



# The future of antivirals: broad-spectrum inhibitors

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## Purpose of review

Potent antivirals are successfully used for the treatment of infections with herpesviruses, hepatitis B and C viruses, HIV, and with some success for influenza viruses. However, no selective inhibitors are available for a multitude of medically important viruses, most of which are (re-)emerging RNA viruses. As it is impossible to develop drugs against each of these viruses, broad-spectrum antiviral agents (BSAA) are a prime strategy to cope with this challenge.

## Recent findings

We propose four categories of antiviral molecules that hold promise as BSAA. Several nucleoside analogues with broad antiviral activity have been described and given the relatively conserved nature of viral polymerases, it may be possible to develop more broad-spectrum nucleoside analogues. A number of viral proteins are relatively conserved between families and may also be interesting targets. Host-targeting antiviral drugs such as modulators of lipid metabolism and cyclophilin inhibitors can be explored as well. Finally, the potent and broad antiviral function of the immune system can be exploited by the development of immune-modulating BSAA.

## Summary

Despite the recent advances, the BSAA field is still in its infancy. Nevertheless, the discovery and development of such molecules will be a key aim of antiviral research in the coming decades.

## Keywords

antiviral therapy, broad-spectrum antiviral agents, host-targeting antivirals, immune modulation, nucleoside analogues

## INTRODUCTION

Several epidemics of (re-)emerging viruses startled the world during the last decennia. The recent ebolavirus outbreak in West Africa and the Middle East respiratory syndrome epidemic in the Middle East and South Korea made clear that antivirals are urgently needed against a number of viruses and viral families, for which no such drugs are currently available. Indeed, potent antivirals (or combinations thereof) are only available to treat infections with a limited number of viruses, that is, herpesviruses, hepatitis B and C viruses (HBV, HCV), HIV, and to some extent for influenza virus. For many other viruses, including neglected and/or emerging RNA viruses that are sometimes highly pathogenic, like the ebolavirus, there are no antiviral treatment options. Furthermore, it can be expected that new, potentially pathogenic viruses will emerge in the future. Because it will not be economically viable to develop specific drugs for each individual virus, the development of broad-spectrum antiviral agents (BSAA) is believed to be essential to address the challenge of viral infections, both common and (re-)emerging. A first major and probably feasible achievement would be the development of

molecules with broad antiviral activity within one virus family (pan-picornavirus, pan-alphavirus, etc.). However, the 'holy grail' would ultimately be the discovery of antiviral molecules that target more than one virus family, or even all RNA viruses or all enveloped viruses. The development of BSAA may be focused on targeting a viral protein, but could also modulate a host cell factor. In this review, we will discuss possible strategies to develop BSAA and highlight promising candidates and strategies.

## DIRECT-ACTING BROAD-SPECTRUM ANTIVIRAL AGENTS

### Nucleoside and nucleotide analogues

Modified nucleosides and nucleotides have been among the earliest marketed antiviral drugs [1].

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## KEY POINTS

- Broad-spectrum antiviral drugs are a prime strategy to cope with the large number of medically important, neglected, and/or (re-)emerging viruses.
- As some nucleoside analogues have proven to target a broad range of viruses, further research into these molecules may yield even more potent and broad-spectrum inhibitors.
- Another strategy to reach a broad spectrum of activity is to target relatively conserved viral enzymes, such as the viral protease.
- Host-targeting antiviral drugs are a third approach, probably with a high barrier to resistance.
- Finally, immune modulation uses specific properties of the host immune system and is one of the most promising strategies for future antiviral therapy.

They are the cornerstone of anti-HIV therapy (e.g., tenofovir, emtricitabine, lamivudine, etc.) and crucial for the treatment of herpesvirus infections (aciclovir, ganciclovir, cidofovir, etc.) [1]. Some nucleoside analogues exert antiviral activity against an elaborate spectrum of viruses. Ribavirin is probably the most well known antiviral nucleoside with a broad antiviral activity and is used to treat chronic hepatitis C and E, respiratory syncytial virus (RSV) infections, and with some success for the treatment of Lassa fever and hantavirus infection [2–6]. Secondly, several 2'-C-methylated nucleosides that had been discovered as HCV inhibitors were found to exert antiviral activity against several positive-sense RNA viruses. Sofosbuvir (a prodrug form of 2'-deoxy-2'- $\alpha$ -fluoro- $\beta$ -C-methyluridine) is now successfully being used for the treatment of HCV infections but has less pronounced or no activity against other RNA viruses [7,8]. By contrast, the prototype inhibitor 2'-C-methylcytidine inhibits a rather broad-spectrum of positive-sense RNA viruses, including flavivirus, norovirus and picornaviruses [9–11]. Moreover, favipiravir, also known as T-705, has antiviral activity against both positive and negative-sense RNA viruses [12]. This molecule is regarded as a nucleobase and is intracellularly metabolized to a nucleotide analogue that inhibits the influenza RNA-dependent RNA polymerase. The compound has been approved in Japan for the treatment of influenza virus infections. Similarly, the adenosine analogue BCX4430 acts as a chain terminator of RNA virus polymerases and elicits in-vitro antiviral activity against most positive and negative-sense RNA viruses. BCX4430 is most potent against filoviruses and protective activity has

