Expanding the activity spectrum of antiviral agents

Aleksandr Ianevski[1], Petter I. Andersen[1], Andres Merits[2], Magnar Bjørås[1,3] and Denis Kainov[1,2]

[1] Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim 7028, Norway
[2] Institute of Technology, University of Tartu, Tartu 50090, Estonia
[3] Department of Microbiology, Oslo University Hospital and University of Oslo, Oslo 0027, Norway

Broad-spectrum antivirals (BSAs) are agents that inhibit replication of several human viruses. Here, we review 108 approved, investigational, and experimental BSAs, for which safety profiles in humans are available. The most effective and tolerable BSAs could reinforce the arsenal of available antiviral therapeutics pending the results of further pre-clinical and clinical studies.

Introduction

Existing and emerging viral diseases are an ever-growing problem, particularly in developing countries. Vaccines and antiviral drugs are powerful tools to combat viral diseases; however, most of these therapeutics target a single virus. By contrast, BSAs are able to inhibit different viral infections, because most viruses follow common pathways to replicate inside a host cell [1]. Academic institutions and pharmaceutical companies have urged the discovery and development of such BSAs to overcome time and cost issues associated with the development of virus-specific drugs and vaccines [2–7].

To identify potential BSAs, we reviewed approved and investigational safe-in-man antiviral agents using drug bank and clinical trials websites, respectively. In addition, we reviewed investigational and approved safe-in-man agents, including antibacterials, antimalarials, antiparasitics, immune-modulating, antidepressants, antiemetics, and so on, for which antiviral activities have been reported in PubMed. By excluding vaccines and interferons, we identified 59 molecules that inhibit replication of several viruses in humans [8]. Recently, some of the experimental antiviral agents entered clinical trials and became investigational. At the same time, novel antiviral activities have been reported for some of these as well as other agents, expanding the list of available BSAs to 108 (Table S1 and Fig. S1 in the Supplemental information online).

Corresponding author: Kainov, D. (denis.kainov@ntnu.no)

108 BSAs target 78 viruses

The BSAs identified target 78 viruses from 24 assigned families, belonging to (–)single-stranded (ss)RNA, (+)ssRNA, double-stranded (ds)RNA, ssRNA-reverse transcriptase (RT), ssDNA, ssDNA-RT, dsDNA, or dsDNA-RT virus groups (Fig. 1; Table S2 in the Supplemental information online). Antiparasitic nitazoxanide has the broadest spectrum of antiviral activity, because it inhibits viruses belonging to the (–)ssRNA, (+)ssRNA, dsRNA, ssRNA-RT, dsDNA, and dsDNA-RT groups. Glycyrrhizin, a sweet-tasting constituent of *Glycyrrhiza glabra* (liquorice) root, and niclosamide, an orally available immunosuppressant used to treat tapeworm infestations, also inhibit several (–)ssRNA, (+)ssRNA, dsDNA and ssRNA-RT viruses *in vitro* and, in some cases, *in vivo* [9–11]. Favipiravir is an anti-influenza drug that has shown antiviral effects against many different (–) and (+)ssRNA viruses.

Importantly, these and other BSAs have been tested against some but not all 78 viruses *in vitro*. In particular, 55 BSAs were recently probed against 13 different viruses [influenza A virus (FLUAV), Rift Valley fever virus (RVFV), Human metapneumovirus (HMPV), echovirus 1 (EV1), chikungunya virus (CHIKV), Ross River virus (RRV), Zika virus (ZIKV), hepatitis C virus (HCV), Sindbis virus (SINV), HIV-1, cytomegalovirus (CMV), hepatitis B virus (HBV), and herpes simplex virus type 1 (HSV-1)]. Novel activities were identified for dalbavancin against EV1; for ezetimibe against HIV-1, SINV, and ZIKV; for azaci-

