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Antiviral Therapy in Human Immunodeficiency Virus Infection

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Contents

Summary

A rapid expansion of our knowledge of drugs that intervene with human immunodeficiency virus (HIV) infection has taken place. This review covers known and potential anti-HIV targets, including receptor blocking agents, membrane stabilisers, reverse transcriptase inhibitors and chain terminators, RNases, agents altering activation, assembly, budding or regulation of transcription and translation, post-transcriptional modifications and other areas. Important or promising agents, such as zidovudine (ZVD; azidothymidine, AZT), dideoxycytidine, dideoxyinosine, foscarnet, interferons, imuthiol, isoprinosine and others that are either on the market or in advanced clinical trials are emphasised.

Four years after the discovery of the aetiological agent, the first drug, zidovudine, has been registered. Many questions about this drug remain, however, owing to the haste with

which it was developed. An unprecedented number of other compounds are under evaluation, making it difficult to assess the relative merits of the different compounds and thus set priorities for their development. The point has been reached where a better economical and intellectual framework is necessary so that researchers and physicians are not overwhelmed by the difficulties of conducting clinical trials during the epidemic and have a reasonable chance of keeping up with laboratory developments.

The therapeutic advances against the human immunodeficiency virus (HIV) have been unprecedented compared with those against other viral diseases. Following the discovery of the virus in 1983 and breakthroughs in laboratory studies in 1984 (Barre-Sinoussi et al. 1983; Levy et al. 1984; Popovic et al. 1984) the first reports of a putative therapy were published in 1984 (Mitsuya et al. 1984), based on analogies with other retroviral systems.

At the time of the previous review of this subject, drugs had been directed at 7 different targets of the HIV replicative cycle and 6 other targets were proposed (Sandström & Kaplan 1987). This represented 12 drugs, 8 of which had been administered to humans.

Taking the Fourth Conference on the Acquired Immunodeficiency Syndrome (AIDS) held in Stockholm in 1988 as a starting point, some current trends can be discerned. Of the approximately 300 abstracts that focused on anti-HIV therapy, 130 dealt with various aspects of the use of zidovudine (ZVD; azidothymidine, AZT). In addition, 70 other nucleoside analogues and 58 compounds or treatment modalities were described in 53 clinical studies and 90 laboratory or animal studies directed at more than 20 targets in the HIV replicative cycle. This mass of information on anti-HIV compounds is staggering considering the paucity of drugs against other viruses.

The rapid increase in knowledge is also illustrated by the finding, made between the submission and revision of this article, that isoprinosine could prevent AIDS-defining conditions in HIV-infected people (see below).

Due to the large number of references to studies presented at the 1988 Stockholm Conference, these are referred to as 'Abstr. . . .' below.

A number of good reviews have been published and may be consulted for further details (Hirsch 1988; Mitsuya & Broder 1987b; Öberg 1988; Tartaglione & Collier 1987). An extremely valuable resource is the publication by the American Foundation for AIDS Research (AmFAR), the *AIDS/HIV Experimental Treatment Directory* (Abrams et al. 1988).

1. Possible Targets in HIV Replication

A number of molecular events in the retrovirus replicative cycle are potential targets for selective inhibitors that would cause minimal toxicity for the host cell (Lowy 1985; Mitsuya & Broder 1987b; Öberg 1988) [see table I].

Early steps in HIV infection include adsorption to a specific cellular receptor on the CD4 molecule (Dalgleish et al. 1984), penetration and uncoating in the cytoplasm. HIV envelope protein gp120 seems to be the viral protein involved in this interaction with CD4, probably bringing the envelope protein gp41 into contact with the cell membrane, a step which is necessary for fusion. The latter activity may occur in the absence of CD4.

An important site for antiviral inhibitors is the HIV-specific enzyme reverse transcriptase, which transcribes the viral genomic RNA into proviral DNA through a complex series of events. These include duplication of the long terminal repeats (LTR), circularisation of the proviral strands and digestion of the viral RNA strand by the viral RNase H. The double-stranded DNA copy, or provirus, is subsequently integrated into host chromosomal DNA, probably through a virally determined function, or it may remain as a cytoplasmic plasmid.

HIV replication is regulated by unique types of genetic control involving at least 3 proteins in-intertwined positive and negative feedback loops

Table I. Targets for anti-HIV activity

Steps in viral replication	Intervention (stage of development[a])
Adsorption to CD4+ molecule	Receptor blocking: CD4 (clinical); sulphated polysaccharides (clinical); peptide T (clinical); antibodies, oligopeptides. Cytotoxic: CD4-toxin (laboratory); antibodies-toxin (laboratory)
Entering cell	Membrane stabiliser: AL-721 (clinical)
Uncoating	?
Reverse transcriptase	Inhibitors: suramin (clinical); HPA-23 (clinical); foscarnet (clinical); rifabutin (laboratory?) Chain terminators: zidovudine (registered drug); other dideoxynucleosides, e.g. ddA, ddI (clinical); fluorothymidine (laboratory)
RNase H	Cleavage of atoxic prodrugs (hypothetical)
RNase L	Ampligen (clinical)
Integration	?
Activation	Cyclosporin A (clinical)
Transcription	*art* inhibitor (hypothetical); oligonucleotides (laboratory)
Translation	Ribavirin (clinical); *tat* inhibitor (hypothetical); penicillamine (clinical)
Post-translational modification	Inhibition of virus-specific protease: avarol (laboratory) Cleavage of atoxic prodrugs (hypothetical)
Glycosylation	Castanospermine (laboratory); deoxynoijmycin (laboratory)
Protein kinase C	Xanthate (laboratory); glycyrrhizin (clinical)
Assembly	Interferons, suramin?
Budding	Hypericine (laboratory)
Cell-bound viral antigens	Toxic CD4 or monoclonal antibodies?
Syncytia formation	CD4+ blocking agents

a See section 2 for description of developmental stages.
Abbreviations: ddA = dideoxyadenosine; ddI = dideoxyinosine.

influencing LTR activation, splicing or mRNA processing (Haseltine 1988).

Proviral DNA is expressed by transcription of the integrated proviral DNA sequences into viral messenger RNA, through splicing mechanisms that are vulnerable to manipulation. The transcribed proteins are modified during post-transcriptional processing, prior to translation into virus-specific proteins. The virus-encoded protease cleaves the gag-pol polyprotein, while the env protein is probably cleaved by cellular enzymes. During maturation, viral envelope proteins are glycosylated and inserted into the plasma membrane. Two copies of single-stranded genomic viral RNA are condensed and assembled with viral core proteins and reverse transcriptase before virus particles are released by budding off the cell membrane. In this process virus-specific proteins are exposed on the surface of the infected cell and gp120 is probably shed by both cells and virus particles. The presence of gp120 on the surface of the infected cell induces fusion (syncytia formation) with uninfected cells bearing the CD4 receptor.

1.1 Drug Classification

Drugs or other modes of intervention can conveniently be classified into 5 groups (table II). Group I includes modalities that are intended to protect uninfected cells from infection either through direct action on the virus or by interfering with the cell in such a way that infection is hindered. Group II covers drugs that interfere with viral or cell functions essential for steps prior to integration of proviral DNA into the chromosome. Group III comprises those drugs or measures that are intended to interfere with the expression of the provirus and the transmission of infectious virus or genomes. Typically, measures that interfere with the fusion of infected cells to non-infected cells will belong here if the primary action is not on the uninfected cell. Group IV comprises interventions that are intended to kill virus-infected cells, and group V covers measures that interfere with actions of the virus without directly affecting virus spread or

Table II. Classification of antiviral modalities

Group	Properties
I	Activity: (a) on virus particle directly, or (b) on uninfected target cell
II	Activity on viral or host cell functions within the infected cell before integration of provirus
III	Activity after integration
IV	Killing of virus-infected cell
V	Inhibition or restoration of secondary viral effects

replication. Such measures include immunorestorative interventions and inhibition of viral factors that cause cachexia, fever, brain dysfunction etc.

2. *Stages of Drug Development*

With the large number of substances that are now under discussion it is often difficult to remember which are merely promising concepts in the laboratory, which have shown more or less substantial promise in patients, which have undergone well-controlled trials, and which have well-documented effects.

The only drug with such clearly proven clinical efficacy that it has warranted approval for widespread use, zidovudine, is thus presented first in this review. In order to aid the reader a conservative method of classification has been used, based on the stage of development that different approaches have reached. Possible ways of interfering with HIV have been labelled 'hypothetical'; compounds that have shown general anti-retroviral and/or anti-HIV activity *in vitro* or in animal models have been labelled 'laboratory'; those which have undergone clinical trials that are either inconclusive because of lack of clear end-points, too few patients, or effects only on surrogate markers etc. are labelled 'clinical', and substances that have been subjected to regular phase I or II trials are labelled appropriately. It should be noted that lack of clinical efficacy and findings of toxicity in the phase I (dose-finding) trials is not necessarily discrediting.

Clinical efficacy should not be judged from dose-finding trials, but should be addressed later in specifically designed trials. The dose level at which toxicity is seen and the type of toxicity will determine the maximal doses that can be justified. If these levels are well above what is expected to be necessary for efficacy further studies may be performed.

3. *Registered Drug*

3.1 Zidovudine

3.1.1 Chemical Structure, History and Development

The only registered drug among the known anti-HIV compounds is 2′,3′-dideoxy-3′-azidothymidine (AZT), now renamed zidovudine (ZVD) and marketed as 'Retrovir'. Zidovudine was synthesised in 1964 (Horwitz et al. 1964), and found to inhibit some retroviruses (Ostertag et al. 1974) and reverse transcriptases (Furman et al. 1986). In a novel assay system for anti-HIV drugs it was found to inhibit HIV replication (Mitsuya et al. 1985). It is generally assumed that the mechanism of action of the drug is its selective capacity to be incorporated by the retroviral reverse transcriptase, rather than by host cell polymerases, causing premature chain elongation termination. A growing number of observations now indicate that there might be other important targets for this drug (Larder et al. 1989).

The group at the National Cancer Institute (NCI) initiated a phase I trial which found that zidovudine had several promising characteristics: oral bioavailability, non-toxic antiviral serum levels, penetration into the CSF, and indications of clinical benefit (Yarchoan et al. 1986). Burroughs-Wellcome acquired the rights to further drug development and organised a multicentre placebo-controlled double-blind trial in 160 patients who had suffered an episode of *Pneumocystis carinii* pneumonia (PCP) within the previous 4 months, and 122 patients who met the criteria for severe AIDS-related complex (ARC). The trial recruited patients from February to the end of June 1986.

This trial was terminated prematurely after a median of 4.5 (range 3.5 to 7) months of study when an independent data and safety monitoring board

found the difference in mortality in the 2 arms (1 in the active drug group *vs* 16 in the placebo group) to be significantly different and thus deemed further blinding unethical (Fischl et al. 1987). Based on this short term experience zidovudine was made available through an emergency treatment protocol from October 1986 to March 1987, during which time 4810 patients with a previous PCP were treated. It was subsequently registered in many industrialised countries for treatment of patients with AIDS-defining infections and ARC in 1987 (Nightingale 1987). Since then many trials have addressed fundamental clinical and pharmacological questions about the drug; in a less urgent situation these would have been documented before registration. For combinations with other drugs, see below. A comprehensive review of zidovudine has recently been published in this journal (Langtry & Campoli-Richards 1989).

3.1.2 Clinical Pharmacological Properties

The plasma half-life of zidovudine is approximately 1.1 hours, and total body clearance 190 ml/min (Blum et al. 1988; Klecker et al. 1987). Based on the plasma half-life, 4-hourly administration was selected for the first clinical trial and carried over to the recommended dosage schedules on registration of the drug. However, the more relevant measures of the pharmacokinetics of the intracellular pools of phosphorylated zidovudine have only been studied superficially (Bhalla et al. 1989; Furman et al. 1986), and several clinical trials have moved into 6- or even 12-hourly schedules (Abstr. 3606). A daily pulse has been shown to be effective *in vitro* (Abstr. 3622).