been demonstrated in filovirus-infected primates [13\*]. Nevertheless, these nucleoside and nucleotide drugs exhibit either toxicity and strong side-effects (e.g., 2'-C-methylcytidine, and ribavirin), limited clinical efficacy against some viruses (e.g., ribavirin against RSV infections), and/or they are still in (early) development [14–16]. Finally, the spectrum of susceptible viral species is often too limited for these drugs to be considered true BSAA.

Despite the drawbacks and limitations of the currently available nucleoside analogues, this class of molecules still holds great promise to deliver *bona fide* BSAA. The replication of the viral genome is an essential part of the viral life cycle and consequently most viruses encode their own polymerases that are significantly divergent from their mammalian counterparts. There is a certain degree of conservation within each type of polymerase family (e.g., DNA or RNA-dependent, etc. [17]), suggesting the possibility to develop BSAA that inhibit virtually all polymerases of a certain type. To identify lead molecules for further development into such nucleoside-based BSAA, a tailored screening approach may be quintessential. An enormous collection of nucleoside and nucleotide analogues has been synthesized to date [18]; a first step in a successful screening campaign would therefore be the rigorous selection of a high-end nucleoside library based on the criteria, such as structural diversity, drug-like characteristics, cell-permeability, solubility, etc. This library can subsequently be tested using a specifically designed screening procedure. A first possibility for such screening would be to use high-throughput enzymatic polymerase assays [19,20] where all selected molecules are tested against several representative viral polymerases of a certain type (e.g., RNA-dependent RNA polymerases from different virus families), searching for molecules that inhibit all tested enzymes. Another approach might be to evaluate such libraries in phenotypic cell-based antiviral assays against a panel of viruses that are representative for particular genera, families, or groups of viruses. A third strategy is the rational design of nucleotide analogues based on a comparison of available polymerase crystal structures and specifically the conserved features in the catalytic site. Further development of hit molecules would include expanding the number of tested viruses and chemical modification of the initial hit (so-called hit explosion) to increase antiviral potency and the number of susceptible agents, but also to improve selectivity and pharmacokinetic parameters. As some of the DNA virus-targeting nucleoside analogues already have a rather broad-spectrum of activity, these efforts should probably be directed towards RNA viruses initially.

### **Viral protease inhibitors**

Besides the viral polymerase, the viral protease is one of the most studied antiviral targets and virus-specific protease inhibitors are used successfully to treat HIV and HCV infections. The development of protease inhibitors targeting multiple virus families might be an interesting strategy. Based on phylogenetic analysis, picornaviruses, caliciviruses, and coronaviruses can be classified in the picornavirus-like supercluster [21]. These viruses all have 3C or 3C-like proteases with a typical chymotrypsin-like fold and a catalytic triad (or dyad) containing a cysteine residue as a nucleophile, making them interesting targets for BSAA development. Rupintrivir for instance is an irreversible 3C-protease inhibitor that was originally developed for the treatment of human rhinovirus infections [22] and antiviral activity has also been shown against other picornaviruses, coronaviruses, and norovirus [23–25]. Other inhibitors of 3C-like proteases were reported with broad-spectrum antiviral activity against both feline coronaviruses and caliciviruses [26]. Therefore, the design of BSAA targeting proteases of different virus families appears feasible and could be a good strategy to develop broader-spectrum antivirals.

