Discovery and Development of Safe-in-man Broad-Spectrum Antiviral Agents

Petter I. Andersen [1#], Aleksandr Ianevski [1#], Hilde Lysvand [1], Astra Vitkauskiene [2], Valentyn Oksenych [1], Magnar Bjoras [1], Kaidi Telling [3], Irja Lutsar [4], Uga Dampis [5], Yasuhiko Irie [3], Tanel Tenson [3], Anu Kantele [6], Denis E. Kainov [1,3,7]

[1] Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology (NTNU), 7028 Trondheim, Norway; petteria@stud.ntnu.no (P.I.A); aleksandr.ianevski@ntnu.no (A.I.); hilde.lysvand@ntnu.no (H.L.); valentyn.oksenych@ntnu.no (V.O.); magnar.bjoras@ntnu.no (M.B.); denis.kainov@ntnu.no (D.E.K.)

[2] Department of Laboratory Medicine, Lithuanian University of Health Science, 44307 Kaunas, Lithuania; astra.vitkauskiene@kaunoklinikos.lt (A.V.)

[3] Institute of Technology, University of Tartu, 50090 Tartu, Estonia; yasuhiko.irie@ut.ee (Y.I.); tanel.tenson@ut.ee (T.T.); kaidi.telling@ut.ee (K.T.); denis.kaynov@ut.ee (D.E.K.)

[4] Institute of Medical Microbiology, University of Tartu, Tartu 50411, Estonia; irja.lutsar@ut.ee (I.L.)

[5] Latvian Biomedical Research and Study Centre, Riga 1067, Latvia. uga.dumpis@gmail.com (U.D.)

[6] Helsinki University Hospital (HUS) and University of Helsinki, Helsinki 00290, Finland; anu.kantele@helsinki.fi (A.K.)

[7] Institute for Molecular Medicine Finland, FIMM, University of Helsinki, 00014 Helsinki, Finland; denis.kaynov@ntnu.no (D.E.K.)

* Correspondence: denis.kainov@ntnu.no; Tel.: +358-40-549-0220

# Contributed equally

Abstract: Viral diseases are one of the leading causes of morbidity and mortality in the world. Virus-specific vaccines and antiviral drugs are the most powerful tools to combat viral diseases. However, broad-spectrum antiviral agents (BSAAs) could provide additional protection of general population from emerging and re-emerging viral diseases reinforcing the arsenal of available antiviral options. Here, we reviewed development process of BSAAs and summarized the information on 119 safe-in-man agents in freely accessible database (https://drugvirus.info/). The number of BSAAs will be increased, their developmental status will be updated, spectrum of their indications will be expanded, as well as BSAA combinations will be approved pending the results of further pre-clinical and clinical studies.

Keywords: virus; antiviral drug; drug discovery and development; broad-spectrum antiviral agents, BSAAs

1. Introduction

Viruses are one of the major causes of morbidity and mortality in the world (1-4). Antiviral drugs and vaccines are used to fight viral infections in human (5, 6). Previously, there has been a focus on "one drug, one virus" dogma, which relied on targeting virus-specific factors. A counterpoint to this is "one drug, multiple viruses" paradigm, which came with the discovery of broad-spectrum antiviral agents (BSAAs), small-molecules that inhibit a wide range of human viruses (7-12). This paradigm was based on the observation that different viruses utilize similar pathways and host factors to replicate inside a cell (13). Although the concept of BSAAs has been around for almost 50 years, the field received a new impetus with recent outbreaks of Ebola, Zika, Dengue, influenza and other viral infections, the discovery of novel host-directed agents as well as development of drug repositioning methodology.

Drug repurposing, also called repositioning, redirecting, reprofiling, is a strategy for generating additional value from an existing drug by targeting disease other than that for which it was originally intended (14, 15). This has significant advantages over new drug discovery since chemical synthesis steps, manufacturing processes, reliable safety, and pharmacokinetic properties in pre-clinical (animal model) and early clinical developmental phases (phase 0, I and IIa) are already available (Fig. 1). Therefore, repositioning of launched or even failed drugs to viral diseases provides unique translational opportunities, including a substantially higher probability of success to market as compared with developing new virus-specific drugs and vaccines, and a significantly reduced cost and timeline to clinical availability (9, 16, 17).

Here, we detail the steps of BSAA repurposing, from discovery of novel antiviral activities in cell culture to post-market studies. Moreover, we summarized currently available information on BSAAs in freely available database, focusing on those antivirals, which have been already tested in human as antivirals, antibacterials, antiprotozoals, anthelmintics, etc. Finally, we discuss future perspectives of using safe-in-man BSAAs for treatment of emerging and re-emerging viral infections as well as viral and bacterial co-infections.
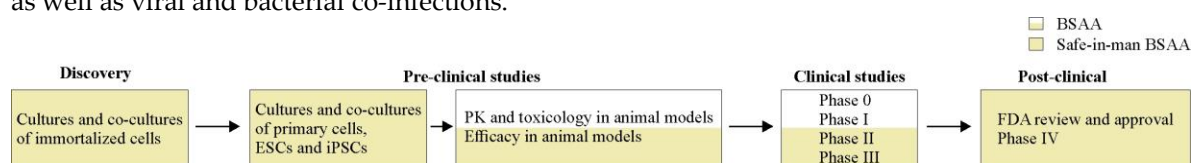
Figure 1. Discovery of novel activities and follow-up development of broad-spectrum antiviral agents (BSAAs). Yellow shading indicates a process of discovery and development of safe-in-man BSAAs, for which pharmacokinetic (PK) properties in pre-clinical (animal model) and early clinical developmental phases (phase 0-IIa trials) are already available. Abbreviations: ESCs, human embryonic stem cells; iPSCs, human induced pluripotent stem cells (iPSCs).

2. Discovery and development of safe-in-man BSAAs

2.1. Discovery of novel BSAA activities in immortalized cell cultures and co-cultures

The discovery of novel activities of BSAAs starts with exposing cells to the candidate antiviral agent at different concentrations and infecting the cells with a virus or mock. Immortalized cancerous cell cultures and co-cultures, which express appropriate viral receptors, are most commonly used in this first step. The half-maximal cytotoxic concentrations (CC_{50}) for a compound is calculated based on their dose-response curves obtained on mock-infected cells. The half-maximal effective concentrations (EC_{50}) are calculated based on the analysis of curves obtained on infected cells. Statistical analyses can help to determine if the differences between CC_{50} and EC_{50} are significant, given the inherent variability of the experiment (18). A relative effectiveness of a drug is defined as selectivity index ($SI = CC_{50}/EC_{50}$).

Cell viability assays and cell death assays are commonly used to assess the cytotoxicity and efficacy of BSAAs (Fig. 2A). Cell viability assays include MTT, MTS, resazurin or similar assays, mitochondrial membrane potential-dependent dyes-based assays, esterase cleaved dye-based assays, ATP-ADP assays, and assays that measure glycolytic flux and oxygen consumption. Other cell death assays include LDH enzyme leakage assays, membrane impermeable dye-based assays, and apoptosis assays, such as Annexin V, TUNEL, and caspase assays (19). For example, the Cell Titer Glo (CTG) assay quantifies ATP, an indicator of metabolically active living cells, whereas Cell Tox Green assay uses fluorescent asymmetric cyanine dye that stains the DNA of dead cells (13, 20-22).

Viral strains or cell lines expressing reporter proteins are also used to assess the efficacy of BSAAs in infected cells. For example, TZM-bl cells expressing firefly luciferase under control of HIV-1 LTR promoter allowed quantitation of BSAA action on HIV-1 infection (tat-protein expression by integrated HIV-1 provirus) using firefly luciferase assay (23, 24). RFP-expressing RVFV, nanoLuc-expressing CHIKV and RRV, as well as GFP-expressing FLUAV, HCV and HMPV also allowed identification of novel activities of several BSAAs (13, 21, 25-31). In addition, qPCR/RT-qPCR, RNA/DNA sequencing, RNA/DNA hybridization, immuno- and plaque assays as well as CRISPR-

CAS9 systems could be used for detection of inhibitory effects of BSAAs (32-39). Interestingly, CRISPR-Cas9, siRNA and shRNA approaches were used for identification of BSAA targets (40, 41).

