Recent developments in anti-herpesvirus drugs

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Background: Herpesviruses notably establish lifelong infections, with latency and reactivation. Many of the known human herpesviruses infect large proportions of the population worldwide. Treatment or prevention of herpes infections and recurrent disease still pose a challenge in the 21st century.

Sources of data: Original papers and review articles, meeting abstracts, a book (Clinical Virology; DD Richman, RJ Whitley & FG Hayden eds) and company web sites.

Areas of agreement: For herpes simplex types 1 and 2 and for varicella zoster, acyclovir (ACV; now increasingly replaced by its prodrug valacyclovir, VACV) and famciclovir (FCV) have greatly reduced the burden of disease and have established a remarkable safety record. Drug-resistance, in the otherwise healthy population, has remained below 0.5% after more than 20 years of antiviral use. In immunocompromised patients, drug resistance is more common and alternative drugs with good safety profiles are desirable. For human cytomegalovirus disease, which occurs in immunocompromised patients, ganciclovir and increasingly its prodrug valganciclovir are the drugs of choice. However, alternative drugs, with better safety, are much needed.

Areas of controversy: Various questions are highlighted. Should the new 1-day therapies for recurrent herpes labialis and genital herpes replace the current standard multi-day therapies? The marked differences between VACV and FCV (e.g. triphosphate stability, effect on latency) may not yet be fully exploited? Do current antivirals reduce post-herpetic neuralgia (PHN)? For immunocompromised patients with varicella zoster virus (VZV) disease, should the first-line treatment be FCV, not ACV or VACV? Should there be more support to explore new avenues for current antivirals, for example in possibly reducing herpes latency or Alzheimer’s disease (AD)? Should primary Epstein–Barr virus (EBV) disease in adolescents be treated with antivirals? How can new compounds be progressed when the perceived market need is small but the medical need is great. FCV was reclassified from prescription-only to pharmacist-controlled for herpes labialis in New Zealand in 2010; should this be repeated more widely? This article reviews new drugs in clinical trials and highlights some of the problems hindering their progress.

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Herpesvirus infections remain a clinical challenge in the 21st century

Herpesviruses are present throughout the animal kingdom having evolved over hundreds of millions of years with their hosts. To date, at least eight distinct human herpesviruses have been identified, assigned to three subfamilies: α-, β- and γ-herpesvirinae. A characteristic of all herpesvirus infections, however, is their ability to establish lifelong infections characterized by periods of latency followed by reactivation. The morbidity produced by these acute and recurrent infections as outlined below produce much human suffering for which there remains an unmet need for control by effective immunization or antiviral therapy.

α-Herpesviruses (herpes simplex and varicella zoster viruses)

Primary infections with herpes simplex viruses (HSV-1, HSV-2) cause localized lesions, generally in orofacial and genital areas, respectively. After the resolution of the primary infection, latent virus resides in sensory neurons. During a recurrence, the HSV in a nerve cell reacts vates, and the virus travels down the axon to infect peripheral tissue to give localized lesions of the skin and mucus membranes. Recurrent lesions are common but the frequency of recurrences can vary widely, e.g. monthly to once per year or less. In recent decades, primary infection of the genital area by HSV-1 has become more common and can be severe, but the recurrence rate is usually much lower than with HSV-2 infections; similarly for HSV-2 infections of the orofacial area. Hence, the two viruses appear to retain their site preferences. Less common clinical manifestations include ocular lesions (e.g. recurrent herpes keratitis) and Bell’s palsy (face drop). Two examples in which HSV gains entry through damaged skin are eczema herpeticum and herpes gladatorium. Antiviral therapy is very effective in these two cases. Rarely, HSV-1 or 2 can cause life-threatening herpes encephalitis in the newborn, if the mother has an active infection at the time of birth, and HSV-1 produces encephalitis in about one per million adults each year. Importantly, in immunocompromised patients, HSV and varicella zoster virus (VZV) disease is both more frequent and severe.

Primary infection with VZV causes chickenpox, commonly during childhood producing widespread characteristic skin lesions. Although normally a self-limiting childhood disease, the economic burden of parents taking time off work has become recognized in the USA, thus making vaccination financially viable. In adults, chickenpox can be more severe, particularly in pregnant women in whom antiviral
therapy is recommended. For ophthalmic zoster, where there is a risk of permanent visual impairment, antiviral therapy is mandatory. Antibody (humoral) immunity normally remains lifelong and this probably protects the individual from systemic spread of cell-free virus with cellular immunity-limiting virus spread from cell to cell. However, as cellular immunity wanes with age, together with a ‘stress’ factor, VZV can reactivate leading to a recurrence of lesions, usually confined to a single dermatome, known as herpes zoster, or ‘shingles’. In contrast to HSV, reactivated VZV can infect satellite and Schwann cells, and the VZV can spread throughout a bundle of nerves serving a dermatome leading to lesions throughout that dermatome but restricted to it. The skin lesions heal relatively quickly, commonly within a month. However, older patients, especially, may experience continuing pain called post-herpetic neuralgia (PHN), a potentially debilitating condition. A reasonable explanation may be that healing of VZV-induced nerve damage is more complex and takes much longer than skin lesions. It has been proposed that afferent C nociceptor nerve fibres are damaged by VZV and regenerate to form aberrant connections. This results in triggering of centrally modulated neuropathic pain by unusual stimuli including touch, heat and cold. This complex healing process may be especially slow in older patients in whom the pain may persist for months or years.

VZV may cause a variety of rarer but potentially serious conditions, e.g. Ramsay–Hunt syndrome.

β-Herpesvirus (human cytomegalovirus) and γ-herpesvirus (Epstein–Barr virus)

The β-herpesvirus, human cytomegalovirus (HCMV), is an extremely common childhood infection that is normally subclinical. Seropositivity is about 70 and >90% in adults living in good and poor socio-economic conditions, respectively. However, infection during pregnancy is important as HCMV is the most common cause of congenital infection in the UK. Primary infection or recurrence in immunocompromised patients produces significant disease including, for example, retinitis or life-threatening pneumonia, or encephalitis. In HIV-infected patients, HCMV diseases were becoming a major medical problem but the introduction of highly active antiretroviral therapy (HAART), now commonly known as antiretroviral therapy (ART), has greatly reduced the threat of HCMV disease. However, there is an unmet need among transplant patients.

The γ-herpesvirus, EBV, is another important human pathogen; >90% of adults worldwide are seropositive. In 1964, Epstein and his student Barr, published their discovery of virus particles in cultured
lymphoblasts from Burkitt’s lymphoma. The links between EBV and various cancers are well known but beyond the scope of this review. Two strains, EBV-1 and EBV-2, are recognized that differ in the genes involved in the latent form of infection. Although both strains are widely distributed geographically, EBV-1 is more common in the USA and Europe, EBV-2 in Africa and an individual may be infected with both strains. During primary infection, EBV infects B cells, some of which are triggered to differentiate into resting, latently infected memory B cells, thus ensuring the lifelong persistence of the virus.

EBV infection causes mild illness in virtually all young (age 2 years) children in poor socio-economic conditions but in only about 50% of children in USA and Great Britain. In adolescents, EBV can cause more serious disease initially known as glandular fever but then renamed infectious mononucleosis (IM). Among USA students entering university, about 50% are EBV seronegative, each year 10–15% of these students will have primary EBV infection of which approximately 60% will have acute IM disease. Thus, about 10% of U.S. university students will have symptomatic IM. The symptoms of IM are commonly malaise, headache, fever, tonsillitis and/or pharyngitis and local lymph node enlargement and tenderness while severe fatigue is often prominent and may last for months.

Transmission is normally through contact with saliva; among adolescents it is commonly known as the ‘kissing disease’; indeed, it is hard to avoid infection. The incubation period is from 30 to 50 days, and infected individuals are likely to be unaware of the transmission risk as intermittent, asymptomatic oral shedding of EBV continues for their lifetime. IM is generally considered to be a self-limiting disease for which only supportive care is needed. The adolescent may recover from the acute symptoms within 1–2 weeks but, in some cases, continue to suffer from fatigue for months and be unable to return to normal life. One of us (A.V.H.) has long used the term ‘career-limiting’ disease, because young persons suffering from IM who are approaching critical exams have been known to fail, putting their careers at risk.

Other β- and γ-herpesviruses, HHV-6, 7 and 8

HHV-6 was initially named human B-lymphotropic virus following its isolation in 1986, from patients with lymphoproliferative disorders. Subsequently, similar viruses were isolated from HIV-infected patients. In 1988, HHV-6 primary infection in children was shown to cause exanthema subitum (ES) or roseola infantum. Subsequently, two variants have been recognized, HHV-6A and HHV-6B, the former being isolated mainly from patients with lymphoproliferative disorders or AIDS.
patients, the later mainly from ES patients. There is an ongoing debate as to whether these variants should be considered as different herpesviruses.