### **HOST-TARGETING ANTIVIRALS AS BROAD-SPECTRUM ANTIVIRAL AGENTS**

Targeting a host protein that is essential in the viral life cycle of different virus families could be a second attractive strategy for broad-spectrum antiviral intervention. However, this strategy is still somewhat controversial since inhibiting the primary roles of host factors may result in toxicity or adverse effects and polymorphisms in a host factor and variable expression levels between patients might be problematic. On the other hand, the selection of viral resistance, which is a major problem for conventional virus-specific drugs, is probably lower for a host-targeting antiviral. The feasibility of host-targeting antiviral strategies will likely depend on which host factor is targeted and how the viruses and the cell depend on its function(s). Modulators of the host lipid metabolism, nitazoxanide, and cyclophilin inhibitors are host-targeting antivirals that are considered as potential BSAA.

### **Modulators of lipid metabolism**

Many viruses are highly dependent on host lipid metabolism for replication [27]. Lipid metabolism is therefore considered as a prime target for BSAA. One of the most widely used classes of lipid modulators is the cholesterol-lowering statins. Statins inhibit

the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, the rate-limiting enzyme involved in cholesterol biosynthesis in the liver. Statins have been reported to possess in-vitro antiviral activity against a variety of viruses, such as HCV, HIV, poliovirus, cytomegalovirus, dengue virus, and RSV [28–35]. Nevertheless, studies evaluating the antiviral efficacy of statins in patients yielded contradictory results. In HCV-infected patients for instance, a modest or even no antiviral effect of statins was observed when used as a monotherapy. However, in combination with the previous standard of care (pegylated interferon- $\alpha$  and ribavirin) a significant increase in sustained virological response rates was observed [36,37]. The potential use of statins as BSAA will require more study to prove their in-vivo antiviral efficacy.

Arbidol is another lipid modulator that is approved in China and Russia for the prophylaxis and treatment of influenza, and other respiratory viral infections. This indole derivative also inhibits the replication of many other enveloped and non-enveloped RNA and DNA viruses, including HBV, HCV, and chikungunya virus [38]. Recent studies suggest that arbidol has a dual mechanism of action: it binds to lipid membranes and interacts with aromatic amino acids in the viral envelope glycoprotein [39]. In this way, it interferes with viral entry and membrane fusion. Arbidol combines good bioavailability with a safe record of use in patients, making it a promising BSAA candidate [40]; however, in-vivo studies confirming its antiviral effect are only sparsely available.

Finally, LJ001 is a lipophilic thiazolidine derivative that is effective against several enveloped viruses, including influenza virus, HIV, and filoviruses [41]. In contrast, the compound has no effect on nonenveloped viruses. LJ001 targets the viral lipid envelope and hampers its ability to mediate virion-cell fusion. Recent studies showed that LJ001 induced lipid oxidation, thereby negatively impacting the biophysical properties of membranes (such as curvature and fluidity) that are needed for viral fusion [41]. Unfortunately, LJ001 itself is unsuitable for further development because of its poor physiological stability and the requirement of light for its antiviral mechanism. New analogues have been developed to overcome these negative characteristics [42]. These analogues have improved antiviral activity, better pharmacokinetic characteristics, and altered light-absorbing properties. However, when evaluated in a mouse infection model of Rift Valley fever virus, these molecules were only able to delay the time of death [42]. Although this particular class of molecules seems to be less suitable for clinical development, the viral membrane could still be a

viable target for BSAs that disturb viral-cell fusion [43,44]. Squalamine is another compound that targets the host membrane, but its mechanism of action differs from LJ001 and analogues. Squalamine is a compound isolated from the dogfish shark and the sea lamprey that inhibits enveloped RNA and DNA viruses both *in vitro* and *in vivo* [45]. The mechanism of antiviral activity is proposed to be the neutralization of the negative electrostatic surface charge of intracellular membranes, thereby making the cellular environment less favorable for viral replication. This disruption of electrostatic potential does not result in structural damage of cellular membranes, as measured by changes in cell permeability [46]. As squalamine can be readily synthesized [47] and has already been studied in humans in several phase 2 clinical trials for cancer and retinal vasculopathies without serious adverse events [48], its potential to be used as a BSAA could be further explored.