After oral administration there is about 70% bioavailability due to first-pass metabolism. The 5′-glucuronide conjugate is the major inactive plasma and urinary metabolite; this is rapidly cleared through the kidneys, with a half-life of 30 minutes. The protein binding is around 35% at clinically relevant plasma concentrations.

Peak plasma levels were 5 μmol/L 1 hour after an oral dose of 5 mg/kg. A concentration of 5 μmol/L zidovudine was found to be inhibitory in the T-cell-derived cell culture system *in vitro*. The dosage used in the clinical trial of zidovudine was 250mg every 4 hours, but for some reason this was altered to 200mg 4-hourly at registration.

The distribution of zidovudine in different body compartments may vary, but 2 localisations are of special interest: the brain and the ano-genital tissues. The initial study indicated that 53% (range 20 to 73%) of the plasma level was attained in the CSF. Later results have found this ratio to be highly variable (9 to 129%) and unrelated to recovery of HIV from the CSF (Abstr. 3653). Conflicting with the evidence of relatively high concentrations of zidovudine in the CSF are findings that indicate that zidovudine does not cross the blood-brain barrier. The CSF concentration is therefore thought to reflect transport through the choroid plexus directly into the CSF (Terasaki & Pardridge 1988). Early studies have indicated a surprisingly rapid (2 weeks) effect on what were thought to be HIV-related CNS symptoms (Yarchoan et al. 1988a), while an effect on opportunistic infections was first demonstrated after 6 to 8 weeks (Fischl et al. 1987).

Although not stated, it is implicit that while a patient is on zidovudine therapy the virus is suppressed and therefore infectivity is diminished. As long as therapy was confined to the very ill this was of little concern. With the drift of usage to less and less symptomatic HIV-infected people, however, it is important to clarify whether there is indeed an effect on infectivity, especially by the sexual route. Recent results indicate that the area under the zidovudine concentration/time curve in semen is 3 times that found in serum (Abstr. 3657; Henry et al. 1988). This might then reduce the number of HIV-infected cells in semen. If HIV is transmitted mainly through infected cells this might be of importance, while it is unlikely to affect infection mediated by viral particles.

With the realisation that other cells than T-helper lymphocytes are infected and that the pools of phosphorylated zidovudine can vary considerably between different types of cells, the minimal effective dose is of concern. In 1 study, primary human monocyte-derived macrophages were not protected by zidovudine 100 μmol/L, probably due to inadequate phosphorylation (Richman et al.

1987b; Abstr. 3132). Conflicting results using more macrophagotropic HIV strains and freshly isolated monocytes have indicated that zidovudine is effective in these cells (Perno et al. 1988; Skinner et al. 1988; Abstr. 3621).

An oncological view of this antiviral substance has directed its use. When adverse effects appear clinicians usually elect to make dose reductions, in order to administer at least some level of drug. This is counter to the practice with antibiotics, where a safe margin over the estimated minimum inhibitory concentration (MIC) is recommended and suboptimal doses are avoided in order not to select resistant variants of the organism. A 2-fold difference in sensitivity to zidovudine between isolates has been observed, and selection for reduced sensitivity to foscarnet has resulted in concomitant reduction of HIV reverse transcriptase sensitivity to zidovudine triphosphate (Abstr. 3655). In 1 study isolates taken from 3 patients late in zidovudine treatment were less sensitive than early isolates (Abstr. 3656). Recent studies have documented the emergence of strains with reduced sensitivity to zidovudine after about 6 months of therapy. The resistance is not correlated to cross-resistance with, for example, dideoxycytidine or foscarnet, nor reflected in an altered effect on isolated reverse transcriptase activity. In these preliminary studies a correlation to the dose of zidovudine, clinical outcome, or level of HIV-antigen has not been observed. The clinical significance of these observations remains to be determined, but they raise concern over administration of zidovudine to asymptomatic HIV-infected patients over long periods of time (Larder et al. 1989).

In clinical practice toxicity has not infrequently led to dose reductions to 400 mg/day, and at least 1 dose-finding trial is evaluating this dose level in single agent treatment. To further complicate clinical management there have been reports of clinical and virological rebound effects on withdrawal of the drug (de Wolf et al. 1988; Helbert et al. 1988b). A toxicity management study is currently under way, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) [Abstr. 3608]. Qualitative routine cell cultures of peripheral blood have been of limited use in the evaluation of the drug response since they might mainly measure cells infected with resting viral genomes that are not expected to be influenced by zidovudine (Jackson et al. 1988b). However, with semiquantitative standardised culture techniques a good correlation with serum HIV-antigenaemia can be obtained (Parks et al. 1988). In an analysis of a subgroup of patients from the phase II study, serum HIV-antigen levels were reduced in patients treated with zidovudine compared with those who received placebo (Chaisson et al. 1988). In patients who discontinued zidovudine, antigen levels rapidly rose to within 83% of pretreatment levels, within 3 weeks (Spear et al. 1988a). Dose reduction to 100mg every 4 hours has also been associated with increases of HIV-antigen (Jackson et al. 1988b).

In a recent trial it appeared that 6 patients who received zidovudine 250mg every 6 hours and later switched to 500mg every 12 hours achieved and sustained depressed levels of HIV antigen (de Wolf et al. 1988). The same group of researchers has documented 2 patients with a slow return of HIV antigen after week 16 while treated with zidovudine 200mg 4-hourly (Reiss et al. 1988). In addition it was found during the assessment of HIV DNA and RNA in blood by the polymerase chain reaction (PCR), that all of the 4 patients on zidovudine treatment had intermediate to low levels of HIV RNA as a sign of HIV replication (Hart et al. 1988).

Patients without HIV antigen or those who had no substantial fall in HIV antigen had a higher rate of mortality (28.3%) than those with a substantial fall in HIV antigen (3.7%) [Abstr. 3596]. However, current methodologies measure free antigen and are thus influenced by the level of anti-p24 antibodies. HIV antigen disappeared in the CSF of patients treated with zidovudine 250mg 6-hourly (Abstr. 3144).

3.1.3 Therapeutic Trials

In the phase II study, 19/137 placebo recipients *versus* 1/145 zidovudine recipients had died when the study was terminated. The probability of de-

veloping an opportunistic infection was significantly higher (43%) among placebo recipients than in the zidovudine group (23%). Zidovudine recipients did not decline in Karnofsky score (controls −5%), gained weight (3kg), and regained reactions to skin test antigens (29%).

After the termination of the double-blind study 229 patients entered an open-label study of 200mg 4-hourly. The 9-month mortality rate for recipients of continuous zidovudine was 8%, while it was 46% for placebo recipients who never converted to zidovudine treatment. After 1 year the mortality in the original 145 patients who received zidovudine was 15% (AIDS 21% and ARC 7%). Among the placebo recipients, most of whom were switched to zidovudine at week 17 of the trial, the mortality after 1 year was 23% from the start of the trial. In zidovudine recipients (n=69) the mortality was 31% after 72 weeks or more (Richman et al. 1988). There was no indication that zidovudine influenced the spectrum of opportunistic infections that occurred during treatment.

A select patient population with poor prognosis seemed to have received the drug under the emergency treatment protocol. Overall, 15% died before week 40 of treatment; however, one-third of these had died during the first 6 weeks.

Recently a large open study of zidovudine in 80 ARC and 285 AIDS patients has been published (200mg 6 times daily in 260 patients or a reduced dose due to initial anaemia and/or leucopenia of 200mg 3 times daily in 105 patients) [Dournon et al. 1988]. The short term beneficial effects corroborate the phase II (Fischl et al. 1987) trial, but the results in patients followed longer than 6 months were disappointing to the authors. 18% of the 80 ARC patients had progressed to AIDS after a mean follow-up of 24.6 weeks, while 37.9% of the 285 AIDS patients had had an opportunistic infection (OI) after a mean follow-up of 33.0 weeks. Cytomegalovirus (CMV) infection was the only clinical prognostic factor associated with death. The CD4+ cell count increased over the first 4 months of treatment, after which there was a progressive reduction. There was a similar fall in HIV antigen in those with p24 antigen levels of less than 200 U/ml on full- or half-dose regimens. Considering the toxicity of zidovudine, the authors suggest that 600 mg/day should be evaluated.

A number of studies have increased our knowledge of the possible advantages of zidovudine. HIV-related thrombocytopenia might in some patients be reversed by zidovudine treatment (Gottlieb et al. 1987b; Hymes et al. 1988; Swiss Group for Clinical Studies on the Acquired Immunodeficiency Syndrome 1988), and preliminary studies show promising results with the use of the drug in neurological disease (Yarchoan et al. 1987, 1988a). An analysis of the patients who took part in the phase II study of Fischl et al. (1987) indicated that the zidovudine-treated patients with AIDS improved in attention, memory and visual motor skills over the first 8 to 16 weeks of the trial. Further analysis was not possible due to the premature termination of the trial (Schmitt et al. 1988). Severe HIV-related psoriasis has been seen to clear during zidovudine treatment in several case reports (Duvic et al. 1987; Feeney & Frazer 1988; Kaplan et al. 1989; Ruzicka et al. 1987), and there also seems to be some benefit in cryptosporidiosis (Abstr. 3672).

It has been a general impression that Kaposi's sarcoma (KS) does not respond to zidovudine treatment. Recently, however, 2 groups have reported stabilisation or regression in about one-third of such patients (Abstr. 3638, 3639).

Recent reports indicate that zidovudine is beneficial in children, especially those with encephalopathy (Pizzo et al. 1988; Abstr. 3145, 3146, 3147).

In an effort to prevent the progression of HIV disease before symptoms and/or complications occur, early administration is being tried in several studies. Preliminary data indicated immunological improvements over a 6-week period, although it is too early to evaluate efficacy (Abstr. 3153). There seems to be a reduction in short term hospitalisation (Abstr. 3673).

3.1.4 Adverse Effects

The preclinical toxicology of zidovudine has recently been summarised by Ayers (1988). Most patients develop nausea and headache over the first

couple of weeks of treatment with zidovudine, but these effects are usually transient. Some develop insomnia, myalgia, fever, asthenia, diarrhoea and abdominal pain. Although the symptoms are common they rarely cause withdrawal of the drug (Fischl et al. 1987).

Anaemia was the most common toxic effect in the phase II study (Richman et al. 1987a), but was reversible when the dose of zidovudine was reduced or discontinued. At least 1 blood transfusion was given to 31% of the patients on zidovudine compared with 11% on placebo. Increases in mean corpuscular volume (MCV) unrelated to B12 or folate deficiency were regularly seen. The anaemia may be evident by the end of the first month. In the follow-up study it was reported that a fairly constant proportion (around 10%) of those on zidovudine experienced anaemia (< 7.5 g/dl) during each 4-week period, however no information is available on the cumulative number of patients who experienced this effect (Richman et al. 1988).

In a study where 6/15 patients developed anaemia (< 100 g/L), the first sign was a fall in reticulocytes. Anaemic patients who showed no rise in MCV seemed to be more prone to rapid development of bone marrow failure. Erythropoietin levels at the time of first transfusion were increased (Walker et al. 1988; Abstr. 3658, 3665).

Neutropenia (500 cells/mm^3) developed in 16% of those on zidovudine and 2% of those on placebo in the phase II trial (Richman et al. 1987a). It has been the clinical impression that zidovudine-induced neutropenia is less often associated with infectious complications (Richman et al. 1987a). Neutropenia was the most common reason (29%) for dose reduction or discontinuation in the follow-up trial, followed by anaemia (23%) [Richman et al. 1988].

Overall, 45% of the zidovudine recipients developed grade 3 bone marrow depression (haemoglobin < 7.5 g/dl, neutrophils < 750/dl or white cells < 1500/dL). Three patients developed hypoplastic marrows. Two patients partially recovered 5 and 6 months after discontinuation of zidovudine, while 1 had not recovered after 9 months. Low initial haemoglobin (Hb), neutrophil or CD4 cell counts, or advanced clinical disease, predisposed toward bone marrow depression (Richman et al. 1987a). Other instances of severe bone marrow suppression have been reported (Forester 1987; Gill et al. 1987; Mir & Costello 1988). In the follow-up study approximately 50% of patients were on the full dose (200mg 6 times daily), 30% were receiving reduced doses, and 20% had temporarily discontinued zidovudine treatment (Richman et al. 1988). It has been proposed that granulocyte-macrophage colony stimulating factor (GM-CSF), uridine or lithium might be used to counteract zidovudine toxicity (Groopman et al. 1987; Roberts et al. 1988; Sommadossi et al. 1988).

