ANTIVIRALS AND ANTIVIRAL STRATEGIES

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Abstract | In recent years, the demand for new antiviral strategies has increased markedly. There are many contributing factors to this increased demand, including the ever-increasing prevalence of chronic viral infections such as HIV and hepatitis B and C, and the emergence of new viruses such as the SARS coronavirus. The potential danger of haemorrhagic fever viruses and eradicated viruses such as variola virus being used as bioterrorist weapons has also increased the profile of antiviral drug discovery. Here, the virus infections for which antiviral therapy is needed and the compounds that are available, or are being developed, for the treatment of these infections are described.

Interest in the development of new antiviral compounds is mainly fuelled by two considerations: what is the requirement for a specific antiviral drug against the virus infection concerned (and, linked to this, could it reasonably be expected that the virus infection would be controlled by using the antiviral drug); and which antiviral drugs are available to treat or prevent the virus infection concerned (or which antiviral strategies could be pursued to meet the demand). The purpose of this article is to examine how different virus infections should be approached from a therapeutic viewpoint — not only those virus infections that we are familiar with, but also new or old virus infections that could emerge or re-emerge, respectively. The basic strategies that are used to design antiviral drugs have been described previously. Here, I will evaluate their usefulness, or potential usefulness, in the control of virus infections. The antiviral drugs that have been formally licensed for medical use are listed in BOX 1. The chemical structures of some of the compounds discussed in this article are shown in FIG. 1; the remaining compounds that are discussed are shown in online supplementary information S1 (figure). The viral and/or cellular targets for antiviral agents and potential antiviral agents are presented in TABLE 1.

Parvovirus infections

The only parvovirus that is pathogenic for humans is B19, which is responsible for so-called fifth disease, or erythema infectiosum, in children. Although complications such as arthritis, aplastic crisis (reticulocytopenia), myocarditis and hydrops fetalis (during pregnancy) can occur after infection with B19 virus, no serious attempts have been made to develop either preventative or therapeutic measures for B19-virus-associated disease and the question of whether any efforts should be made to develop a vaccine or cure for this disease remains open to debate.

Polyomavirus infections

The polyomaviruses JC and BK viruses have been associated with, and are thought to be responsible for, progressive multifocal leukoencephalopathy (PML) and haemorrhagic cystitis, respectively, in patients with AIDS. Several anecdotal case reports have indicated that cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC)] is effective in the treatment of PML in AIDS patients if it is given at the dosage recommended for the treatment of human cytomegalovirus (HCMV) retinitis in AIDS patients — intravenously, 5 mg kg⁻¹ week⁻¹ for 2 weeks then 5 mg kg⁻¹ every 2 weeks, with concomitant probenecid administration. The activity of cidofovir against both primate and murine polyomaviruses has been demonstrated in cell culture in vitro. So far, no other antiviral drugs have been proven to be effective against polyomavirus infections.
these cells and there is evidence that cidofovir restores p53 function in HPV-associated cancers.

Carcinoma cells can be attributed to the induction of apoptosis in cidofovir on the proliferation of HPV-infected cells. Cyclopropyl-2,6-diaminopurine, which selectively inhibits HPV-16-positive cells in organotypic co-cultures of primary normal human keratinocytes with cervical carcinoma cells.

Any recurrences have responded promptly to another course of cidofovir therapy. The inhibitory effect of cidofovir on the proliferation of HPV-infected cells could be attributed to the induction of apoptosis in these cells and there is evidence that cidofovir restores p53 function in HPV-associated cancers.

Two other antiviral agents have specificity for HPV infections — the acyclic nucleoside phosphonate analogues PMEG [9-(2-phosphonylmethoxyethyl)guanine] and cPr-PMEDAP [9-(2-phosphonylmethoxyethyl)-N-cyclopropyl-2,6-diaminopurine], which selectively inhibit HPV-16-positive cells in organotypic co-cultures of primary normal human keratinocytes with cervical carcinoma cells.

Papillomavirus infections
There are several clinical manifestations of human papillomavirus (HPV) infection, which include verruca vulgaris, planar warts, hypopharyngeal, oesophageal, laryngeal and respiratory papillomatosis, genital warts (condylomata acuminata), cervical intraepithelial neoplasia (CIN) (which can develop into cervical carcinoma), vulvar intraepithelial neoplasia (VIN), penile intraepithelial neoplasia (PIN) and perianal intraepithelial neoplasia (PAIN). When injected intravesically or applied topically as a 1% gel or cream, cidofovir has proved highly efficacious in causing regression of many HPV-associated lesions (including laryngeal papillomas and anogenital warts) with no or few recurrences. Any recurrences have responded promptly to another course of cidofovir therapy. The inhibitory effect of cidofovir on the proliferation of HPV-infected cells could be attributed to the induction of apoptosis in these cells and there is evidence that cidofovir restores p53 function in HPV-associated cancers.

Box 1 | Approved antiviral drugs

| HIV infections | Nucleoside reverse transcriptase inhibitors (NRTIs): |
| Zidovudine: 3′-azido-2′,3′-dideoxythymidine (AZT) | Didanosine: 2′,3′-dideoxyinosine (ddI) | Zalcitabine: 2′,3′-dideoxythymidine (ddC) | Stavudine: 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) | Lamivudine: (−)-β-3′-thia-2′,3′-dideoxythymidine (3TC) | Abacavir (ABC): 2-amino-5-cyclopentene-4-carboxamide PMEDAP | Emtricitabine: (−)-β-3′-thia-2′,3′-dideoxy-5-fluorocytidine (TDF) |
| Nucleotide reverse transcriptase inhibitors (NtRTIs): |
| Tenofovir disoproxil: bis(isopropoxycarbonyloxymethyl)ester of (R)-9-(2-phosphonylmethoxypropyl)adenine | Nevirapine | Delavirdine | Efavirenz |
| Protease inhibitors (PIs): |
| Saquinavir | Ritonavir | Indinavir | Nelfinavir | Amprenavir | Lopinavir | Atazanavir |
| Fusion inhibitors (FIs): |
| Enfuvirtide | Pentafuside (T-20) |

HBV infections
Lamivudine | Adefovir dipivoxil: bis[pivaloyloxymethyl]ester of 9-(2-phosphonylmethoxyethyl)adenine

HSV and VZV infections
Aцикловир and its oral prodrug valaciclovir | Пенициловир and its oral prodrug famciclovir | Идохурин: 5-йодо-2′,3′-дидезоктидимидин | Брадувирин: 2′-дезоктидимидин (дидэоктидимидин) |

CMV infections
Ganciclovir and its oral prodrug valganciclovir | Фоскарнет: фосфонофторамидный триповарный тетраэдрид | Цидофовир: (S)-1-(3-гидрокси-2-фосфонометилэтил)цитозин (HPMPC) | Фомивирс: антисенс (фосфоритиолат)

Influenza virus infections
(Прегель) IFN-α | Рибавирин

Adenovirus infections
Adenovirus infections in immunocompetent individuals are generally self-limiting, and neither preventative nor therapeutic measures (vaccination or antiviral therapy) are used. However, in allogeneic haematopoietic stem-cell transplant (HSCT) recipients, adenovirus infections can be severe. In these patients, according to anecdotal reports, cidofovir has been shown to be to be effective in suppressing adenovirus infection, whereas ribavirin and vidarabine have not. At present, cidofovir seems to be the only antiviral drug that could be successfully used to treat adenovirus infections, particularly in HSCT recipients.