In 1990, HHV-7, was first isolated from the CD4+ve lymphocytes from a healthy individual. In 1994, the same virus was isolated from two infants with typical ES. Although not yet well established, it seems that HHV-7 can cause disease similar to ES caused by HHV-6. Throughout the world, most individuals become seropositive for both HHV-6 and HHV-7 during childhood. If an individual escapes being infected with HHV-6 during childhood but is infected as an adolescent, then the virus may cause disease similar to IM.

Since the 1950s, it was suspected that Kaposi’s sarcoma (KS) was caused by an infectious agent and in 1993, KS herpesvirus (KSHV or HHV-8) was identified. Interest in HHV-8 increased as KS developed in AIDS patients. HHV-8 was detected in almost all cases of KS in both HIV-infected and HIV-negative patients. A second B-cell lymphoproliferative disorder, multicentric Castleman’s disease has also been found to be associated with HHV-8 infection.

In contrast to the widespread HHV-6 and -7 seropositivity, HHV-8 seropositive individuals are rare (<3%) in North America and northern Europe, more common in Mediterranean countries and very common in central Africa. With the advent of HAART for HIV-infected patients, the incidence of KS declined dramatically, perhaps by 90%. However, in 2007, there was a report of HIV-associated KS in patients with high CD4 count and low viral load. So, could a second wave of KS emerge? Furthermore, in central African countries, KS is recognized as a major health problem.

Vaccination as an approach to preventing infection and/or disease caused by herpesviruses

Vaccines for α-herpesvirus infections

To date, there has been little success with HSV vaccines. One of the better vaccines (subunit comprising HSV glycoprotein D with adjuvant), initially showed limited activity in preventing HSV-2 genital infections but only in women—there was no apparent effect in men. In a subsequent phase III trial in women only, there was no reduction in HSV-2 infections.

By contrast, VZV vaccination has been successful with the live attenuated strain, Oka developed in Japan in 1974. In the USA, this vaccine was approved in 1995 for universal administration to children. Prior to the introduction of the vaccination programme in the
USA, a whole annual birth cohort of children would have chickenpox each season, mainly in winter and early spring. Since the introduction of vaccination, chickenpox infections have been reduced markedly. There are preliminary indications that the Oka strain is less prone to reactivations than wild-type VZV. For example, in 96 children with leukaemia, those who had been vaccinated were less likely to have herpes zoster than those who had acquired varicella naturally. Therefore, as the current cohort of vaccinees reach old age, a reduction in the incidence of zoster may be anticipated although this expectation is controversial.

The same Oka vaccine, but at a higher dose and given to elderly adults, provides some protection against herpes zoster (shingles). In a large trial, elderly subjects (≥60 years) were given either vaccine or placebo and followed for about 3 years. According to the trial protocol, all patients, who had herpes zoster, were treated with the antiviral, famciclovir (see below). The ‘burden of illness’ (BOI), a measure including both the incidence of shingles and its severity, was reduced by 61%. The incidence of herpes zoster was reduced by 51% and that of PHN by 67% (27 cases versus 80). In this study, PHN was defined as significant pain, score ≥3 on a scale of 10, at 3 months after rash onset. It should be noted that all patients with herpes zoster would have been treated with famciclovir, and so any reduction in PHN was due to vaccine and famciclovir versus famciclovir alone. Reactions at the injection site were more common among vaccinees than with placebo but were generally mild. Being a live vaccine, it is contra-indicated in immunosuppressed patients. In a further large trial in people between 50 and 59 years old, the incidence of herpes zoster was reduced by 70%. Over the years, the database has grown to more than 38,500 subjects. Although the vaccine efficacy for reducing the incidence of herpes zoster has remained at 51%, there was a reduction with age: 64, 41 and 18%, for ages 60–69, 70–79 and ≥80 years, respectively. The apparent reduction of PHN was due largely to the reduction in the incidence of herpes zoster. As a proportion of herpes zoster cases, the reductions of PHN were 5, 55% (P < 0.05) and 26% for the age bands 60–69, 70–79 and ≥80, respectively (https://www.merckvaccines.com/Products/Zostavax/Pages/efficacyfps (efficacySPS.pdf) (5 October 2012, date last accessed)).

In 2006, this vaccine was approved in Europe and USA for those over 60 years old. The later trial data led to an extension of approval to people over 50 years old. In the UK, the advisory committee considered the cost-effectiveness of herpes zoster vaccination and recommended that people aged 70–79 should be vaccinated but that, as more information about the duration of protection became available, earlier vaccination should be considered. To date, protection appears to persist for at least 7 years.
Vaccine for \(\beta\)-herpesvirus (human cytomegalovirus)

There has been some encouraging progress with HCMV vaccines. For example, a vaccine containing glycoprotein gB with the adjuvant MF59 (containing squalene, sorbian trioleate and polysorbate) has been tested in clinical trials by Griffiths et al.\(^\text{13}\) who reported on 140 adults, awaiting kidney or liver transplantation, who were given the vaccine or placebo. Seronegative patients showed a marked rise in HCMV antibody and patients who were already seropositive also showed a significant rise. There appeared to be a benefit because the number of days in which viremia was detected was reduced in the vaccinees, and there was a reduced requirement for ganciclovir (GCV) pre-emptive therapy in those that had received vaccine. In a more recent double-blind, placebo-controlled trial reported by Pass et al.\(^\text{14}\) a similar vaccine (or placebo) was given to 464 seronegative healthy women within 1 year after they had given birth. Subsequently, 19 HCMV infections occurred in the vaccinees compared with 32 among those that were given placebo (14 versus 18%, respectively). None of the women showed any clinical signs at the time of HCMV infection that was confirmed by virus culture or PCR methods. Among the trial population, HCMV infection later led to 1/81 congenital infections in newborn and 3/97 cases occurred among the group that received placebo. Although these results were promising, clearly the incidence of disease was too small to draw firm conclusions. Both trials demonstrate the considerable difficulties in carrying out such studies.

As well as conventional vaccines for HCMV, a DNA vaccine has also shown promise in a Phase II clinical trial. In transplant patients, prophylactic use of current antivirals is limited by their toxicities (see below). Thus, clinical trials have been commenced with a DNA vaccine which contains plasmids which, being non-infectious, pose no threat to the immunocompromised patient.\(^\text{15}\) TransVax\(^\text{TM}\) contains two DNA plasmids corresponding to two HCMV genes encoding viral proteins; pp65 (with four amino acids deleted from the kinase domain) is the immunodominant target for cellular immune response and gB (the extracellular domain) is the major target of neutralizing antibody. The vaccine contains 5 mg total of DNA and is formulated as nanoparticles. Having shown good tolerance in a Phase I trial, a placebo-controlled Phase II trial was completed in 80 HCMV positive haematopoietic stem cell transplant (HCT) patients with leukaemia or lymphoma.\(^\text{16}\) Dosing was at 0, 1, 3 and 6 months starting day 5 to 3 pre-transplant. HCMV load was determined weekly during weeks 3–13, bi-weekly for weeks 14–28 and then monthly for weeks 29–52. T-cell responses to pp65 were enhanced after the second dose. Antibody response to gB remained similar to controls until after the fourth dose, whereas the T-cell response to gB rose after the third dose. These enhanced immune responses were durable up to 1 year.
Importantly, the vaccine reduced various measures of HCMV versus placebo; the time to initial viral detection was prolonged (mean >365 versus 110 days), occurrence of HCMV reactivation was reduced (32.5 versus 61.8%), the duration of viraemia was decreased (mean, relative to time on study, 4.9 versus 7.6 days), and the occurrence of initiating HCMV-specific antiviral therapy was reduced (48 versus 62%). This DNA vaccine appeared to be safe and well tolerated.

Following the success of this Phase II trial, the company, Vical announced that a Phase III trial in HCT patients is planned to start by the end of 2012 and a Phase II trial for solid organ transplant recipients shortly afterwards.

To date, we know of no effective vaccines available for EBV or HHV-6, -7 or -8.

Thus, herpesvirus infections have not, in general, been easy to control by means of vaccination. However, this family of viruses was one of the first for which effective antivirals were discovered. This heralded the era of effective antiviral therapy.

