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*Review*

# Discovery and Development of Safe-in-Man Broad-Spectrum Antiviral Agents

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**Abstract:** Viral diseases are the leading cause of morbidity and mortality in developing countries. Broad-spectrum antiviral agents (BSAA) are key players in control of viral diseases. Here, we reviewed the discovery and development process of BSAAs, focusing on compounds with available safety profiles in human. We summarized the information on approved, investigational and experimental safe-in-man BSAAs in freely accessible database at https://drugvirus.info/. The number of these BSAAs will be increased as well as their spectrum of indications will be expanded pending the results of further pre-clinical and clinical studies. This will ultimately reinforce the arsenal of available antiviral options and provide better protection of general population from emerging and re-emerging viral diseases.

**Keywords:** virus; antiviral drug; drug discovery; drug development; broad-spectrum antiviral, BSAA

## 1. Introduction

Viruses are one of the major causes of morbidity and mortality in developing countries [1-4]. Antiviral drugs and vaccines are used to fight viral infections [5, 6]. Previously, there has been a focus on "one drug, one virus" dogma, which relied on targeting virus-specific factors. An emerging 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 [7-9]. This paradigm was based on the observation that different viruses utilize the same host factors and pathways to replicate inside a cell [10].

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

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intended [11, 12]. 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. Therefore, repositioning of launched or even failed drugs from one disease 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, 13, 14].

Here, we will describe repositioning of BSAAs, focusing on those antivirals, which have been already tested in human as antivirals, antibacterials, antiprotozoals, antiemetics, etc. Moreover, we will detail the steps of drug development process, from discovery of novel antiviral activities in cell culture to post-market studies (Fig. 1). Finally, we will discuss future perspectives of safe-in-man BSAAs and their combinations for treatment of emerging and re-emerging viral infections and co-infections.

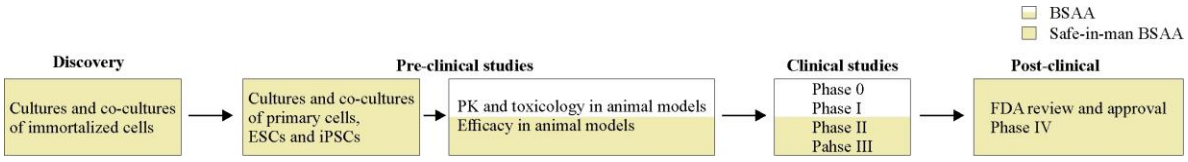


**Figure 1.** Discovery of novel activities and follow-up development of broad-spectrum antiviral agents (BSAAs). Yellow shading indicates a process of discovery and development of safe-in-man BSAAs, for which pharmacokinetic (PK) properties in pre-clinical (animal model) and early clinical developmental phases (phase 0-IIa trials) are already available. Abbreviations: ESCs, human embryonic stem cells; iPSCs, human induced pluripotent stem cells (iPSCs).

## 2. Discovery of novel activities of safe-in-man BSAAs 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 of interest 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 CC50 and EC50 are significant, given the inherent variability of the experiment [15]. 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. 2). 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 (www.abcam.com/kits/cell-health-assays-guide) [16]. For example, the Cell Titer Glo 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 (https://no.promega.com/products/cell-health-assays/cell-viability-and-cytotoxicity-assays/) [10, 17-19].

Viral strains or cell lines expressing reporter proteins in response to viral infections are also commonly used to assess the efficacy of BSAAs. 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 [20, 21]. RFP-expressing RVFV, nanoLuc-expressing CHIKV and RRV, as well as GFP-expressing FLUAV, HCV and HMPV also allow identification of novel activities of several BSAAs [10, 18, 22-27] (10.20944/preprints201909.0128.v1). In addition, qPCR/RT-qPCR, RNA/DNA sequencing, RNA/DNA

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hybridization, CRISPR-CAS immunofluorescence and plaque assays are used to detect inhibitory effects of BSAAs on viral replications [28-35].