### Nitazoxanide

Nitazoxanide was originally developed and commercialized as an antiprotozoal agent and was licensed in the United States as an orphan drug for the treatment of diarrhoea caused by *Cryptosporidium parvum* and *Giardia intestinalis* [49]. It is also widely used in India and Latin America to treat intestinal parasitic infections. In addition to its antiparasitic activity, nitazoxanide inhibits a broad range of unrelated RNA and DNA viruses [50]. Nitazoxanide inhibits the influenza virus by blocking the maturation of the viral hemagglutinin at the posttranslational stage [51]. In HCV-infected cell cultures, nitazoxanide activated protein kinase R, an important component of the innate immune system [52]. The antiviral efficacy of nitazoxanide has also been evaluated in patients. A phase 2b/3 clinical study in patients with laboratory-confirmed influenza showed that nitazoxanide decreased the duration of clinical symptoms and reduced viral shedding compared with placebo [53]. A large phase 3 trial is ongoing. Phase 2 studies also demonstrated that nitazoxanide significantly reduced the duration of symptoms in patients infected with rotavirus or norovirus [54]. For HCV-infected patients, clinical studies showed improved responses when nitazoxanide was combined with pegylated interferon [55]; however, the clinical development for HCV treatment was discontinued because of the recent approval of direct-acting antivirals. The broad-spectrum antiviral activity together with the high barrier for resistance and proven *in-vivo* efficacy for some viral infections make nitazoxanide an attractive candidate to be

developed as a BSAA. Initially, clinical development of this drug will primarily focus on viral respiratory infections and gastroenteritis.

### Cyclophilin antagonists

The cyclophilins are peptidyl-prolyl cis/trans-isomerases that are required for the proper folding of certain host proteins [56] and also play an important role in the life cycles of diverse viruses [57]. Cyclosporine A and sanglifehrin A are immunosuppressive molecules that inhibit cyclophilins [58]. By chemical modification, analogues were generated without immunosuppressive properties. These cyclophilin inhibitors inhibit a broad range of RNA and DNA viruses, both *in vitro* and in animal models [57]. The most advanced molecules are alisporivir and SCY-635. These molecules demonstrated therapeutic efficacy in HCV-infected patients [59,60]. Mechanistically, cyclophilin A (CypA) was shown to interact with the HCV NS5A protein [61,62] and multiple mutations in NS5A were required to confer *in-vitro* resistance to alisporivir, suggesting a high barrier to resistance [62,63]. For HIV, it was reported that CypA binds to the capsid protein p24 [64]. Although cyclophilin inhibitors potentially suppress HIV infection *in vitro* and in the majority of patients, naturally preexisting capsid variants resistant to treatment were observed and preclude the broad therapeutic use of cyclophilin inhibitors against HIV [65,66]. However, as cyclophilins are indispensable for the replication of many viruses, these proteins may be interesting host targets for the development of BSAA. Furthermore, CypA knockout studies in a human cell line and in mice showed that CypA is not essential for basic cell survival [67,68], countering concerns for associated cellular toxicity. However, the *in-vivo* efficacy of cyclophilin inhibitors will need to be demonstrated for other viruses.

### IMMUNE MODULATION

Lastly, the immune system can be regarded as the most known broad-spectrum antiviral mechanism at present. By combining innate and adaptive immune mechanisms, most microbial infections can be cleared successfully. However, in particular cases, the immune system is unable to eliminate the pathogen (e.g., chronic viral hepatitis, herpesvirus latency, HIV infections, etc.) or its actions may be damaging to the host (for instance during RSV infection [69]). Many viruses circumvent or even cooperate with the immune response for their own benefit [70]. Therefore, specific modulation of the immune response is an attractive strategy to address

these persistent, latent or immune-evading viruses, but also to develop novel and potentially broad-spectrum antiviral therapies. The current clinical practice already includes a form of immune modulation-based antiviral therapy: (pegylated) interferon is an important part of chronic viral hepatitis therapy, although it may have severe side-effects.

Immune modulation as an antiviral therapy has distinct advantages, such as the possibility to target a wide array of viruses and other pathogens, and a decreased risk for resistance development. However, there may be certain risks when modulating such a complex system, for instance the possibility to induce autoimmunity or cytokine storms, unwanted effects on commensal microbes or exacerbation of inflammatory diseases. This highlights the need for a very thorough understanding of the underlying immune mechanisms and extensive safety testing.

Modulation of innate immune signaling would be an interesting alternative for the direct use of interferon. One strategy employs the retinoic acid-inducible gene 1 agonist 5'pppRNA [71,72]: this double-stranded RNA (dsRNA) oligomer activates several innate immune pathways and has broad-spectrum antiviral activity against multiple DNA and RNA viruses. In addition, it protects mice from lethal influenza virus infection [71]. Nevertheless, 5'pppRNA still requires parenteral administration. The use of small molecules that trigger an interferon-response, for example, compound C3 [73], provides an interesting possibility to overcome parenteral administration.