Formative years of antiviral chemotherapy

HSV readily replicates in cell culture producing quantifiable cytopathic effects within 2 or 3 days and with relevant laboratory rodent infection models, this enabled the first potential inhibitors to be evaluated. Herpesvirus virions have a large DNA genome encoding more than 70 distinct polypeptides. The virus-coded enzymes, involved in DNA synthesis, are the targets for all effective antiviral agents. Since the discovery of iododeoxyuridine (IDU) in 1959, followed by trifluorothymidine (TFT), nucleoside analogues have been the mainstay of herpesvirus antiviral therapy. IDU, for topical treatment of herpes keratitis (HK), was one of the first effective antiviral compounds to be licensed. Although still in use, IDU and TFT are limited by their toxicities to topical treatment of eye infections (HK). A truly selective, safe and effective antiviral compound seemed to be an impossible dream. Remarkably, over the past 30 years, this situation has been transformed with antiviral therapies for HSV, HIV and HBV continuing for years in individual patients, even decades, with outstandingly few adverse effects.

Compounds to treat α-herpesviruses infections

Overview

The discovery of acyclovir; spelt aciclovir in some countries (ACV), published in 1977, marked a turning point in antiviral therapy.
The Wellcome group showed how ACV could be both effective against HSV and VZV and have an excellent safety profile. ACV itself is inactive, however, in cells infected with HSV or VZV, the viral thymidine kinase phosphorylates ACV to the monophosphate (ACV-MP). Presumably, the flexibility of the acyclic chain allows the purine analogue to be a substrate for a pyrimidine kinase. Cellular enzymes then further phosphorylate ACV-MP to the triphosphate (ACV-TP), which is the active form of the drug, inhibiting viral DNA synthesis. In this way, the virus provides a ‘safety gate’ by which the antiviral compound is activated (Fig. 1B).

Following this lead, various research groups discovered other nucleoside analogues which also use this ‘safety gate’, e.g. brivudin (BVDU) and penciclovir (PCV; Fig. 1C and D). BVDU was approved for therapy of VZV in Germany and then in several other European countries but not in UK or USA where there were safety concerns. Penciclovir is poorly absorbed from the oral route; therefore, an oral prodrug, famciclovir (FCV), was developed. This stimulated the search for an oral prodrug for ACV; valacyclovir (VACV) was selected. As ACV/VACV and PCV/FCV are the only compounds widely approved

Fig. 1 The current nucleoside analogues use a ‘safety gate’ to gain selectivity for herpesvirus-infected cells. (A) Acyclovir (ACV) was the first highly selective antiviral compound and is still used widely for therapy and prevention of HSV and VZV disease. (B) Activation of ACV within cells via the viral thymidine kinase (TK) ‘safety gate’ yielding ACV–triphosphate that interacts with herpes DNA polymerase. (C) Brivudin: bromovinyldeoxyuridine (BVDU). A pyrimidine nucleoside which has not been widely used owing to safety concerns. (D) Penciclovir (PCV) is a guanosine nucleoside analogue that is activated via the viral TK ‘safety gate’. It has poor oral bioavailability and so a prodrug, famciclovir (FCV, Fig. 2) was developed. Like ACV, FCV is widely used for therapy and prevention of HSV and VZV. (E) Ganciclovir (GCV) is a guanosine nucleoside analogue which is activated via HCMV protein kinase. GCV and its oral prodrug valganciclovir (VGCV) are currently the most commonly prescribed drugs for the prevention and therapy of HCMV in immunocompromised patients although they do not have the benign safety profiles of ACV and PCV.
ACV and PCV: pre-clinical comparisons

ACV and PCV share similar modes of action but there are some marked differences. PCV makes good use of the ‘safety gate’, because its preferential affinity for the viral thymidine kinase is about 100-fold that for ACV, leading to 100-fold higher intracellular concentrations of PCV-TP than ACV-TP. The situation is reversed regarding the affinities of their triphosphates for the viral DNA polymerases, ACV-TP being about 100-fold greater than PCV-TP. Both ACV-TP and PCV-TP are not only competitive inhibitors of the viral DNA polymerase but also substrates and the nucleoside analogues are incorporated into the viral DNA. Because ACV does not have a second hydroxyl group, it has been called an ‘obligate’ DNA chain terminator. In a DNA elongation

Fig. 2 The two compounds most often used for suppression and treatment of HSV and VZV disease. Valacyclovir (VACV) and famciclovir (FCV) are acted on by host enzymes as shown to yield ACV and PCV, respectively.
assay, PCV was incorporated into the DNA chain, and further elongation was possible when the template strand had a single deoxycytidine base but not where there were two adjacent deoxycytidine bases.\(^\text{20}\)

Notwithstanding the differences in enzyme affinities, the two compounds have similar potencies as shown by standard cell culture antiviral assays in which the compounds are present continuously. However, such assays do not reflect human pharmacokinetics following oral dosing of ACV/VACV and FCV; high concentrations of the drugs in blood are present for only a short time after each oral dose. Therefore, duration of antiviral effect becomes important. Once phosphorylated, the drugs are trapped within the virus-infected cells but PCV-TP is more stable than ACV-TP. The half-lives in HSV-1, HSV-2 and VZV infected cells are 10, 20 and 9 h compared with 0.7, 1 and 0.8 h, respectively.\(^\text{19,21}\) The high stability of PCV-TP has been shown to give the compound an advantage in both cell culture assays and in animal models.\(^\text{19,22,23}\) For example, in an HSV-1 assay in which the compounds were present for 2 h only, and virus measured 17 h later, ACV had only poor activity, whereas PCV retained good activity. In an HSV-2 assay looking for duration of activity after treatment with ACV or PCV for 18 h, HSV-2 was measured at days 2, 4 and 6. In ACV-treated cultures, virus levels were high at all time points; as expected, ACV-TP levels would have declined rapidly, within a few hours, thus allowing viral replication to recommence. By contrast, no virus was detected in PCV-treated cultures; virus replication never recovered after PCV treatment. A possible explanation may be that, once high levels of PCV-TP are formed in a virus-infected cell, the stability of the PCV-TP may stop virus replication until the cell dies naturally due to the viral infection. A similar explanation may account for the lack of resurgences in an HSV-1 murine immunosuppression model. Following VACV treatment for 5 or 10 days, there was a resurgence of virus replication in both the ear and in the brain stem. Such virus resurgences were not seen following corresponding FCV treatment\(^\text{23,24}\) (Fig. 3).

In a series of experiments investigating the effects of VACV and FCV on latency in a murine infection model, with treatment starting early (times 0–5 days after primary infection) neither compound was able prevent the establishment of HSV latency.\(^\text{25}\) However, there was a marked difference between the compounds when looking at rates of experimental reactivation from explanted dorsal root ganglia. It was shown that VACV had little effect compared with controls. By contrast, FCV commenced from 0 to 5 days after infection significantly reduced reactivations.\(^\text{24}\) With treatment starting either 1 or 2 days after infection, the proportion(%, \(n = 16/\text{group}\)) of mice positive for virus from ganglia (ipsilateral and contralateral) were 60 and 0%, respectively, in
VACV-treated mice and 0 and 0% in FCV-treated mice. With treatment starting 3 or 4 days after infection, 88 and 66% yielded virus in the ipsilateral and contralateral ganglia, respectively, from VACV-treated mice but, with the FCV-treated mice, those virus positive were 7 and 0%, respectively. Delaying treatment to day 5, all ipsilateral and contralateral ganglia yielded virus from VACV-treated mice. By contrast, from FCV-treated mice, only 38% and 0% of ganglia, respectively, yielded virus. Although it is difficult to extrapolate these results to man, especially the time scale, we give two clinical situations in which this unique activity of FCV could possibly lead to clinical benefit (see below).

Resistance of HSV to acyclovir is usually due to a change in the viral thymidine kinase, rarely in the viral DNA polymerase. Often, the mutated thymidine kinase is a protein with no activity, referred to as a TK negative strain. Such resistant strains are cross-resistant to famciclovir. Fortunately, although TK negative strains of HSV are able to establish latency, they are deficient in their ability to reactivate. Therefore, the next recurrence is likely to be the original sensitive virus.

Fig. 3 Taken from Field et al.23 with permission American Society for Microbiology, Washington, DC showing data from an HSV-1 murine infection model, where resurgences of infectious virus occurred in the tissues following cessation of oral VACV but not FCV therapy. (A and C) Virus in ear. (B and D) Virus in brain stem. Therapy from day 1–5 (A, B); day 1–10 (C, D).
Sometimes, the ACV-resistant virus is due to a thymidine kinase with an altered substrate specificity; such strains may, or may not, be sensitive to famciclovir;26 similarly for VZV.27 ACV-resistant virus due to mutations in the polymerase may have reduced (≤ 3-fold) sensitivity to PCV but are rarely highly resistant. Presumably, as virus replication absolutely requires a functional DNA polymerase, potential resistant mutations are restricted.

