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Summary of the III International Consensus Symposium on Combined Antiviral Therapy

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1. Introduction

During the past 2 years the efficacy of combination antiviral therapy has been demonstrated in the treatment of individuals with a human immunodeficiency virus (HIV) infection. Several factors may influence the improved efficacy of combination antiviral treatments as compared to monotherapy. These include: synergistic or additive activity, penetration of combined drugs into a more diverse range of body compartments, prevention of antiviral resistance and targeting of different steps in the viral replication cycle. Moreover, combination therapies, if synergistic, allow for a reduction of potentially toxic components thus, increasing tolerance and hopefully, compliance. On the other hand, therapeutic options for several virus infections are limited. Therefore, research efforts remain aimed at improvement of antiviral therapeutic options by designing and developing new chemotherapeutic agents.

In 1995 and 1996, consensus symposia on combination antiviral therapy were organized to report developments in the field for a wide range of viral diseases and in order to formulate consensus statements. (de Jong et al., 1996, 1997). This

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review provides a summary of the third consensus symposium[1] convened by The Macrae Group (New York) on combination therapy on September 7–9, 1997.

2. Current insights on combination antiviral therapy

2.1. Emerging viruses

With developing molecular technologies, including polymerase chain reaction (PCR), the number of recognized human virus infections increased during the past decade. Several viruses have been identified on the basis of genome sequence even though in vitro isolation and serotyping has not yet been successful. Examples of such viruses are the hepatitis E and G, Bayou virus and the human herpesvirus-8. In addition, ecological factors may influence the emergence of new virus diseases. For example, the resurgence of Dengue in Central and South America could have resulted from ineffective mosquito control. Another example is the deforestation in South America that has been associated with virus diseases transmitted via arthropods (Mayaro virus) or rodents (South American hemorrhagic fever arena viruses such as Machupo virus, the Guanarito virus and the Sabia virus and Hanta viruses like the Sin Nombre virus).

Currently, no effective therapy is available for American hantaviruses or arena viruses, although some success using ribavirin against Asian hantavirus infections (hemorrhagic fever with renal syndrome) has been reported (Huggins et al., 1991). Obviously, more research is required to design specific antiviral agents against Hanta and human arena viruses (Mahy, 1997).

2.2. Filoviruses

Marburg (discovered in 1968) and Ebola (discovered in 1976) viruses have been the cause of several African epidemics with a high mortality rate. The re-emergence of the Ebola virus after 19 years in Kikwit, New Democratic Republic of Congo, Africa (1995) is not understood. The reservoir has not been identified and phylogenetic analysis indicates that an Ebola isolate from the recent outbreak is virtually identical to the virus that caused a similar epidemic in Yambuku, New Democratic Republic of Congo, almost 20 years earlier (Sanchez et al., 1996).

Filoviruses, like other hemorrhagic fever viruses, cause a clinical syndrome characterized by a short incubation period (4–10 days) and high mortality rates. Currently, there is no known specific treatment for filovirus infections and the screening of compounds is difficult because of the stringent biosafety requirements (level 4) for filovirus research. For this reason, the US Army Medical Research Institute uses other viruses with a genetic and molecular organization similar to the filoviruses as a screening model to predict antiviral activity. Using paramyxovirus, a virus phylogenetically related to the filoviruses, several classes of candidate antiviral compounds have been identified, including RNA dependent RNA

[1] Members of the consensus panel were C.A.B. Boucher, Utrecht University, Netherlands; D.A. Cooper, University of New South Wales, Australia; G.J. Galasso, National Foundation for Biomedical Research, USA; F. Hayden, University of Virginia, USA; D.D. Richman, University of California, San Diego, USA; S. Spector, University of California, San Diego, USA; H. Thomas, St Mary's Hospital, London, UK; R. Whitley, University of Alabama, Birmingham, USA. The presenters at the symposium were J. Huggins, US Army Medical Research Institute of Infectious Diseases, USA; R. Whitley, University of Alabama, Birmingham; S. Spector, University of California, San Diego, USA; P. Griffiths, Royal Free Hospital School of Medicine, UK; F. Hayden, University of Virginia, USA; W. Bonnez, University of Rochester, New York, USA; J. Brouwer, Rotterdam University Hospital, Netherlands; D.D. Richman, University of California, San Diego, USA; S. Vella, Instituto Superiore di Sanita, Rome, Italy; G. Moyle, Chelsea and Westminster Hospital, London, UK; A. Haase, University of Minnesota, USA; B. Kerr, Agouron Pharmaceuticals Inc, USA; N. Cammack, Roche Discovery Welwyn, UK; A. Mclean, Oxford University, UK; D.A. Cooper, University of New South Wales, Australia; Y. Benhamou, Hopital de la Pitie-Salpetriere, Paris, France.

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polymerase inhibitors and *S*-adenosyl-homocysteine-hydrolase inhibitors. Prophylaxis against Ebola virus with this latter class of compounds induces a 10^5–10^6-fold reduction in viral replication of the Ebola virus. Recently a new model was created using immunocompetent Balb-C mice infected with a mouse-adapted-Ebola-virus strain. The resulting pathobiology is similar to that witnessed in humans. In this model, prophylaxis with *S*-adenosyl-homocysteine-hydrolase inhibitors appeared promising. The efficacy of these compounds in a primate model was poor due to unfavorable pharmacokinetics. Presently, pharmacokinetic and dose ranging studies with modified *S*-adenosyl-homocysteine-hydrolase inhibitors are in progress.

In conclusion, the results from this new frontier of research are encouraging and may pave the road to an effective therapy against filoviruses.

2.3. Herpes simplex virus

For the treatment of herpes simplex virus (HSV) infections several drugs are available: acyclovir, valaciclovir, penciclovir, famciclovir, foscarnet and cidofovir. Valaciclovir is the L-valyl ester of acyclovir. The oral bioavailability of this acyclovir prodrug is three to five times higher then with the parent drug (Weller et al., 1993; Soul-Lawton et al., 1995). The advantage of valaciclovir as compared to acyclovir is a lower dosing frequency while the clinical efficacy of both drugs for the treatment of mucocutaneous HSV manifestations is comparable. Penciclovir and its prodrug, famciclovir exhibit a similar antiviral effect as acyclovir. Famciclovir, the diacetate ester of penciclovir, was developed to improve oral absorption.

Resistance to acyclovir has been reported almost exclusively in immunocompromised hosts and particularly in patients with AIDS who have less than 100 CD4+ lymphocytes/μl, receiving chronic acyclovir treatment. Both acyclovir and penciclovir require intracellular phosphorylation to the active triphosphate derivatives. The first step, the phosphorylation to the monophosphate derivative, is catalyzed by the viral encoded enzyme thymidine kinase. Resistance against acyclovir and penciclovir is most frequently caused by mutations in this enzyme, resulting in decreased efficacy of the monophosphorylation step (Pottage and Kessler, 1995). Since both foscarnet, a pyrophososphate analogue acting directly on viral DNA polymerase and cidofovir, a monophosphorylated cytosine analogue, do not require viral thymidine kinase for activation both drugs may be useful for therapy of acyclovir resistant HSV strains.

In immunocompetent adults, the clinical manifestations of primary or recurrent mucocutaneous HSV infections are self-limited but can be treated effectively with antiviral monotherapy. Viral drug resistance is a rare event in these patients. Therefore, combination antiviral therapy is not indicated for immunocompetent adults with a non-life threatening HSV disease.

On the other hand, two serious clinical manifestations of HSV are herpes neonatalis and HSV encephalitis, associated with high morbidity and mortality rates. Current monotherapies for these manifestations of HSV are unsatisfactory. In approximately 5% of the babies with neonatal herpes infections of the CNS, the cerebrospinal fluid (CSF) does not become HSV-PCR negative during either acyclovir or vidarabine therapy for 10 days, or CSF becomes HSV-PCR positive after 10 days of treatment. In the same study it was shown that 95% of patients positive for HSV DNA in the CSF after 10 days of treatment experienced significant morbidity and mortality (Kimberlin et al., 1996). Currently, new trials are investigating the efficacy of high dose acyclovir therapy and a prolonged treatment period to assess mortality and long term neurologic outcome in cases of neonatal herpes.

Finally, combination antiviral treatment of both HSV encephalitis and herpes neonatalis warrants evaluation.

2.4. Varicella zoster virus

Three drugs with comparable efficacy are available for the treatment of herpes zoster: acyclovir, valaciclovir and famciclovir. However, in one study it was shown that valaciclovir significantly accelerated the resolution of herpes zoster-associ-

ated pain and also significantly reduced the duration of post-herpetic neuralgia compared with acyclovir (Beutner et al., 1995).

The use of sorivudine, another effective agent against herpes zoster, has not been approved by the US Food and Drug Administration (FDA) due to potentially lethal drug interactions with 5-fluoro-uracil (Okuda et al., 1997).

The efficacy of concomitant administration of acyclovir and prednisone has been investigated (Whitley et al., 1996). In a placebo controlled trial with 208 immunocompetent herpes zoster patients, a combination of acyclovir and prednisone improved the quality of life endpoints (time to return to uninterrupted sleep, time to return to 100% usual daily activity and time for cessation of analgesic therapy). New trials may be performed in order to test whether similar results of quality of life improvements can be achieved administering high doses of valaciclovir or famciclovir without subjecting patients to potential side effects of corticosteroids.

In immunocompromised patients, herpes zoster can be more severe, involving multiple dermatomes, a large surface area of the skin and dissemination. Combination antiviral treatment may be a useful therapy for such patients. However, no clinical or laboratory data support this hypothesis.

