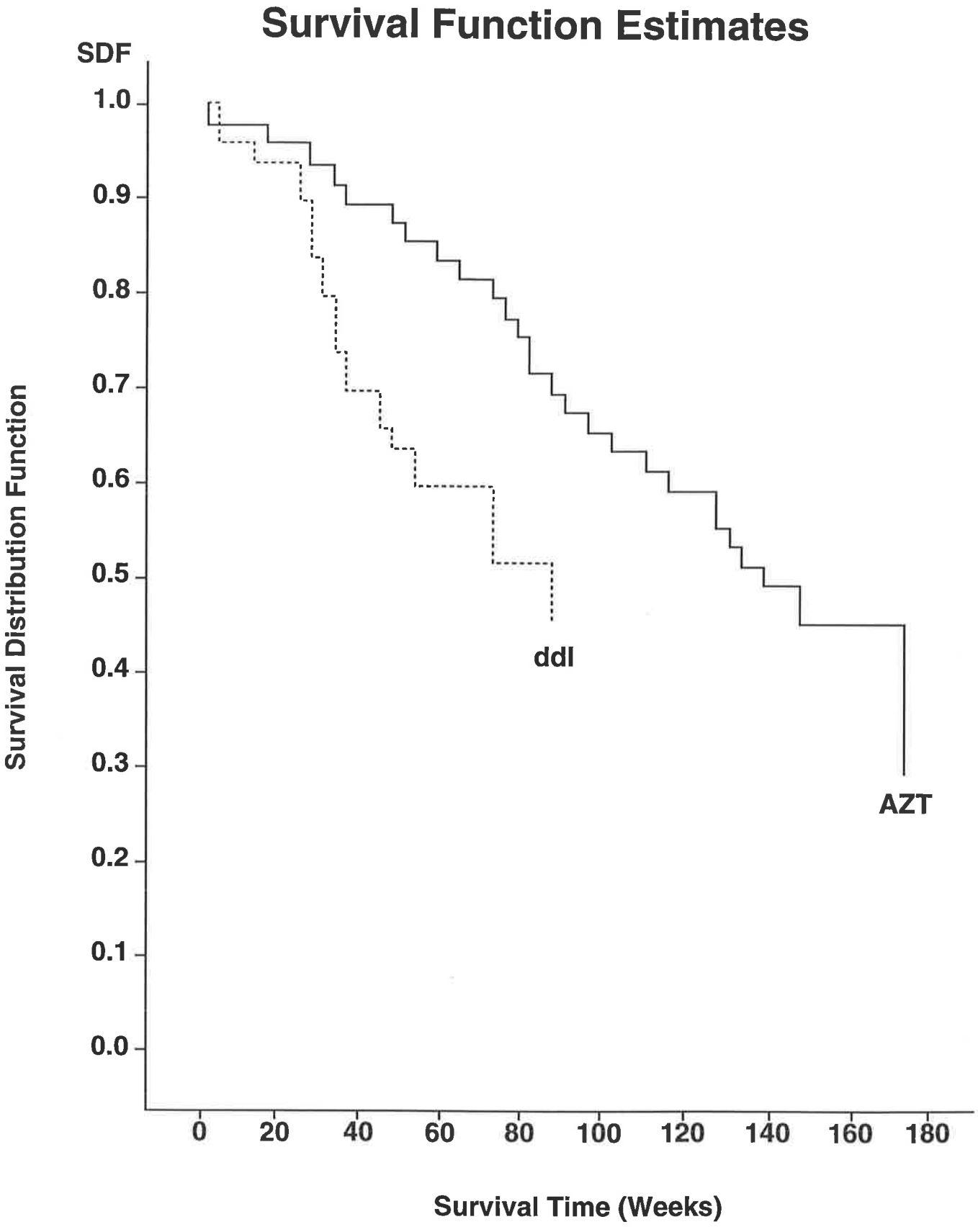
In the AZT arm, 66% of the patients were censored versus 50% in the ddI arm.

The uncorrected mean survival time was longer for the patients in the AZT arm: 85.6 weeks versus 65.4 weeks for the ddI arm.

Figure 22 shows that the survival curve related to the patients from the AZT arm is above the ddI curve.

Without any adjustment with any of the baseline variables, the log rank test used to compare the two treatment arms indicated a significant difference in favor of AZT (p=0.004).

Figure 22


Survival Function Estimates
SDF
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Survival Distribution Function
ddI
AZT
0
20
40
60
80
100
120
140
160
180
Survival Time (Weeks)

COX'S REGRESSION

The subset of baseline variables (see section 3.2) that were selected with the stepwise procedure were: the diagnosis, the baseline CD_4 count (continuous variable) and the hemoglobin level (continuous variable).

The test of the treatment effect after adjusting for those covariates was not significant (p=0.27).

The estimated relative risk of ddI compared with AZT was 1.4 with 95% confidence interval of (0.8, 2.5).

ANALYSIS II

The second analysis examined survival from stopping ZDV for those patients in the 1988 and 1989 cohorts who never received ddI compared with those who did receive ddI. This was essentially a treatment versus no treatment comparison.

The "AZT + no ddI arm" was constituted of patients who started AZT during the year 1988 or 1989 and who did not receive ddI as second line therapy. After exclusions for missing variables at set of 71 patients were identified.

The "AZT + ddI arm" was constituted of patients who started AZT during the year 1988 or 1989 and who subsequently received ddI as second line therapy. After exclusions 53 patients were identified.

Baseline variables were defined at the start of the AZT therapy for both arms.

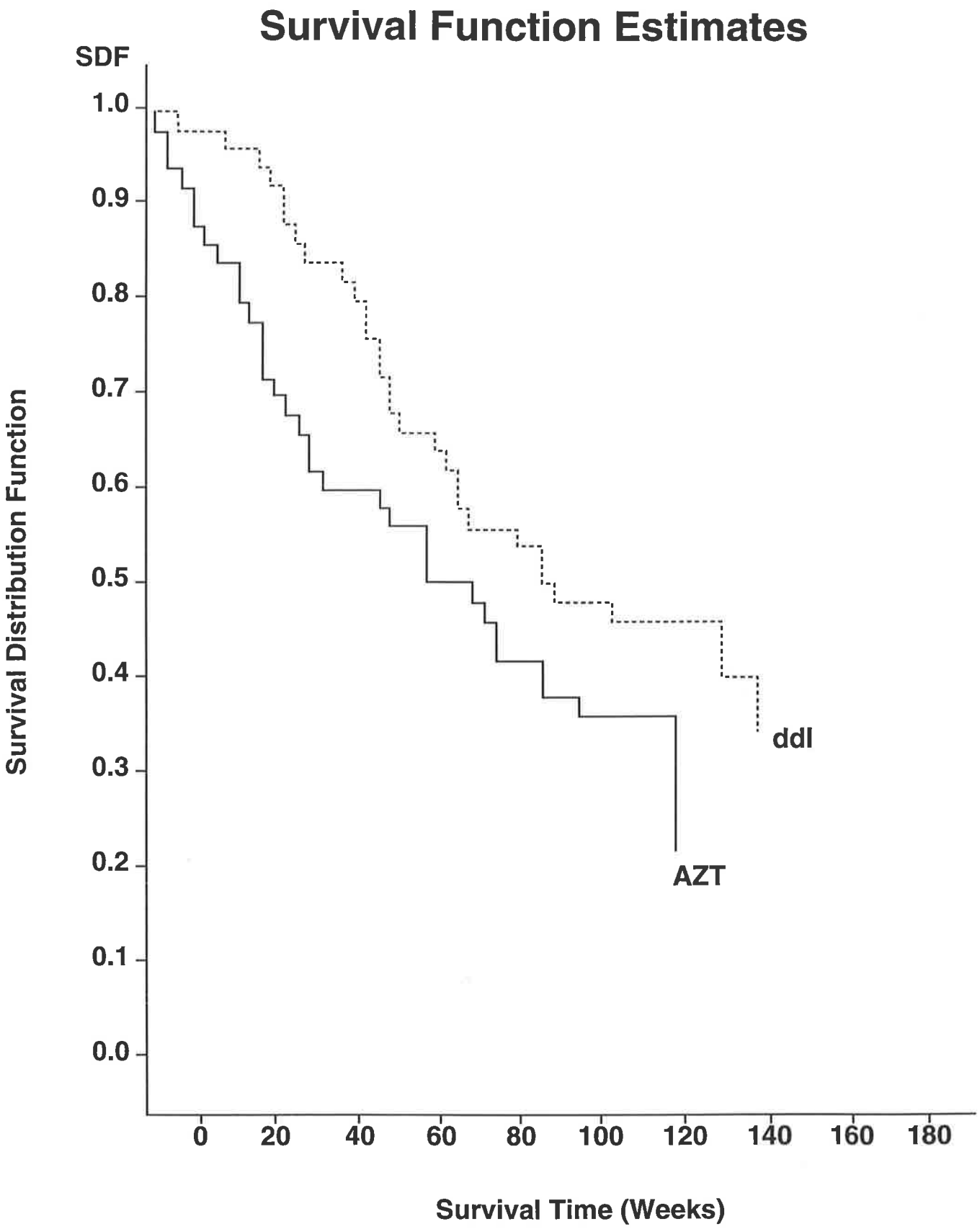
2.4.1.1. *Comparison Between Treatment Arms*

There were no significant differences between treatment arms for any of the baseline variables (Table 39).

Table 39 Baseline Variables by Treatment Arm

	AZT + no ddI arm N=71 (100%)	AZT + ddI arm N=53 (100%)
Diagnosis ARC	38 (54%)	33 (62%)
AIDS	33 (46%)	20 (38%)
CD_4 count (mm^3)		
Mean	147	130
Median	110	102
Range	1 - 600	5-405
<100	29 (41%)	26 (49%)
≥ 100	42 (59%)	27 (51%)
Haemoglobin (g/dl)		
Mean	12.6	13.2
Median	12.8	13.4
Range	8.1 - 15.8	8.9 - 18.1
< 11	12 (17%)	7 (13%)
≥ 11	59 (83%)	46 (87%)

Figure 23


Survival Function Estimates
SDF
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Survival Distribution Function
ddI
AZT
0
20
40
60
80
100
120
140
160
180
Survival Time (Weeks)

SURVIVAL ANALYSIS

For this analysis, the survival time was based on the first AZT stop date.

The censoring was identical for both arms (42-43%).

The uncorrected mean survival time was longer for the patients in the AZT + ddI arm: 90.0 weeks versus 57.9 weeks for the AZT + no ddI arm (Figure 23).

Without any adjustment with any of the baseline variables, the log rank test used to compare the two treatment arms indicated a borderline significant difference between the two treatment arms (p=0.052).

COX'S REGRESSION

The test of the treatment effect after adjusting for the covariates was significant (p=0.002) and in favor of the AZT + ddI arm.

The estimated relative risk of AZT followed by ddI compared to AZT not followed by ddI was 0.46 with 95% confidence interval of (0.28, 0.76).

ANALYSIS III

The third analysis examined the patient group with good baseline characteristics principally CD_4 above 100 cells/mm3 who received only ZDV in the years 1988 and 1989 and those who initially received ZDV and subsequently switched to ddI. All patients included received ZDV for a minimum of 8 weeks to match the entry criteria for the ddI study.

The selected populations for this analysis were similar to the previous one: "AZT + no ddI arm" and "AZT + ddI arm". The difference is that in this analysis, only those patients have been included who had a relatively

good prognosis at the time of initiating AZT and who survived at least 8 weeks (to allow the patient to switch from AZT to ddI).

Following exclusions for missing data the analyzed number of patients were 176 for the AZT + no ddI arm and 40 for the AZT + ddI arm.

Baseline variables were defined at the start of the AZT therapy for both arms.

The same baseline variables as previous were used except the categorical variable for the baseline CD_4 count as there are only patients with CD_4 count above 100.

COMPARISON BETWEEN TREATMENT ARMS

The mean baseline CD_4 count was significantly higher in the AZT + no ddI arm compared to the AZT + ddI arm (p=0.03). The difference between the two means was 56 cells/mm3 (Table 40).

There were no differences between the baseline diagnosis and the mean baseline hemoglobin of the two treatment arms.

Table 40 Baseline Variables by Treatment Arm

	AZT + no ddI arm N=176 (100%)	AZT + ddI arm N=40 (100%)
Diagnosis ARC	142 (81%)	28 (70%)
AIDS	34 (19%)	12 (30%)
CD_4 count (mm^3)		
Mean	263	207
Median	233	183
Range	100 - 1008	100 - 506
<100	0 (0%)	0 (0%)
≥ 100	176 (100%)	40 (100%)
Haemoglobin (g/dl)		
Mean	13.7	14.1
Median	14.0	14-1
Range	8.9 - 17.4	11.3 - 18.1
< 11	8 (5%)	0 (0%)
≥ 11	168 (95%)	40 (100%)

SURVIVAL ANALYSIS

The survival time was calculated from the start date of AZT therapy for both treatment arms.

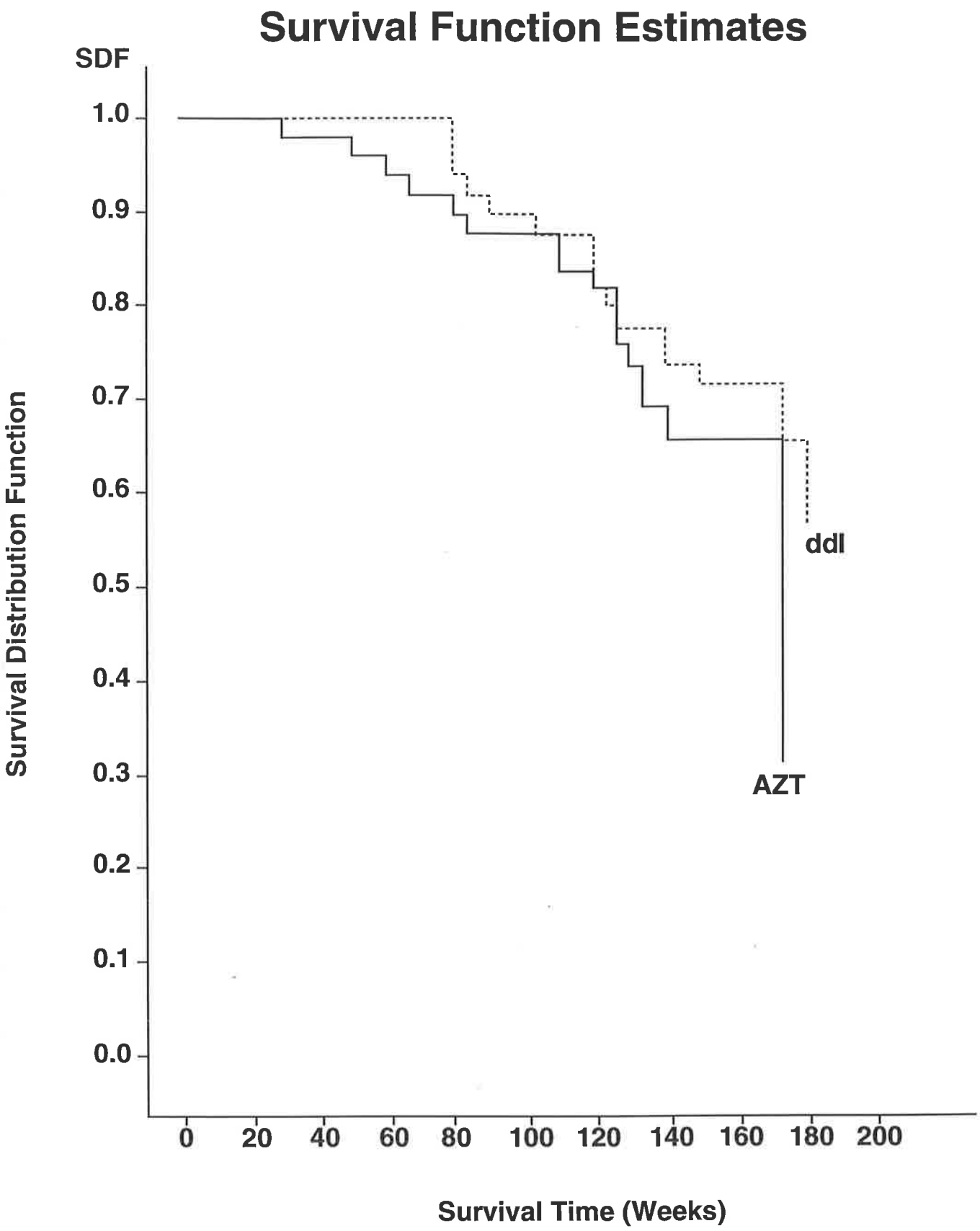
The censoring was quite high and more important for the patients in the AZT + no ddI arm (84%) compared to the AZT + ddI arm (68%).

The uncorrected mean survival time was longer for the patients in the AZT + ddI arm: 151.2 weeks versus 94.4 weeks for the AZT + no ddI arm.

Figure 24 shows that the two survival curves are very close together.

Without any adjustment with any of the baseline variables, the log rank test indicated a not significant difference between the two treatment arms (p=0.36).

Figure 24


Survival Function Estimates
SDF
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Survival Distribution Function
ddI
AZT
0
20
40
60
80
100
120
140
160
180
200
Survival Time (Weeks)

2.4.1.2. *Cox's Regression*

The test of the treatment effect after adjusting for the covariates was not significant (p=0.23).

The estimated relative risk of AZT followed by ddI compared to AZT not followed by ddI was 0.64 with a 95% confidence interval of (0.30, 1.36).

DISCUSSION

Second line ddI does not appear to be as effective in prolonging survival as first line ZDV. However, the samples used show considerable difference in potentially prognostically significant baseline variables. In analyses correcting for these variables switching to ddI after early ZDV intolerance appears as effective in prolonging survival as continued long term ZDV, and receiving ddI after ZDV intolerance provides a significant survival benefit compared with receiving no further antiretroviral therapy. This advantage provided by ddI was shown in small numbers of patients.