An alternative and more specific approach is to selectively activate certain factors of the interferon effector system, thus obtaining a more targeted response and possibly avoiding interferon's side-effects. One example is RNase L-activating molecules with a broad activity against RNA viruses [74]. GSK983 is another small molecule that induces a specific subset of interferon-stimulated genes and has antiviral activity against a number of unrelated viruses, although not all pathogens are susceptible [75]. Finally, two very attractive targets for the development of activating molecules may be the interferon-induced protein with tetratricopeptide repeats and interferon-induced transmembrane protein families which display broad-spectrum antiviral activity [76], but no such activating molecules have been described yet.

Another interesting development in antiviral immune modulation is dsRNA-activated caspase oligomerizers (DRACOs) [77]. These engineered proteins comprise a dsRNA-detection domain, an apoptosis-induction domain and a transduction tag for cellular delivery. Most viruses (including

DNA viruses) produce long dsRNA during their replication cycle which is recognized by the dsRNA-detection domain. By fusing this detector to an apoptosis-inducing domain, DRACOs selectively eliminate virus-infected cells without harming non-infected cells. Consequently, these constructs have antiviral activity against a large spectrum of DNA and RNA viruses [77,78]. An in-vivo proof-of-concept was provided in an influenza mouse model [77]. However, concerns regarding safety and specificity, delivery, cost, and in-vivo efficacy need to be addressed before DRACOs can advance into clinical trials.

Priorities for future research in antiviral immune modulation thus include the discovery and development of more potent molecules with an extensive spectrum of susceptible viruses, the in-vivo validation of the available and novel molecules against clinically relevant pathogens, and the minimization of toxicity and side-effects. A detailed understanding of the fundamental mechanisms governing the innate immune response is imperative in this regard.

## CONCLUSION

Over the past decades, antiviral research has resulted in over 30 marketed antiviral agents, most of them targeting a specific viral species. To cope with the threat of other viral pathogens, particularly (re-)emerging and neglected RNA viruses, the development of BSAA is critical. We discuss different approaches to obtain such molecules, focusing on nucleoside analogues, inhibitors of other conserved sites in viral enzymes, host-targeting antivirals, and finally immune modulators. This list is not exhaustive; other interesting strategies may emerge as our knowledge on viruses expands. Future research efforts should focus on validating these and other approaches and on the early development of candidate BSAA, but they should also be directed at increasing the fundamental understanding of the viral life cycle as this will provide important insights for the development of new broad-spectrum antiviral strategies.