α-herpesvirus infections
The α-herpesviruses include herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella–zoster virus (VZV). They can cause both primary infections (for example, HSV-1 causes gingivostomatitis, encephalitis and eczema herpeticum, HSV-2 causes genital and neonatal herpes, and VZV causes varicella (chicken-pox)) and recurrent infections (for example, HSV-1 causes herpes labialis and herpetic keratitis, HSV-2 causes genital herpes and VZV causes herpes zoster). These viruses can also cause severe, disseminated or progressive mucocutaneous infections in immunosuppressed patients.

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Figure 1 | Structures of selected licensed antiviral drugs and compounds still in clinical or preclinical development. For further detail, see Ref. 8. For structures of the other compounds discussed in this article, see online supplementary information S1 (figure).
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hosts. In this situation, infections should be treated with foscarnet, a pyrophosphate analogue, or the acyclic nucleoside phosphonate cidofovir, neither of which depend on the HSV or VZV TK for their antiviral action (FIG. 3).

Also dependent on the activity of a viral TK, but specifically the VZV-encoded TK, are the bicyclic furo(2,3-d)pyrimidine nucleoside analogues (BCNAs) Cf1368, Cf1369, Cf1743 and Cf1742. These compounds inhibit VZV replication at subnanomolar concentrations and, even at 100,000-fold greater concentrations, are not toxic to host cells.12,13 Unlike the ‘classical’ anti-VZV compounds, the BCNAs are selective for VZV and are therefore inactive against HSV-1 and HSV-2. The alkyl(phenyl) side chain on the furanyl ring of the BCNAs is an important determinant of the specificity of these compounds.14

As acyclic nucleoside analogues require phosphorylation by the virus-encoded thymidine kinase (TK) to exert their antiviral activity (FIG. 2), they do not inhibit the TK-deficient HSV or VZV strains that can occasionally arise, particularly in immunocompromised patients. In this situation, infections should be treated with foscarnet, a pyrophosphate analogue, or the acyclic nucleoside phosphonate cidofovir, neither of which depend on the HSV or VZV TK for their antiviral action (FIG. 3).



| Table 1 | Viral and cellular targets for antiviral agents |
| --- | --- | --- | --- |
| Virus | Viral target | Cellular target | Comments |
| Parvovirus | DNA polymerase | – | Remains to be explored |
| Polymavirus | DNA polymerase | – | Remains to be explored |
| Papillomavirus | DNA polymerase, E6, E7 | – | Remains to be explored |
| Adenovirus | DNA polymerase | Cell-associated factors | Remains to be explored |
| α-herpesvirus (HSV-1, HSV-2, VZV) | Thymidine kinase, DNA polymerase, Helicase-primase | – | Target for activation, Target for inhibition |
| β-herpesvirus (HCMV, HHV-6, HHV-7) | Protein kinase, DNA polymerase, Terminase | – | Target for activation, Target for inhibition |
| γ-herpesvirus (EBV, HHV-8) | DNA polymerase | – | Target for inhibition |
| Poxvirus (for example, variola and vaccinia viruses) | DNA and RNA polymerases | Several* | Remains to be explored |
| Hepadnavirus (HBV) | DNA polymerase (Reverse transcriptase) | – | Target for inhibition |
| Picornavirus (Enteroviruses and Rhinoviruses) | Viral capsid, RNA polymerase | – | Target for inhibition, Remains to be explored |
| Flavivirus (for example, yellow fever and dengue viruses) | RNA polymerase | – | Remains to be explored |
| Arenaviruses (for example, Lassa) | RNA polymerase | Several* | Remains to be explored |
| Bunyaviruses (for example, Crimean–Congo) | RNA polymerase | Several* | Remains to be explored |
| Togaviruses (for example, Western Equine encephalitis virus) | RNA polymerase | Several* | Remains to be explored |
| Rhabdoviruses (Rabies virus) | RNA polymerase | Several* | Remains to be explored |
| Filoviruses (for example, Ebola virus) | RNA polymerase | Several* | Remains to be explored |
| Hepacviruses (HCV) | DNA polymerase, RNA helicase, Viral protease | – | Being investigated |
| Orthomyxoviruses (Influenza) | Matrix (M2) protein, Neuraminidase | – | Target for inhibition, Target for inhibition |
| Paramyxoviruses (RSV) | Fusion polypeptide | Several* | Being explored |
| Coronavirus (SARS-CoV) | Several† | – | Being explored |
| Reoviruses (Rotavirus) | – | Several* | Remains to be explored |
| Retroviruses (HIV) | Several§ | Several¶ | Established or being explored |

*Inosine 5’-monophosphate (IMP) dehydrogenase, S-adenosylhomocysteine (SAH) hydrolase, orotidine 5’-monophosphate (OMP) decarboxylase and cytosine 5’-triphosphate (CTP) synthetase. †Spike (S) protein, RNA polymerase (replicase), RNA helicase and viral protease. ‡Spike (S) protein, RNA polymerase (replicase), RNA helicase and viral protease. § Fusion polypeptide (viral glycoprotein gp41), reverse transcriptase and viral protease. ¶Viral glycoprotein gp120, integrase and transcription transactivator (TAT). ||Integration- and transcription-associated factors.
New anti-HSV agents that target the viral helicase–primase complex$^{15}$ — the thiazolylphenyl derivatives BILS 179BS and BAY 57-1293 — were recently reported to have in vivo efficacy in animal models of HSV-1 and HSV-2 infection$^{16,17}$. These compounds seem to function by enhancing the affinity of the helicase–primase complex for the HSV DNA. This complex comprises three viral proteins — the HSV UL5, UL8 and UL52 gene products (FIG. 3) — which together unwind the double-stranded viral DNA and generate primers for DNA synthesis by the viral DNA polymerase. The antiviral potency of BAY 57-1293 is reported to be superior to all compounds that are currently used to treat HSV infections$^{18}$. These data validate the further pursuit of helicase–primase inhibitors for the treatment of HSV infections.

$β$-herpesvirus infections

Among the $β$-herpesviruses — HCMV, human herpesvirus type 6 (HHV-6), and human herpesvirus type 7 (HHV-7) — HCMV is associated with the primary and subsequent infections CMV mononucleosis and congenital cytomegalic inclusion disease, as well as recurrent infections, such as pneumonitis, hepatitis, retinitis, encephalitis and colitis in immunocompromised hosts.

Five compounds have been licensed to treat HCMV infections — ganciclovir, its oral prodrug valganciclovir, foscarnet, cidofovir and fomivirsen. With the exception of fomivirsen, which targets HCMV immediate-early mRNA, these compounds target the viral DNA polymerase. Ganciclovir must first be phosphorylated by the HCMV-encoded protein kinase, the UL97 gene product (FIG. 2), which is also the main site for mutations that engender resistance towards this compound.

The available anti-HCMV drugs have several drawbacks that limit their clinical utility. Fomivirsen must be injected intraocularly (that is, intravitreally), foscarnet must be given intravenously three times daily and cidofovir is also administered intravenously, albeit once weekly or every other week. Nephrotoxicity is the dose-limiting factor for cidofovir and foscarnet, whereas bone-marrow suppression that results in granulocytopenia and thrombocytopenia is the most common toxic side effect seen with ganciclovir.

Taken together, these considerations justify the search for new anti-HCMV agents that are less toxic and/or more effective. A possible lead compound is 2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV423), which was recently shown to have potent in vitro activity against a wide range of HCMV reference strains and clinical isolates, including those that have acquired resistance to ganciclovir, foscarnet or cidofovir$^{19}$. CMV423 targets a step of the viral replication cycle before the DNA polymerase step and probably coincides with immediate-early antigen expression.