2.5. CMV RETINITIS: FOSCARNET VERSUS GANCICLOVIR

INTRODUCTION

At the time of developing this protocol no comparative study of Foscarnet and Ganciclovir had been published, and, in particular the SOCA study had not commenced. I have included this data in the thesis as Foscarnet is an antiretroviral agent which was known at the time of developing this protocol to suppress HIV replication *in vitro* and *in vivo*. Its use is indicated in patients with AIDS, (specifically with CMV disease) the stage of HIV disease where antiretroviral therapy is best proven to provide survival benefit. Foscarnet is a different chemical class to nucleoside analogue agents and does not require intracellular activation. At that time, HIV or CMV resistant to Foscarnet, either *in vitro* or *in vivo* had not been reported.

This study was, therefore, designed with both comparative efficacy in CMV disease and survival with AIDS in mind. The study was independent of external financial or analytical support, being designed and conducted by collaboration between the HIV unit (Dr Brian Gazzard and myself) and the ophthalmology department (Mr Bruce Mathalone, Mr Martin Harris and Dr Sue Mitchell)

2.5.1. Objectives

The objectives of this randomized open label Foscarnet versus Ganciclovir study was primarily to compare the efficacy and safety of these two agents in the treatment of ophthalmologically confirmed first episode CMV retinitis and as maintenance therapies in preventing

reactivation of disease. Survival was a secondary endpoint, and no quality of life studies were included.

2.5.2. Methodology

The study was divided in to a 3-week-treatment-phase in which patients were randomly assigned to receive either Foscarnet (initial loading dose of 20 mg/kg followed by either a single 16 hour-infusion or three 2 hour-infusions to a total daily dose of 130 - 200 mg/kg (adjusted for creatinine clearance) or Ganciclovir (10 mg/kg per day as two equally divided doses) initially via peripheral venous access until either a Hickman® or Portacath® type central venous access was inserted. Patients in the Foscarnet arm also received two litres of intravenous normal saline per day in an attempt to reduce the frequency of renal impairment (Deray G, et al, 1989 Deray G, 1990).

Inclusion criteria are shown below. Anaemia did not constitute a cause for exclusion and whenever possible was treated by transfusion throughout the study.

INCLUSION CRITERIA WERE:

1) Diagnosis of CMV retinitis by independent ophthalmologist
2) HIV antibody positive as confirmed by two methods
3) over 18 years of age
4) Able to give informed consent
5) Not pregnant or breast feeding and using adequate contraception if a menstruating female.
6) White cell count > 0.8×10^9 cells/l
   Platelets > 50×10^9 cells/l

Urea	< 12 mmol/1
Creatinine	< 130 μlmol/1
Liver Function tests	< 3 x upper limit of normal

7) Not having received either study drug during the last two weeks
8) Not receiving therapy for other active OI concomitantly.

ASSESSMENTS WERE PERFORMED ACCORDING TO THE FOLLOWING SCHEDULE:

EVALUATION AND ASSESSMENT

PRE-TRIAL:

1) Ophthalmological assessment with retinal photography
2) Complete history and examination
3) Blood biochemistry by standard screen including renal function, Calcium and Glucose
4) Full Blood count including white cell differential.
5) Confirmatory HIV test

DURING TREATMENT:

1) Biochemistry-and full blood count twice weekly
2) Ophthalmic assessment weekly with retinal photography
3) Ophthalmic efficacy assessment at end of week 3.

DURING MAINTENANCE:

1) Biochemistry FBC fortnightly
2) Monthly retinal assessment with photography

All patients successfully completing the treatment phase and with laboratory parameters within the inclusion guidelines were continued on their initial therapy as secondary prophylaxis or "maintenance". Drug was

given five times per week with patients self administering via their central venous lines, following a short period of training. Dosages were Ganciclovir 5 mg/kg/day, and Foscarnet 90 - 120 mg/kg/day adjusted for creatinine clearance, with Foscarnet patients encouraged to drink at least 1 litre fluid over the 2-hour-infusion period.

The use of ZDV was encouraged throughout the trial although this drug was stopped in preference to a change in anti CMV therapy if bone marrow suppression occurred.

All patients in the study were followed to the endpoints of disease response at 3 weeks therapy as defined by

a) Complete remission was defined as a 90% reduction in retinal activity with no progression of lesions or development of new lesions

b) Partial responses as less than 90% reduction in lesion activity with no edge progression or new lesions

c) Treatment failure was defined as edge progression and/or the appearance of new retinal lesions

and in the maintenance phase, disease reactivation was defined as the development of new lesions, edge progression or appearance of new activity in previously quiescent area.

Secondary endpoints were death and frequency of an adverse event requiring therapy change. Results were then tabulated and compared by odds ratio and students t test. All patients were followed to death. Patients

who received less than 6 weeks of their primary therapy were excluded from the main survival analysis in an attempt to examine a 'therapy effect'. Intention to treat analysis was thought likely to contain inherent bias due to the anticipated frequency of treatment/maintenance therapy change. Such a group also excluded those patients dying shortly after diagnosis of CMV retinitis who are likely to die of non-CMV related causes and could considerably bias a study involving fairly small numbers.

2.5.3. Statistical Considerations

One hundred and 52 patients were planned to give a two-sided 5% type I error and a 20% type II error in probability given a 15% difference between treatments (i.e. 80% versus 95% response rates). An interim analysis was planned once recruitment was 50% complete.

2.5.4. Ethical Issues

Patients were free to withdraw consent at any time during the study. Those patients failing to comply with administrative requirements of the study were also withdrawn. The study was passed by the Ethical Committee of Riverside Health Authority.

2.5.5. Efficacy In CMV Retinitis

Sixty-two patients were enrolled in the study although five (4 randomized to Ganciclovir, 1 randomized to Foscarnet) were withdrawn within 24 hours as laboratory parameters obtained at time zero failed to meet inclusion criteria although previous recent results had been satisfactory. All 57 patients were Caucasian male homosexuals, six of whom had

received previous treatment for CMV disease at non-retinal sites. None had been treated for CMV in the previous month. A further group of 32 patients diagnosed with CMV retinitis during the recruitment period 1.11.88 - 31.6.90 (Table 41) failed to meet inclusion criteria or declined participation in the trial.

Table 41 Reasons for not entering the study

Declined study	10
Thrombocytopenia	7
Neutropenia	5
Elevated renal function	4
Other OI	6
Total	32

Patients were well matched on baseline characteristics including age (mean 37.4 years), CD_4 positive cell counts, presence of HIV p24 antigen and prior use of ZDV or ddI (Table 42).

Table 42 Patient characteristics

	Mean CD_4 lymphocyte count (range)	HIV p24 positive* (%)	Prior or current antiretroviral use	
			ZDV	ddI
Ganciclovir (26)	20 (2-79)	6 (23)	12	4
Foscarnet (31)	17.4 (2-107)	9 (29)	10	4

* *ELISA (Abbot Laboratories).*

Several patients did not complete 3 weeks of treatment due to either toxicity, consent withdrawal (2 patients decided to decline further therapy) or death due to an unrelated cause (1 patient: death attributed to disseminated Kaposi's sarcoma and Mycobacterium avium complex)(Table 43).

Table 43 Results of treatment

	Not completing therapy	Completed therapy	Complete response at 3 weeks
Ganciclovir (26)	4*	22 (85%)	19/22 (86%)
Foscarnet (31)	6 **	25 (81%)	17/25 (68%)

* *Reasons for not completing Ganciclovir treatment: two patients with WBC <0.8 x10^9/l, two patients withdrew consent before completion of treatment.*

** *Reasons for not completing Foscarnet treatment: one patient had Ca^{2+} <2.0 mmol/l, two had penile ulceration, two had serum creatinine >130 mmol/l, one patient died before completing treatment of an unrelated cause.*

A similar percentage of patients were able to complete 3 weeks therapy, however, more patients completing 3 weeks of Ganciclovir had a complete response at this point, however, this was not significant when analyzed by odds ratio (OR = 2.98 95% confidence interval 0.67-13.1). To show a difference at these 3 week response rates would have required 83 patients per arm (i.e. 166 patients not allowing for withdrawls). Analysis was similar if patients not completing treatment were including (i.e. intention to treat analysis). Furthermore, those patients having an

incomplete response to foscarnet, 5 showed a partial response and 3 were considered treatment failures. The 5 partial responders became complete responders following a further 7 - 10 days of foscarnet at treatment doses. The three non-responders in the Foscarnet group were withdrawn from the study and treated with Ganciclovir. The 3 Ganciclovir incomplete responders showed partial responses and entered the maintenance phase of the study with their next ophthalmological assessment showing complete CMV quiescence. This suggested that both agents were highly effective, particularly when given for sufficient duration.Given these facts, and the slow recruitment to the study we were advised by our statistician, who was not directly involved in the study, to terminate the study.

DISCUSSION OF TREATMENT PHASE

This data suggests that given adequate treatment periods both drugs are highly effective but that Foscarnet shows a slightly slower response.

The 3 week period for treatment chosen at the start of the study is arbitrary and was based on clinical experience, but may be less meaningful in real world practice where decisions on drug use are influenced by many factors including laboratory parameters, concomitant medications, previous tolerance of a medication and retinal appearance.

MAINTENANCE PHASE

Equal numbers of patients entered the maintenance phase. In particular, no patients declined the offer of maintenance therapy despite the potential inconvenience, morbidity and body image difficulties associated with a long term indwelling catheters and daily administration of therapy. Clinical follow-up of all patients for disease and toxicity endpoints continued until

reactivation of retinitis, change of therapy or until death. Similar numbers of patients on each drug regime showed reactivation of retinitis whilst on maintenance. Poor compliance was not thought to be a reason for reactivation.

Patients receiving Foscarnet maintenance showed a trend towards earlier reactivation than those receiving Ganciclovir, however, these differences did not reach statistical significance (Table 44).

Table 44 Results of maintenance

	Number reactivated	Time to reactivation- mean (range)
Ganciclovir (22)	6	25 weeks (9-63)
Foscarnet (22)	7	14 weeks (2-26)

DISCUSSION OF MAINTENANCE PHASE

Both agents appear effective in delaying time to reactivation of treated AIDS related CMV retinitis when compared with published data (Holland GN, et al 1987, Walmsley SL, et al 1988), however, there is a trend towards later reactivations with Ganciclovir.

2.5.6. Adverse Events.

Adverse events occurring during both phases were analyzed together. Adverse events requiring treatment change occurred in more patients receiving Foscarnet than those receiving Ganciclovir, however, this did not reach statistical significance. These events also showed a trend to occur earlier in the Foscarnet group (Table 45).

Table 45 Adverse events (AE) on therapy causing change

	Numbers on Treatment / maintenance	AE causing therapy change	Time to therapy change due to AE - mean (Range)
Ganciclovir	26/22	5	16.6 (8-27)
Foscarnet	31/22	9	8.6 (2-26)

Adverse events seen during the study were consistent with the know toxicities of each medication (Table 46).

Table 46 Adverse events during therapy (causing change or interruption)

Event	Ganciclovir	Foscarnet
Blood transfusion units (number of patients)	94 (13)	38 (5)
Neutrophil <0.8 x 10^9/l	8	0
Creatinine >130 mmol/l	0	10
Penile ulceration	0	6
Calcium <2.00 mmol/l	0	2
Nausea	0	4
Proven line infection	7	4
Suspected line infection	3	0

PENILE ULCERATION

Penile ulceration during Foscarnet therapy had recently been described from our unit and by others and was followed prospectively during this study, with patients being asked daily during treatment and at clinic attendance during maintenance about development of this phenomenon. All patients presenting with penile ulceration had syphilis serology, HSV

and bacterial cultures performed and were advised on routine washing after micturition. Six of 31 patients randomized to Foscarnet (20%) developed penile ulcers thought related to Foscarnet. This incidence was higher than for all patients in East Riverside who received Foscarnet over the same period (Moyle G, et al, 1993). No patients on ganciclovir experienced penile ulceration.

ANAEMIA

Anaemia has been reported as a side effect of both medications, however, a blood transfusion requirement was seen in 13 Ganciclovir-treated patients but only 5 Foscarnet patients. This was statistically significant by odds ratio (OR = 0.339, 95% Confidence interval 0.178-0.645). The number of units required was considerably greater in the Ganciclovir group (98 units) compared with Foscarnet group (38) and this extra cost and patient inconvenience should be taken in consideration when choosing therapy. Furthermore, as more Foscarnet patients received ZDV during CMV therapy this may have increased the incidence of anaemia in that group (see below).

NEUTROPENIA

Neutropenia ($<0.8x10^9/l$) was only seen in the Ganciclovir group. Treatment was generally by interruption of therapy although those patients who had persistent neutropenia changed therapy to Foscarnet. Neutropenia was the only cause of therapy change in the Ganciclovir group. Colony stimulating factors (e.g. GCSF) were not used during this trial, principally due to lack of availability in the UK at that time. These medications could enable Ganciclovir treatment to continue despite neutropenia but at considerable expense and not without side effects,

However, they remain an option in patients unable to tolerate Foscarnet who develop neutropenia on Ganciclovir.

Neutropenia may have also contributed to the increased incidence of proven or suspected line infections occurring in patients receiving Ganciclovir. Suspected line infections were those which were blood culture negative on two samples (line and peripheral cultures) but where fevers and rigors for a minimum 5 days responded to empirical intravenous antibiotics. Ganciclovir-treated patients had more episodes of line infections compared with those receiving Foscarnet hovever this did not reach statistical significance (OR = 3.75, 95% confidence interval 0.952-14.8). In particular, pseudomonas line infections (4 episodes) were only seen in patients receiving Ganciclovir.

OTHER TOXICITIES

Nausea, creatinine elevation and hypocalcaemia all contributed to interruption or change of Foscarnet therapy. Hypoglycemia was not seen with this medication, however, intravenous pentamadine which has been reported to exacerbate this phenomenon was excluded throughout the study. Nausea, although rarely a cause for therapy interruption or change with Foscarnet is often reported in patients receiving Foscarnet together with a non-specific syndrome of 'unwellness' which detracts from patients quality of life whilst receiving this medication.

In summary, both drugs have side effects which frequently lead to interruption or change of therapy. However, the side effect profiles of the two drugs differ enabling switching between the two drugs or concomitant administration of reduced doses of each drug.

2.5.7. Survival

During the study recruitment period 94 patients were diagnosed in East Riverside with CMV retinitis of whom sixty-two patients were enrolled in the comparative Foscarnet versus Ganciclovir study. However, 5 patients were withdrawn from the study within 24 hours as laboratory parameters at time zero had failed to meet inclusion criteria. Of the 57 patients entering the study 36 patients completed at least 6 weeks of therapy with either Foscarnet or Ganciclovir.

All 94 patients diagnosed were followed for survival. At the time of this analysis 3 patients remained alive with CMV retinitis. They have not been included in the listing.

The mean survival for all patients was 37.6 weeks. Figures 24 and 25 show the survival and death rates for all patients with CMV retinitis.

Figure25

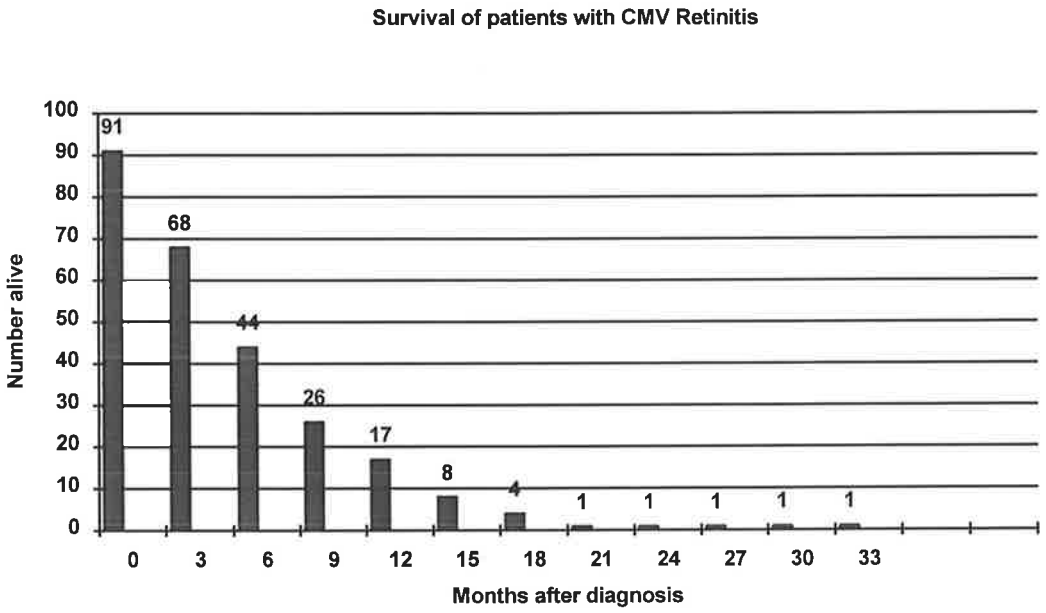
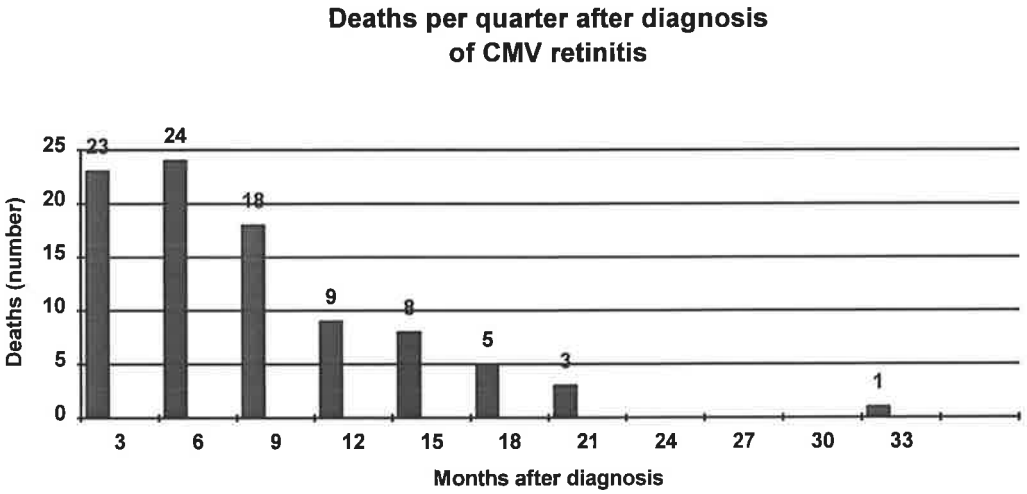


Figure26



Patients with a diagnosis of CMV retinitis may not have died of CMV related diseases and indeed the one trial patient who died during the first 3 weeks of therapy was thought to have died from non-CMV-related causes (Disseminated KS and MAC infection). The one patient who survived to 30 months changed anti CMV therapy multiple times during his care due to both disease reactivation and drug toxicities and also received reduced dose combination of Ganciclovir and Foscarnet. He declined further anti CMV therapy after a second retinal detachment which rendered him blind. He survived a further 3 months after stopping therapy. Analysis of survival data on trial patients has been limited to patients who received a minimum 6 weeks with their primary therapy. In this group of patients survival between Foscarnet and Ganciclovir-treated groups was similar (Table 47) and close to the mean for all CMV patients.