https://doi.org/10.1016/j.drudis.2019.04.006

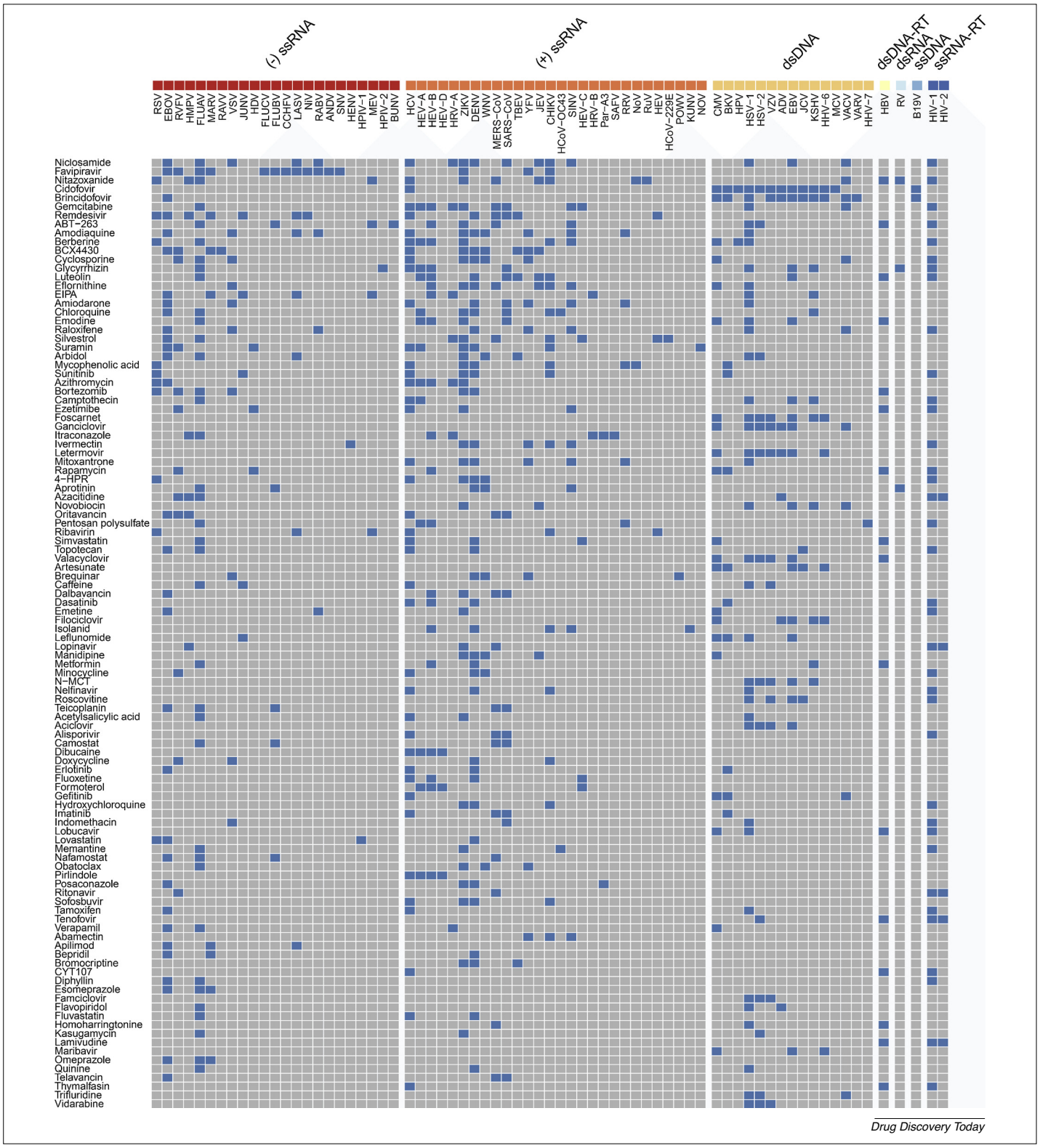

FIGURE 1

In vitro activities of 108 safe-in-man broad-spectrum antivirals (BSAs). Viruses are clustered by virus group. BSAs are ranged from the highest to lowest number of targeted viruses. Blue shading indicates that antiviral activity has been reported for the BSA, whereas gray shading indicates that antiviral activity has not been either reported or studied. Abbreviations: ds, double-stranded; RT, reverse transcriptase; ss, single-stranded.

tidine, cyclosporine, minocycline, and ritonavir against RVFV; for oritavancin against RVFV, HMPV, and HCV; for cidofovir, dibucaine, azithromycin, gefitinib, minocycline, and pirlindole against HCV; and for azacitidine, itraconazole, lopinavir, and nitazoxanide against HMPV [8,12].To further expand a spectrum of these and other BSA activities, the library of 108 BSAs could be readily made available and tested against these and other viruses.