Although no new adverse effects were found in the follow-up study (Richman et al. 1988) there are now several reports indicating that severe polymyositis may develop after extended medication with zidovudine. In 1 study this affected 8/113 patients after 240 to 386 days. Not all patients have reported pain. Proximal muscles such as those in the upper legs and shoulders seem to be most affected. Enzyme levels such as lactose dehydrogenase, asparate aminotransferase, and creatinine phosphokinase are abnormal; the latter enzyme in particular is reported to respond quickly to withdrawal of zidovudine (Besen et al. 1988; Gorard et al. 1988; Helbert et al. 1988a; Abstr. 3598).

Hepatotoxicity seems to be rare during zidovudine treatment; only 3 suspected cases have been reported (Melamed et al. 1988). However, 1 patient who inadvertently took zidovudine 500mg every 5 hours for 16 days had a rise in liver enzymes, which normalised after 2 weeks following dosage reduction to 250mg every 5 hours (Staszewski et al. 1989). Four patients have allegedly taken overdoses of up to 20,000mg of zidovudine without haematological toxicity. Neurological symptoms such as drowsiness, nystagmus and ataxia might have been due to concomitant ingestion of neurotropic drugs. One patient ingesting zidovudine 36,000mg experienced 3 seizures, but had no further consequences (Hargreaves et al. 1988; Pickus 1988; Routy et al. 1988; Spear et al. 1988b). Nail pigmentation has been reported, affecting both hands and feet (Furth & Kazakis 1987; Abstr. 3604).

Although a number of drugs could possibly interact with zidovudine due to suspected interaction with the glucuronidation in the liver (de Simone et al. 1988) and are consequently administered with care, the documentation on drug interactions is still incomplete. In the original study only paracetamol (acetaminophen) could be associated with increased marrow suppression (Richman et al. 1987a; Abstr. 3661).

In view of the growing interest in the combination of zidovudine and acyclovir, it should be noted that 1 case of severe lethargy associated with simultaneous administration of these 2 drugs has been reported (Bach 1987). In a trial of 8 patients given 100mg zidovudine and 800mg acyclovir every 4 hours for 10 weeks, no toxicity in addition to that expected from zidovudine alone was observed (Surbonne et al. 1988). Acyclovir does not appear to interfere with zidovudine plasma levels or glucuronidation (Surbonne et al. 1988). Ganciclovir (5 mg/kg/day) or interferon (> 9 million units) do not seem to be tolerated with full-dose zidovudine (200mg 4-hourly) due to haematological toxicity (Abstr. 3666).

Additional toxicity does not seem to occur with maintenance therapy for cerebral toxoplasmosis (pyrimethamine + sulphadiazine or clindamycin); rather, a significant decrease in mortality occurs with this combination (Abstr. 3667). Zidovudine does not appear to influence coagulation in treated haemophiliacs (Abstr. 3659).

4. Compounds with Potential Clinical Value

4.1 Receptor Blockade

Blockade can be achieved by interfering with either the CD4 molecule or the viral gp120. Thus, intact or specific parts of CD4 could block gp120, or gp120 could, theoretically, block CD4. In addition, specific antibodies raised against these molecules or their respective antibodies (anti-idiotypic antibodies) could be used for these purposes. All these avenues are being actively explored.

During natural infection gp120 is probably shed from both virus and virus-infected cells, although not in high enough quantities to be detected as free antigen, probably due to complex formation with serum antibodies. Furthermore, it can be assumed that cells exposing the CD4 molecule adsorb circulating gp120. This is worrying since it has recently been noted that cells marked with gp120 or certain fragments of the gp120 molecule might be detected by the immune surveillance system as foreign and subsequently be destroyed by specific killer cells. This especially affects cells that are immunologically mobilised for any reason (Germain 1988; Siciliano et al. 1988). This observation restricts the development of gp120 analogues for blocking virus target cells and their potential as vaccine candidates. However, if the mechanism proves to be clinically important, blocking or destruction of the anti-gp120 cytotoxic T-cell might paradoxically be a novel therapeutic approach.

Preliminary work in defining monoclonal anti-idiotypic antibodies, i.e. antibodies against the anti-CD4 antibodies that interfere with the gp120-CD4 interaction, is under way (Abstr. 3061).

4.1.1 Soluble CD4 (Phase I)

With the rapid advances in biotechnology it has been possible to produce peptides from CD4 that are soluble and retain anti-gp120 activity, either as peptides or as recombinant molecules (rsCD4, rsT4). Most interest has focused on the ability of soluble CD4 to block virus adsorption and possibly adsorb circulating gp120 (Deen et al. 1988; Fisher et al. 1988; Hussey et al. 1988; Smith et al. 1987; Traunecker et al. 1988). The gp120 binding site on CD4 has been defined (Jamesson et al. 1988) and synthetic peptides produced (Lifson et al. 1988). Addition of higher concentrations of soluble CD4 as late as 2 hours after infection will inhibit HIV-1 in the syncytial focus assay. There is less inhibition of HIV-2 isolates than HIV-1. U138 astroglioma and RD rhabdomuosarcoma cell lines were not protected by soluble CD4 (Clapham et al. 1989).

The serum half-life is reported as 16 minutes and 6 hours after intravenous or intramuscular injection, respectively, in rhesus monkeys (Lucas et al. 1989; Watanabe et al. 1989). In the latter study virus became more difficult to isolate, and hae-

matological indices tended to be normalised during a 50-day trial of daily 2mg intramuscular doses. Soluble CD4 does not seem to block normal interactions between CD4 and major histocompatibility complex (MHC) class II-bearing cells *in vitro* (Liu & Liu 1988).

In order to facilitate production of properly glycosylated CD4 and prolong the plasma half-life, a hybrid protein with the CD4 gp120 binding site and the Fc immunoglobulin chain has been constructed and expressed in myeloma cell lines. The half-life does indeed seem to be considerably increased, but it remains to be seen what functional properties are added.

The specific blocking of gp120 binding to the CD4 molecule through selected CD4 peptides or soluble CD4 was investigated in 12 papers presented at the Stockholm conference. Phase I studies have been initiated in Boston and San Francisco.

4.1.2 Sulphated Polysaccharides (Phase I/II)

Heparin and dextran sulphate (Mr5000) have been found to inhibit both the *in vitro* formation of syncytia and the activity of reverse transcriptase (Ito et al. 1987). Of this group of compounds (pentosan polyphosphate, carrageenans, fucocidan, ionositol hexasulphate, glucosamine disulphate, chondroitin sulphate), most information is so far available on dextran sulphate. This agent was found to inhibit virus adsorption at 25 μg/ml, to be atoxic at 625 μg/ml, and not interfere with the coagulation process (Baba et al. 1988; Mitsuya et al. 1988; Ueno & Kuno 1987). Data from a phase I trial in San Francisco have been reported (Abrams et al. 1989; Abstr. 3580). In that study 30 patients were given dextran sulphate 900 to 5400mg orally, with 900mg increments. 12 dose reductions occurred during the 8-week trial. Four patients, 1 at 900, 1 at 2700 and 2 at 3600mg, discontinued because of toxicity (diarrhoea requiring therapy, white cells either $< 1.5 \times 10^9$/L or repeatedly below 2.9×10^9/L). Leucopenia of moderate to severe degree was seen in 6 patients. Transaminase elevations (> 2.6 times normal) were seen in 4. One patient had a drop in thrombocytes below 50,000/mm^3. No coagulation abnormalities were noted. Other complaints were hyperexcitability, insomnia, bloating, diarrhoea, and urgency of defecation, but the authors concluded that in general the drug was well tolerated. Recently, concern over the oral absorption and rapid breakdown in plasma has hampered developments of dextran sulphate in HIV therapy.

These studies have been extended to xylanpoly (hydrogen sulphate) disodium salt (HOE/BAY 946), which was reported to inhibit HIV completely at 25 μg/ml in a syncytia assay, and was 4 times more efficient than dextran sulphate 5000 in that study. At 0.04 μg/ml, 50% inhibition of reverse transcriptase activity was seen *in vitro*. Cell toxicity occurred at 30 to 100 μg/ml, ranging up to 400 μg/ml in different systems. Interleukin-1 was induced in a murine macrophage cell line by HOE/BAY 946 to a degree similar to that induced by *Salmonella typhimurium* lipopolysaccharide. Some intraperitoneally injected Friend leukaemia virus-infected mice died of the anticoagulant effect, while oral administration (at approximately 40 mg/mouse) had to be started 4 days before treatment to affect spleen weight (Biesert et al. 1988). Based on observations of a 60-minute plasma half-life after intravenous injection, a 4 to 40% absorption after oral administration and a lower anticoagulant effect than heparin, 2 pilot studies have been initiated (Wagner 1989).

4.1.3 Peptide T (Clinical)

Peptide T is homologous with the vasoactive intestinal peptide (VIP) and is claimed to compete with the binding of gp120 to CD4 (Perth et al. 1986; Ruff et al. 1987), although these *in vitro* findings have been disputed (Sodrosky et al. 1987). Peptide T administered intravenously in doses of 0.033 to 0.8 mg/kg/day in various divided doses seems to be well tolerated in limited trials (4 to 12 patients for 4 weeks) but beneficial effects have so far not been documented (Wetterberg et al. 1987; Abstr. 3102, 3103, 3104, 3105).

4.1.4 Antibodies (Laboratory)

There has been considerable interest in the development of anti-CD4 anti-idiotype antibodies which would interfere with the CD4-gp120 inter-

action or deliver toxic substances. However, none of the monoclonal anti-CD4 antibodies seem to be ideal for this purpose, although some activity can be obtained with, for instance, Leu3a (Abstr. 3061).

4.1.5 Lectin (Laboratory)

The D-mannose-specific lectin from *Gerardia savaglia* effectively blocks HIV infection of H9 cells in a 4-day assay, and inhibits syncytia formation if present before infection. In addition, it interferes with anti-gp120 antibody reactivity with gp120 in western blots, which emphasises the role of gp120 glycosylation (see section 4.15) [Muller et al. 1988a].

4.2 Membrane Stabiliser

4.2.1 AL-721 (Clinical)

The name AL-721 alludes to a mixture of 'active lipids': neutral glycerides, phosphatidylcholine and phosphatidylethanolamine, in a 7:2:1 ratio. It is thought that AL-721 increases membrane fluidity and surface protein organisation through reduction of the cholesterol content of the membrane (Sarin et al. 1985). Although AL-721 and similar variants have been used extensively as nutritional complements the documentation is scarce. In a recently reported trial 10 patients received 10g twice daily for 8 weeks without any effect on the CD4+ cell counts. In this study and 3 other limited studies (55 patients who received 10 to 30 g/day for 2 to 12 months) reported at the Stockholm conference, some patients had a reduction in p24 antigenaemia during AL-721 administration (Abstr. 3529, 3530, 3531). In a dose-finding study (20 to 50 g/day) gastrointestinal disturbances (diarrhoea, nausea, vomiting and abdominal pain) were noted (Abrams et al. 1988).

4.3 Uncoating

No known compound has been found to act against this target.

4.4 Reverse Transcriptase Inhibitors

4.4.1 HPA-23 (Phase I)

Chemical Structure, History and Development

HPA-23 is a mineral condensed heteropolyanion (HPA) with the formula ammonium 21-tungsto-9-antimoniate (MW 6800) [Fisher et al. 1976]. HPA-23 is a competitive inhibitor of reverse transcriptase activities of murine and human retroviruses (Jasmin et al. 1974). HIV reverse transcriptase activity is completely inhibited by 60 μg/ml of HPA-23 (Dormont et al. 1985), however this drug has little effect on the replication of HIV *in vitro* (Balzarini et al. 1986). These findings have been confirmed in a recent detailed analysis of cell-HPA-23 interactions; the authors propose that the main action is through immunomodulation (Dormont et al. 1988).