The ‘non-nucleoside’ 4-hydroxyquinoline carboxamides (PNU-181465$^{20}$ and 4-oxo-dihydroquinolines (PNU-182171 and PNU-183792$^{21}$) interact with the $β$-herpesvirus DNA polymerase. These compounds do not inhibit the cellular DNA polymerases ($α$ and $δ$) or the mitochondrial DNA polymerase $γ$. HSV-1 and HSV-2 viruses that are resistant to these compounds have been found to have a single amino acid change of valine to alanine within conserved domain III of the HSV-1 and HSV-2 DNA polymerases. This valine residue (V823 in HSV-1) is conserved in the DNA polymerases of six of the eight human herpesviruses and seems to have a crucial role in the observed anti-herpesvirus effects of these compounds. The DNA polymerase in HHV-6 contains an alanine at this position and accordingly is not inhibited.

The HCMV terminase, which comprises the UL89 and UL56 gene products, is a target for chemotherapeutic intervention. Together, these gene products cleave the viral high-molecular-weight DNA concatamers into unit-length genomes and package these monomeric genomes into viral procapsids. Intervention at this stage is expected to block viral DNA cleavage and packaging, leading to an accumulation of empty procapsids and unprocessed concatameric DNA, as has been demonstrated with the (naphthylsulphonylamino)phenyl-propanamide BAY 38-4766 (REF. 22). BAY 38-4766 targets a viral DNA maturation step that does not occur in uninfected eukaryotic cells, so targeting the viral terminase is an attractive strategy to combat HCMV infections. In addition to BAY 38-4766, two benzimidazole ribonucleosides — 1-[(β-D-ribofuranosyl)-2-bromo-5,-6-dichlorobenzimidazole (BDCRB) and 1-[(β-D-ribofuranosyl)-2,5,6-dichlorobenzimidazole (TCRB) — have also been shown to inhibit cleavage and encapsidation of viral DNA, and amino acid mutations in both the UL89 and UL56 gene products have been identified in resistant strains of HCMV$^{23}$.

Maribavir (or 1263W94) is another benzimidazole ribonucleoside, in which, in addition to a switch from a $d$ to $L$ configuration, the halogen at position 2 of the benzimidazole is replaced by an isopropylamine, and this compound also has prominent anti-HCMV activity. In
Two phosphorylations are required for activity. Reproduced with permission from Ref. 15 © Nature Medicine (2002) Nature Publishing Group.

\[ \text{HPMPC} \xrightarrow{\text{NH}_2} \text{HPMPC-MP} \xrightarrow{\text{NH}_3} \text{HPMPC-DP} \]

**Figure 3 | The mechanism of action of cidofovir and HSV helicase–primase complex inhibitors.** (a) The mechanism of antiviral action of cidofovir (HPMPC). As an acyclic nucleoside phosphonate, cidofovir is not dependent on activation by a virus-encoded enzyme for activity. Two phosphorylations are required for activity. Reproduced with permission from Ref. 1 © Nature Rev. Drug Discov. (2002) Nature Publishing Group. (b) The mechanism of inhibition of DNA synthesis by HSV helicase–primase complex inhibitors. The helicase–primase complex comprises the viral gene products UL5, -8, -52. This complex unwinds HSV DNA at the replication fork and primes DNA synthesis at both the lagging and the leading strands. The single-stranded-DNA-binding protein ICP8 binds to single-stranded template DNA. HSV DNA polymerase and its accessory protein UL42, promote leading- and lagging-strand DNA synthesis. The TZP (amino-thiazolylphenyl)-containing compounds (red band) enhance binding of the UL5 and UL52 subunits of the helicase–primase complex to both the leading- and the lagging-strand DNA, resulting in inhibition of helicase activity, primase activity and viral DNA synthesis. Modified with permission from Ref. 15 © Nature Medicine (2002) Nature Publishing Group.

that maribavir is rapidly absorbed following oral dosing and achieved marked reductions in HCMV titre in semen\(^2\).

Primary HHV-6 infection, which generally occurs before the patient is 2 years old, is associated with exanthema subitum, and reactivation at a later age is frequent in immunocompromised individuals, particularly bone marrow or solid-organ transplant recipients. At present there is no standardized antiviral treatment for HHV-6 infections. The most potent compounds with the highest antiviral selectivity against HHV-6 are foscarnet, S2242 [2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine] (which is the N\(^7\)-isomeric form of 6-deoxycytidine) and has excellent activity against HHV-6), A-5021 and cidofovir. For HHV-7, which has not been proven to be linked to any disease, except possibly exanthema subitum, the most potent compounds proved to be S2242, cidofovir and foscarnet\(^2\).

**γ-herpesvirus infections**

Among the γ-herpesviruses, Epstein-Barr virus (EBV) is responsible for infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma, lymphoproliferative syndrome, opportunistic B-cell lymphoma and oral hairy leukoplakia. Kaposis’s sarcoma-associated virus (KSHV; which is also known as HHV-8) is associated with Kaposis’s sarcoma, primary effusion lymphoma and Multicentric Castleman’s disease.

Although several of the aforementioned licensed anti-herpetic drugs, such as acyclovir, BVDU and cidofovir, have proven to be effective against the in vitro replication of EBV and, in the case of cidofovir, its clinical manifestations (that is, oral hairy leukoplakia), none of these antiviral drugs has been licensed for the treatment of EBV infections. Recently, BDCRB and maribavir were shown to be active against EBV\(^2\).

For the treatment of HHV-8 infection or associated diseases, several drug candidates have been identified, including HPMPA [(S)-9-(3-hydroxy-2-phosphonylmethoxypyridyl)adenine], cidofovir, S2242 and ganciclovir\(^2\). Also, 4-oxo-dihydroquinolines such as PNU-183792 can inhibit HHV-8 replication due to a direct interaction with the HHV-8 DNA polymerase. It has not yet been established whether any of the compounds that are effective against HHV-8 replication in cell culture in vivo will be efficacious in the treatment of HHV-8-associated diseases in vivo.

**Poxvirus infections**

The family of poxviridae includes orthopoxviruses (such as variola, vaccinia, cowpox, monkeypox and camelpox), parapoxviruses (such as orf) and molluscipoxviruses (molluscum contagiosum virus). The last natural case of smallpox, which is caused by variola virus, occurred in Somalia in 1977, and in 1980 the World Health Organization (WHO) declared smallpox to be officially eradicated. Since then, the only known stocks of variola virus have been held in Atlanta, USA, at the Centers for Disease Control (CDC), and in Koltsso, Russia, in the State Research Centre of Virology and Biotechnology (VECTOR). If illegally
preserved stocks of variola virus were ever used for biological and/or terrorist purposes in a highly mobile and susceptible population, the results could be catastrophic. In fact, variola virus could be considered an ‘ideal’ bioterrorist weapon for several reasons:\(^{10}\): it is highly transmissible by the aerosol route from infected to susceptible persons; the civilian populations of most countries contain a high proportion of susceptible (unvaccinated) persons; smallpox is associated with high morbidity and ∼30% mortality; the initial diagnosis of a disease that has not been seen for 20 years would be difficult; and, at present, other than the vaccinia-based vaccine, which might be effective in the first few days post-infection, there is no formally approved drug for the treatment of smallpox.