BSA structures

C-SPADE and multidimensional scaling analyses [13,14] of BSAs structures revealed that many BSAs have similar scaffolds. These include: acyclovir, famciclovir, filociclovir, ganciclovir and valaciclovir; imatinib, erlotinib, gefitinib, and dasatinib; telavancin, dalbavancin, oritavancin, and teicoplanin; alisporivir and cyclosporine; minocycline and doxycycline; lovastatin and simvastatin; raloxifene and amiodarone; ritonavir and lopinavir; esomeprazole and omeprazole; camptothecin and topotecan; trifluridine and N-MCT; and quinine, brequinar, amodiaquine, hydroxychloroquine, and chloroquine BSAs (Fig. 2). The structural constrains limit the diversity of BSAs. Finding antiviral agents with novel scaffolds could lead to more efficient treatment modalities and provide new knowledge of virus–host interactions. Natural products could represent a source of BSAs with new scaffolds [15].

Mechanisms of actions of BSAs

Unfortunately, we know little about mechanisms of action (MOAs) of these BSAs. In particular, we know potential viral or host targets for only 91 agents (Table S1 and Fig. S2 in the Supplemental information online) [6,8,16,17]. Examples of virus-directed BSAs are nucleotide and nucleoside analogs, which inhibit viral RTs, and RNA or DNA polymerases, given that host factors could be involved in activation of some of the drugs [18]. In particular, tenofovir and lamivudine