Novel anti-HSV-2 and anti-EV1 activities of emetine were discovered recently using CTG/plaque assays in human non-malignant RPE cells. Moreover, novel antiviral activities of the drug were identified using RFP-expressing RVFV, and GFP-expressing HMPV or FLUAV strains in RPE cells (42). Given that emetine also inhibits ZIKV, EBOV, RABV, CMV, HCoV-OC43 and HIV-1 infections (19, 43-46), and that it is an FDA-approved anti-protozoal drug, it may represent a promising safe-in-man BSAA candidate.

2.2. Evaluation of BSAAs in human primary cell culture and co-cultures

Immortalized cell cultures/co-cultures and reporter viral strains represent excellent model systems for the discovery of novel activities of safe-in-man BSAAs. However, these genetically modified systems have certain limitations (attenuated or incomplete virus replication cycle, accumulation of mutations during repeated cell and virus passaging, defective innate immune responses and viral counter-responses, etc.) (47). Thereby, novel antiviral activities of BSAAs should be further validated in primary human cells using different viral strains (including wild-type viruses), different viral loads, different times of compound addition, different endpoint measurements and compound concentration range. Primary cell cultures give more accurate images of drug responses (48-51). They have a low population doubling level and therefore more closely recapitulate the physiological conditions observed in vivo.

Primary cells are cells isolated directly from tissues or blood using enzymatic or mechanical methods. The cells are characterized by their high degrees of specialization, are often fully differentiated and thus require defined culture conditions (serum-free media) in order to preserve their original phenotype. Peripheral blood mononuclear (PBMC), placental, amniotic and fetal primary cultures as well as vaginal/cervical epithelial and male germ cells have been used intensively to validate BSAA activity (49, 52-55). Although primary cell cultures are relevant systems for validation of BSAAs, there are technical difficulties limiting their use, such as ethical issues, purity of population of primary cells, and limited shelf life of the cells. In addition, age, race, sex and other genetic and epigenetic factors of donor cells should be considered to determine common biological effect across a significant number of donors thereby avoiding minor variants (56, 57).

The obstacles associated with use of human primary cell cultures can be bypassed using human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs). ESCs are isolated from surplus human embryos, whereas iPSCs are obtained by reprogramming somatic cells. These cells proliferate extensively and retain multi-lineage activity, which allows to generate virtually any cell type of the body. The ESCs- and iPSC-derived cells have been used successfully to investigate the efficacy of several BSAAs against HBV, ZIKV, CHIKV and HSV-1 infections (Table S1) (58-63).

iPSCs, ESCs and primary tissue cells can be used to generate complex cultures termed organoids. Organoids are miniature and simplified version of organs. Establishing human airway, gut, skin, cerebral, liver, kidney, breast, retina and brain organoids allowed researchers to study toxicity and efficacy of several safe-in-man BSAAs against coronaviruses, influenza, enteroviruses, rotaviruses and flaviviruses (58, 64-70). However, iPSCs, ESCs and iPSCs/ESCs-derived organoids, have the same disadvantages as human primary cells (genetic differences, line-to-line and organoid batch-to-batch variability). On the other hand, these models allow researchers to predict the behavior of viruses in vivo and, therefore, to reduce animal use and in cases where animal models are unavailable to initiate clinical trials.

For example, novel anti-ZIKV activities of enoxacin, amodiaquine and niclosamide were discovered recently using human neural progenitor cells, human pluripotent stem cell-derived cortical neural progenitor cells, and human induced neural stem cells, respectively (58, 71, 72). Enoxacin is an oral broad-spectrum fluoroquinolone antibiotic, which also possesses anti-HCV and anti-HIV-1 activities in immortalized cell cultures (73, 74). Amodiaquine is an anti-malaria drug, which also possesses antiviral activities against DENV, HCV, RRV, SINV, WNV, EFV, EBOV, LASV, RABV, VZV, and HSV-1 in immortalized cell cultures (75-77). Niclosamide is an orally bioavailable anthelmintic drug, which inhibits the broadest range of viruses in vitro and, in some cases, *in vivo* (72, 75, 76, 78-84). These safe-in-man BSAAs represent promising drug candidates.

2.3. Evaluation of BSAAs in animal models

In vitro and ex vivo models do not fully reflect the complexity and physiology of living organisms. Therefore, several in vivo models have been developed to test novel antiviral activities of BSAAs. These include immunocompetent and genetically or chemically immunocompromised mice, guinea pigs, hamsters, ferrets, pigs, macaques and other animals (Fig. 2B) (51, 85-89). PK/PD studies determine drug absorption, dosage and half-life of BSAAs. Toxicological studies determine if the drugs have any adverse effects on the tissues and organs of the animals and defining the dosage of adverse effects (90-92). Studying the efficacy of BSAAs is generally done by treating the animal with the drug or vehicle and infecting it with a virus of interest. Endpoints are usually body weight/ mortality (depending on the virus), histopathology, virus titers in organs, presence of clinical signs and development of immunity (93, 94). Although animal models can give the initial characterization of BSAA, it is important to keep in mind that they differ significantly from humans, with respect to symptoms, disease manifestation, susceptibility, immune responses, pathogenesis, and pharmacokinetics (95, 96).

For example, aminoglycoside antibiotics, kasugamycin and neomycin were successfully tested against ZIKV, FLUAV, and HSV-2 infections in mice (97). Polyether antibiotic salinomycin also showed anti-FLUAV effect in mice (98). In addition, investigational anticancer agent, flavopiridol, was effective against FLUAV in mice (99). These findings support further development of these and other BSAA.

2.4. Clinical trials and post-clinical studies of BSAAs

Clinical trials are the most critical and time-consuming step of a drug candidates' journey to being approved (Fig. 2C). However, safe-in-man BSAAs make this journey relatively short, because they have been already at phase 0, I and sometime at IIa of clinical trials as antibacterial, antiprotozoal, anticancer, etc. agent; i.e. they have been administered at sub-therapeutic doses to healthy volunteers to ensure the drugs are not harmful to the participants. Thus, safe-in-man BSAAs enter phase II and III, which assess the efficacy, effectiveness, safety and side effects of the drugs in clinic. For this, patients with the viral disease in question are invited to join the study, where they are administered the BSAAs at the ideal therapeutic doses. Phase III is the longest of the phases, and include multiple levels of securities to the studies, such as the use of placebos and double-blinded studies, to ensure the data is as unbiased as possible. Upon completing phase III, depending on its performance and efficacy, BSAAs may end either being approved or dropped. The U.S Food and Drug Administration (FDA) estimates that only 25-30% BSAA candidates which enters phase III are approved for use in the public (100). After approval and marketing of the drug, phase IV may be initiated to follow up on the use of the drug in public, to surveil for rare effects (100, 101).

Forty-eight safe-in-man BSAAs undergo clinical studies as antivirals. There are currently 21 compounds in phase I, 34 agents in phase II and 11 compounds in phase III clinical trials. For example, nitazoxanide, remdesivir and brincidofovir are under clinical investigations against different viral infections (NCT03336619, NCT00302640, NCT03605862, NCT03719586, NCT01276756, NCT03905655, NCT01529073, NCT03395405, NCT03216967, NCT01431326, NCT02087306, NCT01769170).

Twenty-one BSAAs were approved by FDA, EMA or other agencies. These BSAAs altogether target 15 viruses. For example, favipiravir, also known as T-705, was approved against FLUAV in Japan; cidofovir is an injectable antiviral medication used as a treatment for CMV retinitis in people with AIDS; ribavirin, also known as tribavirin, is used for treatment of RSV and HCV infections; pleconaril is used against viruses in the picornaviridae family, including enterovirus and rhinovirus; and valacyclovir is used against CMV, EBV, HBV, HSV-1, HSV-2 and VZV infections.

