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# Trends in Antiviral Drug Development

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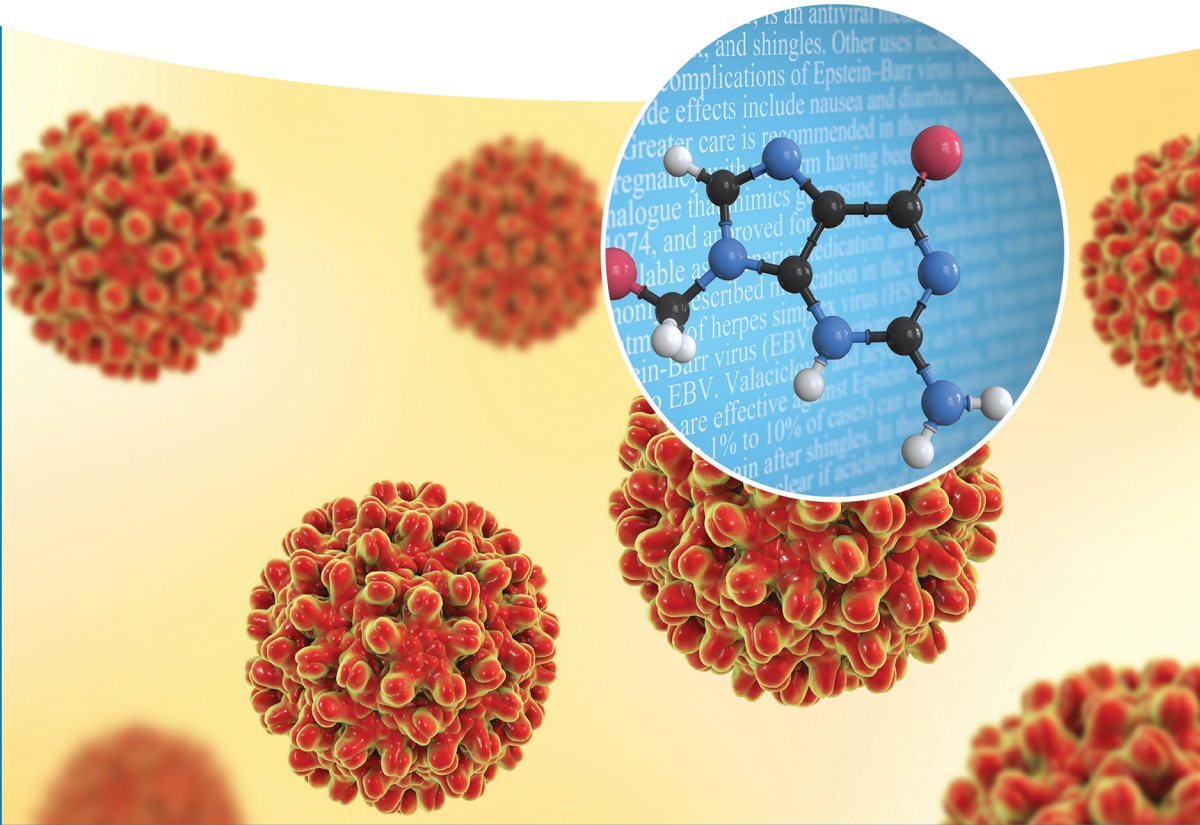

TRENDS IN DRUG DISCOVERY

**Trends in Antiviral Drug Development**

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# Preface

As practitioners in the field of antiviral drug discovery and development, we collectively have observed and contributed to the field for over 47 years, published over 130 papers on antiviral research, introduced 17 antiviral drugs into development, and discovered and developed the first direct-acting antiviral drug to cure a viral disease. We have seen major progress in both treating and curing viral diseases and have observed how advancements in new science and technologies helped expand our knowledge of viruses and provided new insights into how to develop innovative antiviral therapeutics. Yet, we have also seen how cunning viruses are in the way they attack the human host and coopt the human body's own biological processes to survive. We have seen how drug hunters constantly battle to innovate and find ways to combat viruses as they continually change and adapt to the environment around them, and as new viruses emerge by jumping from species to species. Consequently, it was our desire to publish a book that would highlight some of the recent advances in the field of antivirals that occurred over the last 20 years and provide to those who are interested in learning about how antivirals have been developed a window into the thinking and processes used by the scientists who made these discoveries. This book delves into the discovery of drugs to treat HIV, CMV, HSV, VARV, RSV, and SARS-CoV-2, and the breakthrough drugs that cured HCV. It also introduces siRNA therapeutics as a new antiviral modality and the drug candidates in the clinic that are progressing toward achieving an HBV cure. Our hope is that the stories told here in *Trends in Antiviral Drug Development* would expand the readers' understanding of what it takes to develop a drug to treat viral diseases and encourage new ideas and approaches to combat the ever-expanding universe of viral infections.