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## Conflicts of interest

*There are no conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. De Clercq E. Antivirals: past, present and future. *Biochem Pharmacol* 2013; 85:727–744.
  2. Paeshuyse J, Dallmeier K, Neyts J. Ribavirin for the treatment of chronic hepatitis C virus infection: a review of the proposed mechanisms of action. *Curr Opin Virol* 2011; 1:590–598.
  3. Kamar N, Izopet J, Tripon S, *et al.* Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med* 2014; 370:1111–1120.
  4. Pelaez A, Lyon GM, Force SD, *et al.* Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. *J Heart Lung Transplant* 2009; 28:67–71.
  5. Bausch DG, Hadi CM, Khan SH, Lertora JLL. Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever. *Clin Infect Dis* 2010; 51:1435–1441.
  6. Moreli ML, Marques-Silva AC, Pimentel VA, da Costa VG. Effectiveness of the ribavirin in treatment of hantavirus infections in the Americas and Eurasia: a meta-analysis. *Virusdisease* 2014; 25:385–389.
  7. Lawitz E, Mangia A, Wyles D, *et al.* Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; 368:1878–1887.
  8. Dao Thi V, Debing Y, Wu X, *et al.* Sofosbuvir inhibits hepatitis E virus replication in vitro and results in an additive effect when combined with ribavirin. *Gastroenterology* 2015; Submitted.
  9. Lee J-C, Tseng C-K, Wu Y-H, *et al.* Characterization of the activity of 2'-C-methylcytidine against dengue virus replication. *Antiviral Res* 2015; 116:1–9.
  10. Rocha-Pereira J, Jochmans D, Debing Y, *et al.* The viral polymerase inhibitor 2'-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. *J Virol* 2013; 87:11798–11805.
  11. Goris N, De Palma A, Toussaint J-F, *et al.* 2'-C-methylcytidine as a potent and selective inhibitor of the replication of foot-and-mouth disease virus. *Antiviral Res* 2007; 73:161–168.
  12. Furuta Y, Gowen BB, Takahashi K, *et al.* Favipiravir (T-705), a novel viral RNA polymerase inhibitor. *Antiviral Res* 2013; 100:446–454.
  13. Warren TK, Wells J, Panchal RG, *et al.* Protection against filovirus diseases by ■ a novel broad-spectrum nucleoside analogue BCX4430. *Nature* 2014; 508:402–405.
- Recent study describing a new broad-spectrum antiviral nucleoside analogue including in-vivo testing against filoviruses.
14. Poordad F, Lawitz E, Gitlin N, *et al.* Efficacy and safety of valopicitabine in combination with pegylated interferon-alpha (pegIFN) and ribavirin (RBV) in patients with chronic hepatitis C. *Hepatology* 2007; 46:866A.
  15. Russmann S, Grattagliano I, Portincasa P, *et al.* Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. *Curr Med Chem* 2006; 13:3351–3357.
  16. Turner TL, Kopp BT, Paul G, *et al.* Respiratory syncytial virus: current and emerging treatment options. *Clinicoecon Outcomes Res* 2014; 6:217–225.
  17. Bruenn J. A structural and primary sequence comparison of the viral RNA-dependent RNA polymerases. *Nucleic Acids Res* 2003; 31:1821–1829.
  18. Merino P. *Chemical Synthesis of Nucleoside Analogues*. Wiley; 2013.
  19. Ferrari E, Huang H-C. A novel hepatitis C virus NS5B polymerase assay of de novo initiated RNA synthesis directed from a heteropolymeric RNA template. *Methods Mol Biol* 2013; 1030:81–92.
  20. Gong EY, Kenens H, Ivens T, *et al.* Expression and purification of dengue virus NS5 polymerase and development of a high-throughput enzymatic assay for screening inhibitors of dengue polymerase. *Methods Mol Biol* 2013; 1030:237–247.
  21. Fauquet CM, Mayo MA, Maniloff J, *et al.* Virus taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses Virology Division, International Union of Microbiological Societies. Elsevier. 2005.
  22. Patick AK, Binford SL, Brothers MA, *et al.* In vitro antiviral activity of AG7088, a potent inhibitor of human rhinovirus 3C protease. *Antimicrob Agents Chemother* 1999; 43:2444–2450.
  23. De Palma AM, De Palma AM, Pürstinger G, *et al.* Potential use of antiviral agents in polio eradication. *Emerg Infect Dis* 2008; 14:545–551.
  24. Kim Y, Lovell S, Tiew K-C, *et al.* Broad-spectrum antivirals against 3C or 3C-Like proteases of picornaviruses, noroviruses, and coronaviruses. *J Virol* 2012; 86:11754–11762.
  25. Rocha-Pereira J, Nascimento MSJ, Ma Q, *et al.* The enterovirus protease inhibitor rupintrivir exerts cross-genotypic antinorovirus activity and clears cells from the norovirus replicon. *Antimicrob Agents Chemother* 2014; 58:4675–4681.
  26. Kim Y, Shivanna V, Narayanan S, *et al.* Broad-spectrum inhibitors against 3C-like proteases of feline coronaviruses and feline caliciviruses. *J Virol* 2015; 89:4942–4950.
  27. Mazzon M, Mercer J. Lipid interactions during virus entry and infection. *Cell Microbiol* 2014; 16:1493–1502.
  28. Delang L, Paeshuyse J, Vliegen I, *et al.* Statins potentiate the in vitro anti-hepatitis C virus activity of selective hepatitis C virus inhibitors and delay or prevent resistance development. *Hepatology* 2009; 50:6–16.