The first antiviral compound to be used in the treatment and prophylaxis of smallpox, treatment of complications after smallpox vaccination and the treatment of eczema vaccinatum and vaccinia gangrenosa was methisazone (N-methylisatin-3-thiosemicarbazone)\(^{31}\). Severe side effects were reported\(^{31}\) and follow-up studies\(^{32}\) revealed that methisazone has \textit{in vitro} activity against monkeypox virus and variola virus only at concentrations that are too high (∼50 μg ml\(^{-1}\)) to be therapeutically meaningful.

Yet, several potential antiviral therapeutic strategies have proven to be active against orthopoxvirus infections, both in cell culture\(^{15}\) and in animal models\(^{44}\), at concentrations and/or doses that could be used therapeutically. Several potential therapeutic strategies have been designed to target cellular enzymes. These enzymes include inosine 5′-monophosphate dehydrogenase, which is responsible for the conversion of IMP to GMP; xanthosine 5′-monophosphate, which is an important step in the \textit{de novo} biosynthesis of GTP; S-adenosylhomocysteine (SAH) hydrolase, the enzyme responsible for the hydrolysis of SAH, which is the product-inhibitor of S-adenosylmethionine (SAM)-dependent methylation reactions such as those involved in the maturation of viral mRNAs; orotidine 5′-phosphate decarboxylase, the enzyme that is responsible for the conversion of OMP to UMP (uridine 5′-monophosphate), which is an important reaction in the \textit{de novo} biosynthesis of UTP (uridine 5′-triphosphate); cytosine 5′-triphosphate synthetase, which converts UTP to CTP; and viral enzymes such as the poxviral DNA polymerase\(^{35,36}\).

Several nucleoside and nucleotide analogues have been identified as potent anti-poxvirus agents. The nucleoside analogues include S2242 and 8-methyldenosine, whereas the nucleotide analogues include cidofovir, (S)-9-(3-hydroxy-2-phosphonylethoxymethyl)-2,6-diaminopurine (HPMPDAP) and (S)-6-(3-hydroxy-2-phosphonylmethoxypropyl)oxo-2,4-diaminopyrimidine (HPMPO-DAp)\(^{37}\). These compounds have been shown to be effective in various animal models of poxvirus infections\(^{37}\). Cidofovir can protect mice from a lethal respiratory infection with either vaccinia or cowpox, even when administered as a single systemic (intraperitoneal) or intranasal (aerosolized) dose\(^{38}\), and is effective in the treatment of vaccinia virus infection in severe combined immune deficiency (SCID) mice.

In humans, anecdotal reports suggest that both topically and intravenously administered cidofovir can successfully treat recalcitrant molluscum contagiosum and orf infections in immunocompromised patients\(^{39}\). Cidofovir is licensed for clinical use (in the treatment of CMV retinitis in AIDS patients) by intravenous injection. However, it could also be formulated for topical administration (for example, as a gel or cream) or for oral administration in prodrug form. The 1-\textit{O}-hexadecyloxypropyl derivative (HDP-cidofovir), which has increased anti-poxvirus activity relative to cidofovir\(^{39}\) owing to facilitated cellular uptake\(^{40}\), is under investigation as an oral prodrug.

Although the ultimate proof — activity against smallpox in humans — has not been (and, for obvious reasons, cannot be) provided, from the above discussion it can be inferred that cidofovir could be effective in the therapy and short-term prophylaxis of smallpox and related poxvirus infections (that is, monkeypox) in humans, as well as in the treatment of complications of vaccinia that can arise in immunocompromised patients who are inadvertently inoculated with the smallpox vaccine (vaccinia). A murine model that mimics progressive and disseminated vaccinia in humans has recently been developed\(^{41}\). In this model (athymic nude mice inoculated intracutaneously with vaccinia virus), systemic treatment with cidofovir when disseminated vaccinia developed caused the lesions to heal and regress. In most of the animals treated in this way the lesions completely (or almost completely) disappeared within 10–15 days of the start of therapy\(^{41}\). These observations have implications for the therapy of complications of smallpox vaccination.

**Hepadnavirus infections**

More than 350 million people worldwide are chronically infected with the hepadnavirus HBV, and complications of chronic hepatitis B infection, such as cirrhosis, hepatocellular carcinoma and end-stage liver disease, account for approximately 1 million deaths each year. The drugs that have been formally approved for the treatment of chronic hepatitis B are interferon (IFN)-α, lamivudine (3TC) and adefovir dipivoxil [bis(POM)PMEA]. However, IFN treatment is compounded by side effects, such as influenza-like symptoms, anorexia and depression, which require dose adjustment or even discontinuation of therapy. In contrast with IFN, which must be given by parenteral administration, lamivudine can be administered orally and is well tolerated, but leads to resistance in up to 39% of patients after one year of therapy and 66% after four years of therapy.

Adefovir dipivoxil is the oral prodrug of adefovir (PMEA), which, after intracellular conversion to the diphasate form, acts as a competitive inhibitor or alternative substrate for HBV reverse transcriptase and, when incorporated into the DNA, acts as a chain terminator, thereby preventing DNA elongation\(^{42}\) (FIG. 4). In patients with chronic HBV infection who were either positive\(^{43}\) or negative\(^{44}\) for hepatitis B e antigen, 48 weeks of treatment with a dose of adefovir dipivoxil as low as 10 mg day\(^{-1}\) resulted in significant improvement in...
histological liver abnormalities, as well as a significant reduction in serum HBV DNA and alanine aminotransferase concentrations. In these trials, the safety profile of adefovir dipivoxil was similar to that of the placebo, and no HBV polymerase mutations associated with resistance to adefovir were identified. In fact, adefovir dipivoxil proved effective in HBV-infected patients that had developed resistance to lamivudine41. Resistance to adefovir might eventually develop but at a much slower rate and lower frequency than has been observed for lamivudine.

Entecavir and the β-1-nucleoside analogues l-FMAU (1-(2-fluoro-5-methyl-β-1-arabinosyl)uracil; clevudine), β-1-thymidine (β-1-dT), the valine ester of β-1,2′-deoxyctydine (val-β-1-dC) and emtricitabine ((−)-FTC) are under clinical development43 for the treatment of chronic hepatitis B. Emtricitabine is the 5-fluoro-substituted counterpart of lamivudine and has already been licensed for the treatment of HIV infections. Owing to its structural similarity to lamivudine, it is thought that emtricitabine might engender the same resistance mutation (substitution of a methionine residue with leucine or valine residues) as is seen for lamivudine, and thereby produce cross-resistance to lamivudine.

Entecavir is a 2′-deoxyguanosine analogue and has in vitro and in vivo potency that seem to be greater than that of lamivudine — in patients with chronic hepatitis B infection it has proven efficacious at doses as low as 0.1 and 0.5 mg day−1 (REF. 46). Potent efficacy has also been demonstrated with entecavir against woodchuck hepatitis virus (WHV) in woodchucks and duck hepatitis B virus (DHBV) in ducks46. For entecavir to be active against hepadnavirus replication, it must — as for all nucleoside analogues — be converted intracellularly to its 5′-triphosphate form, which, in the case of entecavir, would then compete with dGTP for the viral DNA polymerase. Intracellular entecavir 5′-triphosphate can be expected to accumulate at concentrations that are inhibitory to both wild-type and 3TC-resistant HBV DNA polymerase48.