Clinical Pharmacological Properties

The half-life of tungsten is claimed to be 2.14 hours and antimony 2.13 hours (Kornhauser et al. 1986).

Therapeutic Trials

Results have been reported of a preliminary trial involving 4 patients with ARC or AIDS selected for positive reverse transcriptase activity before the trial (Rozenbaum et al. 1985). No virus reverse transcriptase activity could be measured in virus cultures during treatment, but such activity could be detected in 2 of 4 patients 30 days after the termination of treatment. There was no significant effect on T-cell subpopulations.

Many patients have now been treated in France under different dosage schedules, but only anecdotal data are available (Laporte et al. 1987). In a phase I dose-finding study in which 69 subjects were divided into 4 groups receiving 0.25 to 2 mg/kg/day intravenously 5 days a week for up to 8 weeks, no improvement in immunological parameters was observed (Moskovitz et al. 1988).

Capitalising on these results 15 patients were treated with 1.5 mg/kg intravenously over a 3-minute period twice daily for 14 days. Transient re-

duction in reverse transcriptase activity in co-cultures was noted for 1 to 6 weeks after the treatment (Vittecoq et al. 1988). Only 1 study (Abstr. 3595) demonstrating that HPA-23 inhibited human bone marrow GM-CFU in a dose-dependent manner was presented at the Stockholm conference.

Adverse Effects

Platelet counts have decreased and hepatic transaminases been elevated during trials, but both returned to normal after the drug was discontinued. On high doses (20 mg/kg) 5 of 14 patients discontinued HPA-23 due to headaches, fever, vomiting, cellulitis, localised phlebitis or lymphangitis, or anaemia. 23 of 69 patients reported gastrointestinal symptoms and 7 patients reported CNS symptoms (Moskowitz et al. 1988).

4.4.2 Suramin (Phase I)

Chemical Structure, History and Development

Suramin is discussed in some detail since it was the first anti-HIV drug to be adequately evaluated, and important lessons can be learned from it. Suramin is a hexasodium salt of naphthalenetrisulphonic acid. Originally suramin was used to treat trypanosomiasis and onchocerciasis in Africa. Later it was shown to inhibit the reverse transcriptase activities of such retroviruses as murine leukaemia and sarcoma viruses and avian myeloblastosis virus (De Clercq 1979).

Suramin interferes with many biological functions, including arginine- and lysine-specific ester proteases, some RNA polymerases and virus-mediated cell fusion (De Clercq 1982). It also decreases the binding of immune complexes to erythrocytes.

In reverse transcription it is thought to interact with template and/or primer binding sites and can inhibit the enzyme by 50% in the range 0.1 to 1.0 mg/L (Mitsuya et al. 1984). Suramin also inhibits the replication of HIV in the H9 human T-cell line and in CD4+ lymphocytes. This inhibition occurs in the range 50 to 1000 mg/L and probably reflects a decrease in reverse transcriptase activity. However, the high protein binding makes predictions from *in vitro* experiments difficult.

In a review of 90 suramin analogues synthesised for antifilarial studies, 57 were found to inhibit reverse transcriptase activity. 24 of these had anti-reverse transcriptase activity which surpassed suramin and which did not parallel their antifilarial or antitrypanosomal activity (Jentsch et al. 1987).

Clinical Pharmacological Properties

Plasma concentrations of more than 100 mg/L are often achieved in the treatment of parasitic infections. In this setting suramin has considerable toxicity. Given as an intravenous bolus injection suramin has a very long half-life (approximately 40 days), permitting intermittent dosage schedules. Detailed pharmacokinetics in 4 AIDS patients given suramin 6.2g over 6 weeks demonstrated that plasma levels reached 100 mg/L for several weeks, with a half-life of 44 to 54 days after the last dose. Approximately 99.7% was bound to plasma proteins. Urinary excretion accounted for the elimination of most of the drug (Collins et al. 1986).

Therapeutic Trials

Since suramin was already a registered drug, it could rapidly be evaluated in human studies. In a preliminary open trial, plasma concentrations of >100 mg/L were achieved in 10 patients with AIDS or ARC. Adverse reactions such as transient skin eruptions, fevers, proteinaemia and liver function abnormalities were tolerable (Broder et al. 1985). Most adverse reactions peaked the second week and then subsided. Blood cultures from 4 patients were positive, but became negative during the course of therapy. Once suramin was withdrawn, virus-positive cultures reappeared. Despite an observed virustatic effect during the treatment, there was no significant clinical or immunological improvement. Similar findings were obtained in a trial with 8 German homosexual men using suramin 6.2g over 6 weeks and a maintenance dose of 1 g/week (Busch et al. 1985). The single African individual tolerated suramin better than the other patients, as observed in an earlier study of 5 AIDS patients from Rwanda who were given suramin 20 mg/kg

every 5 days for 35 days. These patients only experienced transient fever and weakness (Rouvroy et al. 1985).

In a multicentre phase I trial, 31 patients received 0.5 g/week, 33 patients 1 g/week and 12 patients 1.5 g/week intravenously for 6 weeks. 40% of the patients became virus-culture-negative. No clinical or immunological benefits were documented (Cheson et al. 1987; Levine et al. 1986).

The 1 suramin study reported at the Stockholm conference (Abstr. 3156) found that 4 of 8 patients treated for 2 to 4 months showed total inhibition of HIV replication.

Adverse Effects

Suramin plasma concentrations of 100 mg/L or greater can suppress T-cell growth. The doses used in the phase I trial caused fever in 78%, and malaise, rashes, nausea, neurological adverse reactions and vomiting in 20 to 43% of patients. In approximately one-third of patients the toxic effects occurred in the second to third week and then resolved. Adrenal insufficiency was identified in 23% of patients and was clinically significant in 14%. Vortex keratopathy was observed in 5 patients in the single centre where slit lamp evaluation was performed. Two of the 98 patients completed the trial. 16 died during the trial or within 3 weeks of its termination. It was concluded that the net effect of suramin alone was harmful in the tested patients. This drug is not currently being considered for single drug treatment modalities (Cheson et al. 1987).

4.4.3 Foscarnet (Phosphonoformate) [Clinical]

Chemical Structure, History and Development

Foscarnet is the trisodium salt of phosphonoformic acid. In 1978, foscarnet was first shown to be a virus inhibitor (Helgstrand et al. 1978). Like phosphonoacetic acid, it is active against herpes simplex, cytomegalovirus and a number of animal retroviruses including the visna lentivirus (Sundquist & Larner 1978).

Foscarnet, a pyrophosphate analogue, is a non-competitive inhibitor of reverse transcriptases (Sundquist & Öberg 1979). In HIV-infected H9 cells a dose of 100 μmol/L inhibited virus replication by 98%, as measured by reverse transcriptase activity, without concomitant cell toxicity. A dose of 680 μmol/L completely inhibited virus replication even if added 4 days after infection, but some suppression of cell growth did occur at that concentration. The reverse transcriptase activity associated with HIV particles is extremely sensitive and is completely abolished by foscarnet 5 μmol/L (Sandström et al. 1985; Sarin et al. 1985). T-Cell colony counts increased in cultures from HIV-infected patients with lymphadenopathy, but not from those with AIDS, when foscarnet 400 μmol/L was added to the medium. The increase did not reach the levels of T-cell colony counts observed using cells from uninfected people (Beldekas et al. 1985). In a system which failed to show inhibition of HIV in macrophages by zidovudine, as observed by Richman et al. (1987b), foscarnet was active presumably because it does not require phosphorylation (Abstr. 3132).

Clinical Pharmacological Properties

The half-life of foscarnet in animals is between 1 and 3 hours after subcutaneous or intravenous injection. About 30% of the dose accumulates in bone and the remainder is excreted unaltered in the urine. No effect on calcium metabolism or bone marrow has been seen. The half-life in bone is biphasic in animals, with a primary phase of 8 days and a subsequent phase of about 1.5 years. During intermittent treatment with 2-hour infusions every 8 hours, mean peak and trough foscarnet levels were 557 μmol/L and 155 μmol/L, respectively (Jacobson et al. 1988). Foscarnet is poorly absorbed when administered orally (Sjöwall et al. 1988).

Therapeutic Trials

The results of 4 pilot studies, with a total of 51 patients, have been published. Foscarnet was given in continuous infusion to 6 ARC and 5 AIDS patients for 21 days, with the intention of achieving concentrations between 75 and 150 mg/L (247 to 495 μmol/L) after a bolus injection of 20 mg/kg and subsequent infusion of 0.05 to 0.11 mg/kg/min

of an 8 g/L solution adjusted after measuring the serum creatinine levels. HIV isolation became more difficult after the trial (Farthing et al. 1987). In another study foscarnet was given as 0.09 to 0.16 mg/kg/ml of a 2.4% solution to achieve a plasma concentration of 150 mg/L (450 μmol/L) for a mean of 14 days to 15 AIDS patients, with dosage adjustments based on serum creatinine levels. The mean steady-state foscarnet level was observed to be 261 (range 189 to 741) μmol/L with this regimen. No effect on the ability to isolate HIV was noted, but there was a decrease in positive CMV cultures and some improvement that was thought to be CMV related (Gaub et al. 1987). There was no alteration in immunological or clinical status. In a study of 11 patients with CMV retinitis, intermittent induction therapy of foscarnet 60 mg/kg 8-hourly was administered in a 2-hour intravenous infusion for 14 days. The dose was adjusted daily, with a 3.5 mg/kg reduction for each 0.1 ml/min/kg decrease below an estimated creatinine clearance of 1.6 ml/min/kg. There was a 58% decrease in HIV antigen, which seemed to be dose related. No statistically significant rise in CD4+ cells was seen (Jacobson et al. 1988). The effect on HIV antigen was confirmed in a study of 14 ARC and AIDS patients with reactivated CMV infection treated on various foscarnet treatment schedules for 28 days (Bergdahl et al. 1988).

If intermittent therapeutic modalities can be worked out, the synergism between foscarnet and zidovudine, and the anti-CMV effect, might add to the treatment possibilities (Heley 1988; Abstr. 3624).

Adverse Effects

More than 140 immunocompromised transplant patients have been treated with foscarnet due to complicating CMV infections. In these patients serum levels of foscarnet of 300 to 450 μmol/L caused a fall in haemoglobin, a rise in serum creatinine and in some instances increased levels of serum calcium. In the pilot studies mentioned above foscarnet has been well tolerated, the main concern being a rise in serum creatinine. In the different studies, 10 of 37 patients had a reversible rise in serum creatinine (Farthing et al. 1987; Gaub et al. 1987; Jacobson et al. 1988). Reversible anaemia in some patients and an axillary thrombosis in a patient administered a high-concentration foscarnet infusion has been reported. Four cases with acute renal failure induced by foscarnet have been reported. It is mandatory to monitor serum creatinine and ensure that the enhanced diuresis is not causing a negative fluid balance (Cacoub et al. 1988). One patient has been reported with acute toxicity consisting of reversible hallucinations and flapping tremor, occurring at 10 times the recommended dose.

4.4.4 Rifabutin (Ansamycin) [Phase I]

Chemical Structure, History and Development

Rifabutin was synthesised as a derivative of rifamycin S. It is active against *Mycobacterium avium-intracellulare* and is currently undergoing clinical trials against this infection in AIDS patients.

Concentrations of between 0.1 and 0.8 mg/L will inhibit HIV replication *in vitro*. The mechanism of action is thought to be inhibition of the DNA-dependent RNA transcriptase in Gram-positive bacteria and some eukaryotic cells and viruses against which it is active (Arora 1983). HIV replication in human peripheral blood lymphocytes (PBLs) *in vitro* can be inhibited, presumably by the binding of rifabutin to reverse transcriptase (Anand et al. 1986). The anti-HIV activity of a combination of rifabutin with heparin or dideoxycytidine (ddC) was claimed to be greater than either drug alone, with no sign of *in vitro* toxicity (Abstr. 3592, 3593).