In a cell culture study of resistance to VZV,28 16 ACV-resistant (ACVr) clones were found to have a non-functional TK. With BVDU, six of six resistant clones were also due to non-functional TK. These TK-negative strains were cross-resistant to PCV. Unexpectedly, the four clones of PCV-resistant (PCVr) virus had no mutations in the TK gene but all four had a single mutation in the VZV DNA polymerase (V666L). Although this is a known resistance mutation for ACV, these PCVr clones retained sensitivity to BVDU and FV-100 (see below). There is a lack of data on PCVr strains of VZV isolated in the clinic. By contrast, ACVr strains have been reported and often shown to be due to TK mutations. The observation that the emergence of resistance is much slower for PCV than ACV can now be understood.29 It is more difficult to get resistance in the VZV DNA polymerase than in the non-essential viral TK.

This work has implications for treating immunocompromised patients presenting with VZV. If treatment is started with VACV, resistance is likely and then FCV becomes ineffective. On the other hand, FCV therapy may be sufficient but if resistance does eventually occur, then BVDU or FV-100 should be considered as a second-line treatment. Rather than changing to another drug, experience with HIV therapy suggests that it may be preferable to continue FCV and add the second drug. In the absence of any clinical data, it would be useful to extend these cell culture VZV-resistance studies to investigate which drug regimen would be best to treat cultures infected with FCV-resistant virus.

Although it has been easy to show differences between ACV and PCV in cell culture assays and in animal models, it has been hard to show differences in clinical studies. In part, this may be due to the very variable nature of herpes simplex and varicella zoster diseases in patients. Now that ACV, VACV and FCV are ‘off-patent’, there seems to be little justification for using ACV except for intravenous therapy in seriously ill, hospitalized patients.

While selection of resistant strains can be readily achieved in tissue culture, ACV- or PCV-resistant strains are rarely encountered in clinical practice except in treatment of HK.30 However, antiviral drug resistance in HSV and VZV can become a serious problem when individual immunocompromised patients need many successive
antiviral treatments. In such cases, the virus isolates may comprise mixtures containing various mutations including mutations that confer resistance to foscarnet (FOS) and cidofovir (CDV; see below) and these infections may be extremely difficult to manage. It is expected that in the future, new agents (e.g. HPI, see below) will be used in combination with the existing nucleoside analogues in these difficult cases.

**VACV and FCV: clinical efficacies**

**HSV-1, herpes labialis (cold sores)**

Primary infection in children is often mild or inapparent but in moderate or severe cases, antiviral treatment has been recommended. Oral ACV suspension (15 mg/kg, five times daily for 7 days) versus placebo reduced duration of lesions (4 versus 9 days) and of virus shedding (1 versus 5 days). Although, for this indication, no trials have been done with famciclovir or valacyclovir, 500 or 1000 mg, respectively, twice daily for 7 days would be logical regimens.31

Initially, it seemed appropriate to treat recurrent herpes labialis topically. The somewhat limited efficacy of ACV was thought to be due to lack of penetration through the skin. PCV cream did show a benefit in various parameters associated with lesions. In this trial, the cream was applied every 2 h while awake—an inconvenient regimen. As the safety of oral ACV and FCV became well established, and as viral resistance to both drugs have remained at a very low level (below 0.5%) in normal healthy subjects after more than a decade of use, trials using oral drugs were initiated. It became clear that early start of therapy was essential to maximize the benefit. In practice, this meant that subjects, prone to herpes labialis, had to be given their medication in advance and instructed to start therapy as soon as they felt the first prodromal signs (e.g. tingling sensation).

Treatments with FCV (either a single 1500 mg dose or 750 mg twice for a single day) or placebo were patient-initiated within 1 h of onset of the prodromal symptoms of herpes labialis.32 One large dose was as effective as two smaller doses. The times to resolution of pain and tenderness were each reduced by 1 day (41%), the times to healing of the primary and of all lesions were each reduced by 2 days (about 30%) and the time to return to normal skin was reduced by 2.5 days (36%).

Similarly, early, short treatments with VACV have been evaluated in herpes labialis. VACV regimens, either 2000 mg twice for one day or the same for the first day followed by 1000 mg twice on the second day, were compared with placebo. The 2-day regimen did not give any additional benefit. The mean duration of the cold sore episode was about 1 day shorter than for placebo.
In these two herpes labialis trials, treatment with either FCV or VACV did not significantly increase the number of ‘aborted’ lesions. Of patients with herpes labialis, only a minority (<10%) have many recurrences and so suppressive therapy is not normally required. However, FCV (500 mg twice daily) or VACV 1000 mg once daily) would be reasonable regimens.

**HSV-2: Primary genital herpes**

The first episode of genital herpes is regarded as a true primary infection when the patient is not already seropositive for HSV-1 or HSV-2. For HSV-1 seropositive patients, the first episode is likely to be less severe than for a true primary infection. In untreated cases of primary genital herpes, virus replication may continue for several weeks.

Initially, ACV was the standard treatment now largely superseded by VACV. In a trial of first-episode genital herpes, VACV (1000 mg twice daily) was compared with ACV (200 mg five times daily) for 10 days; treatment was started within 72 h of symptom onset. For both groups, the median times were similar to cessation of viral shedding (3 days), cessation of pain (5 days) and to healing (9 days).

A dose-ranging study compared FCV (125, 250 and 500 mg doses, three times daily) versus ACV (200 mg five times daily) for 5 or 10 days of therapy. The 250 and 500 mg doses of FCV were comparable to ACV. Although not approved by the Food and Drug Administration (FDA), FCV (250 mg three times daily for 5–10 days) is recommended by the Centers for Disease Control and Prevention (CDC). VACV (1000 mg twice daily for 7–10 days) is both FDA-approved and CDC-recommended.

**HSV-2: Recurrent genital herpes: episodic therapy**

As for recurrent herpes labialis, recent trials for genital herpes have focused on early, short treatment regimens. In a trial comparing FCV (1000 mg twice in a single day) and placebo, patients took a pre-treatment swab for virus shedding, initiated therapy within 6 h of the first symptoms and then attended a clinic within 24 h. The median time to treatment initiation was 2.5 h. FCV reduced median time to healing by nearly 2 days (4.3 and 6.1 days, respectively). The durations of various measures of lesion symptoms were reduced; burning and tingling each by about 30%, pain, itching and tenderness each by about 40%. The proportion of patients with ‘aborted’ lesions was increased by FCV therapy; in intention-to-treat analysis, there was a increase in ‘aborted’ lesions of 23 versus 13%, $P = 0.003$ and in PCR+ve patients, 21 versus 5%, $P = 0.001$.

Using a similar trial design, single-day FCV therapy was compared with a 3-day VACV therapy. The times-to-healing and to resolution...
of symptoms were similar for both groups with a trend to more patients receiving FCV having ‘aborted’ lesions (virus culture+ve: 22 versus 13.5%, \(P = 0.3\)). Thus, FCV (1000 mg twice for a single day) and VACV (500 mg twice daily for three days) were both highly, and equally, effective in reducing the duration of genital symptoms and the proportions of patients with ‘aborted’ lesions appeared to be similar.

**HSV-2: Recurrent genital herpes: suppressive therapy**

A 6-year trial with ACV proved that suppressive therapy was a highly effective option for improving the lives of those patients who had many recurrences a year. During the first year, ACV (400 mg twice daily), compared with placebo, increased the proportion of patients remaining recurrence free (44 versus 2%) and the median time to the first outbreak (246 versus 18 days). During the following years, there appeared to be a gradual improvement with 70% of the ACV-treated patients remaining recurrence free during the fifth year.

In a dose-ranging study, various FCV regimens (125 mg once or twice daily, 250 mg once or twice daily and 500 mg once daily) were compared. FCV (250 mg twice daily) was the most effective regimen for prolonging the time to the first recurrence. In a larger trial comparing FCV (250 mg twice daily) with placebo, the proportions of patients remaining recurrence free at one year were 70 versus 20%.\(^3\) In a further study, FCV (125 mg three times daily, 250 mg three times daily and 250 mg twice daily) was compared with placebo.\(^3\) All FCV dose regimens prolonged the time to first recurrence (222–336 versus 47 days) but the 125 mg dose was less effective than either of the 250 mg regimens.