Michael J. Sofia, PhD, FRSC
Zhengqiang Wang, PhD

# Introduction

# Successes and Challenges in Antiviral Drug Development

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## Introduction

Viral infections have had a profound impact on socioeconomics and life expectancy of human societies throughout human history [1–7]. However, the discovery and development of specific antiviral drugs to treat viral diseases has a relatively short history, largely concomitant with the advent of modern biomedical sciences. Specifically, the FDA approval of idoxuridine (IDU) [8] in 1963, a deoxynucleoside analog first synthesized by Prusoff in 1958 [9], ushered in the era of direct-acting antivirals (DAAs). Since then, many antiviral drugs and drug combinations have been successfully developed and marketed [10], which have largely mitigated many human viral pathogens that historically plagued human societies. Not surprisingly, most of these drugs target viruses that cause chronic infections and/or establish latency. Human immunodeficiency virus (HIV), in particular, has been at the center of antiviral drug development and drug regimen evolution, with dozens of single agents and fixed-dose combinations (FDCs) approved, spanning a wide range of compound classes and molecular targets [11, 12]. The everlasting needs to prevent infection, enhance drug accessibility, and improve adherence have also spurred developments in pre-exposure prophylaxis (PrEP) [13, 14], regimen simplification [15–17], and long-acting injectables (LAIs) [18–20]. Prior to HIV, the earliest antiviral drugs mostly targeted human herpesviruses (HHVs), including herpes simplex virus (HSV), varicella zoster virus (VZV), and human cytomegalovirus (HCMV). Other major chronic viruses targeted by various small-molecule antiviral drugs include hepatitis B virus (HBV) and hepatitis C virus (HCV), both causing viral hepatitis and increasing risk of hepatocarcinoma. Although current antiviral drugs typically do not clear HIV, HBV, or HHVs, the development of DAAs to successfully

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cure HCV represents a major breakthrough [21, 22] in antiviral therapy and provides hope that further development in antiviral drugs could eventually lead to the functional cure of other challenging viral infections. Widespread and seasonal acute viral infections, such as influenza virus, respiratory syncytia virus (RSV), and SARS-CoV-2, have also caused major mortality and morbidity, and have been targeted by successful and ongoing antiviral drug development efforts.

## Antiviral Drugs Targeting Human Herpesviruses

HHVs, a family of eight large DNA viruses, are highly prevalent within human populations [23]. While primary infections are generally associated with low risk, these viruses are all capable of establishing lifelong latency. In immunocompromised individuals, the reactivation of the latent infection causes high morbidity and mortality. Among HHVs, α-herpesviruses HSV and VZV, and β-herpesvirus HCMV, have received particular attention in drug discovery.

The vast majority, if not all, of the earliest HHV drugs belonged to four distinct subclasses of nucleos(t)ide analogs (Table 1): (1) C-5 substituted thymidine analogs, including IDU [8, 24, 25], trifluridine (TFT) [26], and brivudine (BVDU) [27], where the C-5 substituent is mechanistically important by disrupting base pairing; (2) marine sponge *tectitethya crypta* spongonucleoside [28] vidarabine (ara-A) [29], which contains D-arabinose rather than D-ribose; (3) acyclic nucleosides [30] aciclovir (ACV), penciclovir (PCV), and ganciclovir (GCV); and (4) acyclic nucleoside phosphonate (ANP) [31] cidofovir. Intracellularly, all nucleoside analogs (the first three subclasses) are converted to monophosphate (MP) by a virally encoded kinase, and then further phosphorylated by cellular kinases to the active form triphosphate (TP). ANP cidofovir bypasses the function of viral kinase as the MP is already chemically installed, but still uses cellular kinases to form the active TP. Early mechanistic studies showed that these TPs can stop viral DNA synthesis via competitive viral polymerase inhibition or by getting incorporated into the DNA and acting as chain terminators. Of these nucleos(t)ide anti-herpes antiviral drugs, ACV [32, 33] is considered a milestone drug due to its excellent selectivity and low toxicity and has been used to effectively treat infections caused by most known HHVs, particularly HSV and VZV. Another acyclic nucleoside drug GCV [34, 35] has been the first-line treatment for HCMV for decades.

Another mechanistically distinct viral polymerase inhibitor is the pyrophosphate (PPi) mimic foscarnet (PFA), which binds to a site similar to but distinct from the PPi binding site of the viral polymerase to block the pyrophosphate exchange [36]. Notably, PFA does not require phosphorylation and thus is kinase-independent.