Therapeutic Trials and Clinical Pharmacological Properties

In a phase I study reported at the Stockholm conference (Abstr. 3594), 16 patients started on rifabutin 450 mg/day were found to tolerate 2400 mg/day, but skin pigmentation and reversible polyarthritis/arthralgia were commonly observed. Some patients experienced uveitis and hepatitis. No haematological toxicity was observed. Even at 2400 mg/day the observed serum levels of 1000 μg/L were below those required to inhibit HIV *in vitro*.

No clinical or virological effect was apparent when rifabutin was used as monotherapy.

4.5 Reverse Transcriptase Chain Terminators

4.5.1 Dideoxycytidine (Phase I/II)

Chemical Structure, History and Development

In a seminal paper on nucleoside analogues it was shown that several 2′,3′-dideoxynucleosides were inhibitory to HIV replication in ATH8 cells *in vitro*. The most potent among this family of substances was dideoxycytidine, which completely blocked HIV replication at 0.5 μmol/L, with no sign of cellular toxicity or inhibition of T-cell functions up to 5 μmol/L (Mitsuya & Broder 1986). Similar observations were made for dideoxyadenosine (ddA) and dideoxyinosine (ddI). Dideoxycytidine 2 μmol/L (or dideoxyadenosine 50 μmol/L) completely inhibited viral RNA production for 30 days. On removal of the drug viral proliferation did not resume for an observation period of 80 days. The dideoxynucleosides were demonstrated to be DNA chain terminators. At 5 μmol/L of dideoxycytidine (or 100 μmol/L of dideoxyadenosine) normal helper/inducer T-cells retained their capacity to proliferate in response to antigen plus irradiated polymorphonucleocytes (PMN) for 20 days (Mitsuya et al. 1987a). *In vitro* results suggest that intermittent dideoxycytidine together with zidovudine is superior to either drug alone or in constant combination (Abstr. 3620, 3151). As with zidovudine, dideoxycytidine has been found to have a synergistic effect with interferon-α (Vogt et al. 1988). It is also synergistic with heparin and rifabutin (Abstr. 3592). As with zidovudine, there has been debate as to the activity of dideoxycytidine in macrophages (Richman et al. 1987b; see above).

Clinical Pharmacological Properties

Based on the observations that dogs tolerated oral doses of dideoxycytidine giving plasma concentrations of 50 μmol/L for 28 days with virtually 100% absorption after oral administration, and that the drug rapidly penetrated the CSF (although only at 2 to 6% of the serum concentration), dideoxycytidine was considered for human trials (Mitsuya et al. 1987a). In a phase I trial 20 patients were administered intravenous dideoxycytidine 0.03 mg/kg every 8 hours, 0.03, 0.06, or 0.09 mg/kg every 4 hours, or 0.25 mg/kg every 8 hours, for 2 weeks, followed by oral administration of the same dose for another 4 weeks. Peak concentrations of 5 μmol/L were attained with 1-hour infusions of 0.06 mg/kg. The average half-life of the drug was 1.2 hours, and the oral bioavailability between 70 and 80%. Most of the drug seems to be eliminated by renal clearance. CSF samples (n = 9) between 2 and 3.5 hours after the start of the 1-hour infusion contained 9 to 37% of the plasma concentration found at that time (Yarchoan et al. 1988b).

Therapeutic Trials

In the phase I trial transient increases in CD4+ cells were seen at all but the lowest dose levels. No clearcut effect was seen on cutaneous delayed hypersensitivity, and there was no effect on HIV isolation. There was a significant decrease in p24 antigen at week 2 but a rebound effect was seen in most of these patients. Due to the toxicity of dideoxycytidine a limited number of patients (n = 6) subsequently received a weekly alternating regimen of zidovudine 200mg every 4 hours or dideoxycytidine 0.03 mg/kg every 4 hours. The 5 patients who completed 9 weeks of treatment reported a greater feeling of well-being and showed improved immunological and virological test results (Yarchoan et al. 1988b). This trial has been extended to a total of 13 patients on full or half dosages of dideoxycytidine, 8 of whom were still reported to be on therapy at the time of the Stockholm conference (Abstr. 3149). Five of 9 patients on full dose had completed a median of 27 weeks without neuropathy or haematological toxicity.

An extension of the phase I trial with 61 patients given 0.06, 0.03, 0.01 or 0.005 mg/kg every 4 hours has recently been reported (Merigen et al. 1989; Abstr. 3015). Some effect was seen on p24 antigen levels, but CD4+ cell counts did not increase in patients given 0.005 mg/kg.

Adverse Effects

In the phase I trial 9 patients stopped treatment before week 6 (2 due to PCP, 1 high fever, and 6 drug toxicity). During initial therapy a transient symptom complex was seen: cutaneous eruptions, aphthous ulcers, malaise, fever, and to a lesser degree arthralgias, ankle oedema, nail changes and diarrhoea. Toxicity generally appeared after 2 weeks and subsided after 3 days to 3 weeks. Three of the 11 patients on 0.03 to 0.06 mg/kg every 4 hours had dose-limiting thrombocytopenia or neutropenia. Most important, however, was a painful 'stocking-glove' sensimotor peripheral neuropathy that developed in 10 patients who continued beyond week 6. It seemed dependent on the cumulative dose, generally appeared after 10 weeks, and presented with painful dysaesthesia of the feet. The neuropathy generally worsened for up to 5 weeks after the drug was stopped then gradually improved (Yarchoan et al. 1988b). On follow-up of 4 of these patients, 2 were free of their symptoms 3 and 18 weeks, respectively, after the drug was stopped and the other 2 were reported as improved but with ankle jerks still absent (Dubinsky et al. 1988). All patients on the 2 highest doses, 0.03 and 0.06 mg/kg, in the phase I/II trial had dose-limiting neuropathy between weeks 4 and 14. Milder peripheral neuropathy occurred in 8 of 11 patients on 0.01 mg/kg and 2 of 15 on 0.005 mg/kg. The neuropathy took considerable time to resolve. For those on lower doses, all neuropathy had resolved within 6 months, while reduction took longer than 1 year for some patients on the highest doses. Of the 15 patients on 0.005 mg/kg, 7 had mostly mild stomatitis, 7 often mild rashes, 8 arthritis/arthralgia, and 10 fevers (Merrigan et al. 1989).

At this point the therapeutic index seems to be too low for use of dideoxycytidine as a single drug.

4.5.2 Dideoxyadenosine and Dideoxyinosine (Phase I)

Laboratory studies on dideoxyadenosine have confirmed its activity *in vitro* and shown that it is rapidly converted to dideoxyinosine, extra- and intracellularly. Dideoxyinosine is converted to dideoxyadenosine monophosphate (ddAMP) via its monophosphate, ddIMP (dideoxyinosine monophosphate) [Ahluwalia et al. 1987; Johnson et al. 1988; Mitsuya et al. 1987a; Abstr. 3014]. Since dideoxyadenosine is hydrolysed by acid pH in the stomach to dideoxyribose and adenine, and it has been associated with renal toxicity in dogs and rats, direct administration of dideoxyinosine seems preferable (Abrams et al. 1988). Doses of intravenous dideoxyinosine 0.2, 0.4 or 0.8 mg/kg for 2 weeks followed by 4 weeks of oral therapy at twice the intravenous dose have been given in an on-going phase I study to determine the pharmacokinetics and toxicity of the drug. Peak serum levels of 0.6 to 2.5 μmol/L dideoxyinosine were reached with these doses. When given with antacids there was a 40% bioavailability. Some increase in CD4+ cells was seen at the higher doses (Yarchoan et al. 1989).

4.5.3 Fluorothymidine (Laboratory)

Interest in fluorothymidine (3′-fluoro-substituted 2′,3′-dideoxythymidine; FddThd, FLT, 3′-F-TdR, FT or 3′FddTTP) has grown rapidly, as demonstrated by communications from 5 different groups to the Stockholm conference (Abstr. 3001, 3005, 3006, 3007, 3009). Fluorothymidine has been shown to be as effective as zidovudine against the cytopathic effect of HIV *in vitro* and against HIV reverse transcriptase activity. However, several B- and T-cell lines were more susceptible to the cytotoxic effect of this compound than to zidovudine, but less consistently so than to dideoxycytidine (Balzarini et al. 1988; Bazin et al. 1988). These data were corroborated in the reports where sensitivity of simian immunodeficiency virus (SIV) and feline leukaemia virus (FeLV) was also reported to be associated with greater cytotoxicity and inhibition of lymphoproliferative responses (Abstr. 3007).

4.5.4 Acyclic Nucleoside Analogues (Laboratory)

The properties of a new category of acyclic nucleoside analogues, adenallene, cytallene, and 9-(2-phosphonylmethoxyethyl) adenine (PMEA), which inhibit HIV replication and proviral DNA synthesis *in vitro*, open up a new class of experimental

drugs for investigation (Balzarini et al. 1989; Hayashi et al. 1988).

4.5.5 Other Nucleotides (Laboratory)

The pace of investigation into nucleoside analogues is indicated by the discussion of laboratory properties of more than 60 other compounds at the Stockholm conference. In the screening programmes several promising nucleosides have been singled out: carbovir (carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine), CS-87 (3′-azido-2′,3′-dideoxyuridine), D4C (2′,3′-dideoxycytidine-2′-ene), D4T (2′,3′-dihydro-2′,3′-dideoxythymidine). For recent overviews of many of the possible synthetic analogues see Bazin et al. (1989), Chu et al. (1988), Herdewijn et al. (1988), and Vince et al. (1988).

4.6 RNase H (Hypothetical)

Although no compound has yet been described utilising RNase H, for example for cleavage of atoxic prodrugs into toxic compounds, the description of this enzyme might open the way for such activity (Hansen et al. 1988).

4.7 RNase L

4.7.1 Ampligen (Clinical)

Chemical Structure, History and Development

Double-stranded RNA can induce different kinds of interferons, and activate interferon-associated intracellular mediators. It is usually toxic to normal cells but, the polynucleotide poly I-poly C with inserted uracil residues, the so-called mismatched double-stranded RNA, ampligen, has greatly reduced toxicity. It has been investigated in patients with cancer and found to induce interferon levels of 5 to 800 U/ml. Recently it has been shown that ampligen can inhibit HIV in certain cell lines at 250 mg/L (Laurence et al. 1987; Montefiori & Mitchell 1987). Ampligen is synergistic with interferon-α and zidovudine (Hubell 1986; Mitchell et al. 1987; Abstr. 3613).

Clinical Pharmacological Properties

Ampligen has a plasma serum half-life of 23 minutes after a 200mg 30-minute intravenous infusion (Brodsky & Strayer 1987).

Therapeutic Trials

In a preliminary open study, 10 patients received either 200mg or 250mg intravenous ampligen twice weekly for up to 18 weeks. In all patients some initially negative cutaneous delayed hypersensitivity responses turned positive. Unfortunately, due to the small number of patients, changes in T-cell subsets could not be evaluated. HIV RNA disappeared from the blood of 8 of 9 patients. In all patients RNase L, which can degrade macromolecular RNA, including viral mRNA, was initially inactivated. In 3 patients in whom HIV could no longer be detected during the trial RNase L activation was now observed. There was a suggestion that patients with lymphadenopathy syndrome (LAS) or ARC had symptomatic relief (Carter et al. 1987).

In an open trial, 39 patients were treated for a mean of 8.4 months with 100 to 200mg twice weekly. The treatment coincided with clinical improvement, return of cutaneous delayed hypersensitivity, stabilisation of CD4 counts and decrease in HIV antigen levels (Abstr. 3046). Several dose-finding studies are under way in the US (Abrams et al. 1988).

Adverse Effects

Ampligen has been given by intravenous infusion, 10 to 500mg twice weekly, to patients with solid tumours without clinically significant adverse effects. The only adverse effects were mild fatigue, fever and flu-like symptoms.