Table 47 Survival with CMV retinitis by treatment

	Number	Mean Survival	(Standard Deviation)
Foscarnet	21	39.4	(22.2)
Ganciclovir	20	39.6	(19.1) Not significant

Use of Zidovudine was examined within the treatment arms. Patients receiving foscarnet and Zidovudine showed a trend to survive longer than those patients who received foscarnet alone. One patient who was not receiving ZDV at commencement of foscarnet therapy but started within 10 days of CMV diagnosis is included here in the ZDV-treated group (Table 48).

Table 48 Survival of foscarnet patients by ZDV usage

	Number	Mean Survival	(Standard Deviation)
Foscarnet alone	6	27.8	(19.2)
Foscarnet and ZDV	13	43.2	(22.6) Not significant

** excludes 2 patients receiving ddI therapy*

A similar trend to better survival was seen with Ganciclovir patients who were able to tolerate ZDV (Table 49).

Table 49 Survival of ganciclovir patients by ZDV usage

	Number	Mean Survival	(Standard Deviation)
Ganciclovir alone	11	36.6	(20.6)
Ganciclovir and ZDV	6	48.0	(18.7) Not significant

**excludes 3 patients receiving ddI therapy*

When patients with CMV retinitis who continued or commenced ZDV were compared with those who did not receive ZDV regardless of CMV therapy. There was statistical significance in favor of Zidovudine (p=0.05 by T test) (Table 50).

Table 50 ZDV usage and survival

	Number	Mean Survival	(Standard Deviation)
ZDV	19	41.7	(20.1)
No ZDV	17	30.3	(22.1) p=0.05

DISCUSSION

Survival advantage in patients receiving therapy for CMV retinitis appears more dependent on their use of ZDV than on the choice of Foscarnet or Ganciclovir. However, survival following a diagnosis of CMV retinitis remain poor despite initially effective therapy.

3. PART III: DISCUSSION

3.1. INTRODUCTION TO DISCUSSION ON DDI STUDY

Placebo controlled trials of the use of ddI in ZDV-intolerant patients are unlikely to be performed as clinicians generally believe that not to provide antiretroviral treatment for these patients would be unethical and because recruitment to such studies is likely to be poor. For example, the multinational Alpha study, comparing two doses of ddI, closed the study option containing a placebo arm due to slow recruitment (Darbyshire JH, Aboulker JP, 1992). Therefore, the value of ddI and future new antiretrovirals in pre-treated patients will need to be inferred from observational studies, or studies comparing switching to ddI with continuing ZDV therapy.

The choice of ddI as a second line monotherapy in patients with ZDV intolerance is rational given the different toxicity profiles of these drugs and the absence of *in vitro* cross resistance. However, in patients with clinical failure on ZDV but who continue to tolerate this drug the decision to switch to ddI must be compared to the option of adding this drug into combination with ZDV. This gives the potential of synergy between the two drugs and the single codon change which leads to reduced viral sensitivity to ddI may increase sensitivity to ZDV (St Clair M, et al, 1991).

The discussion will examine the importance of the St Stephen's study results and integrate them into recent data to reach towards a clinical algorithm for antiretroviral use.

3.2. SURROGATE AND CLINICAL MARKERS

3.2.1. Surrogate Markers

Changes in surrogate markers occur rapidly after initiation of ZDV therapy and are usually transient, generally returning to baseline within 6 months. More prolonged elevations of CD_4 cells, upto 52 weeks, have been reported in patients commencing ZDV with CD_4 cells between 200 and 500 cells/mm3 (Fischl MA,Richman DD et al, 1990) and those receiving combination nucleoside therapy with ZDV and ddC (Meng T-C, et al, 1992). Rises in CD_4 cells generally coincide with falls in p24 antigen reflecting suppression of viral replication. Timing of development of viral resistance also coincides with clinical deterioration and has recently be suggested as a marker of subsequent disease progression (Montaner JS, et al, 1993).

Results in this study show a limited and generally short-lived response to ddI in both CD_4 count and p24 antigen. In particularly, only 3% of patients with a baseline AIDS diagnosis and no patients with baseline CD_4 count below 50 cells/mm3 had a significant response of 50 cells/50%. In patients with baseline CD_4 counts below 150 cells/mm3 less than one third had any CD_4 count response suggesting that on this criteria, at least, ddI does not represent a "salvage" therapy. However, as response to therapy in terms of CD_4 only explains some of the beneficial effects of ZDV in delaying clinical progression (Lagakos S, 1993, De Gruttola V, 1993), the same is likely to be true for ddI given the same mode of action for these two drugs. Failure to experience CD_4 rise in patients changing to ddI is unlikely to be due to viral resistance as no cross resistance has been demonstrated between these two drugs and p24 antigen, the virological

marker used in this study showed a meaningful response in 67% of patients commencing ddI including 70% of those with a baseline diagnosis of AIDS and 61% with a CD_4 cell count below 100/mm3 at baseline. There is evidence that the load of HIV or HIV-encoded proteins may effect CD_4 cell function and lead to cell death (Chirmule N, et al, 1990 Weinhold KJ, et al, 1989). Patients with late stage HIV disease (e.g. AIDS or CD_4 < 100) have high viral loads and it is well recognized that patients who are p24 antigen negative continue to show evidence of viral replication by more sensitive techniques, even in the presence of nucleoside analogues including ddI (Ho DD, 1989, Aoki S, et al, 1990). It is, therefore, likely that the continued immunosuppression in our patients, despite change to a new therapy was due to inadequate suppression of viral replication by the ddI. This said, it appears that switching to ddI did lead to substantial improvements in CD_4 counts in some patients, most notably about a third of those patients with CD_4 counts greater than 150 cells/mm3. However, as some of these patients had received ZDV for only short periods, having stopped because of early gastrointestinal intolerance, improvements in surrogate markers seen with ddI in some patients may simply be due to a substitution of one antiretroviral for another.

3.2.2. Clinical Markers

Few patients experienced a positive weight response during the study. This may relate to limited efficacy of ddI or, more likely, relates to the fact that many patients experienced gastrointestinal disturbance during therapy and the rules governing administration under 'fasting' conditions disrupted many patients normal eating habits. Periods of weight loss in AIDS patients tend to occur in association with opportunistic infections with weight gain related to recovery of health (C Baldwin, Personal

communication). Patients entering the St. Stephens ddI study were clinically stable suggesting that significant weight gain would not be expected. However, weight gain of upto 2kg has been reported with initiation of ZDV in patients with both advanced HIV disease (who had PCP within the last 120 days, unexplained weight loss of 10% or ARC) (Fischl MA, et al,1987) and mildly symptomatic HIV disease (mostly CD4 counts 200-500 cells/mm3) (Fischl M, Richman DD, et al 1990). Weight gain has not been described in other published adult data of ddI as a second line monotherapy, but has been seen in patients commencing ddI in combination with ZDV or adding ddI to the regimens of patients who had received ZDV for less than 121 days. Patients in this study who were anti-retroviral naive were noted to have greater weight gain than pretreated patients suggesting that this may be an important factor in weight response (Collier AC, et al 1993). As all patients entering the St. Stephens study were ZDV pretreated, this may also have influenced the likelyhood and magnitude of any weight gains.

The high incidence of new and recurrent O.I. seen during the study most likely reflects the poor baseline characteristics of patients entering the study. Consistent with previous data frequency of adverse events was increased in patients with lower CD_4 counts.

3.2.3. Quality of Life

Quality of life (QOL) information is most useful to physicians in patients receiving palliative therapy for a chronic illness such as AIDS and when the favorable effects of a medication need to be balanced against its toxicity profile (Revicki DA. 1990). Furthermore, QOL studies provide a

more 'holistic' or person oriented view of health than that provided by laboratory results and clinical events.

The WHO defines health as a state of 'complete physical mental and social well being not just the absence of disease' (WHO: Constitution of the World Health Organization. Annex I. In: The first ten years of the World Health Organization. Geneva WHO 1958) and this multidimensional definition serves as the basis of quality of life instruments. The Spitzer indexed used in this study incorporates the principle dimensions of physical, emotional and social function, symptoms and outlook (a measure of 'well being'). As it is completed by the patient at each attendance it is more patient oriented and more holistic than the physician completed physical status based Karnofsky index, which has been used in some HIV studies. The scaling of 0-2 is, however, the limiting factor reducing the sensitivity or responsiveness of the measure.

A degree of construct validity was therefore pursued by using the overall assessments which were done by both physician and patient at start of each visit. The scaling of this instrument was oriented toward sensitivity in assessing improvement rather than deterioration and does provide supporting evidence for the results seen with the Spitzer index. Other QOL measures used in HIV studies include the Q-twist, (Gelber RD, et al, 1992) and several abbreviated forms of the Medical Outcome Study (MOS). These instruments are generally much longer than the Spitzer index and hence less convenient for use in a busy clinic but may provide more thorough assessment of QOL through construct validity on each dimension.

Quality of life was generally considered good in most patients entering the study as measured by the Modified Spitzer index therefore improvements with ddI therapy were difficult to measure. Over the first 420 visits, slightly more patients were noted to have improved than deteriorated, as assessed globally by both patients and the trial physician, although at most visits "no change" was reported. Health, the parameter with the most sub optimal scores at baseline improved in 36% of patients by Spitzer index and the global assessment was noted to have improved by 55% of patients. Deterioration was less common in both scoring systems. Arguably the health parameter is less likely than measures such as support and outlook to be influenced by the 'placebo-effects' of trial participation.

Overall more of the quality of life parameters rose than fell, suggesting a quality of life benefit from ddI therapy, at least in the short term. Notably improvements in QOL scores were seen in both ARC and AIDS patients and in patients with baseline CD_4 counts above and below 100 cells/mm^3 at fairly similar rates, contrasting the CD_4 responses which were more frequent in better prognosis patients. It is reasonable to expect that some of these improvements may relate to the availability of a new drug, and therefore potentially new hope as well as the additional support of seeing the study physician and study nurse at more frequent intervals than was routine at our clinics. Similarly, some of those patients reporting deterioration during the first 420 visits may have been experiencing diarrhoea and bloating from the ddI preparation, which was most commonly experienced on starting therapy, and often settled with continued administration. As many patients were in a poor prognostic group, opportunistic infections, unrelated to ddI therapy, were common

and contributed to both global deterioration and lower scores on the Spitzer index.

As follow-up on quality of life data was short and no placebo group was used in this study the data must be interpreted with caution.

Quality of life data has not been published from any other ddI studies and studies with quality of life and ZDV have been limited. Wu, et al, (Wu AW, et al, 1990) used Karnofsky Performance index and the Quality of Well Being scale in 31 patients involved in the BW002 study. When mortality effects are removed Karnofsky scores fell significantly in placebo patients 90.7 to 84.2 ($p < 0.0007$) but not in ZDV patients 91.3 - 88.1 (not significant). Quality of Well Being did not change significantly with placebo and rose, non-significantly, in ZDV patients. The two measures provided construct validity and supported the view that ZDV provides some QOL benefits. Data using the Q-TWIST (Quality adjusted Time without symptoms or toxicity) was obtained from mildly symptomatic patients with HIV (Gelber RD, et al, 1992) in a study comparing 351 placebo and 360 ZDV recipients, and again showed ZDV provided benefit over placebo. Given that the higher than current recommended ZDV dose (1200 mg/day) used in this study contributed to the adverse events in this are it is likely that lower ZDV doses provide a greater Q-TWIST benefit. Analysis by Lenderking and collegues fo QOL data from ACTG protocol 019, again using Q-TWIST, showed that asympomatic patients treated with 500mg of ZDV had a reduction in QOL due to therapy related side-effects which was approximately equivalent to the benefit gained by a delay in disease progession (Lenderking WR, et al, 1994).

In summary, given the limited data in this field and the variety of measurement instruments used it appears that both ZDV and ddI provide Quality of Life benefits for patients with AIDS and symptomatic HIV disease. Quality of Life considerations represent an important facet of informed decision making in the clinic as well as contributing to cost-benefit aspects of drug assessment.

3.3. ADVERSE EVENTS

As there was no placebo arm in this study it is difficult to assess the frequency of events and the causitive relationship of ddI to these events. However, adverse events seen in the St. Stephens study were similar to those previously reported in phase 1 studies, the expanded access programme and more recent studies (Yarchoan R, Pluda J, et al, 1990 Pike IM, 1993 Kahn JO, et al 1992).

3.3.1. Diarrhoea

Diarrhoea was the most frequently reported adverse event. This is thought to be largely related to the citric phosphate buffer used in the sachet preparation of ddI, and may be less of a problem with the chewable preparation now in wider use. Diarrhoea was particularly a problem in patients with pre-existing gastrointestinal dysfunction and was frequently a cause for cessation of ddI therapy. However, in patients who chose to continue therapy the diarrhoea often settled or became more tolerable. Several patients were found to have active gastrointestinal infections on stool culture and therefore all diarrhoea occuring during ddI therapy should not simply be assigned to ddI.

In the ddI expanded access progamme diarrhoea assessed by the investigators as drug-related or possibly drug-related was reported by 16% of patients and was the most frequent drug-related event in this study (Pike IM,1993). This rate is similar to the rate of diarrhoea-related discontinuations in the St. Stephens study (23 patients, 15%). The higher incidence of diarrhoea seen in the St. Stephens ddI study compared with the Expanded access progamme is most likely related to the different definitions used. The St. Stephens definition included all episodes of

diarrhoea regardless of assessed relationship to ddI, and included worsening of diarrhoea in patients with pre-existing gastrointestinal disturbance, and was therefore a more 'catch-all' definition. Furthermore, in 39 of the 90 patients who experienced diarrhoea the diarrhoea resolved within the first month and the monthly follow-up in the Expanded access programme is likely to have missed this group.

The finding that diarrhoea on ddI occured significantly more freqently in AIDS patients is a new finding which may relate to patients with more advanced disease having a higher incidence of AIDS-related enteropathy and gastrointestinal infections some of which was unmasked by the challenge of dealing with the ddI preparation.

3.3.2. Xerostomia

Xerostomia was reported in 25% of patients. AIDS related Sjörgrens syndrome has previously been reported (Schiodt M, et al, 1989), however, the clinical impression was that this problem was related to ddI therapy. Two patients developed overt parotitis during the study and no opportunistic infection was found in the one patient who underwent parotid biopsy for this condition. 6 of 37 patients with Xerostomia, including the 2 with parotitis, were found to have raised amylase, another frequently reported side effect of ddI. Unfortunately, facilities to fractionate the amylase were not available to assess the origin of the amylase, but a previous study has shown raised amylase to be of salivary origin when associated with Xerostomia (Yarchoan R, Pluda J, et al, 1990)

3.3.3. Pancreatic Dysfunction

The incidence of pancreatitis in individuals with HIV infection is unclear but has been reported in association with HIV, opportunistic infections and treatments commonly used during the course of HIV disease (Clas D, et al, 1989, Herer B, et al, 1989). Pancreatitis is clearly increased in frequency in patients receiving ddI compared with other nucleoside analogues, and the use of ddI has been associated previously with fulminant and fatal pancreatitis (Bouret E, et al, 1990). Phase I data, on pancreatitis in 7 patients receiving ddI noted a correlation between cumulative dose of ddI and risk of pancreatitis but not hyperamylasaemia without pancreatitis. No correlation was noted with disease stage or concomitant medications. The authors identified five features supporting the view that ddI is causative in pancreatitis; 1) the frequency of pancreatitis in ddI recipients compared to non recipients; 2) the absence of concomitant medications, cancers or infections associated with pancreatitis; 3) the resolution of pancreatitis on withdrawal of ddI; 4) the recurrence of pancreatitis in some patients on rechallenge and 5) the correlation of pancreatitis with cumulative drug dose (Seidlin M, et al, 1992). A mechanism for ddI pancreatitis has been recently proposed involving release of reactive oxygen species during Xanthine oxidase-catalyzed catabolism of hypoxanthine, a major metabolite of ddI. If this is so, strategies to reduce production of hypoxanthine (e.g. Allopurinol coadministration) may reduce the incidence of this toxicity (Nguyen B-Y T, et al, 1993). In our study, the incidence of this problem peaked within the first 6 months of therapy and was unusual in patients on long-term therapy suggesting the problem may not one of cumulative toxicity alone. Pancreatitis was only seen in this study in patients with a baseline diagnosis of AIDS suggesting that patients with late stage disease may be

more susceptible to this problem. None of our patients who developed pancreatitis were receiving aerosolized pentamadine for *Pneumocystis carinii* prophylaxis. However, raised amylase, possibly suggestive of pancreatic damage, was also seen in some patients with a baseline ARC diagnosis. Patients who had asymptomatic elevation of their amylase showed normalization of this parameter upon discontinuation of ddI, and did not recur in the single patient who was re-challenged.