Although polymerase-targeting nucleos(t)ide analogs and the PPi mimic PFA are largely effective against HHVs, they are limited by dose-related adverse effects and the emergence of drug resistance. In line with expanding the anti-herpes antiviral drug repertoire, efforts in recent years have led to the successful development of three mechanistically novel drug classes: amenamevir (AMNV) and pritelivir (PTV), inhibitors of viral helicase–primase complex, for treating VZV and HSV, respectively; letemovir (LTV), which targets pUL56, a component protein of the

**Table 1** Antiviral drugs against HHVs.

| Drug class and MOA | Drug name | Note | Year approved | Indication approved |
|---|---|---|---|---|
| Nucleos(t)ide analogs: phosphorylated intracellularly, incorporated and act as chain terminators | Idoxuridine (IDU) | Thymidine analog. 5-I blocking base paring. | 1963 | Topical treatment of herpes simplex keratitis |
| | Trifluridine (TFT) | Thymidine analog. 5-$CF_3$ blocking base paring | 1980 | Eye drops for treating keratitis and keratoconjunctivitis caused by HSV |
| | Brivudine (BVDU) | Thymidine analog. 5-Bromovinyl blocking base paring | 2000 | Herpes zoster (VZV) in adult patients |
| | Vidarabine (ara-A) | Marine spongonucleoside analog | 1976 | Ointment for treating HSV; herpes zoster (VZV) in AIDS patients |
| | Aciclovir (ACV) | Acyclic nucleoside analog | 1981 | Treatment of HSV and VZV infections |
| | Penciclovir (PCV) | Competitive viral pol inhibition and chain termination | 1996 | Treatment of zoster (VZV) and genital HSV |
| | Ganciclovir (GCV) | Competitive viral pol inhibition and chain termination | 1988 | First-line treatment of CMV infections |
| | Cidofovir (CDV) | Competitive viral pol inhibition and chain termination | 1996 | ACV-resistant HSV and GCV-resistant CMV infections |
| Pyrophosphate mimic | Foscarnet (PFA) | Binding to the PPi binding site and blocking PPi release | 1991 | ACV-resistant HSV and VZV; GCV-resistant CMV infections |
| Terminase inhibitor | Letermovir (LTV) | Targeting viral terminase component protein pUL56 | 2017 | Prophylaxis of post-transplant HCMV |
| Viral kinase inhibitor | Maribavir (MBV) | Inhibition of viral kinase (pUL97) | 2021 | Treating post-transplant HCMV infections resistant to other drugs |
| Viral helicase–primase inhibitors | Amenamevir (AMNV) | Inhibition of viral helicase–primase complex | 2017 | Herpes zoster (shingles) |
| | Pritelivir (PTV) | Inhibition of viral helicase–primase complex | Phase 3 | HSV infections in immunocompromised patients |

HCMV terminase complex, for HCMV prophylaxis post stem cell transplants; and maribavir (MBV), an HCMV kinase (pUL97) inhibitor for treating post-transplant HCMV resistant to other drugs.

## Antiviral Drugs Targeting Human Immunodeficiency Virus

HIV drug discovery [37, 38] has been particularly successful with the approval of dozens of single agents in the past four decades (Table 2). These drugs, comprising a few distinct drug classes and mechanisms of action, coalesce to a large repertoire for effective antiretroviral therapy (ART), termed the highly active antiretroviral therapy (HAART) [39]. HAART regimens typically consist of two nucleoside reverse transcriptase inhibitors (NRTIs), along with an integrase inhibitor (INSTI), a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI).

### *Drugs Targeting Reverse Transcriptase*

The virally encoded RT [40] is the target of the earliest anti-HIV drug class, nucleos(t)ide reverse transcriptase inhibitors (NRTIs) [41]. Azidothymidine (AZT), initially designed and synthesized by Jerome Horwitz in 1964 [42] as an anticancer drug, was approved by FDA in 1987 as the very first HIV drug [43, 44]. In the following years, numerous other NRTIs were approved, including three other furanonucleoside analogs didanosine (DDI) [45, 46], zalcitabine (DDC) [47], and stavudine (D4T) [48]; the L-nucleoside lamivudine (3TC) [49–51] and emtricitabine (FTC) [52]; the carbocyclic nucleoside abacavir (ABC) [53]; and the acyclic nucleoside phsphonate (ANP) tenofovir (TFV) [54]. These nucleos(t)ide drugs are all converted into triphosphate (TP) intracellularly by cellular kinases, recognized and incorporated by RT into viral DNA, and act as obligate chain terminators by virtue of lacking the 3′-OH. Although generally effective against HIV, NRTIs can cause toxicity by inhibiting human mitochondrial DNA polymerase (Pol γ), leading to depletion of mtDNA and mitochondrial dysfunction [55, 56].