  29. Ikeda M, Abe KI, Yamada M, *et al.* Different anti-HCV profiles of statins and their potential for combination therapy with interferon. *Hepatology* 2006; 44:117–125.
  30. Del Real G, Jiménez-Baranda S, Mira E, *et al.* Statins inhibit HIV-1 infection by down-regulating Rho activity. *J Exp Med* 2004; 2004:541–547.
  31. Giguère J, Tremblay MJ. Statin compounds reduce human immunodeficiency virus type 1 replication by preventing the interaction between virion-associated host intercellular adhesion molecule 1 and its natural cell statin compounds reduce human immunodeficiency virus type 1 replica. *J Virol* 2004; 78:1–5.
  32. Liu S, Rodriguez AV, Tosteson MT. Role of simvastatin and methyl- $\beta$ -cyclodextrin on inhibition of poliovirus infection. *Biochem Biophys Res Commun* 2006; 347:51–59.
  33. Potena L, Frascaroli G, Grigioni F, *et al.* Hydroxymethyl-glutaryl coenzyme A reductase inhibition limits cytomegalovirus infection in human endothelial cells. *Circulation* 2004; 109:532–536.
  34. Martínez-Gutiérrez M, Castellanos JE, Gallego-Gómez JC. Statins reduce dengue virus production via decreased virion assembly. *Intervirology* 2011; 54:202–216.
  35. Gower TL, Graham BS. Antiviral activity of lovastatin against respiratory syncytial virus in vivo and in vitro. *Antimicrob Agents Chemother* 2001; 45:1231–1237.
  36. Zhu Q, Li N, Han Q, *et al.* Statin therapy improves response to interferon alpha and ribavirin in chronic hepatitis C: a systematic review and meta-analysis. *Antiviral Res* 2013; 98:373–379.
  37. Butt AA, Yan P, Bonilla H, *et al.* Effect of addition of statins to antiviral therapy in hepatitis C virus-infected persons: results from ERCHIVES. *Hepatology* 2015; 62:365–374.
  38. Blaising J, Polyak SJ, Pêcheur El. Arbidol as a broad-spectrum antiviral: an update. *Antiviral Res* 2014; 107:84–94.
  39. Teissier E, Zandomenighi G, Loquet A, *et al.* Mechanism of inhibition of enveloped virus membrane fusion by the antiviral drug arbidol. *PLoS One* 2011; 6:e15874.
  40. Liu MY, Wang S, Yao WF, *et al.* Pharmacokinetic properties and bioequivalence of two formulations of arbidol: an open-label, single-dose, randomized-sequence, two-period crossover study in healthy chinese male volunteers. *Clin Ther* 2009; 31:784–792.
  41. Wolf MC, Freiberg AN, Zhang T, *et al.* A broad-spectrum antiviral targeting entry of enveloped viruses. *Proc Natl Acad Sci U S A* 2010; 107:3157–3162.
  42. Vigant F, Lee J, Hollmann A, *et al.* A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. *PLoS Pathog* 2013; 9:e1003297.
  43. Vigant F, Santos NC, Lee B. Broad-spectrum antivirals against viral fusion. *Nat Rev Microbiol* 2015; 13:426–437.
  - A comprehensive review of the broad-spectrum potential of antivirals that target the membrane fusion process.
  44. Song J-M, Seong B-L. Viral membranes: an emerging antiviral target for enveloped viruses? *Expert Rev Anti Infect Ther* 2010; 8:635–638.
  45. Zasloff M, Adams AP, Beckerman B, *et al.* Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. *Proc Natl Acad Sci U S A* 2011; 108:15978–15983.
  46. Sumioka A, Yan D, Tomita S. TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. *Neuron* 2010; 66:755–767.
  47. Zhang X, Rao MN, Jones SR, *et al.* Synthesis of squalamine utilizing a readily accessible spermidine equivalent. *J Org Chem* 1998; 63:8599–8603.
  48. Bhargava P, Marshall JL, Dahut W, *et al.* A phase I and pharmacokinetic study of squalamine, a novel antiangiogenic agent, in patients with advanced cancers. *Clin Cancer Res* 2001; 7:3912–3919.
  49. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of Nitazoxanide. *J Infect Dis* 2001; 184:103–106.
  50. Rossignol J-F. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. ■ *Antiviral Res* 2014; 110:94–103.
  - A complete account on the current knowledge on nitazoxanide, a repurposed broad-spectrum antiviral drug.
  51. Rossignol JF, La Frazia S, Chiappa L, *et al.* Thiazolidines, a new class of anti-influenza molecules targeting viral hemagglutinin at the posttranslational level. *J Biol Chem* 2009; 284:29798–29808.
  52. Elazar M, Liu M, McKenna SA, *et al.* The anti-hepatitis C agent nitazoxanide induces phosphorylation of eukaryotic initiation factor 2 $\alpha$  via protein kinase activated by double-stranded RNA activation. *Gastroenterology* 2009; 137:1827–1835.
  53. Haffizulla J, Hartman A, Hoppers M, *et al.* Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomized, placebo-controlled, phase 2b/3 trial. *Lancet Infect Dis* 2014; 14:609–618.
  54. Rossignol J-F, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. *Aliment Pharmacol Ther* 2006; 24:1423–1430.
  55. Rossignol J-F, Elfert A, El-Gohary Y, Keeffe EB. Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. *Gastroenterology* 2009; 136:856–862.