Unfortunately there is no data which shows that frequent clinical or laboratory monitoring of patients can predict the development of pancreatitis and given the low but important incidence of mortality with this condition it is likely to remain one of the objections to the use of ddI.

The finding of diabetes mellitus in 7 of our patients is new and further supportive evidence of pancreatic damage in patients receiving ddI in this preparation form. Random blood sugars performed prior to therapy were within normal limits in all these patients, none of whom had a history of glucose intolerance. In the patients who stopped therapy, their glucose tolerance returned to normal, suggesting a causal relationship. In those who continued therapy the diabetes was controlled with a standard diabetic diet. This finding has subsequently been reported from the US Expanded Access Programme (Pike IM, 1993)

3.3.4. Peripheral Neuropathy (PN)

Peripheral neuropathy (PN) has been noted as the dose limited side-effect with a number of dideoxynucleoside analogues including ddI, ddC, D4T. A cellular model for this has been created and a mechanism based on

inhibition of mitochondrial DNA polymerase gamma proposed. (Keilbaugh SA, et al, 1991).

Distal symmetrical polyneuropathy (DSP) is the most common form of PN seen in patients with HIV infection. Clinical symptoms and signs have been reported in 18% of AIDS patients not receiving nucleoside analogues (Snider VP, et al, 1983) although electrophysiologic abnormalities suggestive of DSP may be present in upto 35% (So YT, et al, 1988). Reduction in sural nerve conduction amplitudes appear to be the most common abnormality. (So YT, et al, 1988). PN may be caused directly by infectious agents including CMV and HIV (Fuller G, 1989), B12 deficiency and neurotoxic drugs. Some neurotoxic drugs commonly used in persons with HIV infection include nucleoside analogues, vincristine, dapsone and metronidazole.

Nucleoside analogue associated PN occurs almost exclusively in the lower limbs and usually presents bilaterally with pain and dysasthesiae, progressing over 72 hours. It is generally slowly reversible if the drug is stopped immediately, before symptoms have become severe although some patients report an exacerbation of pain in the days following therapy cessation. Patients in whom changes do not reverse generally have numbness rather than pain as the principal long-term symptom of PN. Pain, when present, can be difficult to manage with options including amitriptyline, carbemazepine and opiate analgesia. Transcutaneous electric nerve stimulation (TENS) has been successfully tried in some patients.

A previous study by Yarchoan et al found PN to be the most common dose limiting side effect of ddI. However, nerve conduction studies were

either within normal limits or showed only slight decreases in sural nerve amplitudes. (Yarchoan R, Mitsuya H et al, 1990). However, our study has shown that patients who have subjective symptoms of PN have significantly reduced nerve conduction speeds and amplitudes from their baseline values. Unfortunately baseline values were not predictive of future development of PN. Similarly there were approximately similar proportions of patients with baseline AIDS and ARC suggesting this is not a predictor of PN in individuals receiving ddI. Clinical Status, however, has been noted as a predictor of PN in patients receiving ddC (Snape S, et al 1993) The time to development of PN ranged from 13 to 42 weeks on therapy, with a mean of 27.3 weeks, again similar for both AIDS and ARC patients. Patients with a history of existing PN were excluded from this study as this was found to be a risk factor for developing PN in ddI (Pike IM, 1993). A study using alternating therapy with ZDV and ddC has suggested that rates of PN related to ddC may be reduced with weekly or monthly regimes whilst maintaining efficacy. (Skowron G, et al, 1993). It is likely such an approach would also be useful in reducing ddI related PN. Patients receiving a lower (200 mg/day) dose of ddI in the Alpha study (Darbyshire J, Aboulker JP 1992) had a lower rate of PN than those receiving the higher (750 mg/day) dose, with similar efficacy (or non efficacy) suggesting that lower doses than those used in our studies may be safer for both PN and pancreatitis. However, Kahn et al found equivalent rates of grade 2 or more PN between an intermediate dose (500 mg/day) and the standard (750 mg/day) dose. Interestingly, in this blinded study ZDV-treated patients showed an equivalent rate to those treated with ddI (Kahn J, et al, 1992). Reduced rates of PN seen in more recent studies may reflect increasing physician familiarity with the side effect profile of ddI hence stopping or interrupting therapy as soon as mild

(grade 1) symptoms appear and educating patients to watch for symptoms from the time therapy begins.

3.3.5. Other Adverse Events

Other adverse events occurred with low frequency and given the lack of control group in this study it is difficult to comment on their relationship to ddI therapy. However, events reported from other studies which were also seen in our patient population include elevated hepatic transaminases, seizure disorder, personality change and skin rash. It is possible that some of these infrequent events were related to ddI therapy, however, these problems are also seen in persons with HIV infection not receiving ddI.

Haematological problems were uncommon amongst our cohort. The patients who required transfusion during the study frequently had a prior history of transfusion dependence with Zidovudine, and opportunistic infection associated with haematological dysfunction (e.g. MAI) or were receiving other medications known to cause anaemia (e.g. Ganciclovir, Cotrimoxazole, Dapsone). There were no episodes of WHO grades 3 or 4 neutropenia during the study. Previous studies with ddI have shown that haematological toxicity is infrequent during ddI therapy and this has been one of the reasons ddI has been recommended for patients intolerant to ZDV.

In summary, ddI has a well defined toxicity profile which is distinct from that of ZDV. Gastrointestinal disturbances, such as bloating and diarrhoea, are the most common adverse experience but these may be related to the preparation and the need for buffering agents rather than

the active compound. Peripheral neuropathy and pancreatitis are the major use limiting toxicities, the latter occasionally causing fatalities. The toxicity profile of ddI also makes it suitable for concomitant use with a wide variety of medications used in HIV, including those which may cause additive toxicity with ZDV (e.g. Ganciclovir, Co-trimoxazole). Indeed no major drug interactions were seen during this study.

3.4. HISTORICAL CONTROLS AND SURVIVAL ON DDI

The St Stephen's data was examined in several ways to provide a sense of construct validity to the view of the value of ddI achieved from surrogate markers in the study, to enable some comparison with other, on going, studies with ddI, and to apply the data to real life practice.

The raw survival data reflects the poor prognostic status of patients entering the study. The majority of patients entering the study had a baseline AIDS diagnosis, which was predictive of a poor outcome. However, upto around 24 weeks of follow-up, patients who had received ZDV for less than 6 months and then received ddI show a better survival than those who had received more prolonged periods of ZDV prior to receiving ddI reinforcing the view that these patients may simply have substituted one effective antiretroviral (ZDV) for another (ddI). The appearance of these Kaplan-Meier plots subsequently drawing together would be consistent with the view that a period of survival benefit can be provided by antiretroviral therapy but that this period is similar whether one therapy is used long term or two therapies are used successively, i.e. switching to ddI after ZDV intolerance provides the same survival benefit as long term ZDV, but that benefit is limited. This view is reinforced by the comparative analyses using historical data (see below).

The comparison of the raw ddI data with the ZDV historical control is biased by the fact that ddI is being used as a second line therapy compared with ZDV as first line therapy and, therefore, the 2 groups have different 'time zeros'. This is reflected in the poorer outcomes of the ddI group in all comparisons except those with baseline $CD_4 \geq 100$ cells/mm^3 where few events were seen during the St Stephens study. This patient

group with higher CD4 counts were also more likely to experience an immunological response, which may be predictive of a survival benefit (Graham NMH,et al 1993), and patients with better baseline CD4 values have been noted in other studies of ZDV pre-treated patients to gain greater clinical and immunological benefit than those with lower baseline CD4 counts to change to, or addition of, further nucleoside analogue therapy (Fischl MA,et al 1993).

The 3 corrected analyses were therefore performed to make more reasonable comparison.

The first corrected analysis, comparing 'longterm' ZDV recipients with patients who received ZDV for less than 6 months before receiving to ddI can be seen from the angle of providing some data similar to ACTG studies 116 and 117 (see below) involving 'switch to ddI or continue ZDV' questions and to provide some answers to the question whether use of a further nucleoside analogue is warranted after ZDV intolerance. The groups were poorly matched for baseline characteristics, limiting the value of the unadjusted analysis. The adjusted analysis shows equivalence between the two regimes. This can be interpreted as meaning switching to ddI provides no advantage over continuing ZDV (and similarly no disadvantage) but also that if ZDV intolerance is encountered early in therapy ddI offers the continued benefit ZDV would have offered.

The second corrected analysis is comparable to the original Alpha study design, ddI versus placebo in ZDV-intolerant patients, asking the question if ddI provides survival benefit to these patients. The analysis may be in part biased by the large number of exclusions from the historical control

arm due to missing AZT stop dates. However, the two groups have fairly well matched baseline characteristics. Analysis shows uncorrected a difference of borderline significance favoring use of ddI, and after adjusting for covariates, a strongly significant difference in favor of ddI over no further therapy. This supports the view that ddI is efficacious in this patient group although caution must be exercised in view of the AZT + ddI arm having selected patients with a guaranteed survival time from AZT stop to ddI start.

The third corrected analysis looks at the subgroup of patients with better baseline characteristics, principally CD_4 >100 cells/mm^3 at ZDV initiation and asks the question if switching to ddI after ZDV intolerance in this patient group maintains the ZDV benefits. This again parallels the ACTG 116/117 subgroup analysis. Only patients who received ddI had ZDV intolerance but these patients again had a guaranteed survival to ddI's availability whereas patients in the ZDV + no ddI arm did not receive ddI presumably due to poorer survival (albeit more that 8 weeks after ZDV initiation). This is reflected in the longer mean survival of the ZDV + ddI arm (151.2 weeks) compared with the ZDV + no ddI (94.4 weeks). Adjusting for baseline characteristics no difference is seen between the two regimes. This agrees with the Analysis I data suggesting that ZDV's benefit can be maintained after intolerance in good prognosis patients by the use of ddI.

RECENT COMPARABLE DATA:

Five recently completed studies, from both US and non US groups have been published on the use of ddI. The multinational Alpha study and US. ACTG 118 study, both published in preliminary form (Darbyshire JH,

Aboulker JP, 1992, Allan JD, et al, 1993) have examined various doses of ddI in ZDV-intolerant patients. There were no placebo arms in these studies and comparison was made between arms within the study. Both studies involved similar patient groups to the St Stephen's study. The Alpha study involved 1930 patients receiving two doses of ddI, 750 mg per day or 200 mg per day, weight adjusted for patients weighing less than 60 kg. Analysis was performed on the first 1175 patients. Fifty five percent of patients had received ZDV for greater than 12 months and all had ZDV intolerance for a variety of reasons. The majority of patients in this study, similar to St Stephen's Study, had CD_4 <50 cells/mm^3 (65%) at commencement of ddI, the mean being 26 cells/mm^3 in the high dose group and 23 cells/mm^3 in the low dose group and more than half had AIDS at baseline (61%). Despite considerable statistical power, no differences in survival, or disease progression were noted over the mean 12 months follow-up but adverse events were more common in the higher dose group. CD_4 rises of mean 2.5 cells over the first 6 months were seen only in the high dose group whereas a fall was seen in the low dose group of the same period as measured by area under the curve. ACTG 118 involved 650 patients divided between 3 doses of ddI, 750 mg and 200 mg as in Alpha, plus an intermediate 500 mg dose again all weight adjusted if patients weight was below 60 kg. Baseline characteristics were again similar to the St Stephen's Study with CD_4 counts at a mean of 33 cells/mm^3 with the median duration of prior ZDV being 258 days. Again, no differences were found between groups the primary study endpoint of disease progression or death over the 50.9 week follow-up period. Similarly, no differences in survival alone were seen. Better clinical and CD_4 responses to ddI were seen in patients with higher baseline CD_4 and shorter previous ZDV therapy. CD_4 responses, as measured by a 50% and at least 10 cells rise was seen in 19% of the high dose group 16% of

the intermediate and 8% of the low dose group. However, adverse events tended to be more frequent in the high dose group. A rise in serum amylase was noted to be predictive of development of pancreatitis in this study.

These two studies are very similar to the St Stephen's Study in terms of patient group, baseline characteristics and follow-up. The two studies reach identical conclusions, that higher and lower dose of ddI have no difference in their influence on clinical outcome and that the higher dose, that used in St Stephen's Study, was more likely to provide a modest CD_4 rise at the price of increased toxicity. The Alpha authors question the efficacy of ddI asking if both doses are ineffective or at an appropriate plateau on the dose response curve (Darbyshire JH, Aboulker JP, 1992). Although the second part of this challenge cannot be answered from these or our study, evidence for efficacy is present in each study although the instruments of measurement differ. For CD_4 count Alpha saw an increase in CD_4 over the first 6 months as measured by area under the curve, similarly 19% saw a rise by ACTG 118's 50%/10 cell criteria and at St Stephen's 28% by the 10%/10 cell and 18% by the 25%/25 cell criteria. Virological analysis have not been reported from Alpha or 118 but at St Stephen's up to two thirds of patients achieved a p_{24} antigen response suggestive of anti-viral activity. However, the true measure of efficacy eluded to by Drs. Darbyshire and Aboulker is that of survival.

The mean survival time in our cohort had not been reached, however, given a maximal 108 weeks follow-up patients with < 6 months prior ZDV patients had a survival time of 65.4 weeks, similar to Alpha with 12.9 months (high dose) and 12.4 months (low dose). In the adjusted analysis I comparing ddI second line after 6 months ZDV with our historical cohort of

continuous first line ZDV-treated patients this survival time was not different. However, when the true practice question of 'to give or not to give' ddI to the AZT intolerant patient is asked our data (corrected analyses II + III) support the use of ddI. In particular our analysis 2, where the trends favored the consecutive ZDV, ddI arm (uncorrected survival 151.2 weeks compared to 94.4 weeks) and corrected analysis showed a significantly reduced risk of death when ddI followed ZDV compared with no further antiretroviral after ZDV (p = 0.002 RR 0.46 95% CI 0.28-0.76). The ZDV + ddI arm may have selected a poorer prognosis group as those patients who had rapid Zidovudine intolerance receiving ddI as 'rescue' therapy. Alternatively, the no-ddI group in this analysis may have also had some unfavorable characteristics given patients failed to survive to receive ddI. Overall, however, this data appears to support the view that ddI is efficacious in prolonging survival in ZDV intolerant patients.

Given that ddI is efficacious in ZDV-intolerant patients both in causing minor improvements in immunological and virological parameters and survival, is this the drug of choice in this situation? CPCRA 002 was a randomized open label comparative community based study comparing ddI and ddC in patients who were intolerant to (63%) or had 'failed' ZDV. There was no placebo arm used. Four hundred and sixty seven patients were randomized to receive either 500 mg/day ddI (the intermediate dose of ACTG 118) or 2.25 mg/day ddC, both weight adjusted. Patients were followed for a mean of 16 months. The baseline CD_4 count was again similar to the previous studies at median 37 and a mean around 73. More deaths occurred in the ddI arm compared with ddC-treated patients and in an analysis adjusting for baseline characteristics this was statistically significant (p= 0.003). For the first 6 months of the study the survival curves had been coincident. Rates of adverse events were similar

between the two drugs and consistent with known toxicity profiles. CD_4 count changes in the first 2 months of the trial favored ddI with a mean rise of 8.6 cells/mm^3. The investigators concluded from this that the two drugs had equal efficacy in delaying disease progression but that ddC may provide a survival advantage over ddI (Abrams D, et al 1994) . However, an unpublished comparison with CPCRA's observational data base looked at survival for a group of AZT-treated patients matched for CD_4 count with CPCRA 002 trial participants and found survival was similar to patients in the trial (text from a talk by Don Abrams reproduced in Australian HIV Herald April 1993, p11-21).

Two further studies from the US have examined switching to ddI as compared with continuing ZDV in patients with CD_4 counts less than 300 and, therefore, bear greater similarity to our corrected analyses I and 3. These have been published as ACTG 116A (Notice board, 1993) and ACTG 116B/117 (Kahn J, et al, 1992). These studies compared two doses of ddI, 750 mg/day and 200mg/day, with ZDV. The studies differed in the period of prior ZDV therapy. ACTG 116A, which has only been published in an abbreviated form, looked at patients with 0 - 16 weeks prior ZDV, dividing groups into ZDV naive, <8 weeks and 8 - 16 weeks prior therapy. In the 318 patients who were ZDV naive, ZDV was superior to ddI in preventing disease progression or death. Similar results have been seen when comparing ddC and ZDV (ACTG 114). (Data on file F.Hoffmann-La Roche, Switzerland). However, in patients receiving 8 - 16 weeks prior ZDV, ddI was superior to continued ZDV with significant survival benefit for the 750 mg dose. ACTG 116B/117 involved 913 patients who had received ZDV for at least 16 weeks (mean 13.9 months of prior ZDV). AIDS defining events plus death were less frequent amongst those receiving 500 mg of ddI, but not 750 mg as compared with AZT. However,

for survival alone no advantage or disadvantage for ddI was seen. Similar to our study, in which small CD_4 rises were again seen in the first 8 - 12 weeks in the ddI-treated patients. Subgroup analysis of the 116B/117 study showed the clinical benefits were principally seen amongst the ARC patients (mean baseline CD_4 119 cells/mm^3) rather than AIDS patients (mean baseline CD_4 45 cells/mm^3) . This observation supports the St Stephen's data in which most favorable CD_4 responses were seen in ARC patients and those with baseline CD_4 counts greater than 100 cells/mm^3. The 'switch' after less than 6 months prior ZDV compared with continued ZDV, our corrected analysis I, show equivalence between the two regimes, broadly agreeing with this data. As patients with AIDS and lower CD4 cell counts develop ZDV resistance more rapidly than those with more favourable baseline characteristics, the failure of ddI to result in significant benefit for AIDS patients in the 116B/117 and St Stephens studies and the low mean CD4 count cohorts used in the Alpha and ACTG118 studies may relate to these patients having ZDV resistant virus which led to reduced sensitivity to ddI, as reported by Mayers et al (Mayers DL, et al, 1994).