2.5. Cytomegalovirus

The clinical manifestations of cytomegalovirus (CMV) infections are diverse. In the immunocompetent host CMV infections generally are asymptomatic, although some patients experience a mononucleosis like syndrome. On the other hand, congenital CMV infections may result in the classic cytomomegalic inclusion disease characterized by hepatosplenomegaly, microcephaly and retinitis. The prognosis of symptomatic congenital CMV is poor with a 10% mortality rate, mental retardation and sensorineural hearing loss. Perinatal acquired CMV infection is associated with interstitial pneumonitis (Boppana et al., 1997).

Immunocompromised patients may develop life threatening CMV disease. Patients at greatest risk are solid organ transplant recipients, allogeneic bone marrow transplant recipients and HIV infected individuals with fewer than 50 CD4+ lymphocytes/mm^3. The most common clinical manifestations are CMV pneumonitis in transplant recipients and CMV retinitis in HIV-infected patients. CMV infection in the immunocompromised host may also involve the central nervous system and the gastrointestinal tract (Van der Meer et al., 1996).

In immunocompromised hosts, the optimal approach to CMV infections would be a reconstitution of the immune system. In the treatment of HIV infected individuals, highly active antiretroviral therapy resulted in considerable immune reconstitution, as measured by an increase in the peripheral CD4+ T-cell counts. However, whereas CMV retinitis in HIV infected individuals almost exclusively occurred in patients with <50 CD4 cells/mm^3, recently five HIV infected patients on antiretroviral therapy developed CMV retinitis with CD4 counts >195 cells/mm^3 (Jacobson et al., 1997). These patients started antiretroviral therapy 4–7 weeks before the diagnosis of CMV retinitis and all had a CD4 count <85 cells/mm^3 before initiation of the antiretroviral treatment. However, similar observations have only been made on most rare occasions since this report. Thus, it suggests this original group of patients may have had a low level disease not recognized by their health-care providers.

Three drugs are licensed for the treatment of CMV disease: ganciclovir, cidofovir and foscarnet. These drugs all inhibit CMV DNA polymerase (Van der Meer et al., 1996). Intravenous induction and maintenance therapy with each of the three agents as monotherapy delays the median time to progression of CMV retinitis. Oral administration of ganciclovir (3 g/day) as maintenance therapy for CMV retinitis has been shown to have similar results to those of intravenous use of ganciclovir (5 mg/kg), although some studies reported a slightly shorter time to progression (Oral Ganciclovir European and Australian Cooperative Study Group, 1995). High doses are currently recommended.

Chronic maintenance therapy postpones but usually does not prevent relapses of CMV retini-

tis. Two factors may contribute to the failure of maintenance therapy: suboptimal intraocular drug levels during systemic therapy and the development of viral resistance.

To overcome the first problem, CMV retinitis may be treated by local therapies such as intraocular injections of ganciclovir and foscarnet or with an intraocular sustained-release ganciclovir implant. However, local therapies are not effective in controlling disease of the contralateral eye or extraocular CMV manifestations. In patients with CMV retinitis receiving an intraocular device, it was shown that the risk of developing CMV retinitis in the contralateral eye was approximately 50 and 30% of the patients who developed visceral CMV disease (Martin et al., 1994; Duker et al., 1995; Marx et al., 1996; Charles and Steiner, 1996). A combination of local and systemic therapies may be required to curb both the ocular and extraocular manifestations of CMV infections.

Drug resistance is a major concern and is associated with increased CMV viremia (Boivin et al., 1996). Resistance to the three available drugs has been reported. Moreover, viral strains cross-resistant to the three available drugs have also been isolated from selected patients (Lurain et al., 1994; Baldanti et al., 1996; Van der Meer et al., 1996; Chou et al., 1997; Grossi and Baldanti, 1997; Smith et al., 1997). Two genes of CMV have been found to harbor the resistance mutations: UL97, encoding a viral phosphokinase and UL54, encoding DNA polymerase. Resistance to ganciclovir is conferred by point mutations in the UL97 gene. Ganciclovir is phosphorylated to an active nucleotide in CMV infected cells. The first phosphorylation step of ganciclovir is catalyzed by the UL97 encoded phosphokinase. Resistance to ganciclovir can also be conferred by mutations in the viral DNA polymerase (UL54). Amino acid mutations conferring resistance to foscarnet and cidofovir are exclusively located in the UL54 gene. Since foscarnet does not require phosphorylation and cidofovir is already monophosphorylated. Low-level ganciclovir-resistant isolates ($8\ \mu M < ID_{50} < 30\ \mu M$) were associated with UL97 alterations following short periods of ganciclovir treatment. High-level ganciclovir-resistant isolates ($ID_{50} > 30\ \mu M$) were associated with both UL97 and UL54 alterations and were cultured after extended ganciclovir therapy. The 'high level' ganciclovir resistant isolates were cross-resistant to cidofovir (Smith et al., 1997). The emergence of a virus resistant to ganciclovir seems to select for strains with a diminished susceptibility to foscarnet, suggesting cross resistance.

As a result of suboptimal intra-ocular drug levels and the emergence of resistance with systemic therapy against CMV retinitis, combination antiviral treatment improves efficacy of treatment. Moreover, combination therapy may prevent the emergence of resistant viruses. On the other hand, longer infusion times and enhanced toxicity can be problematic. In a multi-center, randomized, controlled trial, the efficacy of ganciclovir-foscarnet therapy was investigated in 279 AIDS patients with either persistently active or relapsed CMV retinitis. Patients were randomized to therapy (induction and maintenance) with either foscarnet or ganciclovir or a combination of both drugs. The mortality rate was similar among the three groups. Comparison of retinitis progression revealed that combination therapy was the most effective regimen for controlling CMV retinitis. The median times to progression of retinitis were 1.3 months in the foscarnet group, 2.0 months in the ganciclovir group and 4.3 months in the combination therapy group ($P < 0.001$). However, no difference in the visual acuity outcomes was observed. Side effects were similar among the three treatment groups, although combination therapy was associated with the greatest negative impact of treatment on quality-of-life measures (Studies of Ocular Complications of AIDS Research Group, 1996). Currently, the efficacy of combined treatment with foscarnet and ganciclovir is being investigated in the ACTG 228 trial.

In vitro studies have shown that the combination of ganciclovir and human monoclonal antibodies (MSL109) has an additive inhibitory effect on the replication of CMV in human fibroblasts as compared to ganciclovir alone. The combination of foscarnet and MSL109 was found to be synergistic in vitro (Pollard, 1996). A placebo-controlled study comparing the efficacy of ganciclovir alone and in combination with monoclonal

antibody (MSL109) showed increased survival in patients treated with the combination (unpublished data).

Combined administration of antiviral agents may also be indicated since a beneficial effect of monotherapy with ganciclovir, foscarnet or cidofovir on the treatment of CMV colitis, CMV encephalitis and CMV pneumonitis has not been clearly established. Combined treatment with high dose intravenous immunoglobulin and ganciclovir for CMV pneumonitis in bone marrow transplant recipients has resulted in conflicting results. Whereas in two studies an improved survival was observed (Emanuel et al., 1988; Reed et al., 1988), another study reported that this combination has limited value on the outcome of an established cytomegalovirus pneumonia after marrow transplantation (Verdonck et al., 1989). Nevertheless, in some areas, combination of high dose intravenous immunoglobulin and ganciclovir is the standard of care for CMV pneumonitis in bone marrow transplant recipients.

Due to the limitations of the currently available arsenal of antiviral drugs against CMV infection, new drugs are being developed and evaluated to improve therapeutic options. Lobucavir, adefovir dipivoxil and 1263W94 are currently in clinical investigation. The latter drug, a benzimidazole ribonucleoside, is especially promising because it targets a different site in CMV replication, inhibiting a terminase which clips the UL89 endonuclease before packaging of the virion. Another new agent under investigation is ISIS2922, an antisense nucleotide sequence complementary to CMV messenger RNA. As a result of its instability it can only be used for intra-ocular injections. The preliminary results with these new drugs are encouraging and their use, either as monotherapy or in combination with the presently available agents, may be of great use in the control of CMV disease.

2.6. Kaposi sarcoma herpes virus/human herpesvirus-8

Kaposi's sarcoma (KS) is considered to be an angioproliferative disease associated with human herpesvirus-8 (HHV-8) infection. HHV8 is also known as the KS herpes virus (KSHV)(Chang et al., 1994). The precise pathophysiology of the KS lesion remains unclear. In situ hybridization studies have demonstrated the presence of HHV8 DNA in endothelial cells and spindle cells of KS lesions (Staskus et al., 1997; Sturzl et al., 1997). Because of its homology to the Epstein–Barr virus it was initially believed that HHV-8 was capable of cell transformation. However, it was shown that KS lesions are a polyclonal cell proliferation (Delabesse et al., 1997). A clue to the pathophysiologic mechanism of KS lesions might be HHV-8 genes encoding homologs to cellular cytokines such as complement-binding proteins, interleukin 6 (IL-6), dihydrofolate reductase, bcl-2, interferon regulatory factors, IL-8 receptor, neural cell adhesion molecule-like adhesin and a D-type cyclin suggesting that the virus is capable of controlling the cellular micro-environment (Russo et al., 1996).