Twenty BSAAs are undergoing surveillance studies (phase IV). Azithromycin, chloroquine, cyclosporine, ezetimibe, mycophenolic acid, nitazoxanide and rapamycin progressed to phase IV studies without approvals from national or international authorities (NCT01779570, NCT02058173, NCT02564471, NCT00821587, NCT03360682, NCT02328963, NCT02768545, NCT01624948, NCT01770483, NCT02683291, NCT01624948, NCT01469884, NCT03901001, NCT01412515, NCT02990312).
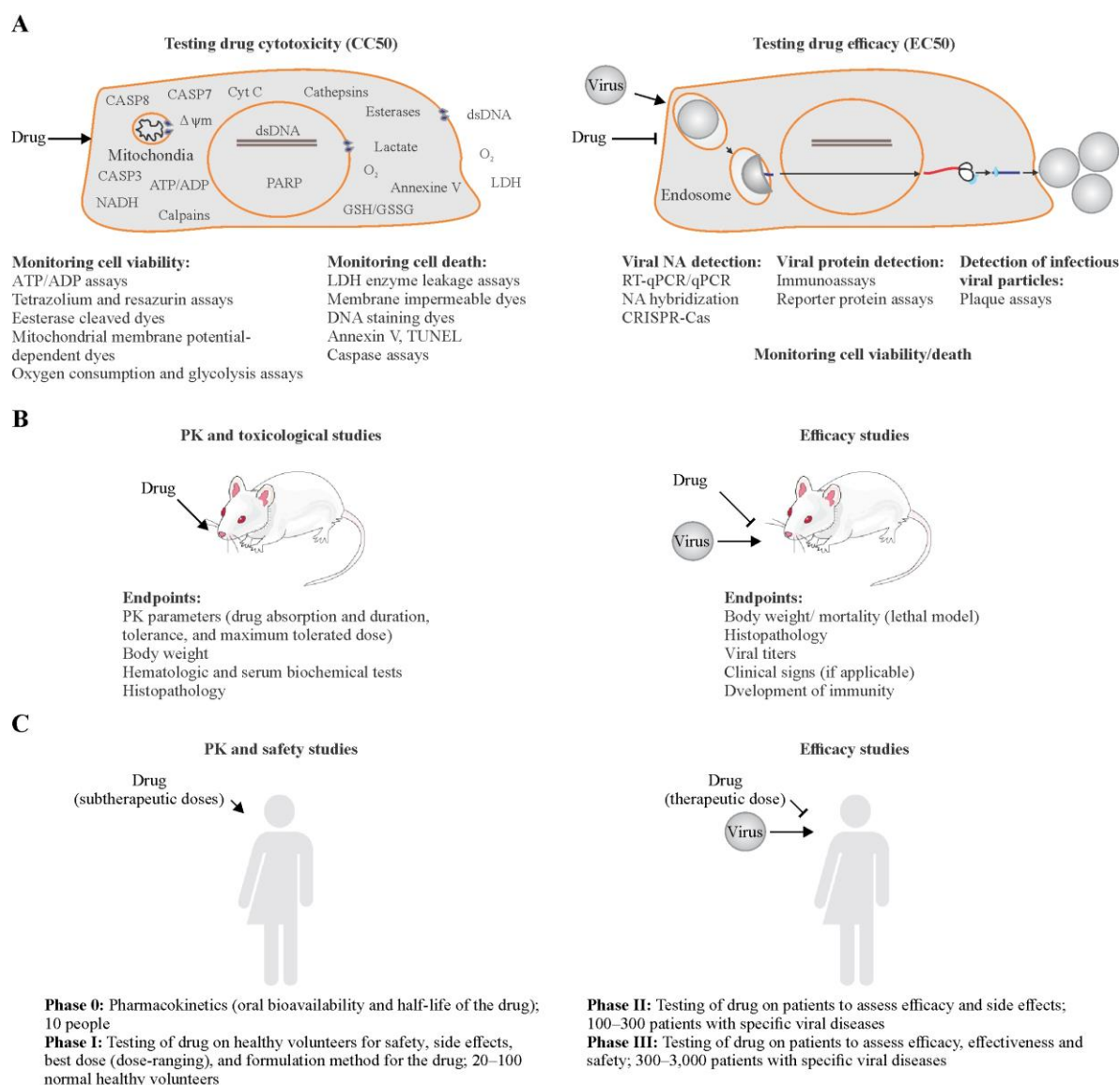
Figure 2. ABC of BSAA development process. (A) Testing BSAA toxicity (left panel) and efficacy (right panel) in immortalized cell cultures and co-cultures. (B) Testing BSAA toxicity (left panel) and efficacy (right panel) in animal models. If BSAA is repositioned from another disease (i.e. its PK/PD and toxicity profiles are available for the animal model) it could bypass the safety studies. (C) Clinical trials of BSAAs. (Left panel) Pharmacokinetics (PK) and safety studies. (Right panel) Efficacy studies. If the drug is repositioned from another disease (i.e. its safety profile in man is available) it could bypass the PK and safety studies in man.

3. BSAA database

We have developed a database for safe-in-man BSAAs, which is available at https://drugvirus.info/ (Fig. 3). The drug annotations were obtained from PubChem, DrugBank, DrugCentral, PubMed and clinicaltrials.gov databases (Table S1) (102-104). The information on virus families were exported from Virus Pathogen Database and Analysis Resource (Table S2) (105). The database summarizes activities and developmental stages of BSAAs. The database allows interactive exploration of virus-BSAA interactions. It also includes information on BSA targets. A feedback form is available on the website. The database will be updated upon request or as soon as a new safe-in-man BSAA emerges or novel activity for an existing BSAA is reported.
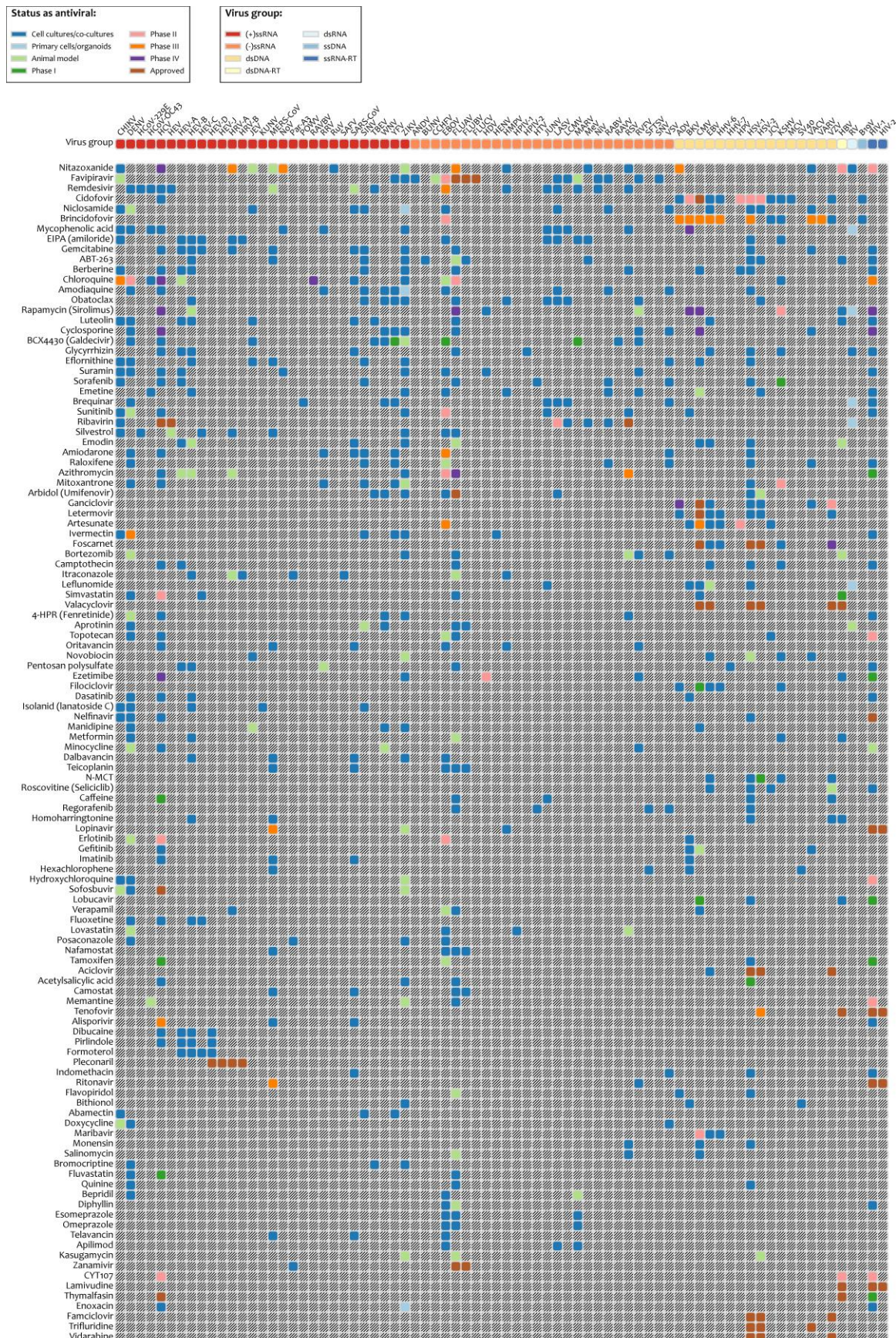
Figure 3. Hundred and nineteen safe-in-man broad-spectrum antiviral agents (BSAAs) and viruses they inhibit. A snapshot is taken from https://drugvirus.info/ website. Viruses are clustered by virus groups. BSAAs are ranged from the highest to lowest number of targeted viruses. Different shadings indicate different development status of BSAAs. Gray shading indicates that the antiviral activity has

not been either studied or reported. Abbreviations: ds, double-stranded; RT, reverse transcriptase; ss, single-stranded.