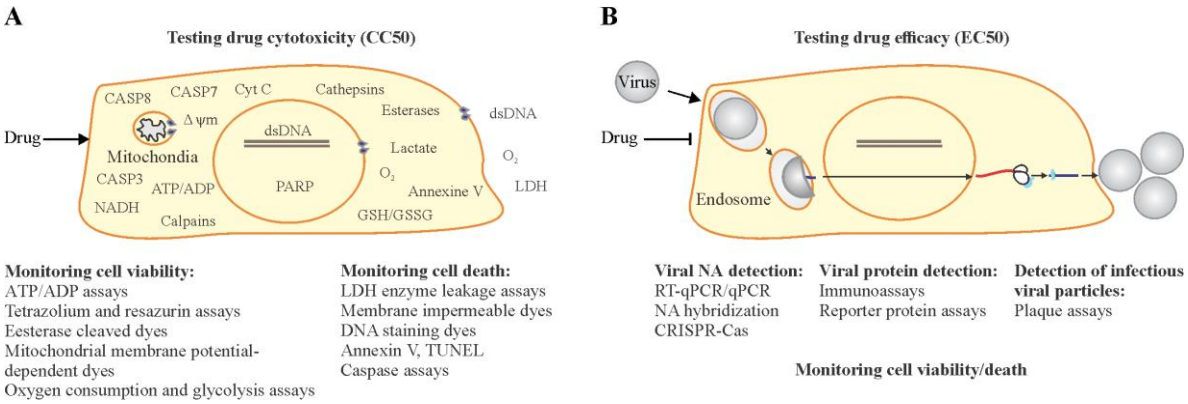


**Figure 2.** Testing BSAA toxicity (A) and efficacy (B) in immortalized cell cultures and co-cultures.

## 3. Pre-clinical evaluation of safe-in-man BSAAs

### *3.1. Evaluation of safe-in-man BSAAs in human primary cell cultures*

Immortalized cancerous 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.) [36]. 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 [37-40]. 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 [38, 41-44]. 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. For example, common genetic variants in IRF7 and IFITM3 gene loci which is associated with innate immune responses to FLUAV infection in monocyte-derived dendritic cells, could influence on the results of BSAA efficacy experiments [45, 46].

### *3.2. Evaluation of safe-in-man BSAAs in human embryonic stem cell and human induced pluripotent stem cell cultures and co-cultures (organoids)*

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) [47-52].

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

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and flaviviruses [47, 53-59] (https://organovir.com/). 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.

*3.2. Evaluation of safe-in-man 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. 3) [40, 60-64]. 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 [65-67]. 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 [68, 69]. 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 and disease manifestation, susceptibility, immune responses, pathogenesis, and pharmacokinetics [70, 71].

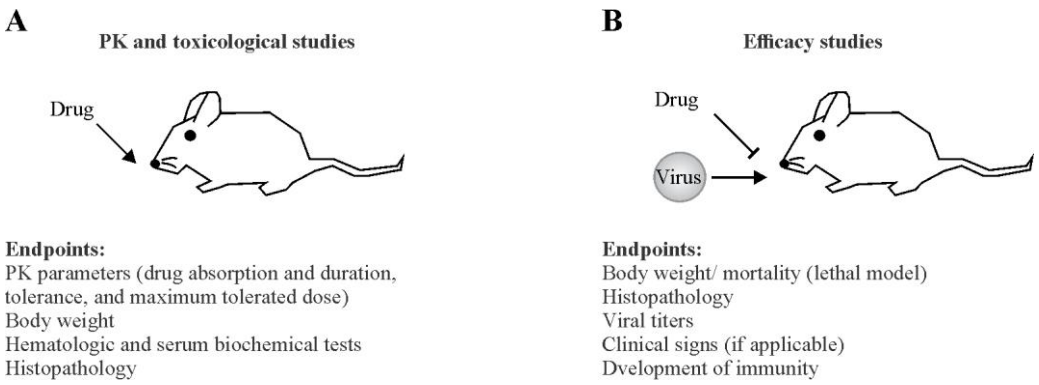


**Figure 3.** Testing toxicity and efficacy of BSAAs in animal models. (A) PK/PD and toxicity studies. (B) Efficacy studies. If BSAA is repositioned from another disease (i.e. its PK/PD and toxicity profiles are available for the animal model) it could bypass the safety studies.

## 4. Clinical trials and post-clinical studies of safe-in-man BSAAs

Clinical trials are the most critical and time-consuming step of a drug candidates' journey to being approved (Fig. 4). 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.: i.e. they have been administered at sub-therapeutic doses to healthy volunteers to ensure the drugs are not harmful to the participants. However, 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 [72]. 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 [72, 73].