In the first trial to evaluate VACV (500 mg once daily) versus placebo for 16 weeks, VACV therapy increased the time to first recurrence (>112 versus 20 days) and increased the proportion of patients remaining recurrence free (69% versus 9.5) at 16 weeks. In a 1-year trial, various doses of VACV (250, 500 and 1000 mg once daily, 250 and 500 mg twice daily) were compared with placebo. The proportions of patients remaining recurrence free were 22, 40, 48, 50 and 49 versus 5%, respectively. When patients with differing baseline recurrences were compared, those with 10 or more per year needed higher-dose therapy relative to those with fewer recurrences.

There has been one trial investigating the potential for antivirals to reduce transmission of HSV from a seropositive subject to a seronegative partner. VACV was compared with placebo and showed about 50% reduction in the risk of transmission. However, in trials with either FCV of VACV in which asymptomatic viral shedding was monitored, neither drug eliminated viral shedding. Therefore, it is important
to advise patients that the risk of transmission remains during suppression therapy.

**VZV: Zoster or shingles**

For many years, ACV was the only oral antiviral therapy widely approved for treatment of herpes zoster. It clearly reduced the acute symptoms of zoster but there was no effect on either the incidence or duration of PHN in a trial including only elderly patients.\(^3\)\(^7\)

VACV (1000 mg tid for 7 or 14 days) was compared with ACV (800 mg five times daily for 7 days) in patients ≥ 50 years with herpes zoster.\(^3\)\(^8\) Skin lesions resolved at similar rates in the three groups. Herpes zoster-associated pain, which includes acute pain and long-term pain, was reduced by VACV treatment (38 and 44 days, respectively, versus 51 days, \(P \leq 0.03\)). It seems that the longer treatment time did not give any extra benefit. In the Kaplan–Meier plots, the difference between the VACV and ACV groups emerged during the acute phase of the disease and did not appear to diverge further during the later part of the trial associated with prolonged PHN. Combining both VACV groups, the proportions of patients with pain at 6 months was reduced from 26% with ACV to 19% with VACV (Fig. 4).

Because there was no accepted treatment for reducing PHN, the first trial of FCV in herpes zoster was versus placebo. FCV therapy (500 or 750 mg tid) reduced the duration of virus shedding and improved the resolution of the acute symptoms. In the Kaplan–Meier plots of proportion of patients with pain over a 6-month period, the lines for the two FCV groups overlap, but these diverge from the placebo line and continue to diverge after 1 month. The median time to resolution of PHN was 53 and 61 versus 119 days). This is even more marked in older patients (over 50 years), the corresponding times for 500 or 750 mg (tid) versus placebo being 63 and 63 versus 163 days, respectively. In these intention-to-treat analyses, PHN was defined as pain continuing after lesion healing. However, there have been various definitions of PHN, for example, pain following healing or pain after 30 days or after 3 months. Following proposals for how pain analyses should be performed in future trials,\(^3\)\(^9\) the data from the FCV trial was re-analysed.\(^4\)\(^0\) Irrespective of which of these three definitions is used, FCV therapy significantly reduced the duration of PHN. In prospectively defined efficacy-evaluable groups, for pain persisting after 30 days, the ratios (values > 1 indicating faster resolution of pain than placebo) were 2.15 (\(P = 0.015\)) and 2.6 (\(P = 0.002\)) and when pain was defined as persisting after 3 months, the ratios were 2.4 (\(P = 0.048\)) and 3.1 (\(P = 0.008\)), respectively. As this trial has given the most clear-cut evidence for antiviral therapy reducing PHN, perhaps FCV should be
considered as the treatment of choice for those patients (i.e. elderly) most at risk of PHN?

Three doses of FCV (250, 500 and 750 mg tid) were compared with ACV (800 mg five times daily).\textsuperscript{41} There were no differences between these groups for cutaneous lesion healing, but FCV treatments gave a greater reduction in zoster-associated pain than did ACV. In the Kaplan–Meier plots, the lines for the FCV and ACV groups continued to diverge after 1 month. This infers that FCV had a greater effect reducing long-term pain than did ACV. In an efficacy-evaluable analysis including only those patients treated within the first 48 h of rash (the group most likely to benefit from antiviral therapy), not only was long-term pain reduced faster in the FCV group than in the ACV group but it was notable that there were only a few patients (<10%) with pain at 180 days in the FCV groups (Fig. 5).

In a trial comparing FCV (500 mg three times daily) with VACV (1000 mg three times daily), patients, these two drugs gave comparable efficacies both in terms of rash healing, time to reduction of zoster-associated pain and safety.\textsuperscript{42}

\textbf{Compounds to treat HCMV}

Because HCMV disease occurs most frequently in immunocompromised patients, their management is complex; this review focuses only on
specific antiviral therapy. Here, we give only a brief summary of current antiviral therapy because we are hopeful that the introduction of new compounds (see below) will radically alter the approach to HCMV management.

Although VACV has some activity against HCMV, the compound needs to be administered at near-toxic doses and is therefore little used. GCV (Fig. 1E) and, more recently its prodrug valganciclovir (VGCV), are the mainstay of therapy for control of HCMV disease. Although HCMV infection is widespread among the population, HCMV disease in adults is normally confined to those who are immunocompromised. As for ACV and PCV, GCV is selectively phosphorylated to the monophosphate in HCMV-infected cells. However, HCMV does not encode a TK but the kinase encoded by the HCMV gene UL97 acts as the ‘safety gate’ for GCV. GCV-TP is the active form that inhibits HCMV DNA synthesis. Unfortunately, GCV does not have the benign safety profile of VACV and FCV, thus limiting its use for prophylactic therapy. For example, in transplant patients, VGCV is not used from the time of transplant but instead patients are closely monitored for HCMV viral load. As soon as HCMV viraemia rises above a predeter-
mined threshold, VGCV therapy is started to prevent the patient progressing to HCMV disease. This is known as pre-emptive therapy.

This approach has been used successfully for many years but, inevitably when treating virus infections in immunocompromised patients, resistance to VGCV does occur. The second-line drugs, the

Fig. 5 FCV compared with ACV for the reduction of herpes zoster-associated pain in efficacy-evaluable patients enrolled within 48 h of rash onset. This is the group of patients most likely to benefit from antiviral therapy. Adapted from the Kaplan–Meier plot Degreef et al.41 with permission from Elsevier Science, BV with only the approved doses shown.
pyrophosphate analogue, FOS, and the nucleotide analogue, CDV, have their own safety issues. So it is particularly encouraging that new HCMV inhibitors and two HCMV vaccines are showing promise in clinical trials.

A barren decade

Since FCV was first approved in the mid 1990s, followed by VACV shortly afterwards, there have been no major products entering the market for the therapy of α-herpesviruses. The main reason for lack of progress has probably been economic rather than scientific. For example valomaciclovir stearate (VALM-S) and valomaciclovir (VALM), prodrugs of H2G (Fig. 6A and B) and FV-100 (prodrug of Cf-1743; Fig. 6C) with promising activities, have passed through pre-clinical toxicology but have faced great difficulties getting backing for clinical trials.

The name, valomaciclovir, was assigned to the prodrug, 4′-valyl-H2G (Fig. 6B) but has commonly, but mistakenly, been used for the mixed diester (Fig. 6A) which should be called valomaciclovir stearate. This has led to confusion in the literature. All the Phase II trials have been with VALM-S which is the prodrug continuing in clinical development. In the two clinical trials reported by Tyring and Balfour, respectively, VALM-S was the prodrug used but it was called valomaciclovir (Dr Bo Oberg, personal communication). For this article, VALM-S refers to valomaciclovir stearate.

In cell culture assays, H2G has good activities against VZV and EBV. Following years of slow progress for VZV therapy, VALM-S was evaluated in a Phase IIb dose-ranging study in patients with herpes zoster. The primary endpoint (time to complete crusting of the shingles rash) was non-inferiority of once-daily VALM-S compared with thrice-daily VACV. The 373 patients enrolled were randomized into three groups, VALM-S (1 or 2 g) or VACV (1 g) with an additional 18 patients having VALM-S (3 g). Compared with VACV, in patients over 50 years old, there were dose-dependent trends to improved pain resolution. Although there were only 18 patients treated with 3 g VALM-S, there was significant superiority to VACV with regard to the primary endpoint, complete crusting (ratio = 2.2, \( P = 0.008 \)) but was similar to VACV for most other parameters.

Clinical efficacy of VALM-S has also been demonstrated against IM due to primary EBV infection. Teenagers and young adults (16 to 24 years old) were randomized to 21 days of VALM-S (4 g daily) or placebo and followed for 6 months. The median time of enrolment of the 21 subjects was 6 days after the onset of IM. VALM-S subjects (\( n = 11 \)) had significantly faster reduction of symptoms than placebo recipients.
(P < 0.05), and significantly faster decrease in EBV load in the oral compartment (oral cell pellet, \( P = 0.03 \), oral supernatant, \( P = 0.001 \)). The proportions of subjects having a 2 log_{10} decrease in EBV load in the oral compartment were significantly different (8/11 versus 2/10, \( P = 0.03 \)). There was no difference in the clearance of EBV viraemia. The authors concluded that this first demonstration of clinical benefit derived from VALM-S therapy of IM should encourage additional studies. However, to our knowledge, none have been carried out.