Also targeting RT, 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) is structurally unique and mechanistically distinct from all other RT inhibitors. As a nucleoside analog, EFdA contains the 3′-OH group, which likely allows more efficient phosphorylation and incorporation. Mechanistically, upon incorporation, EFdA can act as a nonobligate chain terminator as the 4′-ethynyl group distorts the alignment of the primer and the next incoming dNTP, and/or trap RT into a translocation-deficient state where the sliding from N site to P site is inhibited [57, 58]. The latter mechanism renders EFdA the first nucleoside reverse transcriptase translocation inhibitor (NRTTI). Due to the exceptional antiviral potency and the long-lasting PK, EFdA has been developed for PrEP [59].

A conceptually distinct class of RT-targeting drugs is the NNRTIs (Table 2), which allosterically target RT by binding to a largely hydrophobic and well-studied binding pocket ~10 Å away from the active site to induce conformational changes to the active site and distort the positioning of the primer grip to affect its alignment

**Table 2** Antiviral drugs against HIV.

| Drug class and MOA | Drug name | Note | Year approved |
|---|---|---|---|
| NRTIs: intracellularly phosphorylated, incorporated and act as obligate chain terminators | Zidovudine (AZT) | Furanonucleoside; first HIV drug approved | 1987 |
| | Didanosine (DDI) | Furanonucleoside | 1991 |
| | Zalcitabine (DDC) | Furanonucleoside | 1992 |
| | Stavudine (D4T) | Furanonucleoside | 1994 |
| | Lamivudine (3TC) | L-nucleoside | 1995 |
| | Abacavir (ABC) | Carbocyclic nucleoside | 1998 |
| | Tenofovir (TFV) | ANP. Two prodrug regimens (TDF and TAF) approved | 2001 |
| | Emtricitabine (FTC) | L-nucleoside analog | 2003 |
| NRTTI: intracellularly phosphorylated, incorporated and inhibits RT translocation | Islatravir (EFdA) | 3′-OH present. Long acting | Phase 3 |
| NNRTIs: bind to an allosteric site to impact the conformation of the RT active site | Nevirapine (NVP) | First generation | 1996 |
| | Delavirdine (DLV) | First generation; rarely used | 1997 |
| | Efavirenz (EFV) | First generation | 1998 |
| | Etravirine (ETR) | Second generation | 2008 |
| | Rilpivirine (RPV) | Second generation | 2011 |
| | Dapivirine (DPV) | Second generation | 2022 (Africa) |
| | Doravirine (DOR) | Second generation | 2018 |
| PIs: substrate mimics to inhibit viral protease and block the cleavage of viral precursor proteins | Saquinavir (SQV) | Typically used with PK enhancer RTV | 1995 |
| | Ritonavir (RTV) | Used as both an antiviral agent and a PK enhancer (inhibiting CYP3A) | 1996 |
| | Indinavir (IDV) | Typically used with PK enhancer RTV | 1996 |
| | Nelfinavir (NFV) | Inhibits both HIV-1 and HIV-2 | 1997 |
| | Lopinavir (LPV) | Used along with PK enhancer RTV | 2000 |
| | Atazanavir (ATV) | Inhibits UGT1A1 | 2003 |
| | Fosamprenavir (FPV) | Prodrug of amprenavir (APV) | 2003 |
| | Tipranavir (TPV) | Not a substrate mimic. Used along with PK enhancer RTV | 2005 |
| | Darunavir (DRV) | Typically used with PK enhancer RTV. Capable of backbone H-bonding | 2006 |

*(Continued)*

**Table 2** (Continued)

| Drug class and MOA | Drug name | Note | Year approved |
|---|---|---|---|
| INSTIs: bind to IN and viral DNA complex (intasome) and selectively inhibit IN strand transfer | Raltegravir (RAL) | First-in-class | 2007 |
| | Elvitegravir (EVG) | Enhanced by CYP3A inhibitor Cobicistat | 2012 |
| | Dolutegravir (DTG) | Used as a single agent or in FDC along with ABC and 3TC | 2013 |
| | Bictegravir (BIC) | Used in FDC along with TAF and FTC | 2018 |
| | Cabotegravir (CAB) | Long acting. Approved for PrEP | 2021 |
| Entry inhibitors: blocking viral entry at attachment, post-attachment, coreceptor, or fusion | Enfuvirtide (ENF) | Polypeptide (36mer) fusion inhibitor | 2003 |
| | Maraviroc (MVC) | Coreceptor CCR5 antagonist | 2007 |
| | Ibalizumab | Monoclonal antibody targeting CD4 post-attachment | 2018 |
| | Fostemsavir (FTR) | Prodrug of attachment inhibitor temsavir (TMR) | 2020 |
| Capsid inhibitor: binds to viral capsid protein (CA) to impact capsid stability and compete against host factors crucial for virus | Lenacapavir (LEN) | Long-acting injectable (every six months) | 2022 |