Altogether, the database contains 119 approved, investigational and experimental safe-in-man BSAAs, which inhibit 83 human viruses, belonging to 25 viral families. The BSAAs inhibit viral or host factors and block viral replication completely, reduce the viral burden to a level at which host immune responses can deal with it or facilitate apoptosis of infected cells (Table S1). Analysis of BSAA targets and structures (Fig. 4) revealed that the most abundant are nucleotide and nucleoside analogues which inhibit viral RNA and DNA polymerases. Imatinib, erlotinib, gefitinib, and dasatinib, that inhibit tyrosine kinases, are the most abundant host-directed BSAAs. Most of the host targets (except Bcl-xL protein) are essential for viral replication but redundant for the cell, which is critical for reducing putative toxicities associated with blocking cellular pathways. The limited diversity of the targets and scaffolds could slow down the development of BSAA concept.

Emerging BSAAs, such as 5,6-dimethoxyindan-1-one, saliphenylhalamide, and GS-5734 (22, 49, 106-109), whose safety profiles in humans are not yet available, are not included in the database. However, they could serve as valuable antivirals in the future, pending the results of further pre-clinical and clinical investigations.

4. BSAA combinations

BSAAs could be combined with other antiviral agents to obtain synergistic or additive effects against certain viruses (17, 110). Several combination therapies which include BSAAs (such as abacavir/dolutegravir/lamivudine (Triumeq), darunavir/cobicistat/emtricitabine/tenofovir (Symtuza), lopinavir/ritonavir (Kaletra), ledipasvir/sofosbuvir and sofosbuvir/velpatasvir) became a standard for the treatment of HIV or HCV infections. Several synergistic drug combinations such as, obatoclax/saliphenylhalamide and gemcitabine/pimodivir could enter clinical studies and become effective treatment of ZIKV and FLUAV infections (109, 111).

By contrast to individual drugs, combinations of 2-3 BSAAs could be used to target even broader range of viruses (17, 112). Such combinations could serve as front line therapeutics against poorly characterized emerging viruses or re-emerging drug-resistant viral strains. For example, a cocktail of nitazoxanide, favipiravir, and niclosamide could be developed for the treatment of viruses belonging to 11 families.

5. Other activities of BSAAs

Fifty BSAAs possess not only antiviral but also antibacterial activity (Fig. 4; Table S1) (113). Moreover, 13 of the 50 agents are approved as antibiotics (2 withdrawn). These agents with dual activity could be used for treatment of viral and bacterial co-infections or for the protection of patients from the secondary infections. For example, azithromycin could be used against FLUAV and *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* or *Streptococcus pneumoniae* infections (NCT01779570) (114).

In addition, BSAAs showed activity against a wide range of other medically important human pathogens, including fungi, protozoa and parasites (Table S1) (115), pointing out that some pathogens utilize common mechanisms to infect hosts. Moreover, structure-activity relationship analysis of BSAAs suggest that some agents, such as doxycycline, artesunate, omeprazole, nitazoxanide, suramin, azithromycin, minocycline and chloroquine, could have novel antibacterial, antiprotozoal, antifungal or anthelmintic activities (Fig. 3). If confirmed, this could lead to development of broad-spectrum anti-infective drugs.

BSAAs could also serve as treatment of other co-morbidities, therefore, simplifying the therapy and lowering its cost (Table S1). For example, the concomitant actions of ezetimibe and statins could be beneficial for treatment of both hypertension and several viral infections in patients with these co-morbidities (NCT00908011, NCT00099684, NCT00843661, NCT03490097, NCT00994773, NCT00441493).
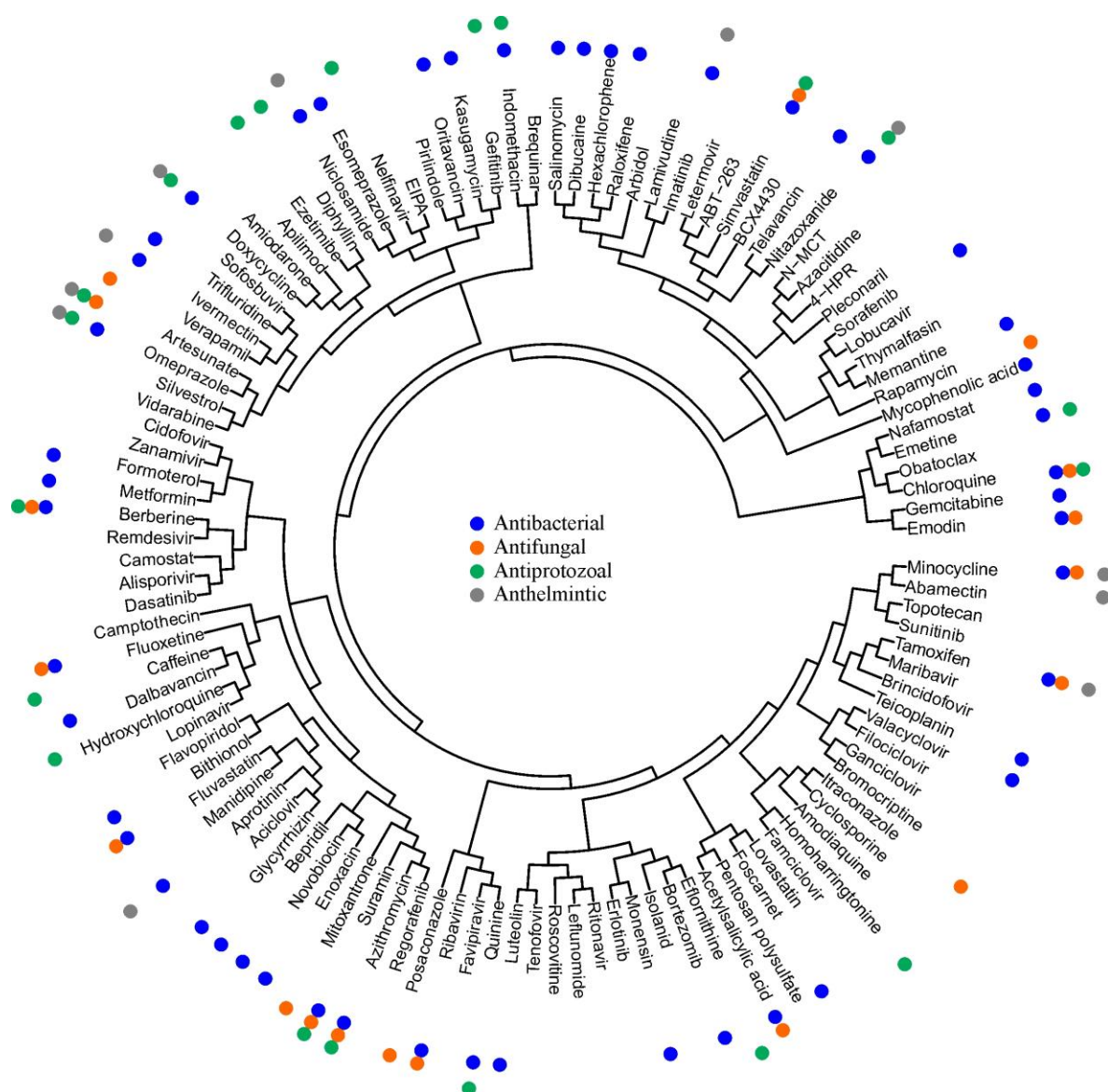
Figure 4. Structure-activity relationship of safe-in-man BSAAs. Web-application serves C-SPADE was used to cluster BSAAs based on their structural similarities and visualize them as a dendrogram of compounds augmented with their functional annotations (https://cspade.fimm.fi/)

4. Conclusion

Here we reviewed a process of BSAA development and summarized information on 119 safe-in-man agents in freely available database. We hope that further pre-clinical and clinical studies on BSAAs will be harmonized, and data collection will be standardized. Furthermore, the follow-up studies as well as the results of on-going, finalized or terminated clinical trials will be made publicly available to allow prioritization and translation of emerging and existing BSAAs into clinical practice. This would allow BSAAs to play a pivotal role in the battle against emerging and re-emerging viral diseases. Discovery of novel BSAAs as well as repositioning existing safe-in-man BSAAs may shorten time and resources, needed for development of virus-specific drugs and vaccines. In the future, BSAAs will have global impact by decreasing morbidity and mortality from viral and other diseases, maximizing the number of healthy life years, improving the quality of life of infected patients and decreasing the costs of patient care.