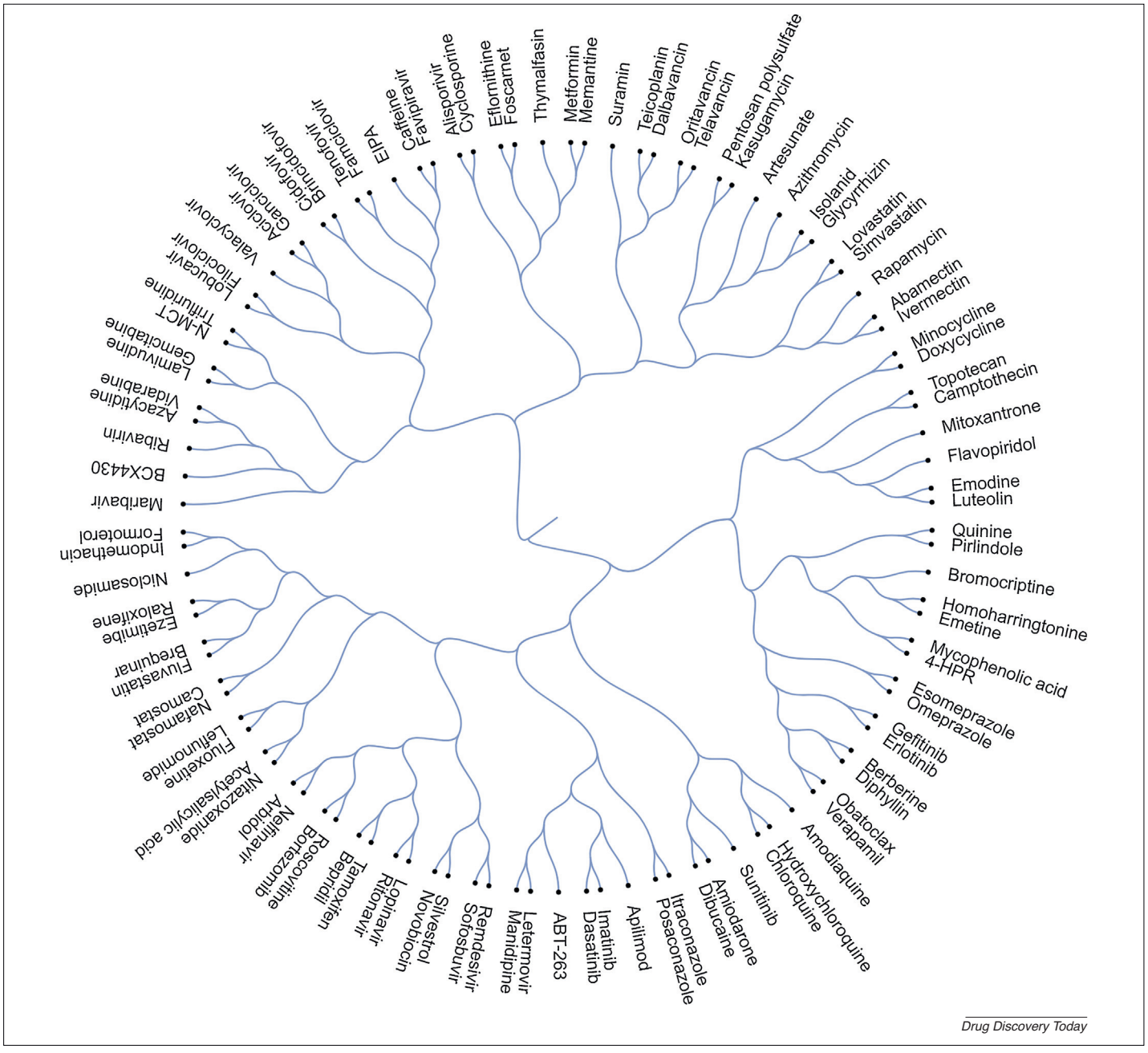

FIGURE 2

Dendrogram showing the structural relationship between 106 out of 108 broad-spectrum antivirals (BSAs). Given their complex structures, CYT107 (IL7) and aprotinin were excluded from the analysis.

inhibit RTs. Ribavirin, favipiravir, sofosbuvir, 4-hydroxyphenylretinamide, remdesivir, and BCX4430 inhibit viral RNA polymerases [19]. Trifluridine, vidarabine, *N*-methanocarbathymidine, maribavir, acyclovir, famciclovir, ganciclovir, filociclovir, valaciclovir, cidofovir, brincidofovir, and tenofovir block viral DNA polymerases [20], whereas azacitidine inhibits RT, and DNA and RNA polymerases. Examples of non-nucleotide/non-nucleoside virus-directed BSAs include inhibitors of viral polymerases (foscarnet), helicases (pirlindole, dibucaine, fluoxetine, and formoterol), terminases (letermovir) and proteases (lopinavir, ritonavir, nelfinavir, and bromocriptine). However, viruses could develop resistance to both nucleotide and nucleoside analogs under selective pressure.

Host-directed BSAs could be subclassified based on the stage of virus replication they inhibit [16,19,21–25]. For example, 4-aminoquinolines (chloroquine, hydroxychloroquine, and amodiaquine), proton-pump inhibitors (omeprazole and esomeprazole), Mcl-1 inhibitors (obatoclax), as well as calcium channel blockers (verapamil and bepridil) prevent virus entry into host cells [26,27]. In addition, glycopeptides (teicoplanin, dalbavancin, oritavancin, and telavancin), serine protease inhibitors (camostat and nafamostat), proteasome inhibitors (bortezomib) and niclosamide prevent endocytosis of viruses [28].

Cyclin-dependent kinase inhibitors (roscovitine and flavopiridol), as well as inhibitors of *de novo* purine and pyrimidine biosynthesis (gemcitabine, mycophenolic acid, leflunomide, and brequinar) alter the transcription and replication of viral genomes. Other kinase inhibitors (dasatinib, imatinib, gefitinib, nilotinib, erlotinib, metformin, apilimod, and sunitinib), as well as importin inhibitors (ivermectin) impair intracellular trafficking of viral components [5,6,29,30]. Emetine, homoharringtonine, indomethacin, rapamycin, and silvestrol inhibit the synthesis and quality control of viral proteins [31]. Mitoxantrone prevents virion assembly [32]. Emodine, CYT107, acetylsalicylic acid, azithromycin, glycyrrhizin, pentosan polysulfate, thymalfasin, camptothecin, topotecan, kasugamycin, and quinine modulate immune responses to viral infections [33–36]. In addition, the Bcl-2 inhibitor ABT-263 accelerates apoptosis of virus-infected cells at concentrations that are nontoxic for non-infected cells and, thus, attenuates virus replication [37].