In summary, multiple studies, in patients with baseline characteristics concordant with the St Stephen's Study have failed to produce a clear clinical algorithm for monotherapy nucleoside use in patients with CD_4 <300 cells/mm^3 but do offer construct validity to our data and subsequent analyses compared to historical controls. ZDV has been shown superior to placebo and to the current nucleoside alternatives ddC (in ACTG 114) and ddI (in ACTG 116A) as first line therapy. However, the question of how long to continue ZDV is somewhat unclear. If continued to intolerance, ddC may be the drug of choice (CPCRA 002) although ddI also appears to provide a survival benefit (St Stephen's Study). Furthermore, patients

have superior responses to ddI if this drug is commenced at higher CD_4 counts and better clinical status (St Stephen's Study, 116B/117). This opens the door for switching therapy before intolerance to ZDV has developed but after its initial clinical and immunological benefits have declined. ddI has been shown to provide benefit as early as 8 weeks after commencement of ZDV (ACTG 116A, 116B/117).

Furthermore, the choice of ddI dosage is poorly defined. As the St Stephen's data supports the assertion of efficacy in survival for ddI then it is likely that the 3 doses used in other studies are on a dose response curve plateau. Therefore, 200 mg ddI/day would be the dose of choice given its lower toxicity incidence as compared with other doses (Alpha, ACTG 118). However, reservations could be expressed regarding this dose as the immunological changes are even less substantial (Alpha).

3.5. FOSCARNET VS GANCICLOVIR

This was the first study to compare the two drugs which had both, in single agent studies, show a high level of efficacy against CMV retinitis. Indeed, in the St Stephen's Study given adequate treatment time both drugs were effective in most patients although there was a strong trend towards a more rapid response with Ganciclovir. Ganciclovir was also found to be better tolerated with only 2 patients stopping therapy during the 3 week treatment phase for drug related events compared with 5 Foscarnet patients. This was also seen overall, with fewer events causing therapy change in the Ganciclovir group and those events occurring later in therapy. The toxicity profiles of the two drugs seen in this study are distinct and were consistent with the known toxicities. No unexpected events occurred during the study. The incidence of penile ulceration was higher than for all patients in East Riverside over the same period (Moyle G, et al, 1993). However, this may be due to the fact that treatment for CMV retinitis was for 3 weeks (and in some patients longer) and the majority of patients received maintenance therapy, whereas for CMV disease at other sites treatment was generally for only 2 weeks and maintenance therapy was infrequently employed. Patients in the East Riverside cohort were more likely to develop penile ulceration during the treatment phase when higher levels of urinary foscarnet were present. Indeed, penile ulceration has been reported to resolve following reduction of Foscarnet dose maintenance levels (Fegueux S, et al, 1990). However, this was not seen in our study.

The only other published comparative study of these two drugs have been the Studies of Ocular Complications of AIDS Research Group (SOCA). This study was a multicentre randomized open comparative trial of

Foscarnet and Ganciclovir in AIDS related CMV retinitis, involving 234 patients with first episode disease. Patients were stratified by disease severity to immediate or delayed therapy prior to randomization. Retina were photographed and independently assessed according to quantitative criteria. Patients were also followed for survival (see below). Doses used were Foscarnet 180 mg/kg/day with saline hyperhydration and adjusted for creatinine clearance and Ganciclovir 10 mg/kg/day each for 2 weeks. Maintenance doses were also similar to those used in the St Stephen's Study. Further treatment cycles could be given with incomplete responses (Studies of Ocular complications of AIDS. Rationale, Design and Methods, 1992.). As in our study the two groups showed similar efficacy in controlling CMV retinitis. Time to disease progression was also similar between the groups although shorter than in our study (Foscarnet 59 days, Ganciclovir 56 days). This study was subsequently terminated by the data safety monitoring board due to mortality differences 19 months after initiation (see below). (Studies of Ocular Complications of AIDS Mortality in patients, 1992).

Maintenance therapy with either drug remains problematic with frequent reactivations of the retinitis, and drug-related adverse events. Reactivation of CMV may relate to the develoment of viral resistance which has been demonstrated both *in vitro* (Biron KK, et al 1986) and *in vivo* (Erice A, et al 1989) with Ganciclovir, and with Herpes simplex virus mutants and Foscarnet *in vitro* (Erikson B, et al 1979). Fortunately, CMV resistant to Ganciclovir remains sensitive to Foscarnet *in vivo* (Parenti DM et al 1990). Clearly, when reactivations on maintenance occur, use of the alternative agent or concomittant administration of both drugs should be considered. Therapy related events leading to interuption of treatment are also frequent and may contribute to reactivations as well as their morbidity and

the potential mortality (particularly with line infections) adversly affecting quality of life. Intolerance to both drugs is not infrequent limiting the therapy options to concomitant administration of reduced doses of both agents or to intra-vitreal administration of Ganciclovir.

Given the similarity in efficacy against CMV retinitis, shown in both the St. Stephens and SOCA studies, between Foscarnet and Ganciclovir, decisions regarding choice of agent are likely to be made on the basis of pre-existing biochemical and haematologial abnormalities, toxicities and their frequencies, concomitant medications which may potentiate toxicity, convenience of administration and any potential survival advantages for either medication.

REPORTED SURVIVAL OF PERSONS WITH AIDS AND CMV RETINITIS

Patients with untreated CMV appear to have a very poor survival with Holland et al reporting none of eight patients with CMV retinitis surviving beyond 6 weeks (Holland et al 1983) and Palestine et al reporting a mean survival 115 days (longest 10 months) in 10 patients (Palestine et al 1984).

Multiple studies, mostly retrospective have examined survival in CMV retinitis both with and without treatment. (Table 51).

Table 51 Reported Survival of Persons With AIDS and CMV Retinitis (After Polis et al,1993)

Reference	Type of Study	No. of Patients	Treatment	Dates Entered	Survival Form Diagnosis of CMV Retinitis (mo) (median)	Survival Form Diagnosis of CMV Retinitis (range)
Holland 1983	Retrospective	10	None	5/81 to 10/82	<11/2	
Palestine 1984	Retrospective	8	None	7/81 to 12/83	4 (mean)	Longest, 10 mo
Henderly 1987	Prospective Retrospective	18 5	Ganciclovir	7/85 to 6/86	6 (mean)	Longest, 15 mo
Jacobson 1988	Retrospective	31	Ganciclovir	1986	5,4	
Jabs 1989	Retrospective	46 15 31 19 12	 None Ganciclovir Responders Non-Resp. or partial Responder	1983 to 4/88	5,5 10.0 2.3	
Holland 1990	Retrospective	10 35 55 43 57	None None/Ganciclovir None/Ganciclovir None Ganciclovir	1981 to 4/84 5/4 to 12/85 1/86 to 12/87 1981 to 12/87 5/84 to 12/87	1 5 4 2 7	0 to 2 mo 1 to 19 mo 0 to 24 mo 0 to 18 mo 1 to 24 mo
Harb 1991	Retrospective	133 61 72 10 56 21	Mixed Mixed Mixed None Ganciclovir Foscarnet	7/85 to 10/89 7/85 to 9/87 10/87 to 10/89 2/87 to 10/89 2/87 to 10/89 2/87 to 10/89	8 5 9 7 8 9	 20% survival >1y 38% survival >1y
SOCA 1992	Prospective	127 107	Ganciclovir Foscarnet	4/90 to 10/91	8.5 12.6	

Despite treatment, prognosis remains poor reflecting the fact that CMV disease generally occurs in patients with late stage HIV disease, CD_4 counts < 50 cells/mm^3 and often on the background of previous or other ongoing opportunistic disease. However, as this table suggests the mean survival times with CMV retinitis have increased substantially and patients surviving > 1 year are increasingly common. Holland et al (Holland GN, et al 1990) retrospectively examined cases of 100 patients with AIDS related CMV retinitis comparing treatment with Ganciclovir (minimum 7 days continuous therapy) with no therapy finding a survival increase from a mean 2 months with no therapy to mean 7 months with Ganciclovir. Suggesting a substantial survival benefit from therapy with this drug.

A second retrospective study by Harb (Harb GE et al 1991) involving records of 133 patients with AIDS related CMV retinitis found survival with retinitis was significantly longer in those diagnosed after September 30, 1987 (9 months) than those diagnosed from July 1985 until that date (5 months). Variables affecting survival included hemoglobin and absolute lymphocyte count at baseline but not concurrent ZDV use, although this cut off date coincides with the introduction of this drug. Comparing patients who commenced therapy with Foscarnet versus Ganciclovir the relative hazard of death for patients receiving Foscarnet was 0.91, not significantly different from Ganciclovir patients. Controlling for prognostic variables including ZDV use produced a relative hazard of 1.0.

The SOCA study (see above) was stopped on the basis of improved survival amongst the 107 patients randomized to Foscarnet compared with the 127 patients who received Ganciclovir. (Relative risk of death for Ganciclovir patients 1.77, adjusted relative risk 1.79 $p = 0.007$; $p = 0.006$

by log rank test of Kaplan-Meier curves). The median survival time for Ganciclovir was 8.5 months and for Foscarnet 12.6 months. One year mortality was maximal for patients who received Ganciclovir and never switched therapy (74 per 100 persons). Ganciclovir patients who subsequently received Foscarnet has similar risk to Foscarnet only patients(46 per 100 and 43 per 100). Patients who initially received Foscarnet but switched to Ganciclovir also had similar risk to the Foscarnet alone patients (47 per 100) suggesting that the Foscarnet effect on survival could be achieved by relatively brief exposure. Interestingly, 2 of the 11 clinics involved in the study showed relative risks favoring Ganciclovir rather than Foscarnet.

On covariate analysis reduced creatinine clearance was shown to increase the relative risk of mortality with Foscarnet.

The use of antiretrovirals differed with 62% of Foscarnet patients receiving ZDV during therapy compared to 36% of the Ganciclovir group. Patients receiving ZDV in the Ganciclovir group also received a lower total daily dose. Use of antiretrovirals was not shown in this study to significantly affect the patterns of mortality within the groups.

Rates of catheter related infections were similar between groups.

A further study by Polis et al (Polis MA, et al, 1993) using prospective data on Foscarnet-treated patients compared with published historical controls also suggested a survival advantage for Foscarnet-treated patients although maximal survival was seen in patients who received both Foscarnet and Ganciclovir over the course of their management.

Our study is not in full agreement with these two publications, showing similar survival between Ganciclovir and Foscarnet (as seen by Harb et al 1991) and ZDV use being the variable which influences survival. The trend towards improved survival with ZDV being seen in both treatment arms whereas no such trends for superior survival were seen between Foscarnet or Ganciclovir. However, given the small numbers in our study there is a considerable Type II error and an advantage for one or other of the drugs may have been missed. Similarly, the potential for a Type I error resulting in a false positive result in favour of Zidovudine exists, but solid trends in favour of the ZDV treated patients were seen in both the Foscarnet and Ganciclovir arm suggesting this result is real. Theoretical reasons for improved survival with Foscarnet focus around this drugs antiretroviral activity (see introduction). Furthermore, Foscarnet may be synergistic with ZDV (Eriksson BFH, Schinazi RF, 1989) whereas Ganciclovir may antagonize the anti HIV effects of ZDV or ddI (Medina DJ, 1992), although recent data suggest ZDV may enhance Ganciclovir's anti CMV activity (Freitas, et al, 1993). This suggests that any ZDV effect on survival should only be seen in the Foscarnet group. Combination of 2 antiretroviral agents may also offer the potential advantage of more effective HIV suppression or delay of the emergence of resistant strains. However, in studies with two nucleoside analogues a combination effect on survival has not been shown in patients with CD_4 counts less than 150 cells/mm^3, those most likely to develop CMV disease (Fischl MA, et al. 1993). ZDV, but not other nucleoside antiretrovirals, is better tolerated by patients receiving Foscarnet compared with those receiving Ganciclovir, principally due to additive or synergistic hematologic toxicity with Ganciclovir. This was seen in both our and the SOCA studies, where more Foscarnet patients received ZDV.

Given these data it is difficult to find full explanation for differences between the two studies, SOCA and St Stephen's. In our cohort it appears ZDV exerts the greatest survival effects, diluting any possible extra Foscarnet benefit or Ganciclovir disadvantage. In the SOCA study 9 of 11 centers, and the overall result suggest the Foscarnet benefit to be the most important. The 6 week treatment period for each initial therapy provided for in the analysis for the St Stephen's Studies should provide maximal opportunity for a Foscarnet 'effect' to be seen, so that the Foscarnet group in our study is similar to the all 'Foscarnet' subgroups analyzed in SOCA study; all of which saw a survival benefit over Ganciclovir.

The conclusion from these two studies is, however, the same. In the SOCA study the choice of Foscarnet for survival is clear. In the St Stephen's Study as Foscarnet patients are more likely to receive ZDV, hence the survival benefit, this makes Foscarnet the drug of choice. Unfortunately, no quality of life measures were included in these studies. However, side effect profiles may give some idea as to the tolerability of the drugs, an important aspect in quality of life. Ganciclovir's side effect profile is limited to hematologic toxicity with neutropenia being the most common and problematic. Increased incidence of line infections, presumably related to this, or additional neutrophil dysfunction, have only been reported from the St Stephen's Study. Anaemia can, as in our study, be readily handled by transfusion although this produces additional costs and extra time at hospital may reduce QOL. Neutropenia can similarly be handled by cytokines such as G-CSF and GM-CSF although again at considerable cost. Exacerbation of these problems by concomitant use of ZDV can be limited by the use of nucleosides such as ddI and ddC which

may provide survival benefits (see ddI study section) without adding hematologic toxicity.

Foscarnet results in a wider spectrum of side effects including renal toxicity, disturbances of calcium and glucose metabolism and penile ulceration. In our study, patients were more likely to stop this medication due to adverse events and tended to stop sooner after initiation of therapy. Similar results were seen in the SOCA study with 39 of 107 Foscarnet patients switching therapy compared with 14 of 127 Ganciclovir patients. As well as adverse events causing change of therapy many patients feel nauseated and non-specifically unwell during Foscarnet therapy. Hyperhydration to limit renal dysfunction (Deray G, et al 1990) and the longer infusion time required for Foscarnet administration may further add to the difficulties of Foscarnet use.

In summary, an algorithm for the treatment of CMV retinitis can be established using the results of our and other studies in this disease. In hematologically stable patients Ganciclovir is the initial treatment of choice providing a more rapid response. Once the CMV is in remission Foscarnet with ZDV may be the most suitable maintenance therapy, given normal renal function, as this combination may offer a survival benefit. Reactivations of CMV may then be treated with Ganciclovir. The use of Foscarnet is dependent on patient tolerability of this drug and it remains to be shown if quality of 'added' survival time is greater with Foscarnet or Ganciclovir.

The development of oral versions of these two drugs or other less toxic antivirals remains critical to the future management of CMV disease. Oral alternatives also provide the opportunity for prophylaxis of CMV disease

which would be a major advance in care given the considerable morbidity and mortality caused by this disease.

3.6. THE FUTURE OF ANTIRETROVIRAL THERAPY

Given that single agent therapy for HIV has thus far not shown dramatic outcome in terms of improved survival, reduction in viral loads or immune restoration it appears likely future therapy will use a number of agents in combination. As HIV replication is a critical event in triggering immune destruction the agents used are likely to focus on this process. Support for the concept of combination therapy's potential utility comes from *in vitro* data showing antiviral synergy between nucleosides (e.g. ZDV/ddI, ZDV/ddC) and nucleosides and other agents (e.g. ZDV/interferon, ZDV/protienase inhibitors) and that different agents may act preferentially on different cell types. Two strategies may be employed in using combination therapy, either 'convergent' or 'divergent' therapies.

Convergent therapy is used to describe the concept of using multiple agents focused on a single site in the viral replicative pathway to induce blockage of that step or select for dysfunctional mutations. The reverse transcriptase enzyme appears the most likely viral protein for this approach given the number of agents currently in development which focus on this enzyme. The alternative, divergent, therapy involves the use of multiple agents to block or interfere at different sites of the viral replication cycle.

Convergent therapy's tenet is that as retroviral drug resistance is conferred by mutations in its own genome (unlike some bacterial drug resistance) the capacity for viral resistance development is limited by the size of the genome. Combining drugs focusing on a single viral protein, hence a single gene, therefore limits the opportunity for multiple drug resistance to develop as the more changes in protein structure selected

for by the treatment, the greater the chance of that protein becoming dysfunctional. (Chow Y-K, et al 1993).

Initial data from Chow, et al, combining ZDV, ddI and nevirapine provided impetus to this concept, showing abrogation of viral replication. However, subsequent reanalysis found a flaw in the data and researchers from other laboratories have reported 'escape mutants' able to replicate in the presence of these combinations.

Studies involving two nucleoside analogues have begun investigating the convergent therapy concept. Combination therapy studies with ddI have thus far been limited and focused only on surrogate markers. Yarchoan and colleagues. (Yarchoan R, et al, 1994) compared alternating ddI and ZDV with combination of ddI and ZDV in 41 symptomatic HIV positive patients with CD_4 < 350 cells/mm^3 and < 3 months of previous nucleoside therapy. After 54 weeks of follow-up CD_4 counts remained elevated in the combination arm but not the alternating arm. P24 antigen and weight changes also favored the combination arm.

Collier et al (Collier AC, et al. 1993) reported on a partially randomized study of 69 patients with CD_4 < 400 cells/mm^3 and less than 4 months prior ZDV comparing combination ddI/ZDV with ddI monotherapy. Larger and more sustained rises in CD_4 were observed in the combination arm and this data was supported by virologic changes.