Supplementary Materials: The following are available online. Table S1: Safe-in-man broad-spectrum antiviral agents; Table S2: Human viruses and associated diseases.

Author Contributions: Conceptualization, D.E.K.; Investigation and Validation, P.I.A., A.I., H.L. and D.E.K.; Data Curation & Visualization, A.I. and D.E.K.; Writing & Editing, all authors; Project Administration and Funding Acquisition, D.E.K.

Funding: This research was funded by Estonian Research Council Mobilitas pluss top researcher grant (contract No. MOBTT31).

Acknowledgments: This study was supported by the European Regional Development Fund, the Mobilitas Pluss Project MOBTT39 (to D.K.). This manuscript has been released as a pre-print (116). We thank Katarzyna Kolasa for illustrations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Disease GBD, Injury I, Prevalence C. 2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392:1789-1858.

2. DALYs GBD, Collaborators H. 2018. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392:1859-1922.

3. WHO. 2015. WHO publishes list of top emerging diseases likely to cause major epidemics. wwwwhoint/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/.

4. Howard CR, Fletcher NF. 2012. Emerging virus diseases: can we ever expect the unexpected? Emerg Microbes Infect 1:e46.

5. Marston HD, Folkers GK, Morens DM, Fauci AS. 2014. Emerging viral diseases: confronting threats with new technologies. Sci Transl Med 6:253ps10.

6. De Clercq E, Li G. 2016. Approved Antiviral Drugs over the Past 50 Years. Clin Microbiol Rev 29:695-747.

7. Bekerman E, Einav S. 2015. Infectious disease. Combating emerging viral threats. Science 348:282-3.

8. Debing Y, Neyts J, Delang L. 2015. The future of antivirals: broad-spectrum inhibitors. Curr Opin Infect Dis 28:596-602.

9. Ianevski A, Andersen PI, Merits A, Bjoras M, Kainov D. 2019. Expanding the activity spectrum of antiviral agents. Drug Discov Today 24:1224-1228.

10. de Clercq E, Montgomery JA. 1983. Broad-spectrum antiviral activity of the carbocyclic analog of 3-deazaadenosine. Antiviral Res 3:17-24.

11. Rada B, Dragun M. 1977. Antiviral action and selectivity of 6-azauridine. Ann N Y Acad Sci 284:410-7.

12. Sidwell RW, Huffman JH, Khare GP, Allen LB, Witkowski JT, Robins RK. 1972. Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 177:705-6.

13. Bosl K, Ianevski A, Than TT, Andersen PI, Kuivanen S, Teppor M, Zusinaite E, Dumpis U, Vitkauskiene A, Cox RJ, Kallio-Kokko H, Bergqvist A, Tenson T, Merits A, Oksenych V, Bjoras M, Anthonsen M, Shum D, Kaarbo M, Vapalahti O, Windisch MP, Superti-Furga G, Snijder B, Kainov D, Kandasamy RK. 2019. Common Nodes of Virus–Host Interaction Revealed Through an Integrated Network Analysis. Front Immunology 4:2186.

14. Nishimura Y, Hara H. 2018. Editorial: Drug Repositioning: Current Advances and Future Perspectives. Front Pharmacol 9:1068.

15. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D, Pirmohamed M. 2019. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18:41-58.

16. Pizzorno A, Padey B, Terrier O, Rosa-Calatrava M. 2019. Drug Repurposing Approaches for the Treatment of Influenza Viral Infection: Reviving Old Drugs to Fight Against a Long-Lived Enemy. 10.

17. Zheng W, Sun W, Simeonov A. 2018. Drug repurposing screens and synergistic drug-combinations for infectious diseases. Br J Pharmacol 175:181-191.

18. Meneghini KA, Hamasaki DI. 1967. The electroretinogram of the iguana and Tokay gecko. Vision Res 7:243-51.

19. Shen L, Niu J, Wang C, Huang B, Wang W, Zhu N, Deng Y, Wang H, Ye F, Cen S, Tan W. 2019. High-Throughput Screening and Identification of Potent Broad-Spectrum Inhibitors of Coronaviruses. J Virol 93.

20. Bulanova D, Ianevski A, Bugai A, Akimov Y, Kuivanen S, Paavilainen H, Kakkola L, Nandania J, Turunen L, Ohman T, Ala-Hongisto H, Pesonen HM, Kuisma MS, Honkimaa A, Walton EL, Oksenych V, Lorey MB, Guschin D, Shim J, Kim J, Than TT, Chang SY, Hukkanen V, Kulesskiy E, Marjomaki VS, Julkunen I, Nyman TA, Matikainen S, Saarela JS, Sane F, Hober D, Gabriel G, De Brabander JK, Martikainen M, Windisch MP, Min JY, Bruzzone R, Aittokallio T, Vaha-Koskela M, Vapalahti O, Pulk A, Velagapudi V, Kainov DE. 2017. Antiviral Properties of Chemical Inhibitors of Cellular Anti-Apoptotic Bcl-2 Proteins. Viruses 9.

21. Ianevski A, Zusinaite E, Kuivanen S, Strand M, Lysvand H, Teppor M, Kakkola L, Paavilainen H, Laajala M, Kallio-Kokko H, Valkonen M, Kantele A, Telling K, Lutsar I, Letjuka P, Metelitsa N, Oksenych V, Bjoras M, Nordbo SA, Dumpis U, Vitkauskiene A, Ohrmalm C, Bondeson K, Bergqvist A, Aittokallio T, Cox RJ, Evander M, Hukkanen V, Marjomaki V, Julkunen I, Vapalahti O, Tenson T, Merits A, Kainov D. 2018. Novel activities of safe-in-human broad-spectrum antiviral agents. Antiviral Res 154:174-182.

22. Muller KH, Spoden GA, Scheffer KD, Brunnhofer R, De Brabander JK, Maier ME, Florin L, Muller CP. 2014. Inhibition by cellular vacuolar ATPase impairs human papillomavirus uncoating and infection. Antimicrob Agents Chemother 58:2905-11.

23. Sarzotti-Kelsoe M, Bailer RT, Turk E, Lin CL, Bilska M, Greene KM, Gao H, Todd CA, Ozaki DA, Seaman MS, Mascola JR, Montefiori DC. 2014. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. J Immunol Methods 409:131-46.

24. Xing L, Wang S, Hu Q, Li J, Zeng Y. 2016. Comparison of three quantification methods for the TZM-bl pseudovirus assay for screening of anti-HIV-1 agents. J Virol Methods 233:56-61.

25. Habjan M, Penski N, Spiegel M, Weber F. 2008. T7 RNA polymerase-dependent and -independent systems for cDNA-based rescue of Rift Valley fever virus. J Gen Virol 89:2157-66.

26. Lee M, Yang J, Jo E, Lee JY, Kim HY, Bartenschlager R, Shin EC, Bae YS, Windisch MP. 2017. A Novel Inhibitor IDPP Interferes with Entry and Egress of HCV by Targeting Glycoprotein E1 in a Genotype-Specific Manner. Sci Rep 7:44676.

27. Kittel C, Sereinig S, Ferko B, Stasakova J, Romanova J, Wolkerstorfer A, Katinger H, Egorov A. 2004. Rescue of influenza virus expressing GFP from the NS1 reading frame. Virology 324:67-73.

28. de Graaf M, Herfst S, Schrauwen EJ, van den Hoogen BG, Osterhaus AD, Fouchier RA. 2007. An improved plaque reduction virus neutralization assay for human metapneumovirus. J Virol Methods 143:169-74.

29. Utt A, Quirin T, Saul S, Hellstrom K, Ahola T, Merits A. 2016. Versatile Trans-Replication Systems for Chikungunya Virus Allow Functional Analysis and Tagging of Every Replicase Protein. PLoS One 11:e0151616.

30. Jupille HJ, Oko L, Stoermer KA, Heise MT, Mahalingam S, Gunn BM, Morrison TE. 2011. Mutations in nsP1 and PE2 are critical determinants of Ross River virus-induced musculoskeletal inflammatory disease in a mouse model. Virology 410:216-27.