Like entecavir, l-FMAU has proven to be a potent inhibitor of both DHBV and WHV replication in acutely infected ducks and chronically infected woodchucks, respectively49. At the highest dose administered (10 mg kg−1 day−1), treatment with l-FMAU led to a reduction in the levels of covalently closed circular WHV DNA and, concomitantly with this, long-lasting suppression of viraemia after withdrawal of therapy49. Also, l-FMAU treatment followed by therapeutic vaccination to break immune tolerance has been advocated as a strategy to control chronic HBV infection in humans49.

The β-1-nucleoside analogues l-FMAU, l-dT and l-dC must also be phosphorylated to their 5′-triphosphate form to interact with HBV DNA polymerase. In fact, l-dT and l-dC have been shown to be rapidly and extensively phosphorylated in both hepatoma cells and primary human hepatocytes43. The 3-phosphoglycerate kinase — a glycolytic enzyme that uses 1,3-bisphosphoglycerate as a phosphate donor to generate ATP during glycolysis — would have an important role in the phosphorylation of the β-1-nucleosides (diphosphates) to their 5′-triphosphate derivatives45.

It is noteworthy that adefovir dipivoxil — a drug that has now been approved worldwide for the treatment of chronic HBV infection — might be a promising adjunctive therapy against anthrax and other human diseases caused by pathogenic bacteria that secrete adenylyl cyclase toxins, such as Bordetella pertussis, Pseudomonas aeruginosa and Yersinia pestis41. Indeed, the active cellular metabolite of adefovir, adefovir dipiphosphate (PMEADP), was recently shown to inhibit the adenylyl cyclase activity of the oedema factor (Ko of 27 nM), which is an important virulence factor in the pathogenesis of anthrax, especially during the early stages of the Bacillus anthracis infection where it contributes to both toxemia and bacteremia.

**Picornavirus infections**

The picornaviruses include the enteroviruses (such as poliovirus, coxsackieviruses A and B, echovirus and hepatitis A virus) and rhinoviruses. Rhinoviruses are the main cause of the common cold, whereas enterovirus infections result in a myriad of disease syndromes, including viral meningitis and viral respiratory infections. Of these viruses, poliovirus has historically received most attention. After a successful worldwide vaccination campaign, the only countries in which polio still remains endemic are Niger, Nigeria, Egypt, India, Pakistan and Afghanistan. Hepatitis A can also be controlled sufficiently by the use of a killed virus vaccine. No vaccines are available for prevention of coxsackieviruses A and B, echovirus and rhinovirus infections.

The compound that has been most extensively studied against both entero- and rhinoviruses is pleconaril,
The mechanism of action of ribavirin and mycophenolic acid, the active component of mycophenolate mofetil. Both these compounds block RNA synthesis by inhibiting the action of inosine 5′-monophosphate (IMP) dehydrogenase — this blocks the conversion of IMP to XMP (xanthosine 5′-monophosphate) and thereby stops GTP and, consequently, RNA synthesis.

This compound binds to a hydrophobic pocket beneath the 'canyon floor' of the VP1 capsid protein of picornaviruses, thereby 'freezing' the viral capsid and preventing its uncoating from the viral RNA genome. Pleconaril is orally bioavailable and achieves plasma concentrations greater than those that are required to inhibit 90% of clinical rhino- and enterviral isolates in vitro. In addition, in the central nervous system (CNS) and nasal secretions the drug is present at concentrations several fold higher than in plasma — a highly desirable feature for an antiviral drug targeted towards viruses that are known to cause CNS and upper-respiratory-tract infections. The clinical efficacy of pleconaril has been assessed in experimentally induced enterovirus (coxsackievirus A21) respiratory infections in adult volunteers and, on a compassionate basis, against potentially life-threatening enterovirus infections. When administered three-times daily, pleconaril reduced the duration and severity of picornavirus-associated viral respiratory illness in adolescents and adults.

Coxsackie B viruses are thought to be the main aetiological agents of viral myocarditis, which is a common cause of idiopathic dilated cardiomyopathy — a severe pathological condition that often requires heart transplantation and are therefore targets for chemotherapy. The immunosuppressive agent mycophenolate mofetil inhibits the development of coxsackie B3 virus-induced myocarditis in C3H mice. Mycophenolic acid (MPA), the active component of mycophenolate mofetil, is a potent inhibitor of IMP dehydrogenase and its antiviral and immunosuppressive effects can be attributed to this interaction, which reduces the supply of GTP (FIG. 5), and therefore, RNA synthesis. The beneficial effect of MPA cannot be ascribed to inhibition of viral replication, as the titre of infectious virus and viral RNA in heart tissue was increased in the mycophenolate-mofetil-treated animals compared with untreated animals.

A more marked inhibitory effect on the development of coxsackie B3-virus-induced myocarditis, corroborated by a marked reduction in the virus titres in the heart, was obtained with the IFN inducers poly(I)-poly(C) and poly(I)-poly(C₆₂U) (also known as ampligen) and, to a lesser extent, with IFN-α2b and pegylated IFN-α2b. Even when the start of treatment with poly(I)-poly(C₆₂U) was delayed until two days post-infection, when lesions had already appeared in the untreated control animals, a marked protective effect on the development of viral myocarditis (assessed six days post-infection) was observed. A combination of an inhibitor of viral replication (such as amplitgen) and an immunosuppressant (such as mycophenolate mofetil) could be an ideal treatment strategy for viral myocarditis. How to implement such a treatment regimen in the clinical setting remains to be addressed.

**Flavivirus infections**

The genus Flavivirus contains more than 70 species, many of which cause disease in humans. Severe flavivirus infections are generally characterized by encephalitis or haemorrhagic symptoms. Mortality rates vary from 1%–2% (in the case of Central European encephalitis virus) to 30%–40% (in the case of Japanese encephalitis virus and tick-borne encephalitis virus, which was previously known as Russian Spring and Summer encephalitis virus). Other important flaviviruses include yellow fever virus, dengue virus, West Nile virus, St Louis encephalitis virus and Murray Valley encephalitis virus. Although feared as a possible bioterrorist weapon, the development of tick-borne flaviviruses as bioweapons might not be practical, as large numbers of infected ticks would be required and it would be difficult to arrange for them to be infected and ready to feed when delivered as weapons.

The use of IFN and IFN inducers might be possible, but, in general, this treatment should be started before or very shortly after infection to have any beneficial effect. An experimental flavivirus encephalitis model has been developed based on infection of hamsters with the murine Modoc virus; during the acute phase, the infection is associated with flaccid paralysis and the neurological sequelae that can develop are similar to those that have been observed in survivors of Japanese encephalitis. This model should be suitable for the evaluation of anti-flavivirus therapies. At present, IFN-α2b, whether pegylated or not, and IFN inducers (poly(I)-poly(C) and ampligen) offer the greatest potential for activity in this model, as they have been shown to significantly delay virus-induced morbidity (paralysis) and mortality (due to progressive encephalitis) in a related model with Modoc virus in SCID mice. It is noteworthy that ribavirin did not provide any beneficial effect in this model, whether given alone or in combination with IFN.

**Arenavirus infections**

Of the 23 arenavirus species that are known, five are associated with viral haemorrhagic fevers — Lassa, Junin, Machupo, Guanarito and Sabia. These viruses are included in the CDC Category A Pathogen List. It is gratifying to note that, as demonstrated with Tacaribe virus and an attenuated Junin virus strain, in vitro...
S-Adenosylmethionine (SAM) → S-Adenosylhomocysteine (SAH) → SAH hydrolase inhibitors

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**Figure 6** | The mechanism of action of adenosine analogues such as 3-deazaneplanocin A. These compounds inhibit the action of SAH hydrolase, which hydrolyses SAH, the product-inhibitor of S-adenosylmethionine (SAM)-dependent methylation reactions. Inhibiting these reactions can affect transcription.

arenavirus replication is susceptible to several compounds, including adenosine analogues (for example, SAH hydrolase inhibitors such as 3-deazaneplanocin A), cytidine analogues (for example, cyclopentenyl cytosine), guanosine analogues (for example, IMP-dehydrogenase inhibitors such as ribavirin) and sulphated polysaccharides (for example, dextran sulphate).