Furthermore, resistance to ddI but not to ZDV may be delayed by using this combination although multidrug resistant mutants showing novel mutations may also be generated (Shafer RW, et al 1994).

Similar observations have also been seen with ZDV/ddC combinations. Meng and colleagues (Meng TC et al, 1992) in a small 6 arm study in 56 patients showed greater and more sustained CD_4 rises with ZDV/ddC combination than those receiving ZDV. However, limited conclusions can be drawn from this study as the ZDV dose used (300 mg/day) in the ZDV-treated comparator arm was below standard dosages. Historical controls from another ACTG study (114) were subsequently used as comparators and continued to show superiority for the ZDV/ddC combination regime on CD_4 count. (Data on file, F.Hoffmann-La Roche, Switzerland).

Similarly, alternating regimes with ZDV and ddC have been shown on CD_4 count and p_{24} antigen changes to be superior to ZDV alone although again this study used doses of ddC higher than those currently recommended. (Skowron G, et al, 1993). However, an alternating regime of ddI and ZDV did not produce CD4 elevations of as great a magnitude as receiving the two drugs concommitantly (Yarchoan R, et al, 1994) and alternating regimes may encourage more rapid selection of drug resistance (Fitzgibbon JE, et al 1993).

The only combination study with clinical endpoints to date is ACTG 155. This large 991 evaluable patient, 3 arm randomized double blind study compared continued Zidovudine with switching to ddC monotherapy or adding ddC to ZDV for combinations all at standard doses. All patients had $CD_4 < 300$ and had received ZDV for at least 6 months (median 18 months). The median follow-up was 17.7 months. Overall, no differences were seen between the three groups, however, as most events occurred in the $CD_4 < 50$ patient group this may have biased the interpretation. Duration of prior ZDV was not noted to be a predictor of response. An unplanned analysis of CD4 substrata showed a survival plus disease

progression advantage to combination therapy over continued ZDV in patients with baseline CD_4 150 - 300 cells/mm^3 but not in patients with lower CD4 counts. Toxicity events were also less frequent in this substrata although overall combination therapy resulted in an additive incidence of toxicity events. (Fischl MA, et al 1993). It appears likely from the preliminary data presented earlier that ddI is at least similar to ddC in combination with ZDV, leaving the choice down to the toxicity profiles.

The concept of 'playing chess' with the reverse transcriptase gene is an appealing one, however, HIV is a highly mutable virus and chronically infected individuals have multiple populations of virus with different genotypic characteristics so only a subset of virus may become dysfunctional while other mutations from those seen in *in vitro* studies may be selected for in the multiple drug environment.

A further problem with nucleoside analogues is the high incidence of adverse events and intolerance to these drugs leads to morbidity and reduction of quality of life. Combination therapy is likely to see at least an additive incidence of toxicity, as seen in ACTG 155, but, potentially, synergistic toxicity. This is particularly true with ddI, ddC and D4T which are all known to cause peripheral neuropathy and are therefore contraindicated for use together. Successive use of ddI in a patient with ddC-induced PN has been reported to lead to an increase in the severity of this problem. (Le Lacheur SF, et al, 1991). The non-nucleoside reverse transcriptase inhibitors, based on limited phase I data, appear to have less severe toxicity problem, however, it remains uncertain if combination with nucleoside analogues will lead to synergistic toxicity.

Foscarnet has also been proposed as a combination agent. Further data from Chow et al (Chow Y-K, 1993) has shown *in vitro* abrogation of HIV replication by a combination of ZDV, ddI and Foscarnet. However, given the problems with intravenous administration and the frequency of adverse events with this agent it is unlikely to be used other than in patients requiring it for specific therapy (e.g. CMV disease).

Many questions still remain from these studies and the results of ACTG 155 have disappointed many who have looked to convergent combination therapy as the next step forward. Further studies such as the Delta study and ACTG 175 may provide answers to the role of nucleoside analogue combination therapy. However, results from these studies appear distant at present and both studies have been troubled by patient withdrawals. Small studies examining triple combination with 2 nucleoside analogues and NNRTIs are also underway and should assist in clarifying the potential of this approach.

Divergent therapy using drugs acting at multiple sites is the approach used in a number of bacterial diseases, most notably tuberculosis, where rapid development of resistant strains commonly occurs. Such an approach is likely also to have merit in the treatment of HIV where resistance to reverse transcriptase inhibitors develop rapidly, particularly in patients with high viral loads. Other critical viral proteins may be less amenable to mutation and/or may be more likely to become dysfunctional following minor changes.

A number of drugs which act at sites of viral replication other than reverse transcriptase are currently in development and are likely to be used in 'divergent combinations' with established therapies. Drugs in at least

phase I/II studies include a number of inhibitors of viral proteinase as well as Interferons which are thought to act at the site of viral assembly and budding. Preliminary data from a trial of monotherapy with a proteinase inhibitor Ro 31,8959 suggest equivalent CD_4 changes to ZDV in patients with baseline counts less than 300 cells/mm3 but with no serious toxic events. This drug shows considerable *in vitro* synergy with ZDV, however, a combination study with this agent and ZDV did not result in dramatic CD_4 rises or complete viral suppression. (Data on file F.Hoffmann-La Roche, Basel, Switzerland). Interferon alpha also shows synergy *in vitro* with ZDV and appears most suitable for use in patients with intermediate or early HIV disease (CD_4 counts >300 cells/mm3) however, dosage levels are not yet clearly determined and the drugs have several drawbacks being suitable only for injectable use and having a high incidence of side effects including 'flu-like' symptoms and, as with ZDV, bone marrow suppression.

A further approach in combination therapy is to combine specific anti HIV agents with drugs which suppress co-factors in progression. Specifically members of the herpes virus family including CMV, Herpes Virus 6 and Herpes Simplex have been suggested to accelerate disease progression. In a recent Wellcome study the antiherpes drug Acyclovir was given at higher than currently recommended doses to patients with CD_4 counts less than 150 cells/mm3 to determine the value of this drug as a prophylaxis against CMV disease. Although this outcome was not achieved the study did show a survival advantage for patients receiving acyclovir (800 mg 6 hourly) over the placebo arm. (Youle M, et al, 1994). This advantage was particularly seen in patients receiving Zidovudine. Data from another study by Cooper et al (Cooper DA, et al, 1993) has supported the view that combination of these two drugs may provide

survival advantage. Acyclovir and ZDV have previously been shown to exhibit synergy against HIV *in vitro* although Acyclovir has no inherent anti HIV activity. As Foscarnet is also an inhibitor of the Herpes virus family some of the survival advantage suggested in some, but not in our study, may be related to inhibition of these cofactors.

The history of medicine contains a number of instances where drugs used as single agents show limited effect but when used in combination lead to cure or palliation (e.g. combination antibiotics for Tuberculosis, combination chemotherapy in Hodgkin's Lymphoma) and this approach holds promise for therapy in HIV. The data on available drugs, the nucleoside analogues, show limited effects in reduction of viral load and short term effects in surrogate measures of immune function. Furthermore, survival benefits from these drugs are very limited and short term quality of life improvements have not been confirmed over longer periods. Resistance to nucleoside analogues develops rapidly and may contribute to the limitations of their benefits.

Studies with current agents require large patient numbers and long follow-up periods to assess differences between treatment regimes reflecting their marginality. Surrogate markers or combinations of markers do not provide ideal predictions of clinical outcome so remain of limited usefulness. However, the future is looking brighter with many new drugs entering into clinical trials and new approaches using established drugs being used. As many of the new agents show, at least in preliminary data, safety profiles more favorable than for drugs presently available we have the promise of both improvement in length and quality of life for persons with HIV infection.

3.7. SUMMARY OF CLAIMS OF ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

3.7.1. ddI Study:

This was the first study to show:

The development of diabetes/glucose intolerance as part of the spectrum of ddI related pancreatic toxicity.

A relationship between time on ddI therapy and development of pancreatic toxicity.

The value of the prospective use of nerve conduction studies in assessing subjective symptomatology in patients with possible nucleoside related peripheral neuropathy.

To show an improvement in quality of life in patients receiving ddI after becoming ZDV-intolerant.

To show a survival advantage for receiving ddI after developing ZDV intolerance as compared with a historical control group treated with Zidovudine alone.

3.7.2. CMV Study

This was the first study to show

The equivalence of therapy for CMV retinitis of Foscarnet and Ganciclovir.

The frequency of penile ulceration in patients receiving Foscarnet.

The survival advantage of continued ZDV use in patients with CMV retinitis regardless of therapy.

G. Moyle 1994

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5. PART V: APPENDICIES

APPENDIX 1

Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome

Reported by
Council of State and Territorial Epidemiologists;
AIDS Program, Center for Infectious Diseases, CDC

INTRODUCTION

The following revised case definition for surveillance of acquired immunodeficiency syndrome (AIDS) was developed by CDC in collaboration with public health and clinical specialists. The Council of State and Territorial Epidemiologists (CSTE) has officially recommended adoption of the revised definition for national reporting of AIDS. The objectives of the revision are a) to track more effectively the severe disabling morbidity associated with infection with human immunodeficiency virus (HIV) (including HIV-1 and HIV-2); b) to simplify reporting of AIDS cases; c) to increase the sensitivity and specificity of the definition through greater diagnostic application of laboratory evidence for HIV infection; and d) to be consistent with current diagnostic practice, which in some cases includes presumptive, i.e., without confirmatory laboratory evidence, diagnosis of AIDS-indicative diseases (e.g., *Pneumocystis carinii* pneumonia, Kaposi's sarcoma).

The definition is organized into three sections that depend on the status of laboratory evidence of HIV infection (e.g., HIV antibody) (Figure 1). The major proposed changes apply to patients with laboratory evidence for HIV infection: a) inclusion of HIV encephalopathy, HIV wasting syndrome, and a broader range of specific AIDS-indicative diseases (Section II.A); b) inclusion of AIDS patients whose indicator diseases are diagnosed presumptively (Section II.B); and c) elimination of exclusions due to other causes of immunodeficiency (Section I.A).

Application of the definition for children differs from that for adults in two ways. First, multiple or recurrent serious bacterial infections and lymphoid interstitial pneumonia/pulmonary lymphoid hyperplasia are accepted as indicative of AIDS among children but not among adults. Second, for children<15 months of age whose mothers are thought to have had HIV infection during the child's perinatal period, the laboratory criteria for HIV infection are more stringent, since the presence of HIV antibody in the child is, by itself, insufficient evidence for HIV infection because of the persistence of passively acquired maternal antibodies < 15 months after birth.

The new definition is effective immediately. State and local health departments are requested to apply the new definition henceforth to patients reported to them. The initiation of the actual reporting of cases that meet the new definition is targeted for September 1, 1987, when modified computer software and report forms should be in place to accommodate the changes. CSTE has recommended retrospective application of the revised definition to patients already reported to health departments. The new definition follows:

1987 REVISION OF CASE DEFINITION FOR AIDS FOR SURVEILLANCE PURPOSES

For national reporting, a case of AIDS is defined as an illness characterized by one or more of the following "indicator" diseases, depending on the status of laboratory evidence of HIV infection, as shown below.

I. Without Laboratory Evidence Regarding HIV Infection

If laboratory tests for HIV were not performed or gave inconclusive results (*See* Appendix I) and the patient had no other cause of immunodeficiency listed in Section I.A below, then any disease listed in Section I.B indicates AIDS if it was diagnosed by a definitive method (*See* Appendix II).

A. Causes of immunodeficiency that disqualify diseases as indicators of AIDS in the absence of laboratory evidence for HIV infection

1. high-dose or long-term systemic corticosteroid therapy or other immunosuppressive/cytotoxic therapy ≤3 months before the onset of the indicator disease
2. any of the following diseases diagnosed ≤3 months after diagnosis of the indicator disease: Hodgkin's disease, non-Hodgkin's lymphoma (other than primary brain lymphoma), lymphocytic leukemia, multiple myeloma, any other cancer of lymphoreticular or histiocytic tissue, or angioimmunoblastic lymphadenopathy
3. a genetic (congenital) immunodeficiency syndrome or an acquired immunodeficiency syndrome atypical of HIV infection, such as one involving hypogammaglobulinemia

B. Indicator diseases diagnosed definitively (*See* Appendix II)

1. candidiasis of the esophagus, trachea, bronchi, or lungs
2. cryptococcosis, extrapulmonary
3. cryptosporidiosis with diarrhea persisting >1 month
4. cytomegalovirus disease of an organ other than liver, spleen, or lymph nodes in a patient >1 month of age
5. herpes simplex virus infection causing a mucocutaneous ulcer that persists longer than 1 month; or bronchitis, pneumonitis, or esophagitis for any duration affecting a patient >1 month of age
6. Kaposi's sarcoma affecting a patient < 60 years of age
7. lymphoma of the brain (primary) affecting a patient < 60 years of age
8. lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child <13 years of age
9. *Mycobacterium avium* complex or *M. kansasii* disease, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
10. *Pneumocystis carinii* pneumonia
11. progressive multifocal leukoencephalopathy
12. toxoplasmosis of the brain affecting a patient >1 month of age

II. With Laboratory Evidence for HIV Infection

Regardless of the presence of other causes of immunodeficiency (I.A), in the presence of laboratory evidence for HIV infection (*See* Appendix I), any disease listed above (I.B) or below (II.A or II.B) indicates a diagnosis of AIDS.

A. Indicator diseases diagnosed definitively (*See* Appendix II)

1. bacterial infections, multiple or recurrent (any combination of at least two within a 2-year period), of the following types affecting a child < 13 years of age:
   septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media or superficial skin or mucosal abscesses), caused by *Haemophilus, Streptococcus* (including pneumococcus), or other pyogenic bacteria
2. coccidioidomycosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
3. HIV encephalopathy (also called "HIV dementia," "AIDS dementia," or "subacute encephalitis due to HIV") (*See* Appendix II for description)
4. histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
5. isosporiasis with diarrhea persisting >1 month
6. Kaposi's sarcoma at any age
7. lymphoma of the brain (primary) at any age
8. other non-Hodgkin's lymphoma of B-cell or unknown immunologic phenotype and the following histologic types:
   a. small noncleaved lymphoma (either Burkitt or non-Burkitt type) (*See* Appendix IV for equivalent terms and numeric codes used in the *International Classification of Diseases*, Ninth Revision, Clinical Modification)
   b. immunoblastic sarcoma (equivalent to any of the following, although not necessarily all in combination: immunoblastic lymphoma, large-cell lymphoma, diffuse histiocytic lymphoma, diffuse undifferentiated lymphoma, or high-grade lymphoma) (*See* Appendix IV for equivalent terms and numeric codes used in the *International Classification of Diseases*, Ninth Revision, Clinical Modification)

   Note: Lymphomas are not included here if they are of T-cell immunologic phenotype or their histologic type is not described or is described as "lymphocytic," "lymphoblastic," "small cleaved," or "plasmacytoid lymphocytic"
9. any mycobacterial disease caused by mycobacteria other than *M. tuberculosis*, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
10. disease caused by *M. tuberculosis*, extrapulmonary (involving at least one site outside the lungs, regardless of whether there is concurrent pulmonary involvement)
11. *Salmonella* (nontyphoid) septicemia, recurrent
12. HIV wasting syndrome (emaciation, "slim disease") (*See* Appendix II for description)

B. Indicator diseases diagnosed presumptively (by a method other than those in Appendix II)

Note: Given the seriousness of diseases indicative of AIDS, it is generally important to diagnose them definitively, especially when therapy that would be used may have serious side effects or when definitive diagnosis is needed

for eligibility for antiretroviral therapy. Nonetheless, in some situations, a patient's condition will not permit the performance of definitive tests. In other situations, accepted clinical practice may be to diagnose presumptively based on the presence of characteristic clinical and laboratory abnormalities. Guidelines for presumptive diagnoses are suggested in Appendix III.

1. candidiasis of the esophagus
2. cytomegalovirus retinitis with loss of vision
3. Kaposi's sarcoma
4. lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child <13 years of age
5. mycobacterial disease (acid-fast bacilli with species not identified by culture), disseminated (involving at least one site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
6. *Pneumocystis carinii* pneumonia
7. toxoplasmosis of the brain affecting a patient >1 month of age

III. With Laboratory Evidence Against HIV Infection

With laboratory test results negative for HIV infection (*See* Appendix I), a diagnosis of AIDS for surveillance purposes is ruled out *unless*:

A. all the other causes of immunodeficiency listed above in Section I.A are excluded; **AND**
B. the patient has had either:
   1. *Pneumocystis carinii* pneumonia diagnosed by a definitive method (*See* Appendix II); **OR**
   2. a. any of the other diseases indicative of AIDS listed above in Section I.B diagnosed by a definitive method (*See* Appendix II); **AND**
      b. a T-helper/inducer (CD4) lymphocyte count <400/mm^3.

COMMENTARY

The surveillance of severe disease associated with HIV infection remains an essential, though not the only, indicator of the course of the HIV epidemic. The number of AIDS cases and the relative distribution of cases by demographic, geographic, and behavioral risk variables are the oldest indices of the epidemic, which began in 1981 and for which data are available retrospectively back to 1978. The original surveillance case definition, based on then-available knowledge, provided useful epidemiologic data on severe HIV disease (*1*). To ensure a reasonable predictive value for underlying immunodeficiency caused by what was then an unknown agent, the indicators of AIDS in the old case definition were restricted to particular opportunistic diseases diagnosed by reliable methods in patients without specific known causes of immunodeficiency. After HIV was discovered to be the cause of AIDS, however, and highly sensitive and specific HIV-antibody tests became available, the spectrum of manifestations of HIV infection became better defined, and classification systems for HIV infection were developed (*2-5*). It became apparent that some progressive, seriously disabling, and even fatal conditions (e.g., encephalopathy, wasting syndrome) affecting a substantial number of HIV-infected patients were not subject to epidemiologic surveillance, as they were not included in the AIDS

case definition. For reporting purposes, the revision adds to the definition most of those severe non-infectious, non-cancerous HIV-associated conditions that are categorized in the CDC clinical classification systems for HIV infection among adults and children (*4,5*).