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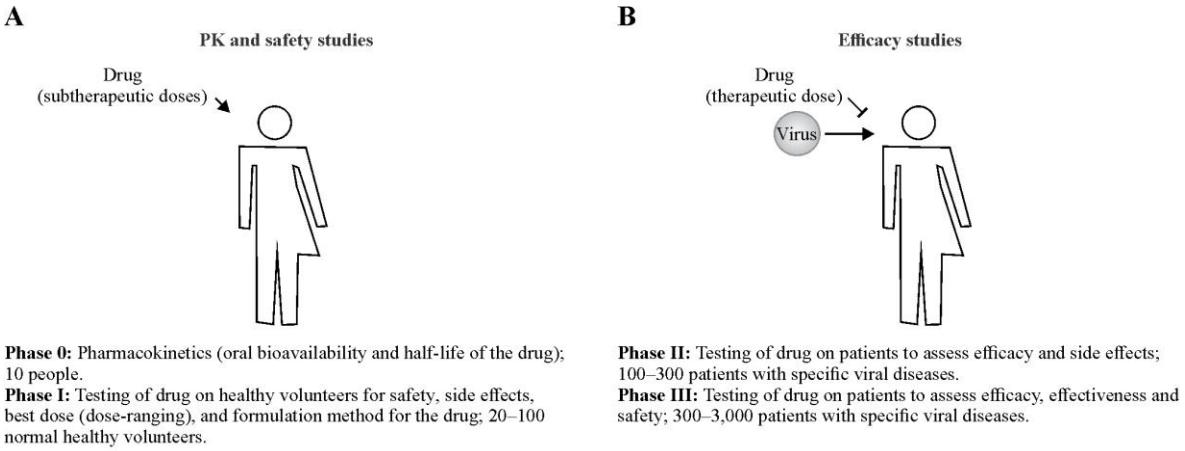


**Figure 4**. Clinical trials of BSAAs. (A) Pharmacokinetics (PK) and safety studies. (B). Efficacy studies. If BSAA is repositioned from another disease (i.e. its safety profile in man is available) it will bypass the PK and safety studies.

## 5. Database of safe-in-man BSAAs

We have developed a database for safe-in-man BSAAs, which is available at https://drugvirus.info/. The drug annotations were obtained from PubChem, DrugBank, DrugCentral, PubMed and clinicaltrials.gov databases (Table S1) [74-76]. The information on virus families were exported from Virus Pathogen Database and Analysis Resource (Table S2) [77]. The database summarizes activities and developmental statuses of 118 compounds which altogether target 83 human viruses (Fig. 5). The database allows interactive exploration of virus-BSAA interactions. A feedback form is available on the website. The website will be updated upon request or as soon as a new save-in-man BSAA emerge or novel activity of existing BSAA is reported.

The database includes 21 BSAAs which 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).

The database also includes 48 safe-in-man BSAAs, which 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).

The rest of safe-in-man BSAAs are still in pre-clinical or discovery stages. Of the drugs in this group, niclosamide is one of the interesting compounds because it showed the broadest spectrum of activities in vitro and in some cases in vivo [57, 78-85] (10.20944/preprints201909.0128.v1). We believe that emetine and gemcitabine could be also pursued as potential BSAA candidates [38, 86] (10.20944/preprints201909.0128.v1). ABT-263, also known as navitoclax, is another interesting BSAA, which is, by contrast to other compounds, facilitates death of infected cells without affecting non-infected cells [17, 87, 88].