HHV-8 sequences have been detected in all forms of Kaposi sarcoma as well as in the blood and in saliva of patients with KS lesions (Koelle et al., 1997). Longitudinal studies indicated that the presence of HHV-8 in the blood is predictive of the subsequent development of KS lesions (Lefrere et al., 1996; Lennette et al., 1996). Whether HHV-8 is present in semen is a controversial issue and needs to be established. In addition to KS lesions, HHV-8 sequences have also been detected in body cavity based lymphoma, multicentric Cattleman's disease, angioimmunoblastic lymphadenopathy and pleural effusion lymphoma. A reported association with multiple myeloma is yet to be confirmed.

The route of transmission of HHV-8 remains to be established. Epidemiologic studies in HIV infected individuals with KS lesions suggest sexual transmission (Gao et al., 1996; Kedes et al., 1996; Lennette et al., 1996; Monini et al., 1996). Furthermore, in the saliva of nine persons with past or current KS lesions, infectious HHV-8 was present, suggesting the possibility of oral transmission (Vieira et al., 1997).

An understanding of the role that HHV-8 plays in the pathogenesis of KS and other HHV-8 associated clinical conditions is crucial for the development of better methods for prevention and

therapy. The pattern of HHV-8 gene expression suggests that most cells in KS lesions are latently infected. Therefore, the utility of antiviral therapy has to be established in patients that already have KS lesions. Uncontrolled studies suggest a possible role for foscarnet in the prevention and remission of KS lesions. Using a culture system with BCLB-1 cells derived from a peripheral effusion lymphoma (Renne et al., 1996), the antiviral activity of several antiviral drugs was tested against HHV-8. In vitro HHV-8 replication was inhibited by foscarnet ($IC_{50} = 80–100$ μM), ganciclovir ($IC_{50} = 2.7–4$ μM) and cidofovir ($IC_{50} = 0.5–1$ μM) but insensitive to acyclovir (Kedes and Ganem, 1997). Prospective, placebo controlled trials are currently being performed investigating the efficacy of foscarnet for the treatment of KS.

Finally, highly active antiretroviral therapy (HAART), containing two reverse transcriptase inhibitors and one protease inhibitor, appears to have a beneficial effect on KS lesions. However, the long term effect of HAART on KS lesions requires evaluation. The beneficial effect of HAART on KS lesions might be due to reconstitution of the cellular immune response. Additionally, since the regulatory tat protein of HIV has been shown to drive proliferation of KS spindle cells in vitro (Barillari et al., 1993; Albini et al., 1995), HAART may reduce tat expression thus indirectly down regulating HHV-8 replication.

2.7. Hepatitis B virus

Acute infection with hepatitis B virus (HBV) is generally asymptomatic but may manifest as jaundice. Subsequent to acute illness, HBV infection may become chronic in those patients that are unable to clear the virus. These patients become chronic carriers of HBV. World-wide, the number of HBV carriers approximates 600 million. After an asymptomatic stage of generally 20 years, chronic HBV infections may lead to serious clinical complications, including cirrhosis and hepatocellular carcinoma. The risk of developing chronic HBV infection after the acute illness depends on the age and immune status at the time of acute infection, among other factors. More than 90% of the neonatal HBV infections become chronic, in contrast to less than 5% of adult acquired HBV infections. Immunocompromised patients have a 20–40% risk of developing chronic hepatitis. Polymorphisms in the MHC class II alleles have also been shown to influence the clinical outcome of HBV infections (Thursz et al., 1995; Thursz, 1997).

HBV related liver damage is mainly due to immune mediated mechanisms directed against infected hepatocytes. Since no effective therapy exists for the acute phase of HBV infections, the goal of treatment is clearance of the virus in patients with chronic HBV infections by virus suppression or boosting the immune response.

Currently, the only approved drug for the treatment of HBV infections is interferon-α (IFN-α). The mechanism of action of IFN-α is unknown but the immunoregulatory and anti-inflammatory properties, as well as the antiviral activity of this cytokine, may play an important role. In patients infected at an adult age with HBV, 15–40% had a sustained response to administration of IFN-α. A poor response rate has been observed in patients with minimal hepatitis often seen after perinatal infection and in immunocompromised individuals. Factors predicting a sustained response to IFN-α therapy are: low serum HBV-DNA concentrations at the onset of treatment, high alanine aminotransferase (ALT) serum levels, adult acquired HBV infection and histologic signs of active inflammation on a liver biopsy and female gender (Anderson et al., 1986; Boivin et al., 1989; Brook et al., 1989).

Administration of IL-12 may be beneficial for patients with chronic HBV infection. IL-12 is an important cytokine against intracellular pathogens. This cytokine promotes cell mediated cytotoxicity and development of T helper cells of Th1 type, which produce IFN-γ and IL- 2. An increase of bioactive IL-12 and Th1 cytokines has been observed in patients who cleared HBV and seroconverted to anti-hepatitis Be (Rossol et al., 1997). Clinical trials investigating the beneficial effect of IL-12 in chronic hepatitis are currently ongoing. Since the Th2 cytokines IL-4 and Il-10 have been shown to inhibit the Th1 mediated responses the effect of down regulating or blocking IL-4 and IL-10 in addition to IL-12 therapy is

currently being explored in a hepadna virus woodchuck model.

Lamivudine, 3′-thiacytidine (3TC), a nucleoside analogue and reverse transcriptase inhibitor with anti-HIV activity, causes a rapid decline in HBV DNA but viremia is observed when therapy is discontinued (Dienstag et al., 1995; Benhamou et al., 1996; Nevens et al., 1997). In a 6 month dose ranging study comprising 51 patients, lamivudine was well tolerated and induced sustained suppression of HBV replication. However, two patients developed temporary hepatic decompensation when treatment was discontinued (Nevens et al., 1997). The data from this study support investigation of lamivudine treatment of longer duration at a dosage of 100 mg once daily. The rapid rebound of viremia after discontinuation of lamivudine treatment can be explained by the stability and persistence of the covalently closed circular intermediate of HBV DNA (cccDNA), which may serve as a template for transcription when treatment is withdrawn. Only lysis of infected cells will eliminate the ccc HBV DNA intermediate, eventually leading to eradication of the virus.

Famciclovir, the well-absorbed oral form of penciclovir, exhibits an inhibitory effect on HBV replication in animal models (duck HBV) and humans. In a double-blind controlled pilot study of famciclovir a fall of $>90\%$ in HBV DNA levels was noted in six of 11 evaluable patients treated with a 10 day course of oral famciclovir (Main et al., 1996). Further studies with more prolonged famciclovir therapy for chronic hepatitis B infection are ongoing.

Reinfection of the graft after liver transplantation secondary to chronic HBV infection is a major problem. Recurrent hepatitis is observed in virtually all HBV DNA-positive patients prior to transplantation. Famciclovir and lamivudine have been administered as a maintenance therapy. The initial experience with these agents in liver transplant recipients has been promising. A number of studies are currently underway to determine whether these drugs are able to prevent post-transplant HBV recurrence.

Development of drug-resistant viral mutants during monotherapy with lamivudine and famciclovir has been observed (Ling et al., 1996; Tipples et al., 1996; Bartholomew et al., 1997). Reduction in susceptibility to these agents appears to be conferred by specific mutations in the HBV polymerase gene and is associated with viral breakthrough during treatment. Drug resistance may be prevented by combination treatment. However, further studies are required to investigate more intensively the efficacy of lamivudine and famciclovir monotherapy and to assess whether combination treatment, either with two antiviral agents or with antiviral and immunoregulatory drugs, is more effective.

Co-infection of HIV and HBV is frequently observed. To date, treatment of HBV infections in HIV infected individuals has not been studied intensively. Several studies involving small numbers of patients showed that IFN-α therapy in HIV/HBV co-infected individuals results in a poor therapeutic response (Marcellin et al., 1993; Pol et al., 1994; Wong et al., 1995; Zylberberg et al., 1996). Currently, studies evaluating the effect of lamivudine on HBV infection in HIV infected individuals are ongoing (Benhamou et al., 1996).

Finally, studies of viral dynamics indicate that the half life of the HBV virion is less than 1 day but the half life of infected cells is between 10 and 100 days (Nowak et al., 1996; Zeuzem et al., 1997) The half life of the infected cells is inversely related to the peak ALT, suggesting that in those patients with the most active inflammatory liver disease the half life of the infected cells is shortest (10 days). In patients with active disease, eradication of HBV infected cells would require approximately 1 year of therapy, in the case of complete suppression of viral replication. However, for those with inactive disease the half life of HBV infected cells is in the order of 100 days. Eradication of HBV infected cells in those patients is estimated to require treatment for many years. Theoretically, patients with minimal to moderate inflammatory activity may benefit from combined administration of viral suppressive agents and aspecific stimulants. These non-specific immunostimulants, like IL-12, might be combined with the administration of various viral antigens to create an antigen specific stimulation of the immune response.

2.8. Hepatitis C

The prevalence of hepatitis C (HCV) in the Western World is 0.2–1.5%. Following acute infection as many as 85% of the patients become chronically infected. HCV infections are generally asymptomatic during the first two to three decades but may then be followed by cirrhosis, liver decompensation or primary liver cancer. In general, chronic HCV infection leads to cirrhosis in at least 20% of patients within two decades after infection. The rate of progression is highly variable. In a large study assessing the natural history of HCV infections, three independent host factors were associated with an increased risk of developing cirrhosis: age of infection >40 years, daily alcohol consumption of 50 g or more and male sex (Poynard et al., 1997). In the same study, the median estimated duration of infection before progression to cirrhosis was 30 years, ranging from 13 years in men infected after the age of 40 to 42 years in women who did not drink alcohol and were infected before the age of 40. Having all three risk factors was associated with a 30% chance of developing cirrhosis in 30 years while no cirrhosis develops in 50 years in patients having none of the risk factors. In another study, histologic evaluation demonstrated that the degree of fibrosis present in a liver biopsy predicts the chance of developing cirrhosis in the ensuing decade. Low grade fibrosis was associated with slow progression whereas all patients with septal fibrosis and incomplete nodularity developed cirrhosis within 10 years (Yano et al., 1996). Because of the variability in clinical outcome of chronic HCV infections, it is important to identify patients that may benefit from treatment.