Another limitation of the old definition was that AIDS-indicative diseases are diagnosed presumptively (i.e., without confirmation by methods required by the old definition) in 10%-15% of patients diagnosed with such diseases; thus, an appreciable proportion of AIDS cases were missed for reporting purposes (*6,7*). This proportion may be increasing, which would compromise the old case definition's usefulness as a tool for monitoring trends. The revised case definition permits the reporting of these clinically diagnosed cases as long as there is laboratory evidence of HIV infection.

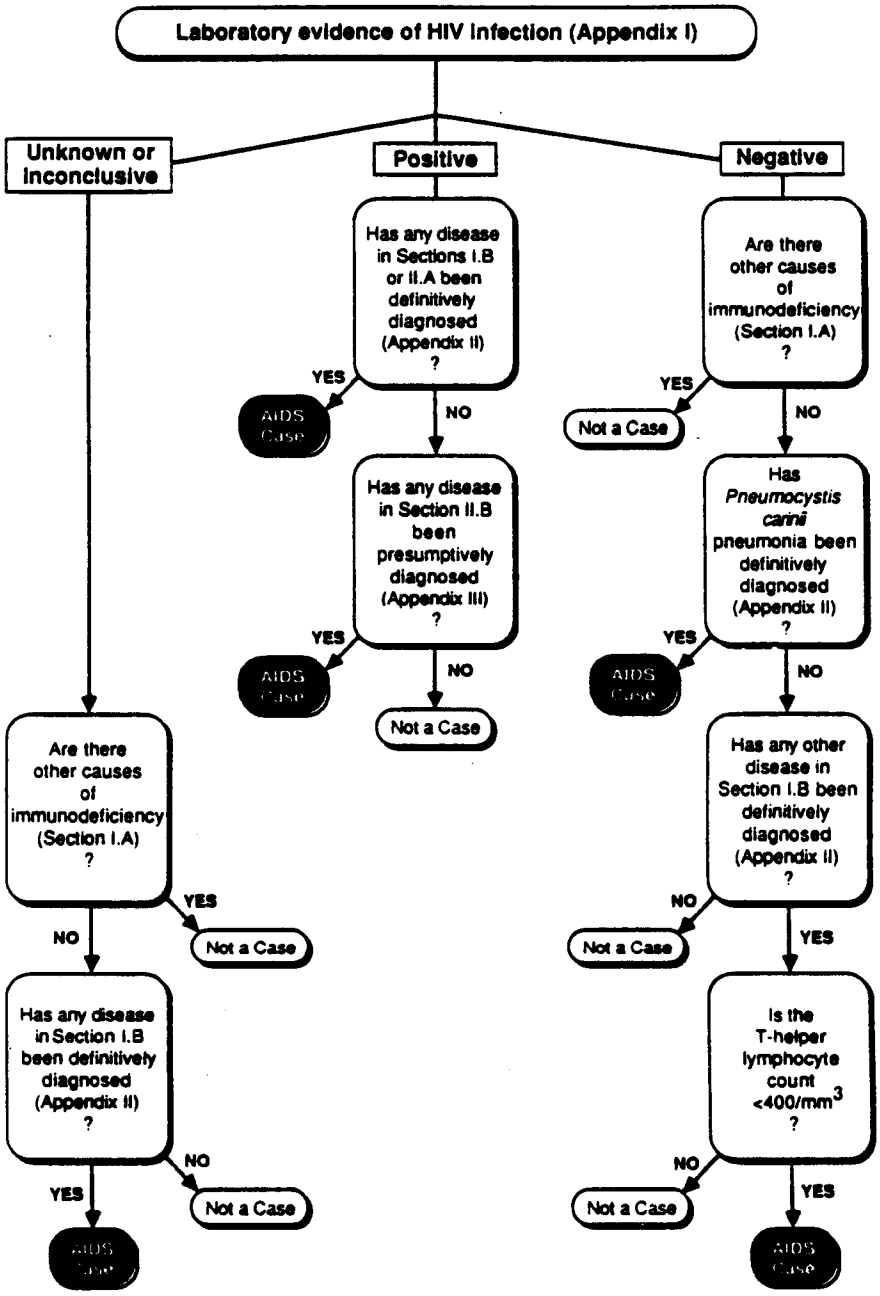
The effectiveness of the revision will depend on how extensively HIV-antibody tests are used. Approximately one third of AIDS patients in the United States have been from New York City and San Francisco, where, since 1985, $<$ 7% have been reported with HIV-antibody test results, compared with $>$ 60% in other areas. The impact of the revision on the reported numbers of AIDS cases will also depend on the proportion of AIDS patients in whom indicator diseases are diagnosed presumptively rather than definitively. The use of presumptive diagnostic criteria varies geographically, being more common in certain rural areas and in urban areas with many indigent AIDS patients.

To avoid confusion about what should be reported to health departments, the term "AIDS" should refer only to conditions meeting the surveillance definition. This definition is intended only to provide consistent statistical data for public health purposes. Clinicians will not rely on this definition alone to diagnose serious disease caused by HIV infection in individual patients because there may be additional information that would lead to a more accurate diagnosis. For example, patients who are not reportable under the definition because they have either a negative HIV-antibody test or, in the presence of HIV antibody, an opportunistic disease not listed in the definition as an indicator of AIDS nonetheless may be diagnosed as having serious HIV disease on consideration of other clinical or laboratory characteristics of HIV infection or a history of exposure to HIV.

Conversely, the AIDS surveillance definition may rarely misclassify other patients as having serious HIV disease if they have no HIV-antibody test but have an AIDS-indicative disease with a background incidence unrelated to HIV infection, such as cryptococcal meningitis.

The diagnostic criteria accepted by the AIDS surveillance case definition should not be interpreted as the standard of good medical practice. Presumptive diagnoses are accepted in the definition because not to count them would be to ignore substantial morbidity resulting from HIV infection. Likewise, the definition accepts a reactive screening test for HIV antibody without confirmation by a supplemental test because a repeatedly reactive screening test result, in combination with an indicator disease, is highly indicative of true HIV disease. For national surveillance purposes, the tiny proportion of possibly false-positive screening tests in persons with AIDS-indicative diseases is of little consequence. For the individual patient, however, a correct diagnosis is critically important. The use of supplemental tests is, therefore, strongly endorsed. An increase in the diagnostic use of HIV-antibody tests could improve both the quality of medical care and the function of the new case definition, as well as assist in providing counselling to prevent transmission of HIV.

FIGURE I. Flow diagram for revised CDC case definition of AIDS, September 1, 1987



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APPENDIX I

Laboratory Evidence For or Against HIV Infection

1. **For Infection:**
   When a patient has disease consistent with AIDS:
   a. a serum specimen from a patient ≥15 months of age, or from a child <15 months of age whose mother is not thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test (e.g., enzyme-linked immunosorbent assay [ELISA]), as long as subsequent HIV-antibody tests (e.g., Western blot, immunofluorescence assay), if done, are positive; **OR**
   b. a serum specimen from a child < 15 months of age, whose mother is thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test (e.g., ELISA), plus increased serum immunoglobulin levels and at least one of the following abnormal immunologic test results: reduced absolute lymphocyte count, depressed CD4 (T-helper) lymphocyte count, or decreased CD4/CD8 (helper/suppressor) ratio, as long as subsequent antibody tests (e.g., Western blot, immunofluorescence assay), if done, are positive; **OR**
   c. a positive test for HIV serum antigen; **OR**
   d. a positive HIV culture confirmed by both reverse transcriptase detection and a specific HIV-antigen test or in situ hybridization using a nucleic acid probe; **OR**
   e. a positive result on any other highly specific test for HIV (e.g., nucleic acid probe of peripheral blood lymphocytes).

2. **Against Infection:**
   A nonreactive screening test for serum antibody to HIV (e.g., ELISA) without a reactive or positive result on any other test for HIV infection (e.g., antibody, antigen, culture), if done.

3. **Inconclusive (Neither For nor Against Infection):**
   a. a repeatedly reactive screening test for serum antibody to HIV (e.g., ELISA) followed by a negative or inconclusive supplemental test (e.g., Western blot, immunofluorescence assay) without a positive HIV culture or serum antigen test, if done; **OR**
   b. a serum specimen from a child < 15 months of age, whose mother is thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test, even if positive by a supplemental test, without additional evidence for immunodeficiency as described above (in 1.b) and without a positive HIV culture or serum antigen test, if done.

APPENDIX II

Definitive Diagnostic Methods for Diseases Indicative of AIDS

Diseases	Definitive Diagnostic Methods
cryptosporidiosis cytomegalovirus isosporiasis Kaposi's sarcoma lymphoma lymphoid pneumonia or hyperplasia *Pneumocystis carinii* pneumonia progressive multifocal leukoencephalopathy toxoplasmosis	microscopy (histology or cytology).
candidiasis	gross inspection by endoscopy or autopsy or by microscopy (histology or cytology) on a specimen obtained directly from the tissues affected (including scrapings from the mucosal surface), not from a culture.
coccidioidomycosis cryptococcosis herpes simplex virus histoplasmosis	microscopy (histology or cytology), culture, or detection of antigen in a specimen obtained directly from the tissues affected or a fluid from those tissues.
tuberculosis other mycobacteriosis salmonellosis other bacterial infection	culture.

HIV encephalopathy* (dementia)	clinical findings of disabling cognitive and/or motor dysfunction interfering with occupation or activities of daily living, or loss of behavioral developmental milestones affecting a child, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection that could explain the findings. Methods to rule out such concurrent illnesses and conditions must include cerebrospinal fluid examination and either brain imaging (computed tomography or magnetic resonance) or autopsy.
HIV wasting syndrome*	findings of profound involuntary weight loss >10% of baseline body weight plus either chronic diarrhea (at least two loose stools per day for ≥ 30 days) or chronic weakness and documented fever (for ≥ 30 days, intermittent or constant) in the absence of a concurrent illness or condition other than HIV infection that could explain the findings (e.g., cancer, tuberculosis, cryptosporidiosis, or other specific enteritis).

*For HIV encephalopathy and HIV wasting syndrome, the methods of diagnosis described here are not truly definitive, but are sufficiently rigorous for surveillance purposes.

APPENDIX III

Suggested Guidelines for Presumptive Diagnosis of Diseases Indicative of AIDS

Diseases	Presumptive Diagnostic Criteria
candidiasis of esophagus	a. recent onset of retrosternal pain on swallowing; **AND** b. oral candidiasis diagnosed by the gross appearance of white patches or plaques on an erythematous base or by the microscopic appearance of fungal mycelial filaments in an uncultured specimen scraped from the oral mucosa.
cytomegalovirus retinitis	a characteristic appearance on serial ophthalmoscopic examinations (e.g., discrete patches of retinal whitening with distinct borders, spreading in a centrifugal manner, following blood vessels, progressing over several months, frequently associated with retinal vasculitis, hemorrhage, and necrosis). Resolution of active disease leaves retinal scarring and atrophy with retinal pigment epithelial mottling.
mycobacteriosis	microscopy of a specimen from stool or normally sterile body fluids or tissue from a site other than lungs, skin, or cervical or hilar lymph nodes, showing acid-fast bacilli of a species not identified by culture.
Kaposi's sarcoma	a characteristic gross appearance of an erythematous or violaceous plaque-like lesion on skin or mucous membrane. (**Note:** Presumptive diagnosis of Kaposi's sarcoma should not be made by clinicians who have seen few cases of it.)
lymphoid interstitial pneumonia	bilateral reticulonodular interstitial pulmonary infiltrates present on chest X ray for ≥2 months with no pathogen identified and no response to antibiotic treatment.
Pneumocystis carinii pneumonia	a. a history of dyspnea on exertion or nonproductive cough of recent onset (within the past 3 months); **AND** b. chest X-ray evidence of diffuse bilateral interstitial infiltrates or gallium scan evidence of diffuse bilateral pulmonary disease; **AND** c. arterial blood gas analysis showing an arterial pO_2 of <70 mm Hg or a low respiratory diffusing capacity (<80% of predicted values) or an increase in the alveolar-arterial oxygen tension gradient; **AND** d. no evidence of a bacterial pneumonia.

toxoplasmosis of the brain	a. recent onset of a focal neurologic abnormality consistent with intracranial disease or a reduced level of consciousness; **AND** b. brain imaging evidence of a lesion having a mass effect (on computed tomography or nuclear magnetic resonance) or the radiographic appearance of which is enhanced by injection of contrast medium; **AND** c. serum antibody to toxoplasmosis or successful response to therapy for toxoplasmosis.

APPENDIX IV

Equivalent Terms and International Classification of Disease (ICD) Codes for AIDS-Indicative Lymphomas

The following terms and codes describe lymphomas indicative of AIDS in patients with antibody evidence for HIV infection (Section II.A.8 of the AIDS case definition). Many of these terms are obsolete or equivalent to one another.

ICD-9-CM (1978)

Codes	Terms
200.0	**Reticulosarcoma** lymphoma (malignant): histiocytic (diffuse) reticulum cell sarcoma: pleomorphic cell type or not otherwise specified
200.2	**Burkitt's tumor or lymphoma** malignant lymphoma, Burkitt's type

ICD-O (Oncologic Histologic Types 1976)

Codes	Terms
9600/3	**Malignant lymphoma, undifferentiated cell type** non-Burkitt's or not otherwise specified
9601/3	**Malignant lymphoma, stem cell type** stem cell lymphoma
9612/3	**Malignant lymphoma, immunoblastic type** immunoblastic sarcoma, immunoblastic lymphoma, or immunoblastic lymphosarcoma
9632/3	**Malignant lymphoma, centroblastic type** diffuse or not otherwise specified, or germinoblastic sarcoma: diffuse or not otherwise specified
9633/3	**Malignant lymphoma, follicular center cell, non-cleaved** diffuse or not otherwise specified
9640/3	**Reticulosarcoma, not otherwise specified** malignant lymphoma, histiocytic: diffuse or not otherwise specified reticulum cell sarcoma, not otherwise specified malignant lymphoma, reticulum cell type
9641/3	**Reticulosarcoma, pleomorphic cell type** malignant lymphoma, histiocytic, pleomorphic cell type reticulum cell sarcoma, pleomorphic cell type
9750/3	**Burkitt's lymphoma or Burkitt's tumor** malignant lymphoma, undifferentiated, Burkitt's type malignant lymphoma, lymphoblastic, Burkitt's type

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1993 revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults*

CD4+ T-cell categories	Clinical categories (A) Asymptomatic, acute (primary) HIV or PGL†	(B) Symptomatic, not (A) or (C) conditions§	(C) AIDS-indicator conditions¶
(1) ≥500/μL	A1	B1	C1
(2) 200-499/μL	A2	B2	C2
(3) <200/μL AIDS-indicator T-cell count	A3	B3	C3

*The shaded cells illustrate the expanded AIDS surveillance case definition. Persons with AIDS-indicator conditions (Category C) as well as those with CD4+ T-lymphocyte counts ≤200/μL (Categories A3 or B3) will be reportable as AIDS cases in the United States and Territories, effective January 1, 1993.
†PGL-persistent generalized lymphadenopathy. Clinical Category A includes acute (primary) HIV infection.
§See text for discussion.
¶See Appendix B.

are listed in Categories B and C must not have occurred.

- Asymptomatic HIV infection
- Persistent generalized lymphadenopathy
- Acute (primary) HIV infection with accompanying illness or history of acute HIV infection[28,30]

Category B consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria: a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples of conditions in clinical Category B include, but are not limited to:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting longer than 1 month
- Hairy leukoplakia, oral
- Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess
- Peripheral neuropathy

For classification purposes, Category B conditions take precedence over those in Category A. For example, someone previously treated for oral or persistent vaginal candidiasis (and who has not developed a Category C disease) but who is now asymptomatic should be classified in clinical Category B.

Category C includes clinical conditions listed in the AIDS surveillance case definition. For classification purposes, once a Category C condition has occurred, the person remains in Category C.

EXPANSION OF THE CDC SURVEILLANCE CASE DEFINITION FOR AIDS: In 1991, CDC, in collaboration with the Council of State and Territorial Epidemiologists (CSTE), proposed an expansion of the AIDS surveillance case definition. This proposal was made available for public comment in November 1991 and was discussed at an open meeting on September 2, 1992.

Based on information presented and reviewed during the public comment period and at the open meeting, CDC, in collaboration with CSTE, has expanded the AIDS surveillance case definition to include all HIV-infected persons with CD4+ T-lymphocyte counts of less than 200 cells/μL or a CD4+ percentage of less than 14. In addition to retaining the 23 clinical conditions in the previous AIDS surveillance definition, the expanded definition includes pulmonary tuberculosis (TB), recurrent pneumonia, and invasive cervical cancer.