Ribavirin has proven to be effective in the post-exposure prophylaxis and therapy of experimental arenavirus infections in animal models, and anecdotal reports suggest that it might also be effective in the treatment of arenavirus infections (Machupo and Sabia viruses) in humans. The most convincing evidence for the efficacy of ribavirin was obtained in the case of Lassa fever, where it was shown to reduce the case-fatality rate, irrespective of the time point in the illness at which treatment was started.

**Bunyam and togavirus infections**

Several togaviruses and bunyaviruses have been described as potential bioterrorism agents — for example, the togaviruses Venezuelan equine encephalitis virus, Eastern equine encephalitis virus and Western equine encephalitis virus, and the bunyaviruses Rift Valley fever virus, Crimean–Congo haemorrhagic fever virus and hantaviruses such as Hantaan virus. However, hantaviruses are unlikely candidates for biological warfare purposes as they are difficult to isolate (and grow) in cell culture, they are not transmitted between humans and there is no evidence that they are infectious by aerosol. Crimean–Congo haemorrhagic fever virus, however, can be readily cultivated, is highly infectious (although so far there is no evidence that it is infectious in aerosol form) and is easily transmitted between humans, giving rise to local epidemics and even nosocomial infections. The case-fatality rate associated with Crimean–Congo haemorrhagic fever is ~30%, which is higher than that of most other viral haemorrhagic fevers.

Bunyaviruses are generally sensitive to ribavirin, and this has also been demonstrated in experimental animal models. IFN and IFN inducers have also proved effective in the treatment of experimental bunyavirus infection, if, as is usual for these compounds, they are administered as early as possible after infection. As for flavivirus infections, ribavirin is of no use in the treatment of togavirus infections. For these infections, IFN (whether pegylated or not) and IFN inducers (such as ampligen) are the recommended therapeutic agents.

**Rhabdo- and filovirus infections**

Rhabdoviruses (such as *Rabies virus*) and filoviruses (such as *Ebola* and *Marburg* viruses) are among the most deadly viruses to infect humans. Rabies is almost invariably fatal, as illustrated by a recent case report; however, other viruses can be contained by repeated administration of specific immunoglobulin and the use of a killed rabies vaccine as soon as possible after the infection has taken place. No vaccine is available for either Ebola or Marburg infections and these viruses are classified as Category A Pathogens. Filoviruses are highly infectious by the airborne route, but can also be transmitted between humans through direct contact with virus-containing body fluids. Although filoviruses could be more difficult for potential bioterrorists to acquire than other biological agents such as *B. anthracis*, their reputation for causing deadly disease might make the effort required seem worthwhile.

Specific immunoglobulin or IFN-α2b are of only limited value in the treatment of experimental Ebola virus infections — for example, rhesus macaques that were treated from the day of infection with Ebola (Zaire) virus experienced a delay of only one day in the onset of illness, viraemia and death. No antiviral drugs that are currently in clinical use, including ribavirin, provide any protection against filoviruses. The most promising therapeutic strategy might be based on the use of SAH hydrolase inhibitors such as 3-deazaneplanocin A. As already described, SAH hydrolase inhibitors interfere with SAM-dependent methylation reactions (FIG.6) such as those involved in the ‘capping’ of viral mRNA.

Some viruses, including rhabdoviruses such as vesicular stomatitis virus (VSV), rely on mRNA ‘capping’, as they are particularly sensitive to inhibition by SAH hydrolase inhibitors. Biochemically, filoviruses are similar to rhabdoviruses — both require 5′-capping of the mRNAs — and, therefore, it could be logically deduced that SAH hydrolase inhibitors such as neplanocin A and 3-deazaneplanocin A, which are highly active both in *vitro* and in *vivo* against VSV, would also be effective in the treatment of Ebola virus infections.

In fact, when administered as a single dose of 1 mg kg⁻¹ on the first or second day after an Ebola Zaire virus infection in mice, 3-deazaneplanocin A reduced peak viraemia by more than 1,000-fold compared with mock-treated controls, and most or all the animals survived. This protective effect was accompanied, and probably mediated, by the production of high...
concentrations of IFN-α in the Ebola virus-infected mice. It can be hypothesized that, by blocking the 5′-capping of the nascent (+)RNA viral strands, 3-deazaneplanocin A prevented the dissociation of these strands from the viral (−)RNA template, thereby leading to an accumulation of replicative intermediates. These replicative intermediates — composed of dsRNA stretches — could then induce the production of high concentrations of IFN.

**Hepacivirus infections**

It is estimated that more than 170 million people worldwide are infected with the hepatitis C virus (HCV), which is a bloodborne virus that is often sub-clinical but which, in up to 85% of cases, leads to a chronic infection that ultimately results in liver fibrosis (cirrhosis), hepatic failure or hepatocellular carcinoma. HCV infection is the most common cause of hepatocellular carcinoma and the main reason for liver transplantation among adults in western countries. The development of effective anti-HCV therapeutics continues to be a daunting challenge owing to the absence of adequate animal models and cell-culture systems for evaluating propagation of the virus and its inhibitors.

At present, the recommended (and approved) therapy for chronic HCV infections consists of pegylated IFN-α2a combined with ribavirin. This therapy is associated with a sustained viral response rate of ~50% among patients infected with HCV genotype 1 and of ~80% in patients infected with HCV of another genotype. Treatment with pegylated IFN-α2a and ribavirin can be individualized by genotype. Patients that are infected with HCV genotype 1 require treatment for 48 weeks (or longer), whereas patients that are infected with HCV genotypes 2 or 3 can be treated for 24 weeks. In addition, for the latter group, the dose of ribavirin can be reduced (from 1,000 or 1,200 mg day⁻¹ to 800 or even 600 mg day⁻¹). Lowering the duration of therapy with a combination of pegylated IFN-α and ribavirin is not a trivial issue owing to both the cost of therapy and its associated toxicities (flu-like syndrome, depression and alterations in red blood cell counts).

Although IFN is generally an immunomodulatory agent and ribavirin is an antiviral agent, when the two agents are used in combination against hepatitis C they appear to act the other way around. Recent work has focused on the development of compounds that interfere with the non-structural (NS) protein-associated NTPase/helicase, serine protease and RNA-dependent RNA polymerase (RNA replicate) activities of HCV. Halogenated benzimidazoles and benzotriazoles have been proposed to be inhibitors of the HCV NTPase/helicase but whether they also inhibit HCV replication remains to be determined. Recently, a NS3 protease inhibitor (BLLN 2061) was reported to reduce the plasma concentrations of HCV RNA when administered orally for 2 days to patients who were infected with HCV genotype 1, thereby providing proof-of-efficacy for the use of HCV NS3 protease inhibitors in humans.