According to the British management algorithm (Booth et al., 1995) and the 1997 NIH consensus statement on the management of HCV, patients eligible for interferon treatment are those at greatest risk for progression to cirrhosis. These patients are characterized by elevated ALT levels, positive serum HCV RNA and on the liver biopsy, fibrosis, moderate inflammation and necrosis without cirrhosis. A liver biopsy is recommended before initiation of therapy to stage the hepatitis and exclude other causes of liver injury. This approach has now been used for 3 years in West London and reproduces these results seen in controlled trials (Foster et al., 1997).

Currently, the drug of choice in the treatment of HCV infections is IFNα. The efficacy of IFNα therapy is measured as normalization of serum ALT and loss of serum HCV RNA. Several studies have investigated the efficacy of IFNα treatment in patients chronically infected with HCV. Studies using IFNα at a dose of 3 MU three times a week for 6 months reported a sustained biochemical response rate (normal ALT levels 6 months after withdrawal of therapy) of 15 to 20% (Carithers and Emerson, 1997; Farrell, 1997; Lee, 1997). At the end of the treatment period, 27–35% of the patients had undetectable HCV RNA levels. At 6 months after withdrawal only 8–12% of the patients had undetectable HCV RNA levels. The response rate in cirrhotic patients has been reported to be lower. Prolonged treatment during 12 months showed a similar initial response as the 6 month regimen but there was a 2-fold sustained response rate of biochemical abnormalities of 19–42% determined 6 months after cessation of treatment (Farrell, 1997; Foster et al., 1997; Lee, 1997). Therefore, the 12 month regimen is recommended.

Three major factors influence the success of interferon therapy: the amount of HCV RNA present in the blood at the start of treatment, the virus genotype (subtype 1a and 1b are associated with low sustained response rate) and evidence of cirrhosis. When cirrhosis is present the response to interferon treatment is poor (Schalm et al., 1997a,b). The initial response to therapy is important for the long term outcome (Kakumu et al., 1997). In a study of 300 patients in Europe less than 1% of the patients with detectable HCV RNA levels after 4 weeks of treatment achieved a sustained response.

With regard to the low response rate and high relapse rate associated with interferon monotherapy, the efficacy of combined antiviral treatment is currently being investigated. To date, the most attention has been focused on the combined administration of interferon with the guanosine analogue ribavirin. Ribavirin can be administered orally and is well tolerated. Ribavirin monother-

apy studies have shown that ribavirin significantly reduces ALT levels but relapses occur in all cases when therapy is withdrawn. The HCV RNA levels do not seem to decline. The mechanism of action of ribavirin is not well understood. The major side effect of ribavirin is hemolytic anemia. Several studies (Brillanti et al., 1994; Schvarcz et al., 1995) and a *meta*-analysis of individual patient data from Europe (Schalm et al., 1997a,b) demonstrated that combined treatment of interferon and ribavirin during 6 months results in an increased (40–50%) sustained response rate (ALT and HCV RNA levels) after treatment withdrawal as compared to interferon monotherapy, both in interferon naive patients and in patients with a relapse after interferon monotherapy. There was no significant difference with respect to tolerance between the combination and interferon monotherapy. These data indicate that the combination of interferon and ribavirin for the treatment of chronic HCV is promising and new data on this combination are evolving.

Finally, the development of improved antiviral agents for HCV is obvious. Unfortunately, there is no in vitro culture system or animal model for HCV but novel agents may be designed based on the structure of viral targets. In addition, several cytokines and immunomodulators have undergone limited study. Combination of antiviral and immunomodulatory agents may play an important role in finding a way to control hepatitis C infections.

2.9. Respiratory viruses

The respiratory viruses constitute a large and varied group of several species that cause upper and lower respiratory tract disease in humans. The spectrum ranges from the mild common cold to a potentially lethal disease, especially in high risk populations such as the elderly, young infants, patients with chronic pulmonary obstructive disease and immunocompromised patients. Respiratory viruses have a considerable impact on morbidity and medical expenditures.

Currently, only three antiviral drugs are available for the treatment of respiratory virus illnesses: amantadine, rimantadine and ribavirin. The first two agents can be used for the prevention and treatment of influenza type A infections. Ribavirin can be administered for severe RSV infections of the lower respiratory tract but is of questionable value. The therapeutic results are unsatisfactory. Because of the limited therapeutic options new drugs directed against respiratory viruses are being developed. However, prevention of respiratory virus infections is cheaper than treatment, more feasible and has a higher success rate.

Three classes of antiviral agents directed against picornaviruses are currently under investigation: capsid binding agents, soluble recombinant intercellular adhesion molecule-1 (sICAM-1) and 3C protease inhibitors. Based on in vitro results the latter class of agents appears to be promising but clinical trials using 3C protease inhibitors have not yet been performed.

Capsid binding agents exert an early antiviral effect by binding to the hydrophobic pocket within VP1 of the capsid of picornaviruses. Two phase II trials have been performed with the capsid binding agent VP63843 (pleconaril). In a double blinded placebo controlled challenge trial VP63843 was orally administered 14 h before inoculation of susceptible volunteers with coxsackie A21 virus. This so-called 'cold' virus primarily causes a respiratory tract infection. Administration of the drug was continued for 6 days at a dosage of 200 mg bid. The proportion of individuals with significant nasal mucus production was reduced from 63% in the placebo group to 0% in the VP63843 group. Symptoms and febrile reaction as well as viral replication in the upper respiratory tract were also reduced (unpublished data).

In another placebo-controlled phase II trial the effect of VP63843 for enterovirus meningitis was investigated. In 39 adults with acute meningitis having a CSF white blood cell count of >5 cells/mm^3 and CSF PCR positive for enterovirus, a substantial beneficial effect was observed in the VP63843 recipients as compared to the placebo recipients. Time for resolution of headache was reduced by 64% and the duration of use of analgesics was reduced by 54% (Weiner et al., 1997). Further studies investigating the efficacy of this

agent in aseptic meningitis are ongoing and VP6834 is available on a compassionate use basis for other enteroviral syndromes. Approximately, 80 of 100 types of rhinoviruses are susceptible in vitro to this agent. Pleconaril is a promising drug for early treatment of respiratory virus infections.

Another antiviral agent against picornaviruses which has been studied is soluble truncated ICAM-1. ICAM-1, a member of the immunoglobulin supergene family is a 55 kDa glycosylated cellular protein which has five extracellular immunoglobulin like domains. The primary function is to bind β-integrins, particularly LFA-1, in immediate cell to cell interactions. However, ICAM-1 is also the cellular receptor for 90% of the rhinovirus serotypes. Recombinant soluble truncated ICAM-1, representing the extracellular domains of ICAM-1 can serve as a competitive antagonist for rhinovirus binding. In vitro studies demonstrated that truncated sICAM-1 specifically inhibits rhinovirus replication. Intranasal application of truncated sICAM-1 (tICAM453) prevented rhinovirus infections in chimpanzees subsequently challenged with infectious rhinovirus serotype 16 (Huguenel et al., 1997). Furthermore, in a placebo controlled trial, 177 susceptible volunteers were challenged with rhinovirus 39. The subjects were treated with sICAM-1 or placebo. Subjects were dosed six times daily for a duration of 7 days beginning either 4 h (prophylaxis model) before or 12 h (early treatment model) after the virus inoculation (total dose 4.4 mg). Infection rates were similar between placebo and sICAM-1 group. The frequency of common cold development was reduced by 34%. Furthermore, the symptom score and the nasal mucus weight were significantly reduced in the sICAM-1 treated group by 45 and 56%, respectively. No difference was observed between the early treatment and the prophylactic group (Turner et al., 1997). These data indicate that use of sICAM-1 reduces severity of symptoms of rhinovirus induced respiratory tract infections. More research is required to investigate the optimal dose and dosing frequency and to develop a formulation that allows less frequent administration dosing.

A new agent has been proved efficacious for influenza A and B virus infections: GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetyl neuraminic acid), zanamivir, a sialic acid analogue which inhibits the viral neuraminidase. Neuraminidase is essential for escape of virus from infected cells and it may also reduce the inactivation of virus by respiratory secretions. Zanamivir was synthesized based on computer analysis of the crystal structure of neuraminidase (Von Itzstein et al., 1993). The agent has a highly selective antiviral activity against numerous influenza A and B viruses in vitro (Woods et al., 1993; Thomas et al., 1994). Pharmacokinetic studies in humans and animals have shown that the oral bioavailability is poor and the half life in humans is only 1.7 h. In a placebo controlled challenge trial, intranasal administration of zanamivir is safe and effective for both prevention and early treatment of experimental infection with influenza A/Texas/91 (H1N1). Compared to the placebo group a 95% reduction in the occurrence of febrile illness was observed in the zanamivir group. Early treatment reduced peak viral titer by 2 logs, reduced the median duration of viral shedding by 3 days and the frequency of febrile illness by 85% (Hayden et al., 1996). Finally, a large randomized placebo controlled trial in the US and Europe using topically administration of zanamivir was performed (Hayden et al., 1997a,b). In this study two routes of administration (dry powder inhalation and a nasal pump spray) were used. The subjects were randomly assigned to one of three treatment arms within 48 h of onset of influenza like symptoms: zanamivir intranasal spray + zanamivir inhalation powder, placebo spray + zanamivir powder or placebo by both routes. For 262 volunteers with documented influenza virus infection, the median duration of symptoms was one day shorter (4 versus 5 days) in both zanamivir treated groups (173 patients) as compared to the placebo group (89 patients). In general the symptoms of the influenza were mild. An analysis of patients having serious symptoms (fever) showed a more substantial difference between placebo and zanamivir groups. When therapy was initiated within 30 h after the onset of symptoms in febrile patients, the median time to alleviation of symptoms was 3 days shorter in the zanamivir group as compared to the placebo group (4 versus 7 days, $P < 0.01$).