31. Andersen PI, Krpina K, Ianevski A, Shtaida N, Jo E, Yang J, Koit S, Tenson T, Hukkonen V, Anthonsen MW, Bjoras M, Evander M, Windisch MP, Zusinaite E, Kainov DE. 2019. Novel Antiviral Activities of Obatoclax, Emetine, Niclosamide, Brequinar, and Homoharringtonine. Viruses 11:pii: E964.

32. Konig A, Yang J, Jo E, Park KHP, Kim H, Than TT, Song X, Qi X, Dai X, Park S, Shum D, Ryu WS, Kim JH, Yoon SK, Park JY, Ahn SH, Han KH, Gerlich WH, Windisch MP. 2019. Efficient long-term amplification of hepatitis B virus isolates after infection of slow proliferating HepG2-NTCP cells. J Hepatol 71:289-300.

33. Fischer C, Torres MC, Patel P, Moreira-Soto A, Gould EA, Charrel RN, de Lamballerie X, Nogueira RMR, Sequeira PC, Rodrigues CDS, Kümmerer BM, Drosten C, Landt O, Bispo de Filippis AM, Drexler JF. 2017. Lineage-Specific Real-Time RT-PCR for Yellow Fever Virus Outbreak Surveillance, Brazil. Emerg Infect Dis 23:1867-71.

34. Laamiri N, Aouini R, Marnissi B, Ghram A, Hmila I. 2018. A multiplex real-time RT-PCR for simultaneous detection of four most common avian respiratory viruses. Virology 515:29-37.

35. Landry ML. 1990. Nucleic acid hybridization in viral diagnosis. Clinical Biochemistry 23:267-277.

36. Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J, Mumford R. 2014. Methods in virus diagnostics: from ELISA to next generation sequencing. Virus Res 186:20-31.

37. Perez JT, Garcia-Sastre A, Manicassamy B. 2013. Insertion of a GFP reporter gene in influenza virus. Curr Protoc Microbiol Chapter 15:Unit 15G.4.

38. Sashital DG. 2018. Pathogen detection in the CRISPR-Cas era. Genome medicine 10:32-32.

39. Zhou L, Peng R, Zhang R, Li J. 2018. The applications of CRISPR/Cas system in molecular detection. Journal of cellular and molecular medicine 22:5807-5815.

40. Puschnik AS, Majzoub K, Ooi YS, Carette JE. 2017. A CRISPR toolbox to study virus-host interactions. Nat Rev Microbiol 15:351-364.

41. Deans RM, Morgens DW, Okesli A, Pillay S, Horlbeck MA, Kampmann M, Gilbert LA, Li A, Mateo R, Smith M, Glenn JS, Carette JE, Khosla C, Bassik MC. 2016. Parallel shRNA and CRISPR-Cas9 screens enable antiviral drug target identification. Nat Chem Biol 12:361-6.

42. Andersen PI, Krpina K, Ianevski A, Shtaida N, Jo E, Yang J, Koit S, Tenson T, Hukkanen V, Anthonsen MW, Bjoras M, Evander M, Windisch MP, Zusinaite E, Kainov DE. 2019. Novel Antiviral Activities of Obatoclax, Emetine, Niclosamide, Brequinar, and Homoharringtonine. Viruses 11.

43. MacGibeny MA, Koyuncu OO, Wirblich C, Schnell MJ, Enquist LW. 2018. Retrograde axonal transport of rabies virus is unaffected by interferon treatment but blocked by emetine locally in axons. PLoS Pathog 14:e1007188.

44. Yang S, Xu M, Lee EM, Gorshkov K, Shiryaev SA, He S, Sun W, Cheng YS, Hu X, Tharappel AM, Lu B, Pinto A, Farhy C, Huang CT, Zhang Z, Zhu W, Wu Y, Zhou Y, Song G, Zhu H, Shamim K, Martinez-Romero C, Garcia-Sastre A, Preston RA, Jayaweera DT, Huang R, Huang W, Xia M, Simeonov A, Ming G, Qiu X, Terskikh AV, Tang H, Song H, Zheng W. 2018. Emetine inhibits Zika and Ebola virus infections through two molecular mechanisms: inhibiting viral replication and decreasing viral entry. Cell Discov 4:31.

45. Mukhopadhyay R, Roy S, Venkatadri R, Su YP, Ye W, Barnaeva E, Mathews Griner L, Southall N, Hu X, Wang AQ, Xu X, Dulcey AE, Marugan JJ, Ferrer M, Arav-Boger R. 2016. Efficacy and Mechanism of Action of Low Dose Emetine against Human Cytomegalovirus. PLoS Pathog 12:e1005717.

46.   Chaves Valadao AL, Abreu CM, Dias JZ, Arantes P, Verli H, Tanuri A, de Aguiar RS. 2015. Natural Plant Alkaloid (Emetine) Inhibits HIV-1 Replication by Interfering with Reverse Transcriptase Activity. Molecules 20:11474-89.

47.   Carter M, Shieh J. 2015. Chapter 14 - Cell Culture Techniques, p 295-310. *In* Carter M, Shieh J (ed), Guide to Research Techniques in Neuroscience (Second Edition) doi:https://doi.org/10.1016/B978-0-12-800511-8.00014-9. Academic Press, San Diego.

48.   Postnikova E, Cong Y, DeWald LE, Dyall J, Yu S, Hart BJ, Zhou H, Gross R, Logue J, Cai Y, Deiuliis N, Michelotti J, Honko AN, Bennett RS, Holbrook MR, Olinger GG, Hensley LE, Jahrling PB. 2018. Testing therapeutics in cell-based assays: Factors that influence the apparent potency of drugs. PLoS One 13:e0194880.

49.   Denisova OV, Kakkola L, Feng L, Stenman J, Nagaraj A, Lampe J, Yadav B, Aittokallio T, Kaukinen P, Ahola T, Kuivanen S, Vapalahti O, Kantele A, Tynell J, Julkunen I, Kallio-Kokko H, Paavilainen H, Hukkanen V, Elliott RM, De Brabander JK, Saelens X, Kainov DE. 2012. Obatoclax, saliphenylhalamide, and gemcitabine inhibit influenza a virus infection. J Biol Chem 287:35324-32.

50.   Koban R, Neumann M, Daugs A, Bloch O, Nitsche A, Langhammer S, Ellerbrok H. 2018. A novel three-dimensional cell culture method enhances antiviral drug screening in primary human cells. Antiviral Res 150:20-29.

51.   Alves MP, Vielle NJ, Thiel V, Pfaender S. 2018. Research Models and Tools for the Identification of Antivirals and Therapeutics against Zika Virus Infection. Viruses 10.

52.   Fink SL, Vojtech L, Wagoner J, Slivinski NSJ, Jackson KJ, Wang R, Khadka S, Luthra P, Basler CF, Polyak SJ. 2018. The Antiviral Drug Arbidol Inhibits Zika Virus. Sci Rep 8:8989.

53.   Robinson CL, Chong ACN, Ashbrook AW, Jeng G, Jin J, Chen H, Tang EI, Martin LA, Kim RS, Kenyon RM, Do E, Luna JM, Saeed M, Zeltser L, Ralph H, Dudley VL, Goldstein M, Rice CM, Cheng CY, Seandel M, Chen S. 2018. Male germ cells support long-term propagation of Zika virus. Nat Commun 9:2090.

54.   Rausch K, Hackett BA, Weinbren NL, Reeder SM, Sadovsky Y, Hunter CA, Schultz DC, Coyne CB, Cherry S. 2017. Screening Bioactives Reveals Nanchangmycin as a Broad Spectrum Antiviral Active against Zika Virus. Cell Rep 18:804-815.

55.   Barrows NJ, Campos RK, Powell ST, Prasanth KR, Schott-Lerner G, Soto-Acosta R, Galarza-Munoz G, McGrath EL, Urrabaz-Garza R, Gao J, Wu P, Menon R, Saade G, Fernandez-Salas I, Rossi SL, Vasilakis N, Routh A, Bradrick SS, Garcia-Blanco MA. 2016. A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection. Cell Host Microbe 20:259-70.

56.   Lee MN, Ye C, Villani AC, Raj T, Li W, Eisenhaure TM, Imboywa SH, Chipendo PI, Ran FA, Slowikowski K, Ward LD, Raddassi K, McCabe C, Lee MH, Frohlich IY, Hafler DA, Kellis M, Raychaudhuri S, Zhang F, Stranger BE, Benoist CO, De Jager PL, Regev A, Hacohen N. 2014. Common genetic variants modulate pathogen-sensing responses in human dendritic cells. Science 343:1246980.