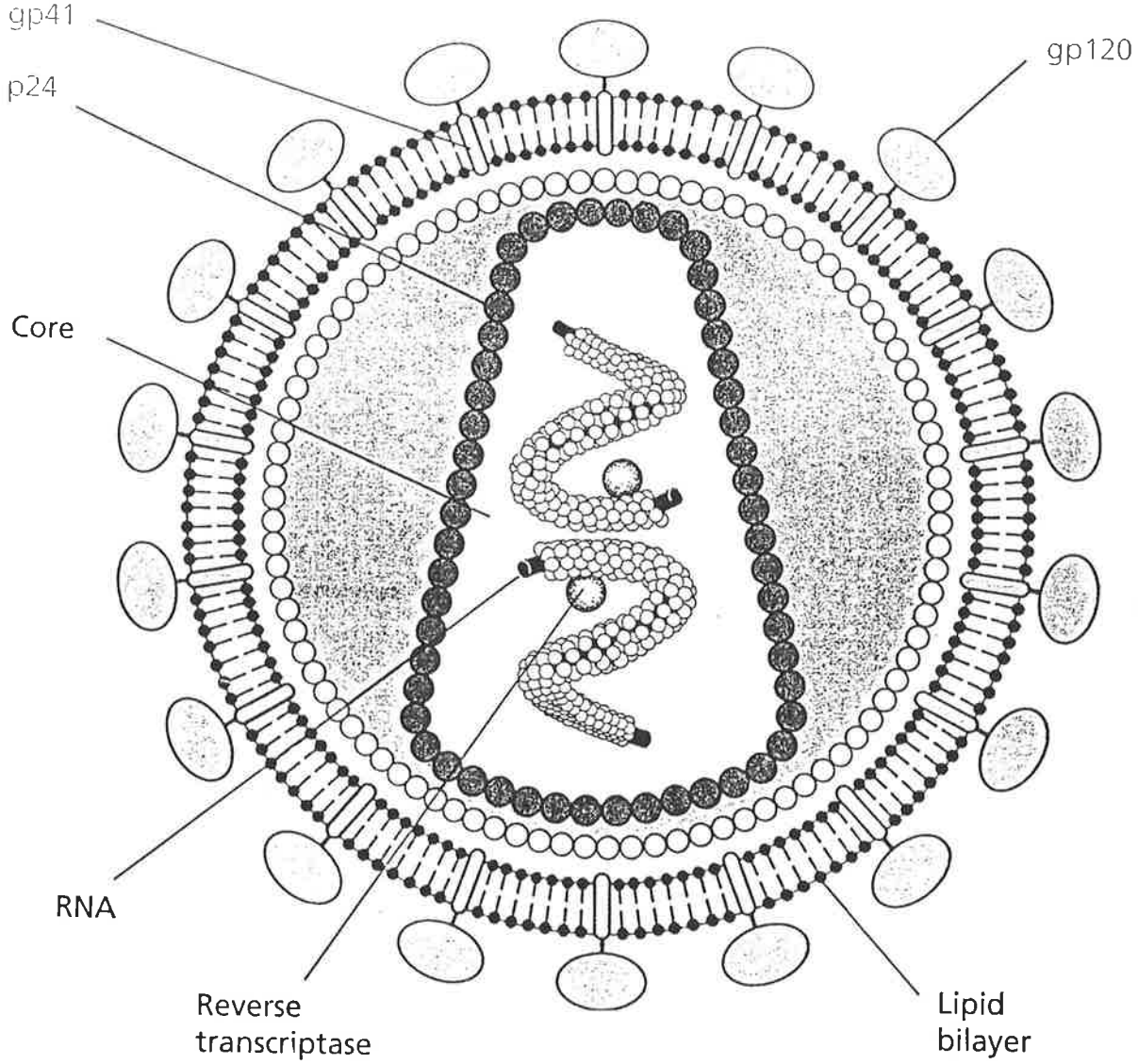
This expanded definition requires laboratory confirmation of HIV infection in persons with a CD4+ T-lymphocyte count of less than 200 cells/μL or with one of the added clinical conditions. This expanded definition for reporting cases to CDC is effective now.

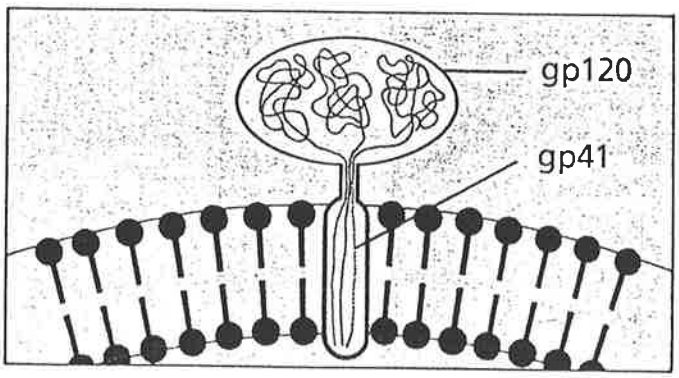
In the revised HIV classification system, persons in subcategories A3, B3, and C3 meet the immunologic criteria of the surveillance case definition, and those persons with conditions in subcategories C1, C2, and C3 meet the clinical criteria for surveillance purposes (Table).

JAMA Editorial Note: Commentary, references, and appendices available.

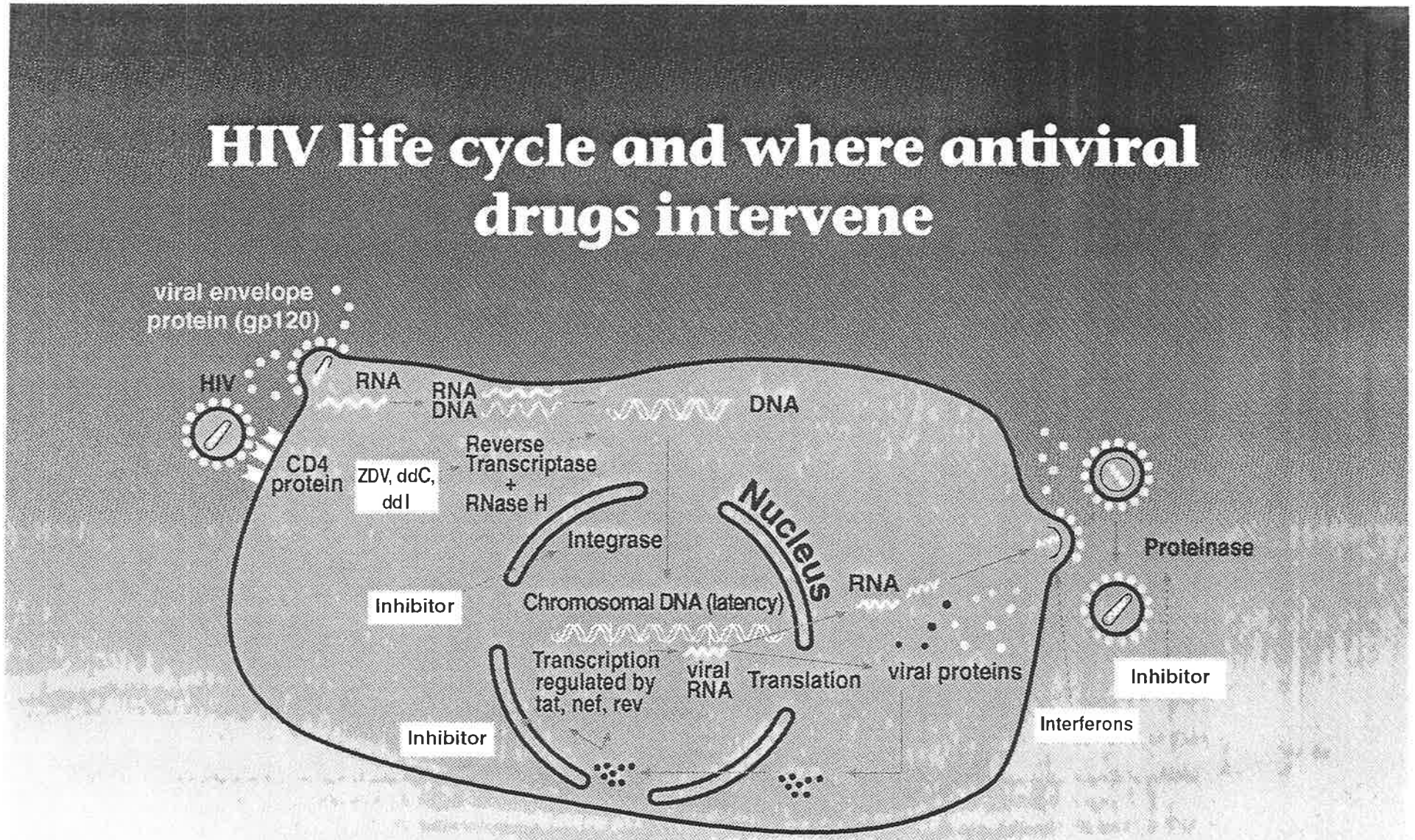
APPENDIX 2

Structure of the human immunodeficiency virus





APPENDIX 3


HIV life cycle and where antiviral drugs intervene
viral envelope protein (gp120)
HIV
RNA
RNA DNA
DNA
CD4 protein
ZDV, ddC, ddI
Reverse Transcriptase + RNase H
Integrase
Nucleus
Inhibitor
Chromosomal DNA (latency)
RNA
Transcription regulated by tat, nef, rev
viral RNA
Translation
viral proteins
Inhibitor
Proteinase
Inhibitor
Interferons

APPENDIX 4

Moyle, G.J. (1995) Resistance to Antiretroviral Compounds: Implications for the Clinical Management of HIV Infection.
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APPENDIX 5

NOTE:

Appendix 5 (questionnaire by Bristol-Myers Squibb Co.) is included in the print copy of the thesis held in the University of Adelaide Library.

6. PART VI:PAPERS BY THE AUTHOR WHICH ARE REFERENCED IN THIS THESIS AND ARE SUBMITTED IN SUPPORT OF CANDITATURE

G. Moyle, C. Harman, S. Mitchell, B. Mathalone, B.G. Gazzard (1992) Foscarnet and Ganciclovir in the treatment of CMV retinitis in AIDS patients: A randomised comparison.
Journal of Infection, v. 25 (1), pp. 21-27, July 1992

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Quarterly Journal of Medicine (QJM), v. 86, pp. 155-163, 1993

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American Journal of Medicine, v. 98 (3), pp. 319–320, March 1995

Easterbrook, P.J., Emami, J., Moyle, G.J. and Gazzard, B.G. (1993) Progressive CD4 cell depletion and death in Zidovudine-treated patients.
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Penile ulceration with foscarnet therapy

Foscarnet is an inhibitor of HIV reverse transcriptase and DNA kinases of a variety of herpesviruses [1]. It is effective in treatment of cytomegalovirus (CMV) retinitis in HIV-positive individuals [2], and maintenance therapy reduces rates of relapse. A recent prospective controlled study [3] reiterated the potential of foscarnet: HIV-seropositive patients with CMV retinitis treated with foscarnet showed improved survival, compared with those given ganciclovir. Side-effects are therefore of considerable importance.

Penile ulcers occurring with foscarnet therapy were described by two groups in 1990 [4,5] and are an important cause of cessation of therapy in some patients. We therefore studied the frequency and natural history of this complication prospectively.

Of 132 patients given a 2- or 3-week course of foscarnet between June 1988 and March 1991, 42 continued on maintenance therapy. All were questioned about penile ulceration on each clinic visit, and specimens were taken from all ulcers for herpesvirus detection and bacterial culture. In addition, syphilis serology was performed on all patients at the time and 3 months later.

Foscarnet was given in two or three divided doses (each at a 2 h infusion with 1 litre normal saline) during the treatment phase (normally 3 weeks), to provide a total dose of 180–200 mg/kg per day. In the maintenance phase, 90–120 mg/kg per day of the drug was administered as a single dose, usually for 5 days per week. Twelve of the 15 patients who developed penile ulceration did so during the initial period of high-dose foscarnet treatment. Three of the 42 patients who received maintenance developed ulceration between weeks 4 and 12 of therapy (Fig. 1).

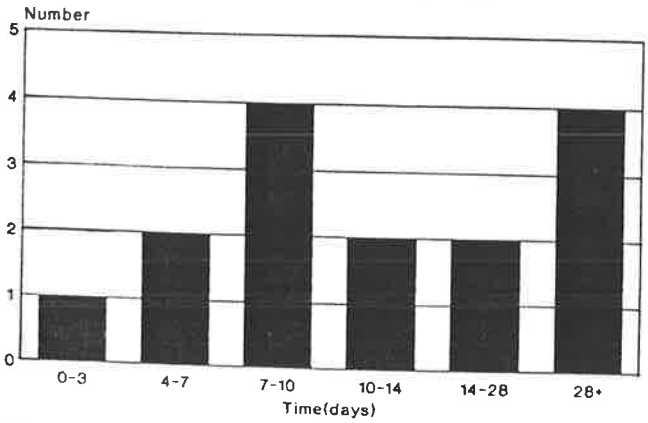

Fig. 1. Time to develop penile ulcers with foscarnet.

A variety of ulcer treatments, including advice on hygiene and saline washing in all patients, thalidomide, acyclovir, hydrocortisone creams and oral antibiotics (three, two, four and two patients, respectively) were used for periods of up to 2 weeks. None produced complete healing, although three patients reported some improvement with retraction of the prepuce and saline washing after each micturition. All ulcers healed within 14 days of stopping foscarnet.

Five of the six patients who were unable to tolerate ganciclovir due to neutropenia developed further ulceration within 7 days of rechallenge with foscarnet. All patients who developed ulceration were uncircumcised, versus 80% of the group as a whole. Two patients had oral ulcers in addition to penile ulcers. No significant differences in age, CD4 cell count, blood urea or blood calcium were observed between those with penile ulcers and those without. No evidence of herpes infection or syphilis was found in any patient. Two patients had positive cultures for *Streptococcus faecalis*, but antibiotic therapy provided only limited benefit, without complete healing.

This study suggests that penile ulcers are the reason for discontinuation of therapy in up to 10% of patients receiving foscarnet, with ulceration developing mostly during the initial 3 weeks of therapy.

The aetiology of the ulceration remains unclear. Histological examination does not suggest that a fixed drug eruption is a likely mechanism [5,6]. Dequalinium has been shown to produce a contact balanitis [7] and a similar mechanism has been suggested for foscarnet [8]. The importance of drug concentration effect is strongly supported by the occurrence of ulceration in uncircumcised patients only. Over 90% of foscarnet is excreted unchanged in the urine and it has been shown that foscarnet-containing creams induce penile ulceration in a 3% mixture [9], but not in a 0.3–1% mixture [10]. Renal excretion of foscarnet is prompt and levels in urine fall rapidly following daily maintenance therapy, which may explain previous reports of resolution of ulcers during this phase [6]. A direct irritant balanitis is less likely as an explanation if the oral ulcers described previously and in two of our patients are genuinely linked to penile ulceration. Certainly, their appearance and disappearance coincided with the genital ulceration. It is not known whether foscarnet is excreted into the saliva.

For the present, all uncircumcised patients taking foscarnet should be advised to retract their prepuce and wash thoroughly following each micturition.

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Zidovudine half-life in haemodialysis patients

Pachon *et al.* [1] recently presented a study of zidovudine pharmacokinetics in a patient with terminal renal insufficiency in *AIDS*. They concluded that serum half-life may be prolonged in patients with chronic renal failure but that treatment with 3 × 100 mg was safe.

Here we describe three patients with end-stage renal failure in whom zidovudine serum levels (all three cases) and dialysate concentrations (patients 1 and 2) were determined between and during haemodialysis sessions. This was performed before initiation of zidovudine treatment in order to assess the safety of treatment. In the first series, serum concentrations were measured at 30-min intervals after oral administration of 200 mg zidovudine between haemodialysis sessions. In a second series (patients 1 and 2), sequential serum and dialysate specimens were drawn during haemodialysis 90 min after oral administration of 100 and 200 mg of zidovudine (every 15 min for the first hour, and then every 30 min). Serum samples (1 ml) were incubated in a high-pressure liquid chromatography (HPLC) assay at 57°C for 30 min. An internal standard solution (A22) was added to 1 ml serum. After 15 min at room temperature, a C18 extraction column was loaded and washed with 2 × 1 ml of methanol water (30 : 75). After centrifugation for 5 min at 3000 **g**, 200 µl were loaded onto a C18 HPLC column (Knauer, Berlin, Germany). Mobile phases were acetonitril (15), H_2O (85), and perchloric acid (0.5); ammonium was 0.3, pH 2.5, and flow rate 0.5 ml/min. Absorption was measured at 278 nm.

The patients (two men and one woman) were aged 42 (patient 1), 50 (patient 2) and 59 years (patient 3). Two were transfusion recipients and one was bisexual. Patient 1 had chronic glomerulonephritis, patient 2 immunoglobulin A nephritis and patient 3 renal failure due to recurrent pyelonephritis. Their CD4+ cell counts were 0.31, 0.15 and 0.11 × 10^6/l, respectively. On enzyme-linked immunosorbent assay, patient 2 was p24-antigen-positive (> 200 pg/ml).

Serum levels reached a maximum after 120, 60 and 30 min (Fig. 1), with peak concentrations of 1.67, 2.8 and 3.2 µmol/l, and initial serum half-lives of approximately 60, 60 and 45 min, for patients 1, 2 and 3, respectively (Fig. 1). These results were not significantly altered by haemodialysis (60 min for patients 1 and 2). Concentrations in dialysate specimens approached 20–33% of the serum values. Zidovudine clearance during haemodialysis was 150 ml/min in patient 2.

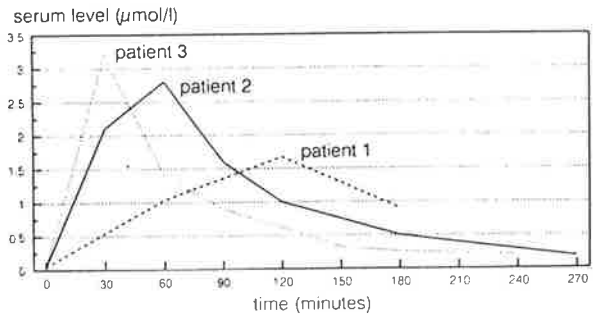

Fig. 1. Zidovudine levels after oral administration of 200 mg in patients with end-stage renal failure.

Our findings support Pachon *et al.*'s conclusion that zidovudine therapy appears safe in this setting. Nevertheless, we did not observe an increased half-life between haemodialysis sessions and thus no normalization on haemodialysis. This supports the hypothesis that the 14–19% of zidovudine excreted renally do not significantly influence its half life in patients with chronic renal failure.

G.J. Moyle, D.A. Hawkins and B.G. Gazzard (1991) Seminoma and HIV infections.
International Journal of STD & AIDS, v. 2 (4), pp. 293-294, July/August 1991