Nasal swabs showed significantly lower viral titers in both zanamivir groups. As a result of these encouraging results, additional phase III trials have been undertaken.

Since topical administration of antiviral drugs may present problems in certain populations like young infants and the elderly, there are efforts to develop oral neuraminidase inhibitors. Pharmacokinetic and efficacy studies have been performed with the oral neuraminidase inhibitor GS4701 (Ro-64-0802) which has been shown to have high and sustained plasma levels in humans following oral administration of the prodrug GS4104 (Ro-64-0796) (Wood et al., 1997). In a placebo controlled, double-blind study using GS4104 as an early treatment of experimental influenza infections in 80 susceptible volunteers, GS4104 treated subjects had lower virus titers, a shorter duration of virus shedding and a shorter time to cessation of influenza symptoms (Hayden et al., 1997a,b). These promising data warrant investigation of GS4104 for the prevention and treatment of natural influenza.

2.10. Papilloma viruses

Human papilloma viruses (HPVs) cause benign tumors (cutaneous warts, anogenital warts and condylomata acuminata) and are occasionally responsible for malignant tumors such as squamous-cell carcinomas. Currently, most therapeutic options comprise destruction of the affected tissue by cytotoxic ablation (podophyllotoxin, trichloroacetic acid and 5 fluoro-uracil), surgical ablation, cryotherapy or laser therapy.

Since IFNα is known for its immunomodulatory and antiviral properties, efficacy has been tested in the treatment of anogenital warts and orolaryngeal papilloma. However, interferon treatment by either the topical, intralesional or systemic route did not result in a high level of cure rate of warts. High recurrence rates were observed indicating that HPV infection is not eradicated (Beutner and Ferenczy, 1997).

Recently, a new drug has become available for treatment of anogenital warts: imiquimod. This immunomodulatory drug has been demonstrated to induce IFNα in both animal and human blood cells in vitro. Moreover, imiquimod also induces TNF-α and enhances the cell mediated cytotoxic response. The efficacy of imiquimod 5% cream was investigated in a multicenter, randomized, placebo controlled trial of 209 patients with genital warts. After 16 weeks of treatment and 12 weeks of follow-up the imiquimod treated group had significantly higher clearance rates than the placebo group (50 versus 11%). The major side effects of imiquimod as compared to placebo were itching (22 versus 7%) and erythema (67 versus 24%) (Beutner and Ferenczy, 1997). These data indicate that imiquimod may be usable as a first line treatment for anogenital warts. As compared to topical podophyllotoxin, imiquimod cream appears more effective in females but not in males and is associated with fewer side effects (unpublished data). However, podophyllotoxin is less costly and a allows shorter duration treatment.

Another therapy for condylomata acuminata currently being investigated is an injectable collagen gel containing 5-fluorouracil (30 mg/ml) and epinephrine (Accusite), which achieves high concentrations of 5-fluorouracil at the site of injection. The gel is applied intralesionally. In a randomized, double blinded study, the efficacy of the 5-fluorouracil/epinephrine gel was compared to 5-fluorouracil gel and the gel itself (placebo) (Swinehart et al., 1997). Lesions were injected once a week for up to 6 weeks. Injection of the gel was associated with pain, burning and stinging in 97% of the patients. In the group treated with fluorouracil/epinephrine gel, the complete response rate (CR) was 61%. In the group treated with fluorouracil gel without epinephrine the CR rate was 43% and in the placebo group 5%. The recurrence rates in the patients with complete responses were 50 and 58% for the 5-fluorouracil/epinephrine group and the 5-fluorouracil group, respectively. No systemic reactions were observed. The results suggest that the injectable 5-fluorouracil/epinephrine gel is a promising tool in the treatment of condylomata acuminata.

The quest for new antiviral agents against HPVs is ongoing. A phase I/II open label trial, using a topical cidofovir (HPMPC) containing gel in the treatment of HPV associated warts in HIV

positive patients, showed that after 2 weeks of observation 23 of 46 (50%) patients had partial wart clearance and seven patients (15%) had a complete response (Van Cutsem et al., 1995). The results warrant further clinical evaluation of topical cidofovir in the treatment of anogenital warts. Recently an antisense DNA compound directed against the E1 gene start codon of HPV6 and HPV11 has been shown very promising results in a mouse model. The E1 gene is responsible for DNA replication and for maintenance of viral episomal DNA. Finally, testing of therapeutic and prophylactic vaccines has been performed in animals the results of which are promising and may be important in controlling HPV associated diseases.

2.11. Human immunodeficiency virus

During the past 2 years, a considerable body of evidence has been collected proving the superior efficacy of combination treatments for HIV infected individuals as compared to monotherapies. For antiretroviral naive patients, combined treatments with two reverse transcriptase (RT) inhibitors and one protease inhibitor result in sustained undetectable levels of plasma HIV RNA ($<$ 500 copies/ml) in a majority of the patients for up to 2 years. One study showed that 85% of the patients with prior zidovudine therapy had reduced levels of HIV RNA to less than 500 copies per milliliter for as long as 1 year following treatment with a combination of indinavir, zidovudine and lamivudine (Gulick et al., 1997). The combination of two RT inhibitors and one protease inhibitor has become the standard of care for the treatment of HIV infected individuals.

Currently, a considerable arsenal of potent antiretroviral agents is available and new compounds are in development. The following nucleoside analogue RT inhibitors are licensed in the US: zidovudine, lamivudine, zalcitabine, didanosine, stavudine and the non-nucleoside RT inhibitors nevirapine and delavirdine. Protease inhibitors available include saquinavir, ritonavir, indinavir and nelfinavir. Compounds currently under clinical evaluation are: ampenavir, abacavir and efavirenz. Clinical studies evaluating and comparing the efficacy of several combinations of three, four and even five antiretroviral agents are currently in progress. In addition, studies are ongoing to evaluate whether, after an induction phase with three or more antiretroviral agents, complete viral suppression can be sustained by a less toxic and cheaper maintenance regimen of only one or two agents. Finally, a study is in progress investigating whether initiation of treatment immediately after primary HIV infection is more beneficial than starting treatment at a later stage of HIV infection.

Insight into the viral dynamics of HIV infection has contributed to the understanding of the favorable results of combined treatment as compared to monotherapy. The plasma levels of HIV RNA are in quasi-steady-state as a result of a dynamic equilibrium of rapid rates of virus production and clearance (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996). Perturbation of this equilibrium by the introduction of potent HIV therapy results in a decline of the HIV RNA levels in a biphasic pattern. The initial phase shows a rapid drop of virus level by (2 logs) 99% in the first 10 days of therapy. With mathematic modeling, the half life of the virus is approximately 6 h and the half life of productively infected lymphocytes is about 1.5 days. The daily virus production is estimated at 10^9–10^{10} virions. About 99% of virus production is attributable to activated infected CD4+ lymphocytes. The compartment of productively infected CD4 lymphocytes is depleted after about 10 days of complete viral suppression. Then, a decrease is observed in the HIV RNA decay rate and a second decay phase begins which varies in duration between 1–4 weeks until HIV RNA disappears below the limit of detection. This second phase has a slower rate of decline and has been attributed to the survival of latently infected cells or antigen presenting cells such as macrophages. The extracellular virus trapped by the follicular dendritic cells of the germinal centers offers an alternative source for this second phase decay. Based on the duration of this second phase of decay calculations suggest that extinction of HIV would require 1–3 years. These calculations were based on the assumption of the absence of a third phase attributable to persistently in-

fected cells and the assumption that the number of latently infected cells is between 10^8 and 10^{12}.

Theoretically, due to the high daily production rate of viruses and the high mutation rate of HIV-RT (3×10^{-5} nucleotides per replication cycle or one mutation per HIV genome), continuous generation of mutations occurs daily including those that confer drug resistance (Coffin, 1995). In untreated patients the presence of pre-existent HIV variants with one drug resistance mutation has been demonstrated. However, viruses with three or more drug resistance mutations theoretically do not pre-exist in untreated patients. Thus, virus variants resistant to a triple combination of drugs are unlikely in untreated patients, creating an important argument for combination treatment with three agents. As compared to monotherapy, combined treatment will also increase the efficacy of viral suppression, thus preventing the emergence of new drug resistance mutations. Drug resistance to a combination of agents will not develop as long as viral replication is completely inhibited.