Several host-directed BSAs inhibit multiple stages of viral replication. For example, lipid-lowering drugs (ezetimibe, atorvastatin, lovastatin, simvastatin, and fluvastatin) attenuate virus entry as well as assembly and budding [38,39]. Cyclophilin inhibitors (alisporivir and cyclosporine) block viral genome replication and virus particle assembly. Cytochrome P450 inhibitors (amiodarone) affect virus binding and entry into target cells [40]. Niclosamide inhibits intracellular trafficking of virus particles and endosomal acidification [9,41]. Polyamine biosynthesis inhibitors (eflornithine) modify transcription and translation rates, and affect signaling pathways in infected cells by binding to RNA and proteins [42]. Nitazoxanide inhibits viral entry as well as the translation of viral proteins [43]. Estrogen receptor modulators (tamoxifen and raloxifen) affect both viral binding to cells and post-binding events, including endocytosis [44]. Suramin targets both viral and host factors during the attachment and release of several viruses [45,46].

The MOAs of remaining BSAs are elusive. However, they could be deciphered using a systems approach, which combines drug toxicity and/or efficacy tests, time-of-compound addition experiments, transcriptomics, proteomics, metabolomics, as well as CRISPR/Cas9 or other techniques that enable the identification and/or validation of antiviral targets [47,48].

Clinical relevance of BSAs

Many safe-in-man BSAs have been shown to have antiviral activity *in vitro*, but only a fraction of these agents has been tested in animal models (Table S1 in the Supplemental information online). Testing activity of BSAs in mouse models could demonstrate whether the drug concentration used for the primary disease indication could be tolerable and effective for secondary disease indication. However, there are no animal models for some viruses. In these cases, the testing of BSAs *in vivo* could be bypassed.

The crucial point for the next step in safe-in-man BSA development is the availability of human clinical, pharmacokinetics and safety data of the repurposed drugs [17,49]. For example, safety data on the anti-influenza drug favipiravir were used in clinical Phase II studies against EBOV (NCT02329054 and NCT02662855). Another interesting example is the antimalarial chloroquine, the safety data of which were used for anti-CHIKV, DENV, HCV, and HIV-1 Phase II studies (NCT00391313, NCT02058173, NCT00308620, and NCT00849602, respectively).

Another option would be to combine BSAs to obtain even broader antiviral effects. For example, a cocktail of nitazoxanide, favipiravir, and niclosamide could be developed for the treatment of infections of viruses belonging to 11 families. Such combinations could also have synergistic or additive effects on a particular viral disease and thereby increase the effectiveness and/or reduce the dosage of antiviral therapeutics. Further clinical investigations could show an effectiveness of such combination therapies and could lead to the development of novel treatment options for emerging and re-emerging viral diseases.

Importantly, BSAs and/or antibiotics, such as oritavancin, teicoplanin, telavancin, kasugamycin, novobiocin, azithromycin, dalbavancin, doxycycline, and minocycline, could be used for the simultaneous treatment of viral and bacterial co-infections, reducing the complexity of the therapy. In addition, other BSAs could be used for the treatment of co-morbidities. For example, ezetimibe is a US Food and Drug Administration (FDA)-approved medication that lowers plasma cholesterol by decreasing its absorption in the small intestine. This drug was successfully tested in combination with antiretroviral therapeutics in patients with HIV [50,51]. The question remains whether ezetimibe alone could lower lipids and reduce HIV titers in patients. This could be addressed in cooperation with clinicians running clinical trials with ezetimibe in patients with HIV (NCT00908011, NCT00099684, and NCT00843661).

Concluding remarks

In conclusion, repurposing existing safe-in-man antiviral agents from one viral disease to another could have a pivotal role in the treatment of viral infections. However, new antiviral activities for existing safe-in-man BSAs have to be discovered in cell cultures, confirmed in animal models (where applicable), and studied in clinical trials. The most effective and tolerable BSAs or their combinations will have global impact, saving time and resources of drug developmental process as well as improving the preparedness and protection of the general population from emerging and re-emerging viral threats.

Acknowledgment

This research was funded by Estonian Research Council Mobilitas Pluss top researcher grant (contract no. MOBTT39).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2019.04.006.