with the polymerase active site [60, 61]. Since NNRTIs do not interfere with cellular kinases or human mitochondrial DNA polymerase, they are generally better tolerated than NRTIs. Although the FDA approval of the first-generation NNRTIs nevirapine (NVP, 1996), delavirdine (DLV, 1997, discontinued), and efavirenz (EFV, 1998) validated NNRTIs as a clinically important drug class, especially in HAART with the NRTIs, these early NNRTI drugs typically exhibit low generic barrier to resistance [62]. The resistance profile was drastically improved with all second-generation NNRTIs, including FDA-approved etravirine (ETR, 2008) [63], rilpivirine (RPV, 2011) [64], and doravirine (DOR, 2018) [65], as well as dapivirine (DPV) recently approved in Africa for use with monthly vaginal rings (VR) for PrEP [66, 67]. Critical to the success of the second-generation NNRTIs is the rationally designed three-ring pharmacophore to confer high binding flexibility and adaptability [68].

### *Drugs Targeting Protease*

HIV aspartyl protease cuts gag and gag-pol polyproteins to enable the maturation of newly assembled HIV virions. Catalytically, two aspartic acid residues, D25 and D125, are essential for its enzymatic activity [69]. Inhibitors are typically

designed to mimick the transition state of viral substrates using nonhydrolyzable dipeptide (Y/F-P) hydroxyethylene isostere substituted for the scissile amide bond [70, 71]. However, peptidomimetics in general are susceptible to liver metabolizing enzyme CYP3A [72]. The only approved nonpeptidomimetic PI [73], tipranavir (TPV), which features a dihydropyrone ring as the central scaffold, strongly induces CYP3A expression. Therefore, all approved PIs are used in combination with a CYP3A inhibitor, such as low-dose ritonavir (RTV), which is both an antiviral agent and a PK enhancer [74], or Cobicistat (COBI), an analog of RTV and a nonantiviral CYP3A inhibitor [75]. Of the many PIs approved, the first-generation PIs saquinavir (SQV), RTV, indinavir (IDV), and nelfinavir (NFV) exhibit low genetic barrier to the development of broad class resistance, whereas the second-generation PIs lopinavir (LPV), atazanavir (ATV), fosamprenavir (FPV), TPV, and darunavir (DRV) demonstrate much improved resistance profiles.

### *Drugs Targeting Integrase Strand Transfer*

Integration of viral DNA into host DNA is a required and signature step in the replication cycle of retroviruses [76, 77]. The stably maintained integrated proviral DNA in resting CD4+ T cells allows persistent infections and poses a major barrier toward eradication via antiviral therapy. Integration is carried out by the virally encoded integrase (IN), which has two distinct functions: the 3′-processing (3′-P) and the strand transfer (ST). The 3′-P nicks the terminal 3′ dinucleotide from viral DNA to generate the free 3′ OH, which serves as the nucleophile for the ST where viral DNA is inserted into cellular DNA [78]. In addition, productive HIV integration also entails IN binding to a cellular cofactor, the lens epithelium-derived growth factor (LEDGF)/p75. Although both enzymatic functions [79] and the allosteric binding to LEDGF/p75 [80] have been targeted for the discovery of IN-targeting antivirals, only selective INST inhibitors (INSTIs) have achieved clinical successes, and validated IN as a viable HIV drug target [81]. The chemical matter of selective INSTIs uniquely and invariably features a chelating triad capable of engaging with two $Mg^{2+}$ ions at the DDE active site [82]. Raltegravir (RAL) [83] was the first-in-class INSTI developed which gained FDA approval in 2007. This milestone success was followed by the successful development and approval of four additional INSTIs, elvitegravir (EVG) [84], dolutegravir (DTG) [85], bictegravir (BIC) [86], and cabotegravir (CAB) [87]. Interestingly, CAB is approved to be used in combination with NNRTI RPV as the first LAI ART option for virologically suppressed adults with HIV-1. In addition, long-acting CAB has the potential for HIV PrEP.