Several studies have been performed investigating the effect of combined treatment with two RT inhibitors and one protease inhibitor on the presence of HIV in different body compartments. Although patients may achieve undetectable plasma HIV RNA levels (below 50 copies/ml) for up to 2 years, HIV RNA is still detectable in the lymphoid tissue of these patients after 6–12 months of treatment (Cavert et al., 1997; Wong et al., 1997a,b). However, a reduction in the amount HIV RNA per gram lymphoid tissue of 3–5 logs was documented after 6–12 months of treatment. No multiply spliced HIV mRNA, indicative of active viral transcription, could be detected in lymph nodes of patients with uninterrupted absence of viremia (< 50 copies/ml), suggesting complete viral suppression. In one study, HIV recovered by co-culture from the lymphoid tissue from two patients with uninterrupted undetectable HIV RNA levels during 1 year of triple therapy, revealed no evidence of evolution towards phenotypic or genotypic resistance. In contrast, in those patients with mono or dual therapy or triple therapy with dose interruptions, that had even the slightest plasma viremia after 1 year of treatment (> 50 copies/ml) a high lymphoid tissue virus burden was detected and associated with the presence of multiply spliced HIV mRNA and genotypic and phenotypic resistance, reflecting ongoing replication (Wong et al., 1997a,b). In conclusion, persistent replication in the presence of antiretroviral selective pressure appears to result invariably in the selection of drug resistant variants.

The effect of highly active antiretroviral therapy on the compartment of latently HIV infected lymphocytes has also been investigated (Chun et al., 1997). Using an ultra sensitive co-culturing system, HIV has been recovered from peripheral blood mononuclear cells from patients who had plasma HIV RNA levels less than 50 copies/ml for up to 2 years. However, by sequence analysis of the co-cultured virus no new drug resistance mutations were detected and phylogenetic analysis revealed no discernible evolution as compared to baseline virus (Finzi et al., 1997; Wong et al., 1997a,b). These data indicate that complete HIV suppression by highly active antiretroviral therapy is feasible but that long term viral latency is a major barrier for HIV eradication if only an antiretroviral strategy is used. Future strategies for HIV eradication may include a combination of potent antiretroviral agents with immunomodulatory drugs that may accelerate the decay of the latently infected cell compartment.

The impact of highly active antiretroviral therapy on the immune system has been intensively investigated. Several studies have demonstrated transient improvement of immunologic parameters with nucleoside and protease monotherapies. The transient nature of the improvement probably reflects the only temporary virologic effect and therapeutic failure of these regimens. Highly active combination therapies seem to result in a more pronounced and sustained improvement, although complete normalization of the immunologic profile is not achieved. An issue of concern is to what extent complete immune reconstitution is possible. The observed rise in CD4 cells upon initiation of antiretroviral therapy is predominantly caused by an increase in the memory (CD45RO +) CD4 cells and only a small increase of naive (CD45RA) CD4 cells. This suggests that

reconstitution of the naive CD4 cell population is a very slow process as is the case in bone marrow transplant recipients (Autran et al., 1997). Moreover, reconstitution will be entirely dependent on sustained substantial viral suppression.

3. Conclusion

In the past years, progress in the field of antiviral therapy has been considerable. On the other hand, no treatment is available yet against some viruses, such as the filoviruses. For several other virus infections, such as chronic hepatitis B and C and the respiratory virus infections, therapeutic options are still limited. To expand therapeutic options many research efforts are invested in the design and discovery of new, improved, generations of antiviral agents, which may be used as monotherapy or in combination with other antiviral agents or immunoregulatory drugs. The results are encouraging. The improved efficacy of combined antiviral treatment as compared to monotherapy has been thoroughly established in the treatment of HIV infected individuals. In addition, evidence accumulates indicating that combination treatment leads to improved antiviral efficacy in other viral infections as well.

References

Albini, A., Barillari, G., Benelli, R., Gallo, R.C., Ensoli, B., 1995. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. Proc. Natl. Acad. Sci. USA 92, 4838–4842.

Anderson, M.G., Harrison, T.J., Alexander, G.J.M., 1986. Randomized trial of lymphoblastoid interferon for chronic active hepatitis B. Br. J. Hepatol. S225–227.

Autran, B., Carcelain, G., Li, T.S., Blanc, C., Mathez, D., Tubiana, R., Katlama, C., Debre, P., Leibowitch, J., 1997. Positive effects of combined antiretroviral therapy on CD4+ T-cell homeostasis and function in advanced HIV disease. Science 277, 112–116.

Baldanti, F., Underwood, M.R., Stanat, S.C., Biron, K.K., Chou, S., Sarasini, A., Silini, E., Gerna, G., 1996. Single amino acid changes in the DNA polymerase confer foscarnet resistance and slow-growth phenotype, while mutations in the UL97-encoded phosphotransferase confer ganciclovir resistance in three double-resistant human cytomegalovirus strains recovered from patients with AIDS. J. Virol. 70, 1390–1395.

Barillari, G., Gendelman, R., Gallo, R.C., Ensoli, B., 1993. The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi sarcoma and cytokine-activated vascular cells, induces adhesion of the same cell types by using integrin receptors recognizing the RGD amino acid sequence. Proc. Natl. Acad. Sci. USA 90, 7941–7955.

Bartholomew, M.M., Jansen, R.W., Jeffers, L.J., Reddy, K.R., Johnson, L.C., Bunzendahl, H., Condreay, L.D., Tzakis, A.G., Schiff, E.R., Brown, N.A., 1997. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 349, 20–22.

Benhamou, Y., Katlama, C., Lunel, F., Coutellier, A., Dohin, E., Hamm, N., Tubiana, R., Herson, S., Poynard, T., Opolon, P., 1996. Effects of lamivudine on replication of hepatitis B virus in HIV-infected men. Ann. Intern. Med. 125, 705–712.

Beutner, K.R., Friedman, D.J., Forszpaniak, C., Andersen, P.L., Wood, M.J., 1995. Valaciclovir compared with acyclovir for improved therapy for herpes zoster in immunocompetent adults. Antimicrob. Agents Chemother. 39, 1546–1553.

Beutner, K.R., Ferenczy, A., 1997. Therapeutic approaches to genital warts. Am. J. Med. 102, 28–37.

Boivin, G., Chou, S., Quirk, M.R., Erice, A., Jordan, M.C., 1989. Randomised controlled trial of IFNα 2A (rbe) (Roferon-A) for the treatment of chronic hepatitis B virus (HBV) infection: factors that influence response. Gut 30, 1116–1122.

Boivin, G., Chou, S., Quirk, M.R., Erice, A., Jordan, M.C., 1996. Detection of ganciclovir resistance mutations quantitation of cytomegalovirus (CMV) DNA in leukocytes of patients with fatal disseminated CMV disease. J. Infect. Dis. 173, 523–528.

Booth, J.C., Brown, J.L., Thomas, H.C., 1995. The management of chronic hepatitis C virus infection . Gut October (37), 449–454.

Boppana, S.B., Fowler, K.B., Vaid, Y, Hedlund, G., Stagno, S., Britt, W.J., Pass, R.F., 1997. Neuroradiographic findings in the newborn period and long-term outcome in children with symptomatic congenital cytomegalovirus infection. Pediatrics 99, 409–414.

Brillanti, s., Garson, J., Foli, M., Whitby, K., Deaville, R., Masci, C., Miglioli, M., Barbara, L., 1994. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 107, 812–817.

Brook, M.G., Karayiannis, P., Thomas, H.C., 1989. Which patients with chronic hepatitis B infection will respond to IFNα therapy? A statistical analysis of predictive factors. Hepatology 10, 761–763.

Carithers, R.L. Jr., Emerson, S.S., 1997. Therapy of hepatitis C: *meta*-analysis of IFNα-2b trials. Hepatology 26 (3S1), 83S–88.

Cavert, W., Notermans, D.W., Staskus, K., Wietgrefe, S.W., Zupancic, M., Gebhard, K., Henry, K., Zhang, Z.Q., Mills, R., McDade, H., Goudsmit, J., Danner, S.A.,

Haase, A.T., 1997. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science 276, 960–964.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M., Moore, P.S., 1994. dentification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266, 1865–1869.

Charles, N.C., Steiner, G.C., 1996. Ganciclovir intraocular implant. A clinicopathologic study. Ophthalmology 103, 416–421.

Chou, S., Marousek, G., Guentzel, S., Follansbee, S.E., Poscher, M.E., Lalezari, J.P., Miner, R.C., Drew, W.L., 1997. Evolution of mutations conferring multidrug resistance during prophylaxis and therapy for cytomegalovirus disease. J. Infect. Dis. 176, 786–789.

Chun, T.W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., Kuo, Y.H., Brookmeyer, R., Zeiger, M.A., Barditch-Crovo, P., Siliciano, R.F., 1997. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387, 183–188.

Coffin, J.M., 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. Science 267, 483–489.

Van Cutsem, E., Snoeck, R., Van Ranst, M., Fiten, P., Opdenakker, G., Geboes, K., Janssens, J., Rutgeerts, P., Vantrappen, G., de Clercq, E., 1995. Successful treatment of a squamous papilloma of the hypopharynx-esophagus by local injections of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. J. Med. Virol. 45, 230–235.

Delabesse, E., Oksenhendler, E., Lebbe, C., Verola, O., Varet, B., Turhan, A.G., 1997. Molecular analysis of clonality in Kaposi's sarcoma. J. Clin. Pathol. 50, 664–668.

Dienstag, J.L., Perrillo, R.P., Schiff, E.R., Bartholomew, M., Vicary, C., Rubin, M., 1995. A preliminary trial of lamivudine for chronic hepatitis B infection. New Engl. J. Med. 333, 1657–1661.

Duker, J.S., Robinson, M., Anand, R., Ashton, P., 1995. Initial experience with an 8-month sustained-release intravitreal ganciclovir implant for the treatment of CMV retinitis associated with AIDS. Ophthalmic Surg. Lasers 26, 442–448.