57.   Zhang YH, Zhao Y, Li N, Peng YC, Giannoulatou E, Jin RH, Yan HP, Wu H, Liu JH, Liu N, Wang DY, Shu YL, Ho LP, Kellam P, McMichael A, Dong T. 2013. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat Commun 4:1418.

58.   Zhou T, Tan L, Cederquist GY, Fan Y, Hartley BJ, Mukherjee S, Tomishima M, Brennand KJ, Zhang Q, Schwartz RE, Evans T, Studer L, Chen S. 2017. High-Content Screening in hPSC-Neural Progenitors Identifies Drug Candidates that Inhibit Zika Virus Infection in Fetal-like Organoids and Adult Brain. Cell Stem Cell 21:274-283 e5.

59. Lanko K, Eggermont K, Patel A, Kaptein S, Delang L, Verfaillie CM, Neyts J. 2017. Replication of the Zika virus in different iPSC-derived neuronal cells and implications to assess efficacy of antivirals. Antiviral Res 145:82-86.

60. Iwasawa C, Tamura R, Sugiura Y, Suzuki S, Kuzumaki N, Narita M, Suematsu M, Nakamura M, Yoshida K, Toda M, Okano H, Miyoshi H. 2019. Increased Cytotoxicity of Herpes Simplex Virus Thymidine Kinase Expression in Human Induced Pluripotent Stem Cells. Int J Mol Sci 20.

61. Simonin Y, Erkilic N, Damodar K, Cle M, Desmetz C, Bollore K, Taleb M, Torriano S, Barthelemy J, Dubois G, Lajoix AD, Foulongne V, Tuaillon E, Van de Perre P, Kalatzis V, Salinas S. 2019. Zika virus induces strong inflammatory responses and impairs homeostasis and function of the human retinal pigment epithelium. EBioMedicine 39:315-331.

62. Ferreira AC, Reis PA, de Freitas CS, Sacramento CQ, Villas Boas Hoelz L, Bastos MM, Mattos M, Rocha N, Gomes de Azevedo Quintanilha I, da Silva Gouveia Pedrosa C, Rocha Quintino Souza L, Correia Loiola E, Trindade P, Rangel Vieira Y, Barbosa-Lima G, de Castro Faria Neto HC, Boechat N, Rehen SK, Bruning K, Bozza FA, Bozza PT, Souza TML. 2019. Beyond Members of the Flaviviridae Family, Sofosbuvir Also Inhibits Chikungunya Virus Replication. Antimicrob Agents Chemother 63.

63. Xia Y, Carpentier A, Cheng X, Block PD, Zhao Y, Zhang Z, Protzer U, Liang TJ. 2017. Human stem cell-derived hepatocytes as a model for hepatitis B virus infection, spreading and virus-host interactions. J Hepatol 66:494-503.

64. Yin Y, Chen S, Hakim MS, Wang W, Xu L, Dang W, Qu C, Verhaar AP, Su J, Fuhler GM, Peppelenbosch MP, Pan Q. 2018. 6-Thioguanine inhibits rotavirus replication through suppression of Rac1 GDP/GTP cycling. Antiviral Res 156:92-101.

65. Watanabe M, Buth JE, Vishlaghi N, de la Torre-Ubieta L, Taxidis J, Khakh BS, Coppola G, Pearson CA, Yamauchi K, Gong D, Dai X, Damoiseaux R, Aliyari R, Liebscher S, Schenke-Layland K, Caneda C, Huang EJ, Zhang Y, Cheng G, Geschwind DH, Golshani P, Sun R, Novitch BG. 2017. Self-Organized Cerebral Organoids with Human-Specific Features Predict Effective Drugs to Combat Zika Virus Infection. Cell Rep 21:517-532.

66. Li C, Deng YQ, Wang S, Ma F, Aliyari R, Huang XY, Zhang NN, Watanabe M, Dong HL, Liu P, Li XF, Ye Q, Tian M, Hong S, Fan J, Zhao H, Li L, Vishlaghi N, Buth JE, Au C, Liu Y, Lu N, Du P, Qin FX, Zhang B, Gong D, Dai X, Sun R, Novitch BG, Xu Z, Qin CF, Cheng G. 2017. 25-Hydroxycholesterol Protects Host against Zika Virus Infection and Its Associated Microcephaly in a Mouse Model. Immunity 46:446-456.

67. Sacramento CQ, de Melo GR, de Freitas CS, Rocha N, Hoelz LV, Miranda M, Fintelman-Rodrigues N, Marttorelli A, Ferreira AC, Barbosa-Lima G, Abrantes JL, Vieira YR, Bastos MM, de Mello Volotao E, Nunes EP, Tschoeke DA, Leomil L, Loiola EC, Trindade P, Rehen SK, Bozza FA, Bozza PT, Boechat N, Thompson FL, de Filippis AM, Bruning K, Souza TM. 2017. The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. Sci Rep 7:40920.

68. Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, Tcw J, Kouznetsova J, Ogden SC, Hammack C, Jacob F, Nguyen HN, Itkin M, Hanna C, Shinn P, Allen C, Michael SG, Simeonov A, Huang W, Christian KM, Goate A, Brennand KJ, Huang R, Xia M, Ming GL, Zheng W, Song H, Tang H. 2016. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med 22:1101-1107.

69. Yin Y, Wang Y, Dang W, Xu L, Su J, Zhou X, Wang W, Felczak K, van der Laan LJ, Pankiewicz KW, van der Eijk AA, Bijvelds M, Sprengers D, de Jonge H, Koopmans MP, Metselaar HJ, Peppelenbosch MP, Pan Q. 2016. Mycophenolic acid potently inhibits rotavirus infection with a high barrier to resistance development. Antiviral Res 133:41-9.

70. Yin Y, Bijvelds M, Dang W, Xu L, van der Eijk AA, Knipping K, Tuysuz N, Dekkers JF, Wang Y, de Jonge J, Sprengers D, van der Laan LJ, Beekman JM, Ten Berge D, Metselaar HJ, de Jonge H, Koopmans MP, Peppelenbosch MP, Pan Q. 2015. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. Antiviral Res 123:120-31.

71. Xu YP, Qiu Y, Zhang B, Chen G, Chen Q, Wang M, Mo F, Xu J, Wu J, Zhang RR, Cheng ML, Zhang NN, Lyu B, Zhu WL, Wu MH, Ye Q, Zhang D, Man JH, Li XF, Cui J, Xu Z, Hu B, Zhou X, Qin CF. 2019. Zika virus infection induces RNAi-mediated antiviral immunity in human neural progenitors and brain organoids. Cell Res 29:265-273.

72. Cairns DM, Boorgu D, Levin M, Kaplan DL. 2018. Niclosamide rescues microcephaly in a humanized in vivo model of Zika infection using human induced neural stem cells. Biol Open 7.

73. Young DD, Connelly CM, Grohmann C, Deiters A. 2010. Small molecule modifiers of microRNA miR-122 function for the treatment of hepatitis C virus infection and hepatocellular carcinoma. J Am Chem Soc 132:7976-81.

74. Kashiwase H, Momota K, Ohmine T, Komai T, Kimura T, Katsube T, Nishigaki T, Kimura S, Shimada K, Furukawa H. 1999. A new fluoroquinolone derivative exhibits inhibitory activity against human immunodeficiency virus type 1 replication. Chemotherapy 45:48-55.

75. Mazzon M, Ortega-Prieto AM, Imrie D, Luft C, Hess L, Czieso S, Grove J, Skelton JK, Farleigh L, Bugert JJ, Wright E, Temperton N, Angell R, Oxenford S, Jacobs M, Ketteler R, Dorner M, Marsh M. 2019. Identification of Broad-Spectrum Antiviral Compounds by Targeting Viral Entry. Viruses 11.

76. Hulseberg CE, Feneant L, Szymanska-de Wijs KM, Kessler NP, Nelson EA, Shoemaker CJ, Schmaljohn CS, Polyak SJ, White JM. 2019. Arbidol and Other Low-Molecular-Weight Drugs That Inhibit Lassa and Ebola Viruses. J Virol 93.

77. Boonyasuppayakorn S, Reichert ED, Manzano M, Nagarajan K, Padmanabhan R. 2014. Amodiaquine, an antimalarial drug, inhibits dengue virus type 2 replication and infectivity. Antiviral Res 106:125-34.