### *Drugs Targeting Viral Entry*

HIV entry consists of three major steps [88]: attachment, coreceptor binding, and fusion, all of which have been successfully targeted by FDA-approved drugs (Table 2). The infection starts with binding of the viral glycoprotein gp120 to the CD4 receptor on host cells. Pharmacologically disrupting this binding

interaction led to the development of recently approved fostemsavir (FTR), the prodrug of attachment inhibitor temsavir [89, 90]. Significantly, since FTR works in a step prior to coreceptor binding, it inhibits both CCR5-tropic and CXCR4-tropic HIV.

Upon attachment, the binding to CD4 causes gp120 conformational changes to allow HIV to stably bind to the CCR5 (R5-tropic) or CXCR4 (X4-tropic) coreceptor, which is a required step for viral entry. The approved HIV drug maraviroc (MVC) [91, 92] is a CCR5 antagonist that binds to a CCR5 transmembrane pocket to allosterically alter the conformation of the extracellular domains to block viral gp120 binding. This specific mechanism also renders MVC ineffective against X4-tropic HIV and necessitates a tropism assay prior to treatment. Although resistance via tropism switch is rare due to large genetic barrier, high-level resistance to MVC was still selected, which is due to viral entry using MVC-bound CCR5 [93]. Alternatively, the outgrowth of the preexisting X4-tropic viral population can also confer resistance to MVC [94].

The sequential binding of the receptor and the coreceptor triggers fusogenic conformational changes in viral gp41 to expose a fusion peptide that inserts into the cellular plasma membrane to initiate fusion. The fusion-targeting enfuvirtide (ENF) was the first approved HIV entry inhibitor [95]. A polypeptide (36mer) derived from HIV-1 gp41 C-terminal heptad repeat (CHR), ENF interferes with CHR interaction with the highly conserved N-heptad repeat (NHR) to inhibit the formation of the required six-helix bundle (6HB) and subsequent viral entry. ENF is not orally available due to the intrinsically unfavorable physicochemical properties of peptides [96], especially enzymatic degradation and poor membrane permeability, and is administered via subcutaneous injection.

Ibalizumab [97, 98] is another HIV entry inhibitor approved. It is a humanized monoclonal antibody and a post-attachment inhibitor. Mechanistically, ibalizumab binds to a conformational epitope on domain 2 of the extracellular portion of CD4, and blocks HIV from binding to the CCR5 or CXCR4 coreceptors. Similar to the attachment inhibitor FTR, ibalizumab is active against both R5-tropic and X4-tropic HIV isolates.

### *Drugs Targeting Viral Capsid*

The multifunctional HIV capsid protein (CA) is the target of the most recently approved HIV drug lenacapavir (LEN) [99]. Mechanistically [100, 101], LEN binds to a pocket at a conserved interface between CA protomers to interfere with the binding of host cofactors essential for multiple phases of the viral replication cycle, including NUP153 and CPSF6, which are required for viral nuclear entry. LEN shows antiviral potency at picomolar concentrations against all subtypes of HIV-1 tested, and exhibits high synergy and no cross-resistance with other classes of HIV drugs. The exceptional antiviral profile, along with the low in vivo systemic clearance and slow-release kinetics from the subcutaneous injection site, renders LEN an excellent LAI drug [102]. Remarkedly, LEN is approved to be used via injection every six months.

## Antiviral Drugs Targeting Hepatitis B Virus

Hepatitis B virus (HBV) chronically infects an estimated 240–350 million people worldwide and remains a major global health threat [103, 104]. Although an effective vaccine is widely available, each year there are still around 1 million people newly infected. Individuals chronically infected with HBV are at high risk of severe liver diseases typically progressing through fibrosis, cirrhosis, and eventually hepatocellular carcinoma. HBV-associated liver diseases account for approximately 750 000 deaths annually. Fortunately, multiple drugs have been developed and marketed against chronic HBV (Table 3) [105]. Although these drugs rarely lead to functional cure, their lifelong use effectively suppresses HBV replication and reduces the risk of liver cancer. Current HBV drugs consist of two classes (Table 3): the immunomodulatory interferon, including IFN alpha-2b and PegIFN alpha-2a [106]; and direct acting nucleos(t)ide analogs (NAs) targeting reverse transcriptase (RT), the viral polymerase. Similar to HIV, HBV-encoded RT has been successfully targeted by various NAs, including cytidine L-nucleoside analog lamivudine (3TC) [107], adenosine analogs adefovir (AFV) [108] and tenofovir (TFV) [107], guanosine carbocyclic analog entecavir (ETV) [109], and L-thymidine telbivudine (TBV) [110] and its 2′-β-F analog clevudine (CLV) [111]; the latter was approved in South Korea only. Notably, while close analogs AFV and TFV were initially approved as ester prodrugs, the recent ProTide prodrug of TFV, tenofovir alafenamide (TAF) [112, 113],