4.7.2 Oligodeoxynucleotides (Laboratory)

It has been found that nucleotides that are protected from degradation by modification to phosphorothioate analogues are efficient inhibitors of HIV replication. This property is not limited to *art/trs* anti-sense oligomers, but is associated with the guanosine and cytosine content and length (up to 28-mer, S-dC28). S-dC28 gave complete inhibition

of HIV replication at 0.5 μmol/L without toxic effects on cells not exposed to the virus. *De novo* synthesis of proviral DNA was inhibited, but not viral adsorption or translation of viral proteins. No induction of interferon-γ, as found with double-stranded RNA, was seen.

Synergy was noted with dideoxyadenosine (Matsukura et al. 1987), and these findings were extended to show that an anti-sense *art/trs* 28-mer inhibited p24 *gag* protein production in chronically HIV-infected cells *in vitro* (Abstr. 3027). While zidovudine, dideoxyadenosine and dideoxycytidine failed to protect macrophages from the HIV-induced defect in antigen presentation, S-dC28 permitted such infected cells to function and suppress HIV replication (Abstr. 3028). A means of large scale production of DNA analogues is now available (Abstr. 3029).

While oligonucleotides as described above may have both a specific and a nonspecific anti-HIV effect, it has been demonstrated that short (8-mer) anti-sense oligodeoxyribonucleoside methylphosphonates directed at the first splice acceptor site of the *tat*-3 gene have a more potent inhibitory effect than the corresponding sense oligomer or an unrelated herpes simplex anti-sense sequence. However, although the sense construction did not influence the viral cytopathic effect or reverse transcriptase activity it did reduce viral RNA production (Zaia et al. 1988).

4.8 Integration (Hypothetical)

The existence of an HIV-specific integrase might be utilised for specific intervention in the integration step.

4.9 Activation

4.9.1 Cyclosporin A (Clinical)

Chemical Structure, History and Development

Cyclosporin is a widely used cytostatic drug in transplantation therapy due to its selective effect on helper T-cells. It has been considered in HIV therapy on 2 grounds: inhibition of helper T-cells would diminish the substrate for HIV replication and/or reduce the effects of autoimmunity that develop in some patients.

Recently it has been shown that cyclosporin has an effect on HIV infection of H9 cells and peripheral blood-derived lymphocytes *in vitro* if added within the first 2 hours of infection. If added 24 hours before infection it seemed to completely inhibit infection, It does not affect proliferation of these cells or the CD4 antigen expression. No effect on infection in U937 or primary monocytes/macrophages could be demonstrated (Wainberg et al. 1988).

Therapeutic Trials

A follow-up of the first open pilot study was reported at the Stockholm conference (Andrieu et al. 1988; Abstr. 3579). In the first pilot study, 15 patients received 8.4 mg/kg/day. After 3 months treatment was stopped in 8 who were considered non-responders (1 of these developed AIDS); the remaining 7 were considered responders due to marked and persistent increase in CD4+ cells, and these patients have been followed for 24 to 28 months. In the second pilot study 9 patients received 6.4 mg/kg/day and seemed to have stable CD4+ and CD8+ cell counts during 5 to 12 months of follow-up. However, the design of the study and the low numbers make it inadequate to evaluate the efficacy of cyclosporin.

Adverse Effects

The principal adverse reaction with systemic cyclosporin treatment is nephrotoxicity which is usually reversible. Mild hepatotoxicity and hypertension may also occur. The drug is not myelotoxic.

4.10 Regulation of Transcription and Translation

It is now clear that an intricate web of positive and negative feedback loops regulate HIV. The full extent of this network is not yet clear, but it offers several avenues for interference (for review see Haseltine 1988).

4.11 *Tat*-Mediated Inhibition of Transcription

4.11.1 Penicillamine (Clinical)

The cysteine analogues D- and L-penicillamine have been investigated due to their affinity for proteins with high cysteine content, such as the HIV nucleotide-binding protein. *In vitro* data utilising a transfection system with an LTR-CAT and *tat* on 2 different plasmids indicated that D-penicillamine directly interfered with some part of that regulatory loop, for example chelation of Zn^{2+} essential for *tat*-protein dimer formation, or directly interfered via cysteine bonds (Chandra et al. 1988). A concentration of 40 mg/L was required to totally inhibit HIV infection of H9 cells, while concentrations above 100 mg/L had no cellular toxicity (Chandra & Sarin 1986). Since a 1986 trial with 13 patients where doses of 0.5 to 2g were reported to inhibit HIV isolation, with concomitant suppression of T-cell functions, these drugs have not received much attention (Schulof et al. 1986). That trial was prematurely terminated due to induction of decreased MLR responses in the first 5 patients after the planned 6 weeks of treatment. Three patients experienced transient rashes and 1 stopped the drug due to a skin reaction, otherwise the drug was well tolerated at 2 g/day for 1 to 5 weeks (Schulof et al. 1986).

4.11.2 Imuthiol (DTC) [Phase I/II]

Chemical Structure, History and Development

Although it has been claimed that the main action of imuthiol is through thymic hormone-like activity, it is also conceivable that through its chelating properties it could inhibit the metal-dependent *tat* gene (Frankel et al. 1988).

Clinical Pharmacological Properties

Imuthiol is related to disulfiram, which breaks down into 2 molecules of imuthiol which retain the 'Antabuse' effect of the parent compound when alcohol is ingested (Lange & Sönnichsen 1988). Disulfiram is also prescribed in HIV infection and collected data on oral disulfiram 750mg weekly or 500mg twice weekly were claimed to show an increase in CD4+ cells and reduction of clinical symptoms (Abstr. 3042).

Therapeutic Trials

Initial pilot trials indicated that imuthiol 10 mg/kg orally once a week was well tolerated, and associated with a rise in CD4+ cells and restoration of cutaneous delayed type hypersensitivity. On the basis of these observations 83 patients were enrolled in a 16-week placebo-controlled double-blind study, with a later 16-week crossover phase. Due to the limited number of patients and/or time, the impact on progression to AIDS (occurring in 0 of 38 patients receiving imuthiol and 3 of 39 receiving placebo) could not be evaluated (6 patients could not be followed, due to loss of follow-up of 4, intolerance in 1, and patient's choice in 1 case). Furthermore, events within the first 4 weeks of the trial were not reported. There was a significant decrease in constitutional symptoms (persistent diarrhoea, weight loss and persistent fever), in 8 of 11 imuthiol recipients *vs* 0 of 5 on placebo. At the last time point, week 16, there was a significant difference in CD4+ cell counts for patients receiving imuthiol. However, these patients had higher counts at baseline and showed a less pronounced increase than those on placebo. The reaction to recall antigens can not be evaluated since only the end results are presented; 3 of 29 were anergic on imuthiol *vs* 9 of 32 on placebo. Peak reverse transcriptase activity in co-cultures or HIV antigen was not affected. Considering the adverse effects it is doubtful if the trial was blinded (Lang et al. 1988). Similar results were obtained by the same group of researchers in an open study of 26 patients treated with imuthiol 10 mg/kg weekly for 1 year (Abstr. 3055).

In an open dose-escalating trial of 14 patients the imuthiol dose was doubled every 4 weeks, starting at 200 mg/m^2/week, administered intravenously, and reaching 800 mg/m^2 twice a week after 16 weeks. In patients with > 200 CD4+ cells the CD4+ cell counts stabilised, and the symptom score and lymph node size decreased. In patients with < 200 CD4+ cells there was no difference in progression (Abstr. 3041).

Adverse Effects

An unpleasant taste was noted in 32%, abdominal discomfort in 26%, and nausea in 15% of patients on imuthiol in the placebo-controlled trial. No placebo effect was greater than 6%. However, all adverse effects were mild and the drug is claimed to have been tolerated for 3 years in HIV patients and 5 years in cancer patients. No adverse effect on blood cell counts or platelets has been seen.

4.12 *art/trs* Inhibitor

See oligonucleotides above.

4.13 Translation

4.13.1 Ribavirin (Phase I/II)

Chemical Structure, History and Development

Ribavirin is a nucleoside consisting of D-ribose attached to a 1,2,4 triazole carboxamide. Synthesised in 1972, ribavirin was found to be active against a number of RNA and DNA viruses including influenza A and B, respiratory syncytial virus (RSV), measles, herpes simplex, and varicella/zoster (Chang & Hell 1981; Witkowski et al. 1972).

Topical administration by small particle aerosol has been promising in influenza and respiratory syncytial virus infections. Ribavirin has also been of some benefit in the treatment of Lassa fever.

The mechanism of the antiviral effect of ribavirin is poorly understood and probably not the same for all viruses. In 1977 the drug was found to inhibit murine retrovirus replication *in vitro* and *in vivo* (Shannon 1977). Its mechanism of action is thought to involve alterations of the intracellular guanosine pool and the guanylation step required for 5′-capping of viral messenger RNA. Ribavirin suppressed HIV replication in continuous cell lines and human peripheral blood leucocytes (PBLs). Exposure of cultures to doses of 50 to 100 mg/L for periods of 8 to 9 days inhibited reverse transcriptase activity and HIV immunofluorescence. If additional drug was added after 5 days, virus expression was further delayed but not prevented (McCormick et al. 1984). Ribavirin has been shown to act antagonistically with pyrimidine analogues such as zidovudine, but not with purine analogues (Baba et al. 1987; Vogt et al. 1987).

Clinical Pharmacological Properties

Ribavirin can be administered orally. It is eliminated slowly via the kidneys, with a terminal serum half-life of 1 to 2 days (Laskin et al. 1987). A considerable amount of drug is retained in red blood cells and other tissues. After administration for more than 2 weeks the plasma half-life is in the order of 2 weeks. Mean trough levels after 2 weeks were 5.0, 11.1 and 20.9 μmol/L for groups (n = 3) of patients on 200, 400 and 800mg 3 times daily, respectively (Roberts et al. 1987). Plasma concentrations have been maintained at between 6 and 15 mg/L in ARC/AIDS patients. Levels of ribavirin in the CSF of 3 treated AIDS patients were 67 to 115% of that found in plasma (Crumpacker et al. 1986).

Therapeutic Trials

Initial studies of ribavirin 600 to 800 mg/day have been equivocal. One major trial of 163 patients randomised to ribavirin 600 or 800 mg/day or placebo was reported at the Stockholm conference. A transient effect on reverse transcriptase activity in co-cultures was seen at 800 mg/day (Abstr. 3571).

Adverse Effects

The primary adverse effect has been a reversible inhibition of haemoglobin synthesis in the cancer trial referred to above.

In HIV-infected patients 600 mg/day administered orally in 3 divided doses was well tolerated for the first 2 weeks, with mild symptoms by week 4. CNS complaints occurred within 2 weeks in 2 of 3 patients on 1200 mg/day orally. All 3 patients who received 2400 mg/day complained of moderate to severe CNS symptoms and fatigue. Minor reactions included a metallic taste, dry mouth, increased thirst, flatulence, fatigue, headache, irritability, mood lability and insomnia (Roberts et al. 1987).

4.13.2 Avarol (Laboratory)

Following the observation that the sponge product avarol, a sesquiterpenoid hydroquinone, has a potent anti-HIV effect *in vitro* (Sarin et al. 1987), it has recently been shown that this is due to inhibition of tRNA UAG-termination codon suppressor. This causes a read-through of the termination codon at the *gag-pol* junction and allows protease synthesis in Mo-MuLV-infected NIH-3T3 cells (Kuchino et al. 1988). These findings were recently extended to HIV-infected H9 cells (Muller et al. 1988b; Abstr. 3568). In an H9/HTLV-IIIB *in vitro* system avarol demonstrated a synergistic antiviral effect with zidovudine. An antagonistic cytotoxic effect was seen at doses 10 times higher than those necessary for the antiviral effect. As with interferon-α this synergy is found between compounds belonging to 2 different antiviral modality groups (table II) [Schröder et al. 1988].

4.14 Post-Translational Modification

4.14.1 Virus-Specific Protease (Laboratory)

It has been proposed that with the characterisation of the 99-amino-acid (9kD)-long aspartic protease that preferentially cleaves in hydrophobic domains, a mechanism of inhibition is feasible (Billich et al. 1988). The enzyme is similar to renin, for which inhibitors exist (Öberg 1988). Studies of avian sarcoma-leucosis virus protease indicate that small peptides can be constructed that compete with the natural substrate or act as inhibitors (Kotler et al. 1988).