Emanuel, D., Cunningham, I., Jules-Elysee, K., Brochstein, J.A., Kernan, N.A., Laver, J., Stover, D., White, D.A., Fels, A., Polsky, B., 1988. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Ann. Intern. Med. 109, 777–782.

Farrell, G.C., 1997. Therapy of hepatitis C:IFNα-n1 trials. Hepatology 26 (3S1), 96S–100.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D., Siliciano, R., 1997. Identification of a reservoir of HIV-1 in patients on highly active antiretroviral therapy. Science 278, 1295–1300.

Foster, G.R., Goldin, R.D., Main, J., Murray-Lyon, I., Hargreaves, S., Thomas, H.C., 1997. Management of chronic hepatitis C: clinical audit of biopsy based management algorithm. Br. Med. J. August 23 (315), 453–458.

Gao, S.J., Kingsley, L., Li, M., Zheng, W., Parravicini, C., Ziegler, J., Newton, R., Rinaldo, C.R., Saah, A., Phair, J., Detels, R., Chang, Y., Moore, P.S., 1996. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat. Med. 2, 925–928.

Grossi, P., Baldanti, F., 1997. Treatment of ganciclovir-resistant human cytomegalovirus infection. J. Nephrol. 10, 146–151.

Gulick, R.M., Mellors, J.W., Havlir, D., Eron, J.J., Gonzalez, C., McMahon, D., Richman, D.D., Valentine, F.T., Jonas, L., Meibohm, A., Emini, E.A., Chodakewitz, J.A., 1997. Treatment with indinavir, zidovudine and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. New Engl. J. Med. 337, 734–739.

Hayden, F.G., Treanor, J.J., Betts, R.F., Lobo, M., Esinhart, J.D., Hussey, E.K., 1996. Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. J. Am. Med. Assoc. 275, 295–299.

Hayden, F.G., Lobo, M., Treanor, J.J., Miller, M., Mills, R.G., 1997. Efficacy and tolerabilty of oral GS4104 for early treatment of experimental influenza in humans. 37th ICAAC, Toronto, Ontario, Canada, September 28–October 1, 1997, Abstract LB-26.

Hayden, F.G., Osterhaus, A.D., Treanor, J.J., Fleming, D.M., Aoki, F.Y., Nicholson, K.G., Bohnen, A.M., Hirst, H.M., Keene, O., Wightman, K., 1997b. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenzavirus infections. GG167 Influenza Study Group. New Engl. J. Med. 337, 874–880.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373, 123–126.

Huggins, J.W., Hsiang, C.M., Cosgriff, T.M., Guang, M.Y., Smith, J.I., Wu, Z.O., LeDuc, J.W., Zheng, Z.M., Meegan, J.M., Wang, Q.N., 1991. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. J. Infect. Dis. 164, 1119–1127.

Huguenel, E.D., Cohn, D., Dockum, D.P., Greve, J.M., Fournel, M.A., Hammond, L., Irwin, R., Mahoney, J., McClelland, A., Muchmore, E., Ohlin, A.C., Scuderi, P., 1997. Prevention of rhinovirus infection in chimpanzees by soluble intercellular adhesion molecule-1. Am. J. Respir. Crit. Care. Med. 155 (4), 1206–1210.

von Itzstein, M., Wu, W.Y., Kok, G.B., Pegg, M.S., Dyason, J.C., Jin, B., Van Phan, T., Smythe, M.L., White, H.F., Oliver, S.W., 1993. Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 363 (6428), 418–423.

Jacobson, M.A., Zegans, M., Pavan, P.R., O'Donnell, J.J., Sattler, F., Rao, N., Owens, S., Pollard, R., 1997. Cy-

tomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 349 (9063), 1443–1445.

de Jong, M.D., Boucher, C.A., Galasso, G.J., Hirsch, M.S., Kern, E.R., Lange, J.M., Richman, D.D., 1996. Consensus symposium on combined antiviral therapy. International Society for Antiviral Research and the National Institutes of Allergy and Infectious Diseases. Antiviral Res. 29, 5–29.

de Jong, M.D., Boucher, C.A., Cooper, D.A., Galasso, G.J., Gazzard, B., Lange, J.M., Montaner, J.S., Richman, D.D., Thomas, H.C., 1997. Summary of the II International Consensus Symposium on Combined Antiviral Therapy and implications for future therapies. Antiviral Res. 35, 65–82.

Kakumu, S., Aiyama, T., Okumura, A., Iwata, K., Ishikawa, T., Yoshioka, K., 1997. Earlier loss of hepatitis C virus RNA in interferon therapy can predict a long-term response in chronic hepatitis C. J. Gastroenterol. Hepatol. 12, 468–472.

Kedes, D.H., Operskalski, E., Busch, M., Kohn, R., Flood, J., Ganem, D., 1996. The seroepidemiology of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nat. Med. 2, 918–924.

Kedes, D.H., Ganem, D., 1997. Sensitivity of Kaposi's sarcoma-associated herpesvirus replication to antiviral drugs. Implications for potential therapy. J. Clin. Invest. 99, 2082–2086.

Kimberlin, D.W., Lakeman, F.D., Arvin, A.M., Prober, C.G., Corey, L., Powell, D.A., Burchett, S.K., Jacobs, R.F., Starr, S.E., Whitley, R.J., 1996. Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. J. Infect. Dis. 174, 1162–1167.

Koelle, D.M., Huang, M.L., Chandran, B., Vieira, J., Piepkorn, M., Corey, L., 1997. Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. J. Infect. Dis. 176, 94–102.

Lee, W.M., 1997. Therapy of hepatitis C: IFNα-2a trials. Hepatology 26 (3S1), 89S–95.

Lefrere, J.J., Meyohas, M.C., Mariotti, M., Meynard, J.L., Thauvin, M., Frottier, J., 1996. Detection of human herpesvirus-8 DNA sequences before the appearance of Kaposi's sarcoma in human immunodeficiency virus (HIV)-positive subjects with a known date of HIV seroconversion. J. Infect. Dis. 174, 283–287.

Lennette, E.T., Blackbourn, D.J., Levy, J.A., 1996. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. Lancet 348, 858–861.

Ling, R., Mutimer, D., Ahmed, M., Boxall, E.H., Elias, E., Dusheiko, G.M., Harrison, T.J., 1996. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 24, 711–713.

Lurain, N.S., Spafford, L.E., Thompson, K.D., 1994. Mutation in the UL97 open reading frame of human cytomegalovirus strains resistant to ganciclovir. J. Virol. 68, 4427–4431.

Mahy, W.J., 1997. Human viral infections: an expanding frontier. Antiviral Res. 36, 75–80.

Main, J., Brown, J.L., Howells, C., Galassini, R., Crossey, M., Karayiannis, P., Georgiou, P., Atkinson, G., Thomas, H.C., 1996. A double blind, placebo-controlled study to assess the effect of famciclovir on virus replication in patients with chronic hepatitis B virus infection. J. Viral. Hepat. 3, 211–215.

Marcellin, P., Boyer, N., Colin, J.F., Martinot-Peignoux, M., Lefort, V., Matheron, S., Erlinger, S., Benhamou, J.P., 1993. Recombinant alpha interferon for chronic hepatitis B in anti-HIV positive patients receiving zidovudine. Gut 34 (2S), 106.

Martin, D.F., Parks, D.J., Mellow, S.D., Ferris, F.L., Walton, R.C., Remaley, N.A., Chew, E.Y., Ashton, P., Davis, M.D., Nussenblatt, R.B., 1994. Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. A randomized controlled clinical trial. Arch. Ophthalmol. 112, 1531–1539.

Marx, J.L., Kapusta, M.A., Patel, S.S., LaBree, L.D., Walonker, F., Rao, N.A., Chong, L.P., 1996. Use of the ganciclovir implant in the treatment of recurrent cytomegalovirus retinitis. Arch. Ophthalmol. 114, 815–820.

Monini, P., de Lellis, L., Fabris, M., Rigolin, F., Cassai, E., 1996. Kaposi's sarcoma-associated herpesvirus DNA sequences in prostate tissue and human semen. New Engl. J. Med. 334, 1168–1172.

Nevens, F., Main, J., Honkoop, P., Tyrrell, D.L., Barber, J., Sullivan, M.T., Fevery, J., De Man, R.A., Thomas, H.C., 1997. Lamivudine therapy for chronic hepatitis B: a 6-month randomized dose-ranging study. Gastroenterology 113, 1258–1263.

Nowak, M.A., Bonhoeffer, S., Hill, A.M., Boehme, R., Thomas, H.C., McDade, H., 1996. Viral dynamics in hepatitis B virus infection. Proc. Natl. Acad. Sci. USA 93, 4398–4402.

Okuda, H., Nishiyama, T., Ogura, K., Nagayama, S., Ikeda, K., Yamaguchi, S., Nakamura, Y., Kawaguchi, Y., Watabe, T., 1997. Lethal drug interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. Drug Metab. Dispos. 25, 270–273.

Oral Ganciclovir European and Australian Co-operative Study Group, 1995. Intravenous versus oral ganciclovir: European/Australian comparative study of efficacy and safety in the prevention of cytomegalovirus retinitis recurrence in patients with AIDS. AIDS 9, 471–477.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span and viral generation time. Science 271, 1582–1586.

Pol, S., Wesenfelder, L., Dubois, F., Roingeard, P., Carnot, F., Driss, F., Brechot, C., Goudeau, A., Berthelot, P., 1994. Influence of human immunodeficiency virus infection

on hepatitis delta virus superinfection in chronic HBsAg carriers. J. Viral Hepat. 1, 131–137.

Pollard, R.B., 1996. CMV retinitis: ganciclovir/monoclonal antibody. Antiviral Res. 29, 73–75.