**Table 3** Antiviral drugs against HBV.

| Drug class and MOA | Drug name | Note | Year approved |
|---|---|---|---|
| Nucleoside polymerase inhibitors: intracellularly phosphorylated, incorporated and act as chain terminators | Lamivudine (3TC) | L-nucleoside | 1998 |
| | Adefovir (AFV) dipivoxil | ANP prodrug | 2002 |
| | Entecavir (ETV) | Carbocyclic nucleoside | 2005 |
| | Telbivudine (TBV) | Thymidine L-nucleoside | 2006 |
| | Tenofovir (TFV) disoproxil fumarate (TDF) | Prodrug of ANP | 2008 |
| | Tenofovir (TFV) alafenamide (TAF) | ProTide prodrug of ANP | 2016 |
| | Clevudine (CLV) | L-nucleoside; approved in Korea | 2006 |
| Interferons (IFNs): immunomodulators | IFN alpha-2b | Three times a week; injection | 1991 |
| | PegIFN alpha-2a | Long acting (once weekly, injection) | 2005 |

allows much lower dosing and improved safety profile due to the liver-specific delivery. Interestingly, HBV and HIV share two prominent therapeutic commonalities: (1) many NAs approved for treating HBV are also HIV drugs, including 3TC, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide TAF and (2) these drugs do not cure the infection, presumably because they do not impact the viral reservoirs. Adefovir was also initially being developed as an HIV drug; however, its toxicity at the doses required for systemic usage limited its use, compared to a much lower dose required for a liver-tropic drug. Current efforts in the field are looking to identify a combination regimen that suppresses viral replication, reduces the production of HBV surface antigen, and reactivates an HBV-specific immune response with the hope of achieving high rates of functional cure with a short duration of therapy.

## Antiviral Drugs Targeting Hepatitis Delta Virus

Hepatitis D virus (HDV) is a single-stranded (−)-RNA virus whose genome folds into a rod-like structure with no nucleocapsid. HDV requires the presence of HBV to propagate because it uses the HBV surface antigens (HBsAg) to complete its viral coat. It is the HBsAg in the HDV viral coat that is recognized by the sodium taurocholate cotransporter polypeptide (NTCP) receptor on the surface of an hepatocyte facilitating viral entry. The HD antigen (HDAg) expressed as two isoforms (small and large) is the only protein expressed by HDV. Since HDV produces no other proteins, it uses cellular DNA-dependent RNA polymerases to replicate its genome. Post-translational modification of HDAgs modifies their function and therefore enables modulation of the viral life cycle. Coexistence of HDV and HBV leads to more severe liver disease and a more rapid progression to cirrhosis and liver cancer [114, 115].

Current therapies to treat HDV infection consist of pegylated interferon-α (PEG-IFN) and newly introduced bulevirtide (Myrcludex B, BLV), which blocks viral entry (Table 4). Pegylated interferon has shown limited efficacy [116]. However, BLV, which is a myristolated synthetic lipopeptide mimicking the N-terminal region of the pre-S1 domain of HBsAg, has demonstrated promising efficacy and enhanced efficacy has been observed in combination with PEG-IFN [114].

**Table 4** Antiviral drugs against HDV.

| Drug class and MOA | Drug name | Note | Year approved |
|---|---|---|---|
| Therapeutic protein<br>Innate immune activation | Pegylated interferon-α (PEG-IFN) | Subcutaneous delivery; limited efficacy | Mid-2000s |
| Myristolated synthetic polypeptide | Bulevirtide (BLV, Myrcludex B) | Inhibits viral entry by blocking the NTCP receptor; only approved by the EMEA in the European Union and not by the US FDA | 2020 |

## Antiviral Drugs Targeting Hepatitis C Virus

The hepatitis C virus (HCV) is a liver tropic virus that chronically infects over 75 million individuals worldwide and is a leading cause of chronic liver disease and liver cancer [117]. This (+)-sense RNA virus replicates in the cytoplasm of hepatocytes, does not integrate into the host genome, nor does it appear to leave a reservoir of genetic material in the infected cell [118].

Drugs targeting the hepatitis C virus (HCV) can be classified into two categories: immunomodulators and direct-acting antivirals (Table 5). The first drug developed for curing HCV was the immunomodulator interferon-α (IFN). The evolution of interferon therapies ultimately led to the development of more effective pegylated interferons, PEG-INTRON and PEGASYS. The use of ribavirin (RBV) in conjunction with IFN was shown to increase the cure rates of IFN therapies to 40%; however, significant adverse side effects and genotype coverage limited the use of IFN regimens [119].