In pre-clinical studies, FV-100 was shown to be highly active (EC_{50} about 1 nM) against VZV and uniquely specific for only this virus.\(^4^5\) FV-100 requires phosphorylation by the VZV TK and so uses the same ‘safety gate’ as for the other nucleoside analogues, ACV, PCV and H2G. The next steps remain a mystery, certainly FV-100 TP is formed
in such small, trace amounts, it cannot be the active form of the compound. Hence, it should retain activity against VZV resistant strains with mutant viral DNA polymerase. In Phase I trials, single doses of FV-100 (from 100 to 800 mg) were evaluated in young and elderly patients. Even the 200 mg dose gave plasma levels of drug exceeding the active levels for 24 h. With the high potency and favourable pharmacokinetics, it was hoped that FV-100 would be more effective than the current drugs, FCV and VACV in treating herpes zoster patients.

FV-100 regimens (200 and 400 mg once daily) were compared with VACV (1000 mg three times daily) in a Phase II dose-ranging study in herpes zoster patients aged 50 years and older. The primary endpoint was a reduction in herpes zoster associated pain and severity (BOI) 30 days after the start of treatment; a 20–25% difference between the FV-100 cohorts and VACV would be significant. Secondary endpoints included BOI after 90 days, incidence of PHN, mean time to lesion healing and use of concomitant pain medications.

This trial missed the primary endpoint, the reductions in BOI being 3 and 7% for the FV-100 cohorts (200 and 400 mg) versus VACV, respectively. For the BOI 90-day endpoint, the reductions were 4 and 14%, respectively. There was also an apparent dose–response trend in the reductions (12 and 39%) of PHN incidence, (17.8 and 12.4 versus 20.2%), respectively. Although somewhat disappointing, this result points the way to testing FV-100 at 800 mg once daily.

Both VALM-S and FV-100 have shown promising efficacy against herpes zoster (shingles) and VALM-S has also shown efficacy against IM for which there are no approved antiviral drugs. However, their further progression seems to remain in doubt; why should this be so? During the period when ACV was available generically but not FCV and VACV, ACV was commonly prescribed even though the prodrugs had better efficacies. Perhaps, the improved efficacies were perceived to be modest. VALM-S and FV-100 now face a similar barrier for shingles therapy given the availability of generic VACV and FCV. For IM therapy, the situation is rather different. There is a perception that antiviral therapy can reduce viral replication effectively but not give clinical benefit. In our view, this could be due to the short duration of the courses used to date. Indeed, in the above 3-week VALM-S trial, treatment did give short-term clinical benefit but did not enhance the rate of EBV clearance. It seems that a lesson can be learnt from the experience with HBV therapies. These treatments are continued long after the HBV load has been reduced to below the limit of detection. A serological marker is used as a guide to when antiviral treatment can be withdrawn safely. With EBV, IgG antibodies to the viral nuclear antigens (EBNA IgG) appear only during the recovery period (Fig. 7).
Because long-term fatigue is the most serious factor limiting young adolescents’ return to normal life, we suggest that antiviral therapy should be continued until the long-term fatigue has resolved or the EBNA IgG levels are high, whichever is the later.

If the need for therapy of IM becomes accepted, there is already a choice of drugs. In cell culture assays, ACV, PCV and H2G have similar activities against EBV. FCV and VACV are already widely available and so could be used clinically. A reasonable regimen would be 500 mg twice or three times daily during the acute phase of IM, say 3 weeks, followed by 500 mg once daily for the remaining period, as suggested above. Because PCV-TP is likely to have a much longer half-life than ACV-TP in EBV-infected cells, FCV may be the preferred option.

**New approaches to anti-herpesvirus chemotherapy**

*Introduction*

Current therapies ultimately target herpes DNA polymerase. Recently, there has been a paradigm shift. While inhibition of DNA synthesis remains the goal, the helicase–primase complex is inhibited by several compounds (HPI) active against HSV and VZV. Another new approach is directed at HCMV DNA terminase, a viral enzyme with no mammalian equivalent and this is the target for letermovir.
There are at least seven distinct virus proteins that are essential for the replication of herpesvirus DNA and, before the DNA polymerase can function, the double-stranded genome is first subjected to unwinding (helicase) and short RNA priming-strand synthesis (primase) functions.\(^4\) These functions are carried out by ‘helicase–primase’, a heterotrimeric complex comprising the products of HSV genes \(UL5\), \(UL52\) and \(UL8\) corresponding to the helicase, primase and ancillary proteins, respectively. Several potent inhibitors of HSV and VZV have been found to bind to this complex and effectively block HSV DNA synthesis and stop the production of infectious virus. The mechanism of action of several different compounds was confirmed to be inhibition of helicase–primase by the selection of drug-resistant viruses. The resistance mutations all mapped to functional regions of \(UL5\) or, occasionally \(UL52\) genes.\(^4\)

From several independent lines of research, two series of compounds with HPI activity have progressed to clinical trials. The first lead compound (ASP 2151 or amenamevir; Fig. 8A) is highly active against both HSV and VZV in vitro.\(^5\) It was shown to be effective against HSV in laboratory animal infection models\(^6\) and two Phase II clinical trials were completed.\(^7,8\) Although a late Phase I trial was terminated for safety reasons, the proof of principle was established and further compounds in the same series are likely to emerge. The second compound BAY 57-1293 or AIC316 (recently named pritelivir; Fig. 8B) was discovered in the laboratories of the Bayer pharmaceutical company and further developed by AiCuris, Germany. Again the compound is highly active against both HSV-1 and HSV-2 (\(EC_{50}\) in tissue culture <1 \(\mu\)M for both viruses) but, in this case, not VZV. Oral administration of BAY 57-1293 was found to be effective in murine and guinea pig HSV-infection models in both normal animals (reviewed\(^9\)) and immunologically compromised mice.\(^10\)

Several surveys of clinical isolates show that these are universally sensitive to pritelivir (BAY 57-1293). However, resistance mutations are readily selected in cell culture. Furthermore, clinical isolates are found to contain a small proportion of polymorphisms in the critical residues at low concentration (usually of the order \(10^{-6}\)) that are resistant.\(^11\) A common mutation occurs in HSV helicase at residue lysine 356 just downstream from the fourth functional domain in the enzyme.\(^12\) Resistance selection is not anticipated to be a serious problem in the short-to-medium-term future except in immunocompromised patients. In this case, resistance following monotherapy is likely as for the current nucleoside analogues.

Phase I dose-escalation studies\(^13\) evaluated pritelivir (5–200 mg). The higher doses of pritelivir maintained serum concentrations well
above that required to inhibit HSV with a terminal half-life of up to 80 h (i.e. much longer than ACV). No safety issues were encountered and, to date, no significant side effects have been reported. Subsequently, a Phase II trial investigated HSV-2 mucocutaneous shedding with a treatment for 28 days with 5 mg once daily (od), 25 mg od, 75 mg od or 400 mg once weekly versus placebo. There was a dose-dependent reduction in virus shedding rates (21.2, 9.2, 2.0 and 5.2 versus 16.5%, respectively). The corresponding lesion rates were 13.3, 4.6, 1.1 and 1.3 versus 9.1%, respectively. No safety issues were identified. There were no cases of resistant virus. Thus, these are extremely encouraging results albeit the number of subjects is small.59

Finally, the question remains as to whether or the HPI can impact on latency, i.e. the time to the next lesion and/or the frequency of lesions

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**Fig. 8** Three non-nucleosides inhibitors of herpesviruses. (A) Amenamivir: the first helicase–primase inhibitor to enter clinical trials for treatment HSV and VZV. Development terminated following safety concerns during a Phase I trial.50 (B) Pritelivir (BAY 57-1293 or AIC316) is a potent oral helicase–primase inhibitor that is undergoing Phase II clinical trials against HSV.49 (C) Letermovir is showing promise for control of HCMV. To date, this compound appears to have a better safety profile than current HCMV therapies.62
following cessation of therapy. A claim has been made that the recurrent lesions were less frequent in guinea pigs following experimental therapy; however, few data were published.60 We should remain sceptical as no mechanism for an effect on latent virus has been proposed—the question clearly requires further research.