56. Göthel SF, Marahiel MA. Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol Life Sci* 1999; 55:423–436.
57. Frausto SD, Lee E, Tang H. Cyclophilins as modulators of viral replication. *Viruses* 2013; 5:1684–1701.
58. Davis TL, Walker JR, Campagna-Slater V, *et al.* Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. *PLoS Biol* 2010; 8:e1000439.
59. Hopkins S, Dimassimo B, Rusnak P, *et al.* The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. *J Hepatol* 2012; 57:47–54.
60. Flisiak R, Feinman SV, Jablkowski M, *et al.* The cyclophilin inhibitor debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naïve hepatitis C patients. *Hepatology* 2009; 49:1460–1468.
61. Kaul A, Stauffer S, Berger C, *et al.* Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. *PLoS Pathog* 2009; 5:e1000546.
62. Coelmont L, Hanouille X, Chatterji U, *et al.* Deb025 (Alisporivir) inhibits hepatitis c virus replication by preventing a cyclophilin a induced cis-trans isomerisation in domain ii of NS5A. *PLoS One* 2010; 5:e13687.
63. Delang L, Vliegen I, Froeyen M, Neyts J. Comparative study of the genetic barriers and pathways towards resistance of selective inhibitors of hepatitis C virus replication. *Antimicrob Agents Chemother* 2011; 55:4103–4113.
64. Gamble TR, Vajdos FF, Yoo S, *et al.* Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* 1996; 87:1285–1294.
65. Chatterji U, Bobardt MD, Stanfield R, *et al.* Naturally occurring capsid substitutions render HIV-1 cyclophilin A independent in human cells and TRIM-cyclophilin-resistant in Owl monkey cells. *J Biol Chem* 2005; 280:40293–40300.
66. Galloway Pa, Ptak RG, Bobardt MD, *et al.* Correlation of naturally occurring HIV-1 resistance to DEB025 with capsid amino acid polymorphisms. *Viruses* 2013; 5:981–997.
67. Colgan J, Asmal M, Neagu M, *et al.* Cyclophilin A regulates TCR signal strength in CD4+ T cells via a proline-directed conformational switch in Itk. *Immunity* 2004; 21:189–201.
68. Braaten D, Luban J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *EMBO J* 2001; 20:1300–1309.
69. Openshaw PJM, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. *Clin Microbiol Rev* 2005; 18:541–555.
70. Nagy P, Pogany J. The dependence of viral RNA replication on co-opted host factors. *Nat Rev Microbiol* 2012; 10:137–149.
71. Goulet M-L, Olagnier D, Xu Z, *et al.* Systems analysis of a RIG-I agonist inducing broad spectrum inhibition of virus infectivity. *PLoS Pathog* 2013; 9:e1003298.
72. Olagnier D, Scholte FEM, Chiang C, *et al.* Inhibition of dengue and chikungunya virus infections by RIG-I-mediated type I interferon-independent stimulation of the innate antiviral response. *J Virol* 2014; 88:4180–4194.
73. Martínez-Gil L, Ayllon J, Ortigoza MB, *et al.* Identification of small molecules with type I interferon inducing properties by high-throughput screening. *PLoS One* 2012; 7:e49049.
74. Thakur CS, Jha BK, Dong B, *et al.* Small-molecule activators of RNase L with broad-spectrum antiviral activity. *Proc Natl Acad Sci U S A* 2007; 104:9585–9590.
75. Harvey R, Brown K, Zhang Q, *et al.* GSK983: a novel compound with broad-spectrum antiviral activity. *Antiviral Res* 2009; 82:1–11.
76. Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. *Nat Rev Immunol* 2013; 13:46–57.
77. Rider TH, Zook CE, Boettcher TL, *et al.* Broad-spectrum antiviral therapeutics. *PLoS One* 2011; 6:e22572.
78. Guo C, Chen L, Mo D, *et al.* DRACO inhibits porcine reproductive and respiratory syndrome virus replication in vitro. *Arch Virol* 2015; 160:1239–1247.