The evolution of HCV curative therapies eventually focused on the development of direct-acting antivirals (DAAs) and the elimination of IFN in clinical practice (Table 5). DAA therapeutic development targeted three viral nonstructural proteins, NS3/4a protease, NS5A replication complex protein, and the NS5B RNA-dependent RNA polymerase [22, 120]. The launch of the NS3/4a PIs telaprevir and boceprevir each in combination with IFN not only led to improved cure rates but also added to the undesirable side effect profile already seen with IFN use. Approval of the liver-targeted NS5B polymerase inhibitor sofosbuvir ushered in the era of IFN-free HCV curative therapies with short 8–12-week treatment duration, pan-genotypic profile, high barrier to resistance, minimal side effect profile, and cure rates surpassing 95% [121, 122]. Combinations that included sofosbuvir with either RBV, the NS3/4a PI, simeprevir or NS5A inhibitors, ledipasvir or daclatasvir soon became the regimens of choice for genotypes 1, 2, and 3 HCV patients [123–126]. Eventually, DAA regimens that included combinations of NS5A inhibitors and NS3/4a PIs with or without a non-nucleoside NS5B inhibitor (paritaprevir/ombitasvir/dasabuvir, grazoprevir/elbasvir) were introduced but had limited uptake because of their poor genotype coverage and resistance profile [127, 128]. However, these were eventually surpassed by the highly potent and pan-genotypic regimes sofosbuvir/valpatasvir and glecaprevir/pibrentasvir which dominate the market providing cure rates of over 95% [22, 129, 130]. To address any patients who exhibited breakthrough resistance or relapse, primarily from the NS5A component of these regimens, the triple combination regimen sofosbuvir/valpatasvir/voxilaprevir was introduced [131].

## Antiviral Drugs Targeting Acute RNA Respiratory Viruses

### *Drugs Targeting Human Influenza Viruses*

Seasonal flu epidemics are caused by human influenza A virus (IAV) and influenza B virus (IBV). IAVs are classified into many subtypes based on the two viral glycoproteins

**Table 5** Antiviral drugs against HCV.

| Drug class and MOA | Drug name | Note | Year approved |
|---|---|---|---|
| **Therapeutic protein Innate immune activation** | Interferon-α (IFN) | Subcutaneous delivery; efficacy was improved with the development of pegylated IFN | 1991 |
| Nucleoside NS5B pol inhibitors: intracellularly phosphorylated, incorporated and act as chain terminators | Ribavirin (RBV) | Additional MOAs include inhibiting IMPDH; inducing Th2 to Th1 switch; lethal mutagenesis; and inhibiting RNA capping. Used in combination with PegIFN | 1999 |
| | Sofosbuvir (SOF) | ProTide prodrug; liver-targeted delivery. | 2013 |
| Non-nucleoside NS5B pol inhibitor: allosterically inhibit pol | Dasabuvir | Genotype 1; binds to HCV NS5B palm I site | 2014 |
| | Beclabuvir (BCV) | Binds to NS5B thumb domain site I | Post marketing |
| NS3/4 protease inhibitors: bid to the active site inhibit the cleavage of HCV polyproteins | Boceprevir (BOC) | Genotype 1; carbonyl amide warhead; withdrawn in 2015 | 2011 |
| | Telaprevir (TVR) | Genotype 1; carbonyl amide warhead; discontinued in 2014 | 2011 |
| | Simeprevir (SMV) | Genotype 1 | 2013 |
| | Paritaprevir (PTV) | Genotype 1; discontinued in 2018 | 2014 |
| | Grazoprevir (GZR) | Genotypes 1 and 4 | 2015 |
| | Asunaprevir (ASV) | Genotype 1 | 2014 |
| | Voxilaprevir | Pan-genotypic; part of combination with SOF and velpatasvir | 2017 |
| NS5A inhibitors: bind to HCV NS5A to impact genome replication and virion assembly | Daclatasvir (DCV) | In combination with ASV; genotype 1 | 2014 |
| | Ledipasvir (LDV) | In combination with SOF; genotype 1 | 2014 |
| | Ombitasvir (OBV) | Used in combination therapy for genotype 4 | 2014 |
| | Elbasvir (EBR) | Used in combination with GZR for genotype 1 or 4 | 2016 |
| | Velpatasvir (VEL) | Used in combination with SOF for all major genotypes | 2020 |
| | Pibrentasvir (PIB) | Used in combination with Glecaprevir for all major genotypes | 2017 |