An attractive approach for the development of HCV inhibitors is to target the NS5B RNA-dependent RNA polymerase (RdRp). The impetus for such an approach comes from the fact that VP 32947 or 3-[(2-dipropylamino)ethyl]thio]-5H-1,2,4-triazino(5,6-b)indole was found to suppress the replication of bovine viral diarrhea virus (BVDV) through an inhibitory effect on the NS5B RdRp. Infections with this pestivirus have an economic impact, but can also be considered as a surrogate virus for HCV. Other compounds that have been identified as highly selective inhibitors of BVDV replication owing to a specific inhibitory effect on the BVDV RNA replicate are compound 1453 (REF. 86) and compound ‘22’ (REF. 87). Although these compounds, in their own right, could be pursued for the treatment of pestivirus infections in domesticated livestock, they could also be model compounds for the development of non-nucleoside HCV RdRp inhibitors. As non-nucleoside RdRp inhibitors such as compound ‘22’ are active at nanomolar concentrations, they seem, at first glance, much more potent than the (ribo)nucleoside analogues, such as N4-hydroxycytidine, that have been reported to block the replication of BVDV and HCV.

**Orthomyxovirus infections**

Of the orthomyxoviruses, influenza A and influenza B viruses cause epidemics in humans. Influenza A viruses, which have been isolated from a wide variety of avian and mammalian species, can cause widespread human epidemics or pandemics with high mortality rates because these viruses are readily and rapidly transmitted between humans by the aerosol route. Whereas influenza B virus only undergoes antigenic drift based on relatively minor changes (transition and/or transversion mutations) in the viral surface glycoproteins haemagglutinin (HA) and neuraminidase (NA), influenza A virus is prone to both antigenic drift and antigenic shift, the latter resulting from major antigenic changes owing to reassortment of genomic fragments between influenza viruses of different animal species.

The high virulence of some influenza A virus strains, such as H5N1, which emerged in Hong Kong in 1997, and the fact that lethal influenza A viruses can be generated in the laboratory by reverse genetics, have accentuated the fear of influenza A viruses being used as bioterrorist weapons. Additionally, highly pathogenic avian influenza A viruses — for example, subtype H7N7 — that are responsible for fowl plague in poultry, can be transmitted to people who handle infected poultry and be further transmitted from person to person; a fatal course of pneumonia in association with acute respiratory distress syndrome has been noted in an individual infected with the avian H7N7 virus.

For many years, amantadine and rimantadine have been used for the prophylaxis and therapy of influenza A virus infections, but they have not gained wide acceptance for three reasons. First, these agents do not have activity against influenza B viruses, as these viruses lack the matrix protein M2 that determines the anti-influenza-virus activity of amantadine and rimantadine; second, the prospect of rapid emergence of drug-resistant virus
hMPV was first isolated in 2001 from young children with respiratory-tract disease. The clinical symptoms are similar to those caused by RSV, and range from upper-respiratory-tract disease to severe bronchiolitis and pneumonia. hMPV is similar to RSV in that infection usually occurs during the winter months and is common in young children, elderly people and immunocompromised individuals. In a study carried out on hospitalized patients with respiratory-tract illness, hMPV was the second-most-detected viral pathogen (RSV being the first) during two successive winter seasons. There is no specific antiviral treatment for hMPV infection.

A significant number of patients who are diagnosed with influenza-like illness harbour RSV and, as influenza and RSV infections occur at approximately the same time, there is a need to distinguish between the two to prescribe specific antiviral treatment. As mentioned above, specific treatment for influenza consists of the NA inhibitors (zanamivir and oseltamivir), whereas for RSV infections the only approved therapy is aerosol administration of ribavirin. In practice, however, ribavirin is rarely used owing to the technical burden of delivery by aerosol inhalation. Attempts have been made to develop RSV inhibitors that target the viral fusion (F) protein and therefore block virus–cell fusion and syncytium formation. An example is 4,4′-bis-[4,6-bis-(3-(bis-carbamoylmethylsulphamoyl)-phenylamino)-1,3,5-triazin-2-ylamino]-biphenyl-2′,2″-disulphonic acid (RFI-641), which has proved to be efficacious when administered prophylactically (or up to 24 hours post-infection) by the intranasal route in mice, cotton rats and African green monkeys intranasally infected with RSV.

Coronavirus infections

In the past, human coronavirus infections, such as infection by 229E virus, were not considered sufficiently serious to be controlled by either vaccination or
specific antiviral therapy. This has now changed markedly with the emergence of severe acute respiratory syndrome (SARS), which has been unequivocally associated with a newly discovered coronavirus — SARS-associated coronavirus (SARS-CoV)\textsuperscript{99–104}. The disease is mainly characterized by influenza-like symptoms, high fever, myalgia, dyspnea, lymphopenia and lung infiltrates (pneumonia) leading to acute breathing problems, with an overall mortality rate of about 10% (in the elderly this can be as high as 50%).

The genome structure, life cycle and phylogenetic relationships of SARS-CoV have been addressed previously\textsuperscript{105}. There are several proteins encoded by the SARS-CoV genome that could be considered targets for chemotherapeutic intervention: the spike (S) protein, the coronavirus main proteinase (3Clpro), the NTPase/helicase, the RNA-dependent RNA polymerase and, possibly, other viral-protein-mediated processes.

The coronavirus S protein mediates infection of permissive cells through interaction of its S1 domain with angiotensin-converting enzyme 2 (ACE2), which is a functional receptor for SARS-CoV\textsuperscript{106}. A 193-amino-acid fragment of the S protein (corresponding to residues 318–510) binds ACE2 more efficiently than the full S1 domain and, in fact, the 193-residue fragment blocks S-protein-mediated infection with an IC\textsubscript{50} of <10 nM (the IC\textsubscript{50} of the full S1 domain is ~50 nM)\textsuperscript{107}. Also, human monoclonal antibodies to the S1 protein block the association of SARS-CoV with ACE2, indicating that the ACE2-binding site of S1 could be a target for drug development\textsuperscript{108}. The first small-molecular-weight inhibitor that was found to interact with the ACE2 active catalytic site, (S,S)-2-[(1-carboxy-2-(3-(3,5-dichloro-benzyl)-3H-imidazol-4-yl)-(ethylamino)-4-methyl-pentanoic acid (MLN-4760), has already been described\textsuperscript{109}. Whether MLN-4760 inhibits SARS-CoV infection remains to be ascertained.

The coronavirus main proteinase, Mpro, also known as 3Clpro, is a target for the design of anti-SARS-CoV drugs\textsuperscript{110}. It was proposed that compounds such as AG7088, which have proven to be active against the rhinovirus 3C proteinase, could be modified to make them active against coronaviruses\textsuperscript{110}. A first modification of AG7088 that removed the methylene group of the p-fluorophenylalanine residue created KZ7088. KZ7088 has been modelled into the structure of the SARS-CoV 3Clpro protein\textsuperscript{111}, and further work in this area could advance structure-based drug design against SARS\textsuperscript{112}. Another potential target for the development of anti-SARS agents is the SARS-CoV-associated NTPase/helicase\textsuperscript{113}.

The SARS-CoV RNA-dependent RNA polymerase is also a potential target for anti-SARS therapy\textsuperscript{114}. This enzyme does not contain a hydrophobic pocket for non-nucleoside inhibitors such as those that have proven active against HCV polymerase or HIV-1 reverse transcriptase\textsuperscript{114}. Of the many nucleoside analogues that are expected to target the SARS-CoV RNA polymerase and for which efficacy has been determined, only N\textsuperscript{4}-hydroxycytidine — incidentally, the same compound that has been accredited with anti-HCV activity\textsuperscript{73} — showed activity, albeit at a low level (EC\textsubscript{50} of 10 μM; selectivity index of ≥10), against SARS-CoV replication in cell culture\textsuperscript{115}.