78. Kao JC, HuangFu WC, Tsai TT, Ho MR, Jhan MK, Shen TJ, Tseng PC, Wang YT, Lin CF. 2018. The antiparasitic drug niclosamide inhibits dengue virus infection by interfering with endosomal acidification independent of mTOR. PLoS Negl Trop Dis 12:e0006715.

79. Huang L, Yang M, Yuan Y, Li X, Kuang E. 2017. Niclosamide inhibits lytic replication of Epstein-Barr virus by disrupting mTOR activation. Antiviral Res 138:68-78.

80. Wang YM, Lu JW, Lin CC, Chin YF, Wu TY, Lin LI, Lai ZZ, Kuo SC, Ho YJ. 2016. Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission. Antiviral Res 135:81-90.

81. Fang J, Sun L, Peng G, Xu J, Zhou R, Cao S, Chen H, Song Y. 2013. Identification of three antiviral inhibitors against Japanese encephalitis virus from library of pharmacologically active compounds 1280. PLoS One 8:e78425.

82. Jurgeit A, McDowell R, Moese S, Meldrum E, Schwendener R, Greber UF. 2012. Niclosamide is a proton carrier and targets acidic endosomes with broad antiviral effects. PLoS Pathog 8:e1002976.

83. Stachulski AV, Pidathala C, Row EC, Sharma R, Berry NG, Lawrenson AS, Moores SL, Iqbal M, Bentley J, Allman SA, Edwards G, Helm A, Hellier J, Korba BE, Semple JE, Rossignol JF. 2011. Thiazolides as novel antiviral agents. 2. Inhibition of hepatitis C virus replication. J Med Chem 54:8670-80.

84. Wu CJ, Jan JT, Chen CM, Hsieh HP, Hwang DR, Liu HW, Liu CY, Huang HW, Chen SC, Hong CF, Lin RK, Chao YS, Hsu JT. 2004. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother 48:2693-6.

85. Morrison TE, Diamond MS. 2017. Animal Models of Zika Virus Infection, Pathogenesis, and Immunity. J Virol 91.

86.   Haese NN, Broeckel RM, Hawman DW, Heise MT, Morrison TE, Streblow DN. 2016. Animal Models of Chikungunya Virus Infection and Disease. J Infect Dis 214:S482-S487.

87.   Taylor G. 2017. Animal models of respiratory syncytial virus infection. Vaccine 35:469-480.

88.   Thangavel RR, Bouvier NM. 2014. Animal models for influenza virus pathogenesis, transmission, and immunology. J Immunol Methods 410:60-79.

89.   Louz D, Bergmans HE, Loos BP, Hoeben RC. 2013. Animal models in virus research: their utility and limitations. Crit Rev Microbiol 39:325-61.

90.   Alabaster V., In Vivo Pharmacology Training Group. 2002. The fall and rise of in vivo pharmacology. Trends Pharmacol Sci 23:13-8.

91.   Rizk ML, Zou L, Savic RM, Dooley KE. 2017. Importance of Drug Pharmacokinetics at the Site of Action. Clinical and translational science 10:133-142.

92.   Parasuraman S. 2011. Toxicological screening. Journal of pharmacology & pharmacotherapeutics 2:74-79.

93.   Smee DF, Barnard DL. 2013. Methods for evaluation of antiviral efficacy against influenza virus infections in animal models. Methods Mol Biol 1030:407-25.

94.   Oh DY, Hurt AC. 2016. Using the Ferret as an Animal Model for Investigating Influenza Antiviral Effectiveness. Frontiers in Microbiology 7.

95.   Barré-Sinoussi F, Montagutelli X. 2015. Animal models are essential to biological research: issues and perspectives. Future science OA 1:FSO63-FSO63.

96.   Shanks N, Greek R, Greek J. 2009. Are animal models predictive for humans? Philosophy, ethics, and humanities in medicine : PEHM 4:2-2.

97.   Gopinath S, Kim MV, Rakib T, Wong PW, van Zandt M, Barry NA, Kaisho T, Goodman AL, Iwasaki A. 2018. Topical application of aminoglycoside antibiotics enhances host resistance to viral infections in a microbiota-independent manner. Nat Microbiol 3:611-621.

98.   Jang Y, Shin JS, Yoon YS, Go YY, Lee HW, Kwon OS, Park S, Park MS, Kim M. 2018. Salinomycin Inhibits Influenza Virus Infection by Disrupting Endosomal Acidification and Viral Matrix Protein 2 Function. J Virol 92.

99.   Soderholm S, Kainov DE, Ohman T, Denisova OV, Schepens B, Kulesskiy E, Imanishi SY, Corthals G, Hintsanen P, Aittokallio T, Saelens X, Matikainen S, Nyman TA. 2016. Phosphoproteomics to Characterize Host Response During Influenza A Virus Infection of Human Macrophages. Mol Cell Proteomics 15:3203-3219.

100.   U.S Food and Drug Administration. 2018.   The Drug Development Process: Step 3. https://www.fda.gov/patients/drug-development-process/step-3-clinical-research. Accessed

101.   Umscheid CA, Margolis DJ, Grossman CE. 2011. Key concepts of clinical trials: a narrative review. Postgraduate medicine 123:194-204.

102.   Ursu O, Holmes J, Bologa CG, Yang JJ, Mathias SL, Stathias V, Nguyen DT, Schurer S, Oprea T. 2019. DrugCentral 2018: an update. Nucleic Acids Res 47:D963-D970.

103.   Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. 2019. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res 47:D1102-D1109.

104.   Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, Assempour N, Iynkkaran I, Liu Y, Maciejewski A, Gale N, Wilson A, Chin L, Cummings R, Le D, Pon A, Knox C, Wilson M. 2018. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 46:D1074-D1082.

105. Pickett BE, Greer DS, Zhang Y, Stewart L, Zhou L, Sun G, Gu Z, Kumar S, Zaremba S, Larsen CN, Jen W, Klem EB, Scheuermann RH. 2012. Virus pathogen database and analysis resource (ViPR): a comprehensive bioinformatics database and analysis resource for the coronavirus research community. Viruses 4:3209-26.

106. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 9.

107. Patil SA, Patil V, Patil R, Beaman K, Patil SA. 2017. Identification of Novel 5,6-Dimethoxyindan-1-one Derivatives as Antiviral Agents. Med Chem 13:787-795.

108. Muller KH, Kainov DE, El Bakkouri K, Saelens X, De Brabander JK, Kittel C, Samm E, Muller CP. 2011. The proton translocation domain of cellular vacuolar ATPase provides a target for the treatment of influenza A virus infections. Br J Pharmacol 164:344-57.

109. Kuivanen S, Bespalov MM, Nandania J, Ianevski A, Velagapudi V, De Brabander JK, Kainov DE, Vapalahti O. 2017. Obatoclax, saliphenylhalamide and gemcitabine inhibit Zika virus infection in vitro and differentially affect cellular signaling, transcription and metabolism. Antiviral Res 139:117-128.

110. Cheng YS, Williamson PR, Zheng W. 2019. Improving therapy of severe infections through drug repurposing of synergistic combinations. Curr Opin Pharmacol 48:92-98.

111. Fu Y, Gaelings L, Soderholm S, Belanov S, Nandania J, Nyman TA, Matikainen S, Anders S, Velagapudi V, Kainov DE. 2016. JNJ872 inhibits influenza A virus replication without altering cellular antiviral responses. Antiviral Res 133:23-31.

112. Foucquier J, Guedj M. 2015. Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect 3:e00149.

113. Schor S, Einav S. 2018. Combating Intracellular Pathogens with Repurposed Host-Targeted Drugs. ACS Infect Dis 4:88-92.

114. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Jr., Musher DM, Niederman MS, Torres A, Whitney CG, Infectious Diseases Society of A, American Thoracic S. 2007. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44 Suppl 2:S27-72.

115. Montoya MC, Krysan DJ. 2018. Repurposing Estrogen Receptor Antagonists for the Treatment of Infectious Disease. MBio 9.

116. Andersen PI, Ianevski A, Lysvand H, Vitkauskiene A, Oksenych V, Bjoras M, Telling, K, Lutsar I, Dumpis U, Irie Y, Tenson T, Kantele A, Kainov DE. 2019. Discovery and Development of Safe-in-Man Broad-Spectrum Antiviral Agents. Preprints. 2019100144 (doi: 10.20944/preprints201910.0144.v2)