References

1 Bekerman, E. and Einav, S. (2015) Infectious disease. Combating emerging viral threats. *Science* 348, 282–283
2 Yuan, S. *et al.* (2019) SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. *Nat. Commun.* 10, 120
3 Check, H.E. (2018) Experimental drugs poised for use in Ebola outbreak. *Nature* 557, 475–476
4 Jaishankar, D. *et al.* (2018) An off-target effect of BX795 blocks herpes simplex virus type 1 infection of the eye. *Sci. Transl. Med.* 10 eaan5861
5 Schor, S. and Einav, S. (2018) Repurposing of kinase inhibitors as broad-spectrum antiviral drugs. *DNA Cell Biol.* 37, 63–69
6 Debing, Y. *et al.* (2015) The future of antivirals: broad-spectrum inhibitors. *Curr. Opin. Infect. Dis.* 28, 596–602
7 Yu, F. *et al.* (2013) Approaches for identification of HIV-1 entry inhibitors targeting gp41 pocket. *Viruses* 5, 127–149
8 Ianevski, A. *et al.* (2018) Novel activities of safe-in-human broad-spectrum antiviral agents. *Antiviral Res.* 154, 174–182
9 Kao, J.C. *et al.* (2018) The antiparasitic drug niclosamide inhibits dengue virus infection by interfering with endosomal acidification independent of mTOR. *PLoS Negl. Trop. Dis.* 12 e0006715
10 Song, J.H. *et al.* (2017) Antiviral activity of gemcitabine against human rhinovirus *in vitro* and *in vivo*. *Antiviral Res.* 145, 6–13
11 Shen, Z. *et al.* (2015) Antiviral effects of cyclosporine A in neonatal mice with rotavirus-induced diarrhea. *J. Pediatr. Gastroenterol. Nutr.* 60, 11–17
12 Bosl, K. *et al.* (2019) Critical nodes of virus-host interaction revealed through an integrated network analysis. *BioRxiv* . http://dx.doi.org/10.1101/548909 Published online February 19, 2019
13 Ravikumar, B. *et al.* (2017) C-SPADE: a web-tool for interactive analysis and visualization of drug screening experiments through compound-specific bioactivity dendrograms. *Nucleic Acids Res.* 45, W495–W500
14 Backman, T.W. *et al.* (2011) ChemMine tools: an online service for analysing and clustering small molecules. *Nucleic Acids Res.* 39, W486–W491
15 Martinez, J.P. *et al.* (2015) Antiviral drug discovery: broad-spectrum drugs from nature. *Nat. Prod. Rep.* 32, 29–48
16 Kaufmann, S.H.E. *et al.* (2018) Host-directed therapies for bacterial and viral infections. *Nat. Rev Drug Discov.* 17, 35–56
17 Pizzorno, A. *et al.* (2019) Drug repurposing approaches for the treatment of influenza viral infection: reviving old drugs to fight against a long-lived enemy. *Front. Immunol.* . http://dx.doi.org/10.3389/fimmu.2019.00531 Published online March 19, 2019
18 De Clercq, E. (2015) Curious (old and new) antiviral nucleoside analogues with intriguing therapeutic potential. *Curr. Med. Chem.* 22, 3866–3880
19 McKimm-Breschkin, J.L. *et al.* (2018) Prevention and treatment of respiratory viral infections: Presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV Antiviral Group conference. *Antiviral Res.* 149, 118–142
20 Hartline, C.B. *et al.* (2018) A standardized approach to the evaluation of antivirals against DNA viruses: orthopox-, adeno-, and herpesviruses. *Antiviral Res.* 159, 104–112
21 Li, C. *et al.* (2019) Repurposing host-based therapeutics to control coronavirus and influenza virus. *Drug Discov. Today* 24, 726–736
22 Shim, J.M. *et al.* (2017) Influenza virus infection, interferon response, viral counter-response, and apoptosis. *Viruses* 9 E223
23 Soderholm, S. *et al.* (2016) Multi-omics studies towards novel modulators of influenza A virus-host interaction. *Viruses* 8 E269
24 Muller, K.H. *et al.* (2012) Emerging cellular targets for influenza antiviral agents. *Trends Pharmacol. Sci.* 33, 89–99
25 Saiz, J.C. *et al.* (2018) Host-directed antivirals: a realistic alternative to fight Zika virus. *Viruses* 10 E453
26 Al-Bari, M.A.A. (2017) Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. *Pharmacol. Res. Perspect.* 5 e00293
27 Sakurai, Y. *et al.* (2015) Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. *Science* 347, 995–998