In addition to N\textsuperscript{4}-hydroxycytidine, some calpain inhibitors (N-(4-fluorophenylsulphonyl)-L-valyl-L-leucinal) inhibit SARS-CoV replication (EC\textsubscript{50} of 1 μM; selectivity index of ≥100)\textsuperscript{115}. The target of the calpain inhibitors remains to be elucidated. Inhibitory effects on SARS-CoV (again with selectivity indexes of up to ~100 and EC\textsubscript{50} values as low as 1 μg ml\textsuperscript{-1}) have been observed for a variety of compounds, including vancomycin, erythromycin and teicoplanin aglycon derivatives\textsuperscript{116}, and mannose-specific plant lectins, derived from Galanthus nivalis (snowdrop), Hippeastrum hybrid (amaryllis)\textsuperscript{117} or Allium porrum (leek)\textsuperscript{118}, which might all owe their antiviral activity to an interaction with components of the viral entry machinery. Glycyrrhizin has also been shown to inhibit the replication of SARS-CoV\textsuperscript{119}, but only at concentrations (EC\textsubscript{50} of 300–600 μg ml\textsuperscript{-1}) that could not be achieved in the target tissue or organs.

An effective agent, at least for the prophylaxis and early post-exposure management of SARS, would seem to be human IFN, either α, β or γ\textsuperscript{120}. Pegylated IFN-α was recently shown to reduce viral replication and excretion, viral antigen expression by type 1 pneumocytes and the attendant pulmonary damage in cynomolgus macaques that were infected experimentally with SARS-CoV\textsuperscript{121}. These preliminary results warrant further studies with pegylated IFN-α, which is commercially available, in the prophylactic or early post-exposure treatment of SARS should it re-emerge.

Reovirus infections

Rotavirus, which is associated with worldwide epidemics of viral gastrointestinal infections, is the most clinically important of the reoviruses. Although several attempts have been made, and are still being, made to develop an effective vaccine for rotavirus infections, the current treatment for this infectious diarrhoea is mainly based on the administration of fluids (physiological saline) to prevent dehydration. Yet, it should be pointed out that SAH hydrolase inhibitors such as 3-deazaneplanocin A (see above) offer great promise for the treatment of reo- (or rota-) virus infections. In cell culture\textsuperscript{122}, 3-deazaneplanocin A was found to inhibit rotavirus replication at an EC\textsubscript{50} of 0.04 μg ml\textsuperscript{-1} and a selectivity index of 10,000.

Retrovirus infections

Since the identification of HIV as the causative agent of AIDS more than 20 years ago, so many efforts have been made to keep this disease under control that 19 compounds have been formally approved as anti-HIV drugs, and they can be used in a variety of combinations\textsuperscript{122}. They can be divided into five categories: the nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine; the nucleotide reverse transcriptase inhibitors (NtRTIs), such as tenofovir disoproxil fumarate; the non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as nevirapine, delavirdine and efavirenz; the protease inhibitors (PIs),
Several noteworthy compounds have proceeded through clinical trials\textsuperscript{124} including the following compounds. The bicyclam AMD3100 (REF. 125), which specifically targets CXCR4, the co-receptor that is used by T-lymphotropic (or X4) HIV strains to enter cells, and SCH-C (SCH 351125), which specifically targets CCR5, the co-receptor used by macrophage (M)-tropic (or R5) HIV strains to enter cells. New NRTIs such as β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (which is marketed as Reverset\textsuperscript{TM}) and amdoxovir (which is also known as DAPD; (−)-β-D-2,6-diaminopurine dioxolane), which are active against HIV isolates with resistance to other NRTIs such as zidovudine and lamivudine. New NNRTIs such as capravirine and etravirine, which are effective against HIV-1 strains carrying the K103N and Y181C mutations that make them resistant to the ‘classical’ NNRTIs (nevirapine and efavirenz). S-1360, a diketo-acid derivative targeted at the integrase-mediated strand-transfer reaction and the first integrase inhibitor to reach clinical studies. Finally, tipranavir (PNU-140690), which is the first non-peptidomimetic inhibitor of the HIV protease to be brought to the clinic and which is expected to retain activity against HIV-1 isolates that have developed resistance to the ‘classical’ peptidomimetic PIs. Several other therapeutic strategies that are still at the preclinical stage are being pursued for the chemotherapy of HIV infections\textsuperscript{124}. These include the following strategies. Downmodulation of CD4 — which is the main cellular receptor used by HIV to bind to its target cells — by cyclotriazadisulphonamides (CADAs). The use of compounds that specifically bind to the viral envelope glycoprotein gp120, such as BMS-378806 [4-benzoyl-1-(4-methoxy-1\textsubscript{H}-pyrrolo-(2,3-b)pyridin-3-yl)oxoacetyl-2-(R)-methylpiperazine], and which thereby block interaction of gp120 with CD4. Using plant lectins derived from snowdrop agglutinin (GNA), amaryllis lectin (HHA) and aglycons of the glycopeptide antibiotics vancomycin, teicoplanin and eremomycin, which specifically interact with gp120. Developing pyranodipyrimidine inhibitors of HIV integrase, such as V-165, that inhibit integration in a manner that is different from the diketo-acid derivatives (such as S-1360) in that they do not show cross-resistance with the diketo-acid derivatives; and finally, two new classes of compounds (N-aminoimidazole derivat es (NAIMS) and pyridine oxide derivatives), which seem to inhibit a post-integration, transcription transactivation event, the exact nature of which remains to be elucidated. Recently, a new class of anti-HIV compounds called maturation inhibitors were identified\textsuperscript{126}. PA-457 [3-O-(3′,3′-dimethylsulfonyl)betulinic acid] disrupts a late step in HIV-1 Gag processing that involves conversion of the capsid precursor (p25) to mature capsid protein (p24). Virions released from PA-457-treated cultures are no longer infectious, and resistance mutations have been identified in the p25 to p24 cleavage site\textsuperscript{126}. Further studies are required to assess whether PA-456 (and similar compounds) have therapeutic potential for HIV infections.

**Figure 8** | Schematic representation of the HIV life cycle, depicting the targets for anti-HIV agents. So far, 19 compounds have been licensed for use in HIV therapy. The five main drug categories are nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and fusion inhibitors. New agents include viral adsorption inhibitors and co-receptor antagonists. Modified with permission from REF.1 © Nature Rev. Drug Discov. (2002) Nature Publishing Group.
Conclusion

At present, 37 chemicals, plus IFN-α in both pegylated and unpegylated forms, have been formally approved for the treatment of viral infections, at least half of which are intended to treat HIV infections. A similar number of compounds are also under preclinical or clinical development, at least half of which can be expected to reach the antiviral drug market. Overall, antiviral strategies seem to target the inhibition of viral DNA polymerase for the treatment of DNA virus infections, helicase/NTPase for the treatment of HSV, HCV or SARS-CoV infections, IMP dehydrogenase for the treatment of HCV and some (-)RNA virus (for example, arena- and bunyavirus) infections, SAH hydrolase for the treatment of other (-)RNA virus infections such as Ebola and Marburg virus or (+)RNA virus infections such as rotavirus, and RNA-dependent RNA polymerase for the treatment of other (+)RNA virus infections (flavivirus and hepatitis C). Finally, IFNs seem to be good therapeutic agents for those viral infections like those caused by coxsackieviruses, hepatitis B and C viruses and SARS-CoV, that, as yet, cannot be sufficiently curbed by other therapeutic or prophylactic approaches.

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