4.15 Glycosylation

4.15.1 Glycosylation Modifiers (Laboratory)

It has been shown that castanospermine and deoxynoijrimycin, inhibitors of glycosidases in glycoprotein processing, have anti-retroviral activity in model systems (Sunkara et al. 1987). Subsequently it has been demonstrated that castanospermine inhibits the replication of HIV and syncytia formation *in vitro*. Since HIV produced by castanospermine-treated cells can bind to CD4+ cells it is assumed that a later step (membrane fusion?) is inhibited (Walker et al. 1987). Cells treated with 7.5 mmol/L, 3 times the antiviral dose, grew normally (Tyms et al. 1987). Reports of further *in vitro* studies of castanospermine and other glycoprotein inhibitors were presented in 3 communications at the Stockholm conference. Cell surface protein seemed unaffected by treatment. Synergy with zidovudine and soluble CD4 was found in 1 study (Johnson et al. 1989; Abstr. 3540, 3541, 3618).

A broad inventory of different aminosugars has been made in the Karpas cell assay with cytopathic effect of HIV as an end-point. Notably, deoxynoijrimycin was only marginally active, while its N-butyl derivative, an α-glucosidase I inhibitor like castanospermine, combined efficacy with lack of toxicity (Fleet et al. 1988). Using many of these compounds it is concluded that they interact with HIV infections on the level of virus infectivity and cytopathogenicity, but leave replication unaffected (Montefiori et al. 1988).

4.16 Protein Kinase C

4.16.1 Xanthate (Laboratory)

Xanthate inhibits shedding of HIV from chronically infected Ke37-III lymphoma cells and blocks *de novo* infection by inhibition of the cellular protein kinase C (Abstr. 3573).

4.16.2 Glycyrrhizin (Clinical)

Glycyrrhizin from the licorice root has been postulated to inhibit HIV replication by protein kinase C inhibition. Two AIDS patients received 800 or 1600 mg/day by continuous intravenous infusion for 8 to 16 weeks. HIV antigen is claimed to have been reduced and lymphocyte proliferation to have shown good recovery (Abstr. 3533, 3536).

4.17 Assembly

4.17.1 Interferons (Clinical)

At the time of our previous review there was considerable interest in the interferons following the report of the interferon-α anti-HIV effect (Ho et al. 1985). For review see Sandström & Kaplan 1987).

There is no convincing evidence that interferon-α alone in the dose range 3 to 36 million units (MU) is of benefit in AIDS (Interferon Alpha Study Group 1988). There is some indication that interferon-α (starting with 35 MU/day) given to patients with Kaposi's sarcoma and a relatively intact immune system will result in tumour regression and suppression of HIV antigen, although there is no effect on CD4+ cell counts (Lane et al. 1988). In another high-dose (27 to 36 MU/day) study a significant increase in CD4+ cells and decrease in HIV antigen was seen in patients with regression (12/26) of their Kaposi's sarcoma lesions. The patients in the latter study had higher initial CD4+ cell numbers (de Wit et al. 1988). The clinical relevance of these observations remains to be established. However, interferon-α which is weakly active alone against HIV *in vitro,* has nonetheless demonstrated synergy with zidovudine, foscarnet, and dideoxycytidine (Hartshorn et al. 1986, 1987; Vogt et al. 1988). One case report describes positive effects of low-dose (2 to 4 U/kg) oral interferon-α (Hutchinsson & Cummings 1987).

Several attempts have been made to combine interferon-α with zidovudine in clinical trials. In most trials the high doses of interferon-α required for treatment of Kaposi's sarcoma have been used (> 9 MU), and have been found to be incompatible with full-dose zidovudine treatment due to bone marrow toxicity (Abstr. 3627, 3628, 3631). No clinical benefits have been seen. However, an effect on HIV antigen has been claimed with lower doses (1.5 to 3 MU) of subcutaneous interferon-α combined with zidovudine 100mg 4 times daily (Abstr. 3626).

Interferon-β has also demonstrated *in vitro* anti-HIV activity, and further human trials with this agent are proposed, following evidence of less toxicity in preliminary trials in humans (Michaelis & Levy 1988; Abstr. 3510, 3515).

4.18 Budding

4.18.1 Hypericin (Laboratory)

Extracted from the plant *Hypericin triquetrifolium*, the aromatic polycyclic diones hypericin and pseudohypericin were found to interfere with Friend leukaemia virus (FV) infection in mice without apparent toxicity (50 μg/mouse). These substances are reported to be toxic to human cells at concentrations > 10 mg/L. There was no effect on radiation leukaemia virus (RadLV) RNA production, directly on the activity of the reverse transcriptase or on viral cell surface proteins, and the mechanism of action is thus assumed to be at a later stage of virus production. Some researchers have claimed that hypericin and pseudohypericin have increased efficacy when combined with zidovudine. They are claimed to cross the blood-brain barrier and have been given to humans as antidepressants (Meruelo et al. 1988).

4.19 Cell-Bound Viral Antigens

4.19.1 CD4-Pseudomonas Exotoxin (Laboratory)

Exploiting the knowledge of soluble CD4 discussed above, the gp120 binding portion of CD4 has been coupled to the active portion of Pseudomonas exotoxin A on an *E. coli* pV403 plasmid. The resulting hybrid protein was selectively toxic to cells displaying gp120 on their surface. Class II major histocompatibility (MHC II) expressing cells that normally interact with CD4 were not sensitive to the hybrid protein (Chaudhary et al. 1988).

4.19.2 CD4-Ricin A (Laboratory)

Soluble recombinant CD4 can be used to deliver the ricin A chain to infected cells expressing gp120. Such cells are killed, while non-infected cells are unaffected. Daudi cells expressing high levels of histocompatibility class II antigens were not killed by the complex (Till et al. 1989).

4.19.3 Monoclonal Antibody-Ricin A (Laboratory)

A monoclonal antibody conjugated to the ricin A chain and directed against the neutralising epitope of gp120 was toxic to infected cells *in vitro* which were exposed to pulses as short as 20 minutes. Activity was seen on acutely and chronically infected cells, but not on uninfected cells. Blocking antibodies from patients only partially inhibited the

effect. Resistant cell variants arose at a frequency of 0.1 to 1%, but did not produce infectious viruses (Pincus et al. 1989).

4.20 Syncytia Formation

See glycosylation modifiers above.

4.21 Inactivation of Virus

4.21.1 Passive Immunisation (Clinical)

The feasibility of passive serotherapy has also been explored (Zolla-Pazner et al. 1987).

Plasma, 55 to 500ml, from 2 symptom-free HIV seropositive donors with high titres against p24, was given to 6 patients with advanced AIDS. HIV antigen disappeared for a period of time that was linearly related to the volume of plasma given. There was a significant improvement in symptom score, Karnofsky score and bodyweight during the antigen-free month after plasma infusion compared to the period prior to infusion and the following antigenaemic month. T-Cell subsets seemed to be affected favourably, and there seemed to be fewer OIs during the antigen-free period. There was a significant difference in success of HIV isolation from plasma or blood mononuclear cells during the antigen-free time compared to before treatment and after return of antigenaemia (Jackson et al. 1988a). Similar results with regard to antigenaemia during treatment were obtained in a study where 4 ARC and 5 AIDS patients received plasma from healthy HIV-infected people with high neutralising antibody titres, 500 ml/month for 3 months. In this study no effect was seen on CD4+ cell counts. The size of the study did not permit clinical evaluation (Karpas et al. 1988).

A technique for production of human IgG monoclonal antibodies (anti-gp41 and anti-gp120) was presented at the Stockholm conference (Abstr. 3577).

4.22 Other

4.22.1 Somatostatin (Laboratory)

Somatostatin has been used in the treatment of persistent diarrhoea (Cook et al. 1988). Observation of an increase in white cell counts in 1 such trial led to investigation of the antiviral activity of the long-acting analogue SMA 201-995. It was found to have *in vitro* inhibitory properties at a concentration (10^{-10} mol/L) corresponding to physiological doses without *in vitro* toxic effects (Abstr. 3548).

4.22.2 AS101 (Clinical)

As with other compounds which are contemplated for use in HIV infection, such as immunomodulators (imuthiol, isoprinosin), it has also been found that AS101 (an ammonium salt of tellurium) has an anti-HIV effect *in vitro*. Reverse transcriptase activity was reduced by 62% in chronically infected HUT78 cells after 7 days' exposure to 5 μg/ml AS101 (Abstr. 3036). AS101 enhances production of interleukin-2, colony stimulating factor, interferon-γ and tumour necrosis factor *in vitro* (Abstr. 3037). Two open studies were presented at the conference (Abstr. 3038, 3039), involving a total of 53 patients for up to 61 weeks. 36 of these were assigned to receive a 2, 3 or 5 mg/m^2 infusion 3 times a week for 12 weeks, followed by a maintenance dose of 3 mg/m^2. Compared to historical controls there appeared to be a dose-dependent effect on survival, incidence and days/months of opportunistic infection. Adverse effects were limited to a garlic-like body odour.

4.22.3 Fusidic Acid (Clinical)

It has been claimed that fusidic acid is active against HIV *in vitro* and affects HIV disease (Faber et al. 1987; Abstr. 3067). Other studies have refuted this (Abstr. 3527, 3563, 3564, 3565).

5. Immunological Reconstitution

At the Stockholm conference 12 biological response modifiers were reported in 21 clinical studies. However, the studies reported at the conference were too small or not sufficiently advanced to assert a clinical benefit of any of these substances (i.e. ampligen, acemannan, AS101, cyclosporin, imuthiol, enkephalin, IMREG-1, isoprinosine, methisopinol, naltrexone, polyantigenic stimulator and thymostimulin). Only a few studies (of ampligen and isoprinosine) addressed the com-

bination with antiviral compounds, i.e. zidovudine.

A broad range of different immunotherapies were investigated – monoclonal antibodies that can deliver a toxin to HIV-infected target cells, infusion of plasma with high titres of anti-p24 (Jackson et al. 1988), *in vitro* inhibitory anti-p18 monoclonal antibodies (Abstr. 3071), and expansion of autologous CD8+ cells (Abstr. 3066). Single large doses of intravenous immunoglobulins to adults were found to be ineffective.

The different interferons have been studied intensively (see above), although the numbers of patients were small in the various studies. There was no clear evidence of benefit from interferons alone, although the anti-HIV activity was confirmed for interferons α and β in *in vitro* studies. Following the reported *in vitro* synergy between interferon-α and zidovudine, it has been found that bone marrow toxicity is dose-limiting for the combination, but some clinical benefit might be gained. A number of *in vitro* studies addressed the tumour necrosis factor (TNF), alone or in combination with interferon-γ. Granulocyte-macrophage colony stimulating factor might find a therapeutic niche in zidovudine-induced bone marrow suppression, since it interferes with the triphosphate levels of zidovudine and natural nucleosides (Bhalla et al. 1989). Although it stimulates HIV replication on its own, it will potentiate the effect of zidovudine in monocytes and macrophages (Hammer & Gillis 1986; Perno et al. 1989). An additive effect has recently been reported between the *Streptomyces olivaceogriseus*-extracted tripeptide FK-565, *N*-(N^2-(*N*-hepatonyl-gamma-D-glytamyl)-meso-2-(l),2′-(D)-diamino-1-pimeloyl)-D-alanine, and zidovudine on FV infection in mice, as well as by FK-565 alone (Yokota et al. 1988).

Most immunomodulatory treatment modalities that were discussed in detail in the previous review have not been much studied over the last 2 years (see Sandström & Kaplan 1987).

Plasmapheresis over protein A-containing columns seems to have found a treatment niche in removal of autoimmune antibodies in HIV-related disease, as evidenced by only 1 communication to the Stockholm conference (Abstr. 2124).

Thymic hormones have received little continued attention. The 3 studies on thymostimulin (TP-1) at the Stockholm conference did not demonstrate any clinical benefit (Abstr. 3072, 3073, 3074).