28 Zhou, N. *et al.* (2016) Glycopeptide antibiotics potently inhibit cathepsin L in the late endosome/lysosome and block the entry of Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV). *J. Biol. Chem.* 291, 9218–9232
29 Bekerman, E. *et al.* (2017) Anticancer kinase inhibitors impair intracellular viral trafficking and exert broad-spectrum antiviral effects. *J. Clin. Invest.* 127, 1338–1352
30 Nelson, E.A. *et al.* (2017) The phosphatidylinositol-3-phosphate 5-kinase inhibitor apilimod blocks filoviral entry and infection. *PLoS Negl. Trop. Dis.* 11 e0005540
31 Amici, C. *et al.* (2015) Inhibition of viral protein translation by indomethacin in vesicular stomatitis virus infection: role of eIF2alpha kinase PKR. *Cell Microbiol.* 17, 1391–1404
32 Deng, L. *et al.* (2007) Identification of novel antipoxviral agents: mitoxantrone inhibits vaccinia virus replication by blocking virion assembly. *J. Virol.* 81, 13392–13402
33 Malakar, S. *et al.* (2018) Drug repurposing of quinine as antiviral against dengue virus infection. *Virus Res.* 255, 171–178
34 Gopinath, S. *et al.* (2018) Topical application of aminoglycoside antibiotics enhances host resistance to viral infections in a microbiota-independent manner. *Nat. Microbiol.* 3, 611–621
35 Lee, N. *et al.* (2017) Anti-inflammatory effects of adjunctive macrolide treatment in adults hospitalized with influenza: a randomized controlled trial. *Antiviral Res.* 144, 48–56
36 Rialdi, A. *et al.* (2016) Topoisomerase 1 inhibition suppresses inflammatory genes and protects from death by inflammation. *Science* 352 aad7993
37 Bulanova, D. *et al.* (2017) Antiviral properties of chemical inhibitors of cellular anti-apoptotic Bcl-2 proteins. *Viruses* 9, E271
38 Bernal, E. *et al.* (2017) Statins in HIV-infected patients: potential beneficial effects and clinical use. *AIDS Rev.* 19, 59–71
39 Enserink, M. (2005) Infectious disease. Old drugs losing effectiveness against flu; could statins fill gap? *Science* 309, 1976–1977
40 Salata, C. *et al.* (2018) Amiodarone affects Ebola virus binding and entry into target cells. *New Microbiol.* 41, 162–164
41 Marrugal-Lorenzo, J.A. *et al.* (2019) Repositioning salicylanilide anthelmintic drugs to treat adenovirus infections. *Sci. Rep.* 9, 17
42 Mounce, B.C. *et al.* (2016) Inhibition of polyamine biosynthesis is a broad-spectrum strategy against RNA viruses. *J. Virol.* 90, 9683–9692
43 Hickson, S.E. *et al.* (2018) Inhibition of vaccinia virus replication by nitazoxanide. *Virology* 518, 398–405
44 Murakami, Y. *et al.* (2013) Selective estrogen receptor modulators inhibit hepatitis C virus infection at multiple steps of the virus life cycle. *Microbes Infect.* 15, 45–55
45 Coronado, M.A. *et al.* (2018) Zika virus NS2B/NS3 proteinase: a new target for an old drug - suramin a lead compound for NS2B/NS3 proteinase inhibition. *Antiviral Res.* 160, 118–125
46 Albulescu, I.C. *et al.* (2017) Suramin inhibits Zika virus replication by interfering with virus attachment and release of infectious particles. *Antiviral Res.* 143, 230–236
47 Soderholm, S. *et al.* (2016) Immuno-modulating properties of saliphenylhalamide, SNS-032, obatoclax, and gemcitabine. *Antiviral Res.* 126, 69–80
48 Zusinaite, E. *et al.* (2018) A systems approach to study immuno- and neuro-modulatory properties of antiviral agents. *Viruses* 10, E423
49 Strittmatter, S.M. (2014) Overcoming drug development bottlenecks with repurposing: old drugs learn new tricks. *Nat. Med.* 20, 590–591
50 Saeedi, R. *et al.* (2015) Lipid lowering efficacy and safety of ezetimibe combined with rosuvastatin compared with titrating rosuvastatin monotherapy in HIV-positive patients. *Lipids Health Dis.* 14, 57
51 Wohl, D.A. *et al.* (2008) Ezetimibe alone reduces low-density lipoprotein cholesterol in HIV-infected patients receiving combination antiretroviral therapy. *Clin. Infect. Dis.* 47, 1105–1108