Whether or not HPIs make a lasting impact on HSV therapy and prophylaxis remains to be seen. The compounds appear to have greater potency and the potential for less frequent dosing. However, nucleoside analogues such as ACV and PCV and their prodrugs have an, as yet, unrivalled safety record and, as discussed above for VALM-S and FV-100, it remains to be seen if our improved understanding of these HPIs will provide sufficient confidence in this new family of antivertex drugs as the clinical trials progress. Furthermore, there is also an economic reality in that VACV and FCV are now available generically which may be a barrier to the further development of HPI monotherapies.

Letermovir - a promising new therapy for HCMV

As discussed above, the current standard therapy for HCMV disease relies on GCV or its oral prodrug, VGCV. These compounds are associated with much dose-related toxicity, and there are also serious safety issues, including myelotoxicity or nephrotoxicity associated with the second-line treatments; pyrophosphate analogue, FOS and the nucleoside phosphonate (i.e. nucleotide analogue) CDV. There is much interest, therefore, in alternatives with improved safety.

A new compound, letermovir, has recently entered the HCMV clinical arena and is currently attracting considerable interest. Letermovir (Fig. 8C) is a 3,4 dihydroquinozoline that has an entirely novel antiviral mechanism of action. In contrast to GCV, this compound does not interfere with DNA synthesis. Instead, letermovir has been shown to inhibit a later stage of virus DNA processing; the terminase enzyme that is required to cleave newly replicated virus DNA into genome lengths for packaging into virions. This activity is performed by an enzyme complex comprising three HCMV proteins; the products of HCMV genes UL56, UL89 and UL104.61 The antiviral target for letermovir was confirmed by the selection of drug-resistant mutants that contain single base-substitutions in HCMV UL56 at amino acid residues L241 and R369 and the mutations proved to confer resistance.62 Letermovir was reportedly more potent than GCV with EC50 values of approximately 5 nM, furthermore, it was shown to be highly effective in a murine xenograft HCMV infection model.
Phase I studies confirmed that the compound is well tolerated. Furthermore, the relatively long half-life (approximately 10 h) means that once daily dosing is feasible. In a randomized, controlled and open-label study, letermovir (40 or 80 mg daily for 14 days) was given to transplant patients as pre-emptive therapy compared with VGCV. The primary end-point was decline in HCMV DNA from baseline to day 15. No patient receiving letermovir developed HCMV disease and, in this small trial, the efficacies of letermovir and VGCV were comparable. A Phase-2B, multicentre safety and efficacy study for the prevention of cytomegalovirus reactivation in bone marrow transplant recipients in USA and Germany was completed at the end of 2011. A total of 133 patients were enrolled. Letermovir (60, 120 or 240 mg once daily) or placebo (for 84 days) was started in patients who had no detectable HCMV replication for 5 days before commencing prophylaxis. The incidence and time-of-onset of HCMV prophylaxis failure were the two primary endpoints. The overall failure rates (including patients who discontinued treatment for any reason) were 49, 32, 29 versus 64%, respectively. For viral failures, the results were 27, 19, 6 versus 36%, respectively and, when patients who had detectable HCMV on day 1 of therapy were omitted from the analysis, the proportions were 15, 6, 0 versus 27%, respectively. The safety profile of letermovir was similar to placebo at all doses.

In summary, the more effective control of HCMV in immunocompromised patients remains an important clinical need among this relatively small patient population. This enabled letermovir to be granted ‘orphan drug status’ in USA that has important implications for funding its further clinical development. Clearly, there are several exciting innovations in this corner of the antiviral field and clinicians should be aware that the scene is undergoing rapid change with a protein vaccine, a DNA vaccine and a new compound showing promising efficacies.

Old drugs—new avenues

ME-609: topical combination therapy for herpes labialis

Reactivation of latent HSV commonly leads to labial lesions (cold sores) that are estimated to occur in 20–40% of the population. However, the extent of tissue damage directly resulting from virus replication is probably minimal with most of the clinical manifestations (e.g. itching, redness, swelling and pain) resulting from the host secondary immune response and associated acute inflammation. Based on this premise, a research programme was initiated to examine the
possibility of combining a specific antiviral agent with an anti-inflammatory or local anaesthetic agent in one topical formulation. The idea was investigated by means of a murine HSV infection model where HSV-1-induced skin lesions were aggravated by transfer into the mice of primed immune cells. A large series of formulations comprising FOS or ACV combined with a steroidal or non-steroidal anti-inflammatory agent or, in some cases, a local anaesthetic were tested in this model. Most combinations either had no effect or exacerbated disease with the exception of ACV combined with hydrocortisone (HC). The relative concentrations were optimized leading to a formulation containing 5%ACV-1% HC.66,67

The selected combination (now known as ME-609) was then tested in humans with herpes labialis following experimental reactivation of their latent HSV by means of UV light.68 In their study, ME-609 was compared with placebo. Treatment started 2 days after the UV light stimulus; 120 of 380 patients developed classical sores following the reactivation stimulus. Treatment with ME-609 reduced lesion incidence, healing time, lesion size and lesion tenderness. Healing time (time to restoration of normal skin) was reduced to 9.0 days from 10.1 days for the placebo-treated controls although, reportedly, there was no effect on lesion pain. Nonetheless, these encouraging results led to clinical trials in patients suffering natural recurrent herpes lesions. In the recent trial reported by Sailer et al., 2437 healthy subjects with a previous history of herpes labialis were enrolled from 51 sites in USA and four sites in Canada.69 The patients were given ACV-HC cream, ACV cream alone or placebo (vehicle) and instructed to apply the cream five times daily starting as soon as possible after noting a prodromal symptom. Of 1443 patients who initiated treatment during the study period, the reported number of ‘aborted lesions’ was 42 versus 35 versus 26% for ACV-HC, ACV and placebo, respectively. Duration of ulcerative lesions to ‘loss of hard crust’ was 5.7 versus 5.9 versus 6.5 days with a 1.5 day reduction in time to lesion healing to normal skin versus placebo but a modest 0.2 day reduction relative to ACV alone. The 5% ACV-1% HC under the name Xerese was approved for marketing in USA in July 2009 for recurrent herpes labialis and, more recently, in Europe as Xereclear. There are extensive reviews describing the rationale and work leading up to this interesting combination therapy.70,71 It should be stated, however, that this approach is controversial. Although steroid therapy has long been used by some ophthalmologists to control severe stromal damage, resulting from recurrent HSV or VZV eye infections, there is concern that steroids prolong virus shedding with the potential for further virus-induced damage. Furthermore, prolonged virus replication would be likely to increase the risk of antiviral resistance development. These are serious concerns
that only further research in suitable laboratory infection models and by means of monitoring infections in the clinic will help to resolve. In conclusion, the use of the proprietary combination therapy is strongly advised rather than prescribing the single generic components (steroid plus ACV) to avoid detrimental outcomes. Although many patients may prefer to use the 1 day (one or two doses) of oral treatment rather than the topical treatment, perhaps the topical cream could eventually become available as a non-prescription therapy?

**New opportunities for FCV and VACV**

As mentioned above, VACV reduced but did not prevent transmission from HSV seropositive subjects to their partners. Based on the observation in mice that early FCV therapy reduced the subsequent ability of latent HSV to reactivate,

perhaps the seronegative partner should take FCV daily? This would not be expected to prevent transmission of HSV nor of the virus becoming latent but it might reduce the probability of recurrent lesions? For couples who do not want to use barrier contraceptive methods, this could provide an alternative approach.

Adult and neonatal HSV encephalitis, although very rare, is a devastating, life-threatening disease. The routine clinical practice is to use ACV (i.v.) at its maximum tolerated dose, followed by VACV during the recovery phase. Although not FDA approved, the generally recommended regimen is ACV, 20 mg/kg i.v. every 8 h for 14 to 21 days in neonatal patients and similarly in adults but using 10–15 mg/kg. Undoubtedly, ACV therapy is hugely beneficial but far from perfect. In various studies published in the past decade, the adult mortality has been about 10% (much less than the 50% expected without therapy) and the majority of patients are left with neurological sequelae. It seems that, with such a devastating disease, clinicians are reluctant to try different therapies. However, PCV can be given at higher doses than ACV since PCV is not so prone to crystallization in the kidneys as is ACV. Also, in the mouse model described above, there were resurgences of viral replication in the brain stem of mice treated with VACV but not in those treated with FCV (Fig. 3). These two factors suggest that PCV (i.v.) followed by FCV therapy could potentially give a better outcome than ACV i.v. followed by VACV.