Interleukin-2 alone in dose-escalating trials has not demonstrated clinical effects, but has been associated with considerable toxicity. At least 5 trials are under way to explore its value in combination with zidovudine (Abrams et al. 1988).

Tumour necrosis factor (TNF) has been found to be up-regulated in HIV-infection and is proposed to cause symptoms such as cachexia, bone marrow suppression and chronic inflammation (Abstr. 3502, 3503, 3505). With its availability in recombinant form (rTNF) it is also being administered in preliminary studies due to its *in vitro* anti-HIV activity (Abstr. 3501, 3507).

There have been no developments with regard to *transplantation* of homologous or heterologous bone marrow, although there is still some interest in transfusion of autologous *in vitro* expanded CD8+ lymphocytes, as indicated above.

Imuthiol and *AS101* are discussed above.

5.1 Isoprinosine (Phase II)

5.1.1 Chemical Structure, History and Development

Isoprinosine was formulated in about 1970 as the p-acetamidobenzoic salt of N,N-dimethylamino-2-propanol and inosine in a 3 : 1 molar ratio. In animal models and in humans it has been claimed to have activity against a number of viral diseases. These reports have often been challenged, however, and only low levels of *in vitro* antiviral activity have been detected. Isoprinosine has consequently been viewed principally as an immunomodulator due to its capacity to increase NK cell cytotoxicity, restore interleukin-2 production and augment the anti-tumour activity of interferon in animal models (for review see Campoli-Richards et al. 1986; Gordon & Ginsberg 1988).

Isoprinosine has increased the mitogen-induced biastogenesis in PBL from patients with AIDS or

LAS, and stimulated *in vitro* interleukin-2 production in PBLs from AIDS patients (Tsang et al. 1984).

In early studies isoprinosine demonstrated weak antiviral activity. Recently it has been shown that isoprinosine partially inhibits HIV at a dose of 200 μg/ml (Pompidou et al. 1985). *In vitro* it does not seem to interfere with the action of zidovudine (Schinazi et al. 1988).

5.1.2 Clinical Pharmacological Properties

The half-life of the inosine moiety is 50 minutes following an oral dose, and the major excretion product is uric acid. The other components are oxidised or glucuronidated and excreted. Coadministration of isoprinosine with zidovudine seemed to elevate the serum concentrations of the latter drug in 8 AIDS patients; however, the standard deviation was large. It was speculated that this was probably due to interference with glucuronidation of zidovudine (de Simone et al. 1988).

5.1.3 Therapeutic Trials

Isoprinosine was administered in a placebo-controlled double-blind trial in patients at risk for AIDS at doses of 1 or 3 g/day for 28 days. An augmentation was noted in natural killer (NK) cell activity as well as total number of T-cells, and T-helper cells at 3 g/day (Wallace & Bekesi 1986).

Due to the laboratory results, preliminary clinical findings, lack of toxicity and the self-administration of isoprinosine that was taking place in the US, a phase II clinical trial was carried out involving 21 Danish and Swedish clinics. 866 mostly asymptomatic HIV-infected patients were randomly assigned to isoprinosine 1g every 3 hours or placebo matching in taste and appearance in a 24-week double-blind trial. 17 and 16 of those assigned isoprinosine or placebo, respectively, were excluded from analysis due to a duration of treatment of less than 2 weeks (26) or violation of major inclusion criteria (2 placebo patients with AIDS at entry, 5 without serological confirmation of HIV infection). Those remaining were analysed according to the intention-to-treat principle. Of the 412 who received isoprinosine, 2 developed CDC-defined AIDS (PCP), while 17 of the 419 placebo recipients met the same criteria (10 PCP, 3 Kaposi's sarcoma, 1 oesophageal candidiasis, 1 herpes simplex > 4 weeks, 1 wasting, 1 encephalopathy; $p < 0.001$). A similar, but non-significant, trend was noted in patients in all the 3 substrata with < 200, 200 to 500 and > 500 CD4+ cells. There was no corresponding difference in minor end-points such as thrush, hairy leucoplakia or herpes zoster. No significant difference in the decline of CD4+ cell was observed (Court Pedersen, Scandinavian Isoprinosine Study Group, personal communication.)

5.1.4 Adverse Effects

No serious adverse effects have been observed. Since inosine is broken down to uric acid, gout or renal dysfunction are relative contraindications. Although uric acid levels increased by week 2 and remained elevated in the phase II trial, no significant adverse effects were seen.

5.2 IMREG-1 (Clinical)

IMREG-1 has been discussed for several years in conjunction with HIV infection (for review see Gottlieb et al. 1987). Four communications were presented at the Stockholm conference. IMREG-1 has now been shown to consist of at least 2 peptides, Tyr-Gly and Tyr-Gly-Gly, that are the same as the N-terminal sequences of enkephalins. The peptides are claimed to enhance interleukin-2, interferon-γ and macrophage or leucocyte inhibiting factors (MIF, LIF) *in vitro* (Abstr. 3049). IMREG-1 enhanced and/or modified the expression of interleukin-2 receptors on CD4+ cells from HIV-infected patients *in vitro* (Abstr. 3052). No enhancement of HIV-replication *in vitro* was observed (Abstr. 3051). A placebo-controlled trial in 150 patients with ARC or Kaposi's sarcoma has just been concluded (Abstr. 3048).

5.3 Cimetidine (Clinical)

Early on the H_2-receptor antagonist cimetidine was reported anecdotally to influence HIV infection. Administration of 1200 mg/day for 5 months

Table III. Reported drug interactions [data from Hirsch 1988; Öberg 1988; and abstracts of the Fourth International Conference on AIDS, Stockholm, 1988 as cited in the text (section 6)]

	ZVD	ddA	ddC	PFA	CD4	Sur
Acyclovir	S					S?
Castanospermine	S				S	
Ribavirin	A	S	A	S		
Interferon	S		S	S		
Ampligen	S/A					
GM-CSF	S					
PFA	S					
ddC	+	+				
ddA	+					
Avarol	S					
Soluble CD4	S					
Dextran sulphate	S					

Abbreviations and symbols: ZVD = zidovudine; ddA = dideoxyadenosine; ddC = dideoxycytidine; PFA = foscarnet; Sur = suramin; GM-CSF = granulocyte-macrophage colony-stimulating factor; S = synergy; A = antagonism; + signifies combinations positive.

with an interruption of 3 weeks after 3 months in 33 ARC patients has now been reported to favourably influence CD4 counts, cutaneous delayed hypersensitivity and clinical parameters (weight, fever, performance status) [Brockmeyer et al. 1988].

6. Combinations of Drugs

To reduce toxicity and increase efficacy there is a search for drugs that have synergistic therapeutic effects with different toxicity spectra (Hirsch 1988; table III). Most interest has naturally focused on combinations with zidovudine. Reports of synergism have been published or reported for interferon-α (Hartshorn et al. 1987; Abstr. 3133, 3625, 3626, 3627, 3629, 3630, 3631), acyclovir (Mitsuya & Broder 1987; Abstr. 3134, 3135, 3136, 3137, 3139), ampligen (Mitchell et al. 1987; Abstr. 3140, 3613), granulocyte-macrophage colony stimulating factor (Hammer & Gillis 1976), castanospermine (Johnson et al. 1989; Abstr. 3618), amphotericin methyl ester (Abstr. 3128), 9-(2-phosphonylmethoxyethyl)adenine (PMEA) [Abstr. 3148], dideoxycytidine (Abstr. 3149, 3151, 3150, 3620), avarol (Schröder et al. 1988), dextran sulphate (Ueno & Kuno 1987) and foscarnet (Abstr. 3624). Antagonism has been reported for ribavirin (Baba et al. 1987; Vogt et al. 1987) and ampligen (Abstr. 3141). Dideoxycytidine has been shown to be synergistic with interferon-α (Vogt et al. 1988), oligocytidine with dideoxyadenosine (Matsukura et al. 1987), and castanospermine with soluble recombinant CD4 (Abstr. 3618). Although many of the above studies claim synergy, strict criteria are rarely applied (Chou & Talay 1987).

7. Conclusion

With the enormous expansion of possible therapeutic substances it may seem moot to dwell on methods of laboratory drug evaluation. However, as can be seen above a wide array of methods are indeed used to demonstrate anti-retroviral or anti-HIV effects *in vitro* and it is difficult to judge the relative merits of different compounds. The necessity of addressing this will become more apparent when an array of specially synthesised similar compounds are to be compared, i.e. nucleoside analogues. Although the standardisation of the available preclinical laboratory methods is less than satisfactory, the major bottleneck does not seem to lie in laboratory capacity or ingenuity. If there are deficiencies in our methods of measuring anti-retroviral effects, this is even more true of the evaluation of toxicity. The simian immunodeficiency virus/macaque model may offer some hope. There are severe shortcomings in the clinical trials. Hope, desperation, and insufficient background information and critical planning have often lead to studies with ill-defined end-points, and too few patients to address the stated hypothesis.

Criteria need to be adopted setting out the properties that merit a drug entering phase I studies, and adherence to scientifically sound protocols is necessary when studying the effects of these drugs in the first human volunteers. It is important to enrol a homogeneous group of subjects and not settle for the 'x' ARC and 'y' AIDS patients that are immediately available. There are many arguments in favour of offering these new drugs to patients

Table IV. Principles for treatment development in HIV disease

Stage of HIV disease	Efficacy	Toxicity
Asymptomatic, no laboratory abnormalities	Better than isoprinosine	Atoxic
Asymptomatic, CD4+ cells 200-400	Better than isoprinosine or zidovudine	Low
ARC or AIDS	1. Better than zidovudine	Same/less than zidovudine
	2. Same as zidovudine	Better TI or different toxicity than zidovudine
	3. Combination with zidovudine (ideal – synergy)	Different toxicity than zidovudine
AIDS with bone marrow suppression	Same as zidovudine	Different toxicity than zidovudine

Abbreviation: TI = therapeutic index.

who only fulfill the minimal criteria for zidovudine therapy. In these patients there is some motive for intervention. They are not critically ill and may later benefit from zidovudine, but may, in the case of low toxicity and a subjective sense of effect, want to continue on the study drug, adding valuable information on extended use of such drugs.

A more analytical approach to therapeutic development now seems possible. There are a number of clinical stages in HIV infection that now require separate consideration in terms of drug development (table IV). Two things are obvious from table IV. First, that zidovudine is the standard drug in symptomatic HIV disease against which all other therapies should be evaluated. In most symptomatic stages it is no longer ethical to pursue placebo-controlled double-blind studies. Secondly, the primary objective of most studies of promising drugs should be to document carefully any toxicity prior to evaluation of which category of patients is appropriate for trials with that particular drug. Thus, for patients without symptoms or laboratory signs of their infection, only very low toxicity is permissible in view of the number of patient-months necessary to document clinical efficacy and for the proposed clinical use. Similarly, for asymptomatic individuals with laboratory signs of immunological damage, only low toxicity is permissible, and an expected effect superior to zidovudine required. For patients who are now prescribed zidovudine several options require study; those that focus on a better efficacy than zidovudine, better therapeutic index, or the possibility of combination with zidovudine. It is possible that it will be desirable to combine drugs belonging to the different groups in table II. Lastly, there is an immediate need for drugs with a different toxicity profile to zidovudine, for those patients who can not tolerate this drug.

The latter studies will be further complicated in evaluation of both toxicity and efficacy, since the effects and end-points will be influenced by other concomitant drugs such as aerosolised pentamidine in primary and secondary prophylaxis, and so on.

In various countries different bodies have formed the necessary collaborative networks to tackle these very laborious trial programmes, for example in the US the AIDS Treatment Evaluation Units (ATEU), in Britain the Medical Research Council (MRC), in France CITRAS, and in Scandinavia the Nordic Medical Research Councils (NMRC).

However, considering the multitude of patients, investigators and possible drugs, such functions need to be expanded and generously supported. The slow nature of HIV infection and the heavy clinical burden it carries places emphasis on the need for many centres to collaborate to assemble large enough homogeneous groups of patients under conditions where it is possible to uphold high data quality. Lack of funding contributes to a difficult state of affairs and leads to tensions between clinicians, pharmaceutical companies and funding agencies.

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