Pottage, J.C. Jr., Kessler, H.A., 1995. Herpes simplex virus resistance to acyclovir: clinical relevance. Infect. Agents Dis. 4, 115–124.

Poynard, T., Bedossa, P., Opolon, P., 1997. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. Lancet 349, 825–832.

Reed, E.C., Bowden, R.A., Dandliker, P.S., Lilleby, K.E., Meyers, J.D., 1988. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. Ann. Intern. Med. 109, 783–788.

Renne, R., Zhong, W., Herndier, B., McGrath, M., Abbey, N., Kedes, D., Ganem, D., 1996. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in culture. Nat. Med. 2, 342–346.

Rossol, S., Marinos, G., Carucci, P., Singer, M.V., Williams, R., Naoumov, N.V., 1997. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J. Clin. Invest. 99, 3025–3033.

Russo, JJ., Bohenzky, R.A., Chien, M.C., Chen, J., Yan, M., Maddalena, D., Parry, J.P., Peruzzi, D., Edelman, I.S., Chang, Y., Moore, P.S., 1996. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proc. Natl. Acad. Sci. USA 93, 14862–14867.

Sanchez, A., Trappier, S.G., Mahy, B.W., Peters, C.J., Nichol, S.T., 1996. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proc. Natl. Acad. Sci. USA 93, 3602–3607.

Schalm, S.W., Fattovich, G., Brouwer, J.T., 1997a. Therapy of hepatitis C: patients with cirrhosis. Hepatology 26 (3S1), 128S–132.

Schalm, S.W., Hansen, B.E., Chemello, L., Bellobuono, A., Brouwer, J.T., Weiland, O., Cavalletto, L., Schvarcz, R., Ideo, G., Alberti, A., 1997b. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. *Meta*-analysis of individual patient data from European centers. J. Hepatol. 26, 961–966.

Schvarcz, R., Ando, Y., Sonnerborg, A., Weiland, O., 1995. Combination treatment with IFNα-2b and ribavirin for chronic hepatitis C in patients who have failed to achieve sustained response to interferon alone: Swedish experience. J. Hepatol. 23 (S2), 17–21.

Smith, I.L., Cherrington, J.M., Jiles, R.E., Fuller, M.D., Freeman, W.R., Spector, S.A., 1997. High-level resistance of cytomegalovirus to ganciclovir is associated with alterations in both the UL97 and DNA polymerase genes. Infect. Dis. 176, 69–77.

Soul-Lawton, J., Seaber, E., On, N., Wootton, R., Rolan, P., Posner, J., 1995. Absolute bioavailability and metabolic disposition of valaciclovir, the L-valyl ester of acyclovir, following oral administration to humans. Antimicrob. Agents Chemother. 39, 2759–2764.

Staskus, K.A., Zhong, W., Gebhard, K., Herndier, B., Wang, H., Renne, R., Beneke, J., Pudney, J., Anderson, D.J., Ganem, D., Haase, A.T., 1997. Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J. Virol. 71, 715–719.

Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group, 1996. Combination foscarnet and ganciclovir therapy versus monotherapy for the treatment of relapsed cytomegalovirus retinitis in patients with AIDS. The Cytomegalovirus Retreatment Trial. Arch. Ophthalmol. 114, 23–33.

Sturzl, M., Blasig, C., Schreier, A., Neipel, F., Hohenadl, C., Cornali, E., Ascherl, G., Esser, S., Brockmeyer, N.H., Ekman, M., Kaaya, E.E., Tschachler, E., Biberfeld, P., 1997. Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma. Int. J. Cancer 72, 68–71.

Swinehart, J.M., Sperling, M., Phillips, S., Kraus, S., Gordon, S., McCarty, J.M., Webster, G.F., Skinner, R., Korey, A., Orenberg, E.K., 1997. Intralesional fluorouracil/epinephrine injectable gel for treatment of condylomata acuminata. A phase 3 clinical study. Arch. Dermatol. 133, 67–73.

Thomas, G.P., Forsyth, M., Penn, C.R., McCauley, J.W., 1994. Inhibition of the growth of influenza viruses in vitro by 4-guanidino-2,4-dideoxy-*N*-acetylneuraminic acid. Antiviral Res. 24, 351–356.

Thursz, M.R., Kwiatkowski, D., Allsopp, C.E., Greenwood, B.M., Thomas, H.C., Hill, A.V., 1995. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. New Engl. J. Med. 332, 1065–1069.

Thursz, M.R., 1997. Host genetic factors influencing the outcome of hepatitis. J. Viral Hepat. 4, 215–220.

Tipples, G.A., Ma, M.M., Fischer, K.P., Bain, V.G., Kneteman, N.M., Tyrrell, D.L., 1996. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 24, 714–717.

Turner, R.B., Wecker, M.T., Pohl, G., Witek, T.J., Marlin-McNally, E., Hayden, F.G., 1997. Efficacy of soluble ICAM-1 (sICAM) for prevention of Rhinovirus infection and ilness. 37th ICAAC, Toronto, Ontario, Canada, September 28–October 1, Abstract H-85.

Van der Meer, J.T., Drew, W.L., Bowden, R.A., Galasso, G.J., Griffiths, P.D., Jabs, D.A., Katlama, C., Spector, S.A., Whitley, R.J., 1996. Summary of the International Consensus Symposium on Advances in the Diagnosis, Treatment and Prophylaxis and Cytomegalovirus Infection. Antiviral Res. 32, 119–140.

Verdonck, L.F., de Gast, G.C., Dekker, A.W., de Weger, R.A., Schuurman, H.J., Rozenberg-Arska, M., 1989. Treatment of cytomegalovirus pneumonia after bone marrow transplantation with cytomegalovirus immunoglobulin combined with ganciclovir. Bone Marrow Transplant 4, 187–189.

Vieira, J., Huang, M.L., Koelle, D.M., Corey, L., 1997. Transmissible Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in saliva of men with a history of Kaposi's sarcoma. J. Virol. 71, 7083–7087.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373, 117–122.

Weiner, L.B., Rotbarth, H.A., Gilbert, D.L., Hayden, F.G., Mynhardt, J.H., Dwyer, D.E., Trocha, H., Rogers, J.M., Mckinlay, M.A., 1997. Treatment of enterovirus meningitis with pleconaril (VP63843), an antipicornaviral agent, 37th ICAAC, Toronto, Ontario, Canada, September 28–October 1, Abstract LB-27.

Weller, S., Blum, M.R., Doucette, M., Burnette, T., Cederberg, D.M., de Miranda, P., Smiley, M.L., 1993. Pharmacokinetics of the acyclovir pro-drug valaciclovir after escalating single- and multiple-dose administration to normal volunteers. Clin. Pharmacol. Ther. 54, 595–605.

Whitley, R.J., Weiss, H., Gnann, J.W. Jr., Tyring, S., Mertz, G.J., Pappas, P.G., Schleupner, C.J., Hayden, F., Wolf, J., Soong, S.J., 1996. Acyclovir with and without prednisone for the treatment of herpes zoster. A randomized, placebo-controlled trial. The National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Ann. Intern. Med. 125, 376–383.

Wong, D.K., Yim, C., Naylor, C.D., Chen, E., Sherman, M., Vas, S., Wanless, I.R., Read, S., Li, H., Heathcote, E.J., 1995. Interferon alfa treatment of chronic hepatitis B: randomized trial in a predominantly homosexual male population. Gastroenterology 108, 165–171.

Wong, J.K., Hezareh, M., Gunthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., Richman, D.D., 1997a. Recovery of replication competent HIV despite prolonged suppression of plasma viremia. Science 278, 1291–1295.

Wong, J.K., Gunthard, H.F., Havlir, D.V., Zhang, Z., Haase, A.T., Ignacio, C.C., Kwok, S., Emini, E., Richman, D., 1997b. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc. Natl. Acad. Sci. USA 94, 12574–12579.

Wood, N.D., Aitken, M., Sharp, S., Evison, H., 1997. Tolerability and pharmacokinetics of the influenza neuraminidase inhibitor Ro-0802 (GS4071) following oral administration of the prodrug Ro 64-0796 (GS4104) to healthy male volunteers. 37th ICAAC, Toronto, Ontario, Canada, September 28–October 1, Abstract A-123.

Woods, J.M., Bethell, R.C., Coates, J.A., Healy, N., Hiscox, S.A., Pearson, B.A., Ryan, D.M., Ticehurst, J., Tilling, J., Walcott, S.M., 1993. 4-Guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetylneuraminic acid is a highly effective inhibitor both of the sialidase (neuraminidase) and of growth of a wide range of influenza A and B viruses in vitro. Antimicrob. Agents Chemother. 37, 1473–1479.

Yano, M., Kumada, H., Kage, M., Ikeda, K., Shimamatsu, K., Inoue, O., Hashimoto, E., Lefkowitch, J.H., Ludwig, J., Okuda, K., 1996. The long-term pathological evolution of chronic hepatitis C. Hepatology 23, 1334–1340.

Zeuzem, S., de Man, R.A., Honkoop, P., Roth, W.K., Schalm, S.W., Schmidt, J.M., 1997. Dynamics of hepatitis B virus infection in vivo. J. Hepatol. 27, 431–436.

Zylberberg, H., Jiang, J., Pialoux, G., Driss, F., Carnot, F., Dubois, F., Brechot, C., Berthelot, P., Pol, S., 1996. IFNα for chronic active hepatitis B in human immunodeficiency virus-infected patients. Gastroenterol. Clin. Biol. 20, 968–971.