Recent research has provided evidence linking HSV to Alzheimer’s disease (AD). If AD is due to slowly reactivating latently infected brain cells, not leading to an acute infection but, perhaps via cell-to-cell spreading, leading to new latently infected cells, then the work investigating latency in mice (see above) emphasizes that VACV and FCV
have key differences making it important to test both compounds in clinical trials. As both compounds have good safety records with long-term therapies (to prevent HSV recurrences), clinical trials have little to lose and much to gain. To date, funding for clinical trials has been lacking. Perhaps, the U.S. ‘Orphan drug’ route should be explored for helping development.

With an increasing worldwide population living in good hygienic conditions, the number of young adolescents having symptomatic IM (glandular fever) may be expected to increase. As mentioned above, either FCV or VACV could be used to treat IM, possibly FCV being the preferred option due to the anticipated long half-life of the active form, PCV-TP?

**CMX001: a potential new therapy for HCMV in immunocompromised patients**

There has been much interest recently in a CDV oral prodrug known as CMX001, which has a long intracellular half-life, potentially allowing once-weekly dosing. It is currently being developed for prevention of HCMV disease in transplant patients in whom HCMV virus has been detected (pre-emptive prophylaxis).

CMX001 administered orally, is readily taken up by cells and catabolized to CDV-diphosphate which is the active inhibitor of HCMV DNA-polymerase. Persistence of antiviral activity *in vivo* is due to the slow washout of CDV diphosphate giving a half-life of 8–10 days. A key point, however, is the claimed improved safety profile of the prodrug compared with that of the parent compound. So far, no nephrotoxicity or renal complications have been reported for CMX001; the only safety signal, to date, being reversible diarrhoea. A Phase II, double-blind, placebo-controlled trial in haemopoietic stem cell transplant recipients was completed last year (http://clinicaltrials.gov/ct2/show/NCT01241344 (5 October 2012, date last accessed)). Some 230 patients were enrolled. At baseline, patients had to be CMV DNA-negative. CMX001 was administered either once (100 and 200 mg) or twice (100 and 200 mg) weekly versus placebo for about 10 weeks (range 9–11 weeks) to week 13 after transplantation. Proportions of patients with CMV viraemia (>1000 copies/ml) at any time were 6, 7, 4 and 2 versus 25%, respectively. Considering only those patients who did not have viraemia within 24 h after the start of treatment, the proportions were 2, 2, 0 and 0 versus 15%, respectively (*P < 0.002* for twice weekly dosing). However, diarrhoea was the most common adverse event and was dose-limiting at the highest dose (200 mg twice weekly). Thus, the Phase II clinical trial of CMX001
was very encouraging and Chimerix Inc. has announced their intention to progress to Phase III trials. It remains to be seen whether there are remaining safety issues with this CDV derivative.

Concluding remarks

It is timely to review herpes antiviral therapy, as new and varied avenues of research are currently breathing new life into this area. In the field of HSV and VZV antiviral chemotherapy, the nucleoside analogue oral prodrugs, FCV and VACV, are very well established. We make four observations, which are generally accepted:

1. The existing compounds, FCV and VACV, are effective in reducing burden of disease.
2. Notwithstanding their short plasma half-lives, they are convenient to use in oral formulations leading to good compliance.
3. Over more than 20 years, there has been no obvious trend to antiviral drug resistance among immunocompetent patients (<0.5%) apart from those with ocular herpes.
4. Above all, during this time, no significant safety issues have emerged, even during long-term suppression therapy.

However, there remain some unresolved issues:

1. For treating HSV recurrences, either herpes labialis or genital herpes, the 1-day therapies offer the best results. Should they replace the current standard multi-day therapies?
2. The marked differences between VACV and FCV (e.g. triphosphate stability, effect on latency) may not yet be fully exploited?
3. Do current antivirals reduce PHN? This is a hotly debated issue (see below).
4. For immunocompromised patients with VZV disease, should the first-line treatment be FCV, not ACV or VACV?
5. Should there be more support to explore new avenues for current antivirals, for example in possibly reducing herpes latency or AD?
6. Should IM, caused by primary EBV, in adolescents be treated with antivirals?
7. How can new compounds be progressed when the perceived market need is small but the medical need is great.
8. FCV was reclassified from prescription only to pharmacist-controlled for herpes labialis in New Zealand in 2010 (see comment below). Should this be repeated more widely?
Regarding the debate on PHN, some, including the UK regulatory authorities, do not allow any claim for FCV or VACV/ACV reducing PHN. In contrast, it is accepted as unethical to compare compounds versus placebo in herpes zoster patients ≥50 years old. Clearly, these contrasting views are incompatible. Certainly, it is reasonable to assume that PHN is due to nerve damage and that VZV damages the nerves during the prodromal pain experienced before the rash appears. Therefore, starting treatment after the appearance of the rash could be too late. However, it seems unlikely that the immune system is able to stop virus replication in cells of the nervous system when clearly there is continuing virus replication in skin tissue, as shown by new vesicles forming. Perhaps, one should follow the experience gained with HSV recurrences in which high dose, early treatments, have been beneficial. For example, FCV at 1000 mg three times for the first day followed by 5 days at the standard 500 mg dose would cost the same but may possibly give a better result than the standard regimen. Severe prodromal pain has been associated with an increase risk of PHN. Although unilateral dermatomal pain is a poor predictor of herpes zoster, both FCV and VACV have sufficiently benign safety records that antiviral therapy could be started if the physician is consulted by an elderly patient with severe unilateral dermatomal pain. Therefore, could elderly patients be better informed of the need to seek urgent medical help when they experience unexplained, one-sided severe pain, not wait a couple of days to see if the pain goes away.

In 2001, Tyring who has reported on both FCV and VACV trials, wrote ‘Oral famciclovir has potent antiviral activity against HSV-1, HSV-2 and VZV and is the only antiviral proven to reduce the duration of PHN’. Considering the evidence presented here, it seems probable that both FCV and VACV have some effect on reducing PHN but that the evidence for such activity is stronger for FCV because it was compared directly with placebo, an option not now open for VACV, VALM-S or FV-100.

Against this background, it is therefore very difficult to progress new compounds (e.g. valomaciclovir stearate; FV-100). It may not be sufficient to demonstrate non-inferiority or even slight advantage. In order to replace current therapies and become standard therapy, this will require the demonstration of sufficient superiority to alter perceptions. This is a major hurdle. Because of the highly variable nature of herpes infections the demonstration of superiority is likely to require large and necessarily expensive, multicentre trials. For herpes zoster, the monitoring period must be at least 6 months (preferably a year) to assess the very long-term pain in the oldest patients. These factors make it very
difficult to progress new classes of antiviral such as HPI for HSV or VZV.

Regarding the reclassification of FCV from prescription only to pharmacist-controlled for recurrent herpes labialis, various factors need to be considered. Possible misuse could lead to overdose, risking potential harm, or under dosing which could allow virus resistance to emerge. Experience with FCV suggests that these risks are acceptable. The dosing regimen for herpes labialis is simple (one day either one or two doses) which would reduce the risk of misuse. However, the initial diagnosis of herpes labialis should be made by a physician so that the patient can be advised on the best use of FCV, for example to carry the first dose at all times so that treatment can be started as soon as the first prodromal sign is noticed. The change of classification is only for herpes labialis, not genital herpes. Because of the stigma associated with genital herpes, patients may well opt to self-treat having obtained FCV to treat herpes labialis. Although the treatment regimens for recurrent episodes are similar for herpes labialis and genital herpes, primary genital herpes requires a longer course of treatment. Perhaps even more importantly, the patient will miss the advice about genital herpes, including the potential for virus transmission. The fact that this is most likely to happen could be regarded either as a reason for not allowing the reclassification or for allowing it for both herpes labialis and genital herpes, in which case FCV would be issued with advice about both indications.

In contrast to the treatment of α-herpesviruses infections, the situation for HCMV is different since all the current drugs are associated with significant toxicity. In this case, the affected population is relatively small; comprising new-born and immunocompromised patients. However, the standard care often involves monitoring for the appearance of virus in the patient before commencing therapy to prevent the development of disease. This means that testing new compounds for suppression to prevent the appearance of virus, in comparison with placebo, is seen to be ethically acceptable. Two new compounds in the clinical arena (letermovir, CMX-001) have much longer tissue half-lives and offer potential for much less frequent dosing with the associated benefits. Furthermore, experience with long-term therapy for HIV and viral hepatitis over the past decade, including a variety of novel non-nucleosides, has provided a much better understanding of drug-resistance, safety issues and their management. Also, new HCMV vaccines are showing promising efficacies. We therefore remain generally optimistic that at least some of the exciting developments described in this article will translate into routine clinical practice in the not-too distant future.
Declaration of conflicting interests

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