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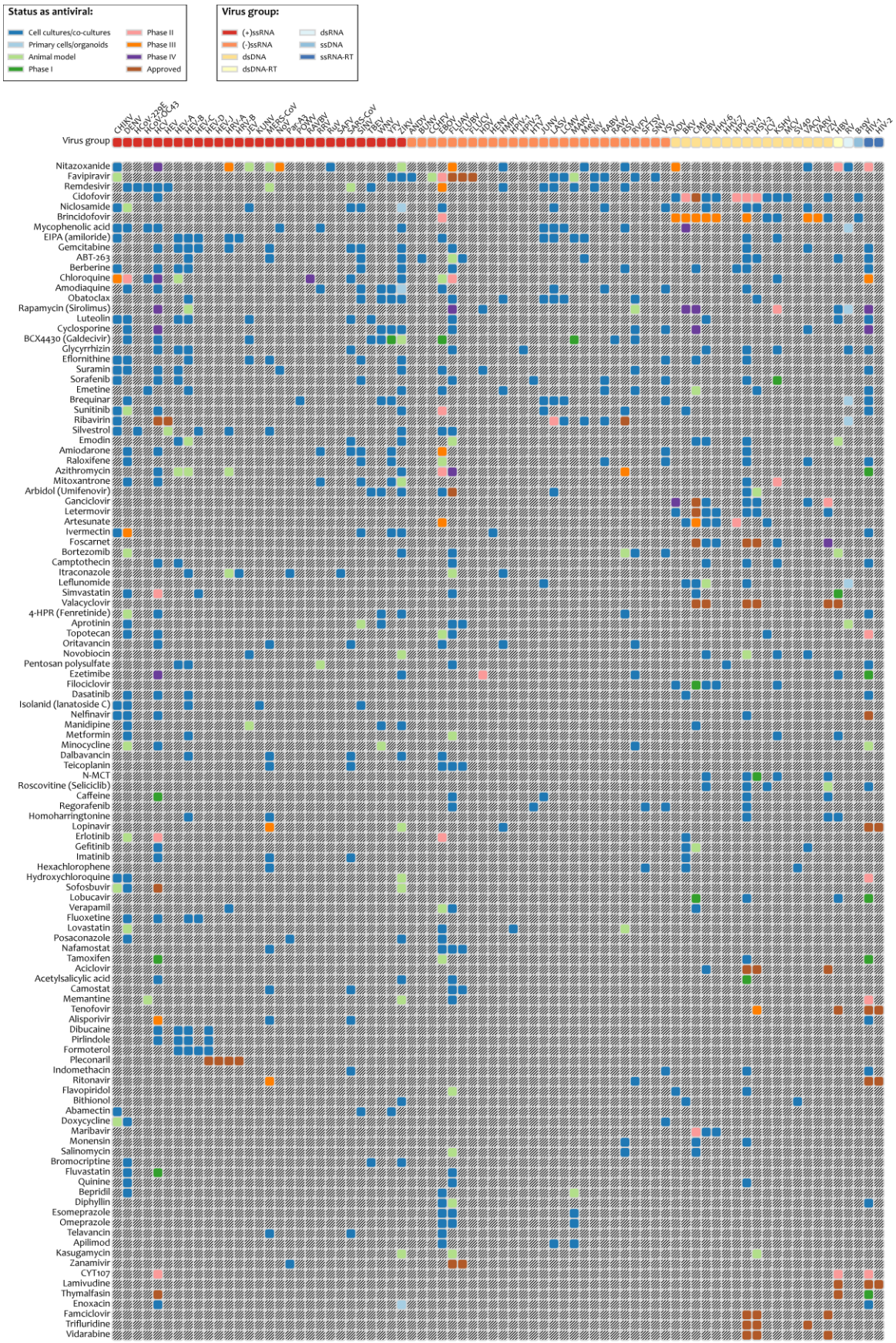

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**Figure 5**. Hundred and seventeen safe-in-man broad-spectrum antiviral agents (BSAAs) and viruses they inhibit. A snapshot is taken from https://drugvirus.info/ website. Viruses are clustered by virus groups. BSAAs are ranged from the highest to 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.

## 6. Conclusions and future perspectives

Here, we reviewed the processes of discovery and development of safe-in-man BSAAs. However, emerging BSAAs, such as 5,6-dimethoxyindan-1-one, saliphenylhalamide, and GS-5734 [19, 38, 86, 89-91], whose safety profiles in humans are not yet available, could serve as valuable antivirals in the future, pending the results of further pre-clinical and clinical investigations.

BSAAs could be combined with other antiviral agents to obtain synergistic or additive effects [14, 92]. For example, it was reported that obatoclax and saliphenylhalamide, as well as gemcitabine and pimodivir (JNJ872) possessed synergistic effects against ZIKV and FLUAV infections in vitro, respectively [86, 93]. Moreover, many combination therapies, which include BSAAs, became a standard for the treatment of HIV and HCV infections. These include abacavir/dolutegravir/lamivudine (Triumeq), darunavir/cobicistat/emtricitabine/tenofovir (Symtuza), lopinavir/ritonavir (Kaletra), ledipasvir/sofosbuvir, sofosbuvir/velpatasvir, Ombitasvir/paritaprevir/ritonavir+dasabuvir+ribavirin [94-96].

By contrast to individual drugs, combinations of 2-3 BSAAs could be used to target even broader range of viruses [14, 97]. 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 infections of viruses belonging to 11 families.

Fifty BSAs possess not only antiviral but also antibacterial activity (Fig. 6; Table S1) [98]. 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 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) [99].

In addition, BSAAs showed activity against a wide range of other medically important human pathogens, including fungi, protozoa and parasites (Table S1) [100], 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. 6). If confirmed, this could lead to development of broad-spectrum anti-infective drugs.

BSAAs could also serve as treatment of other co-morbidities simplifying the therapy and lowering its cost (Table S1). For example, the concomitant actions of ezetimibe could be beneficial for treatment of both hypertension and HCV, HDV and HIV infections in patients with these co-morbidities (NCT00908011, NCT00099684, and NCT00843661).

In conclusion, BSAAs could play a pivotal role in the battle against emerging and re-emerging viral diseases. Development 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.

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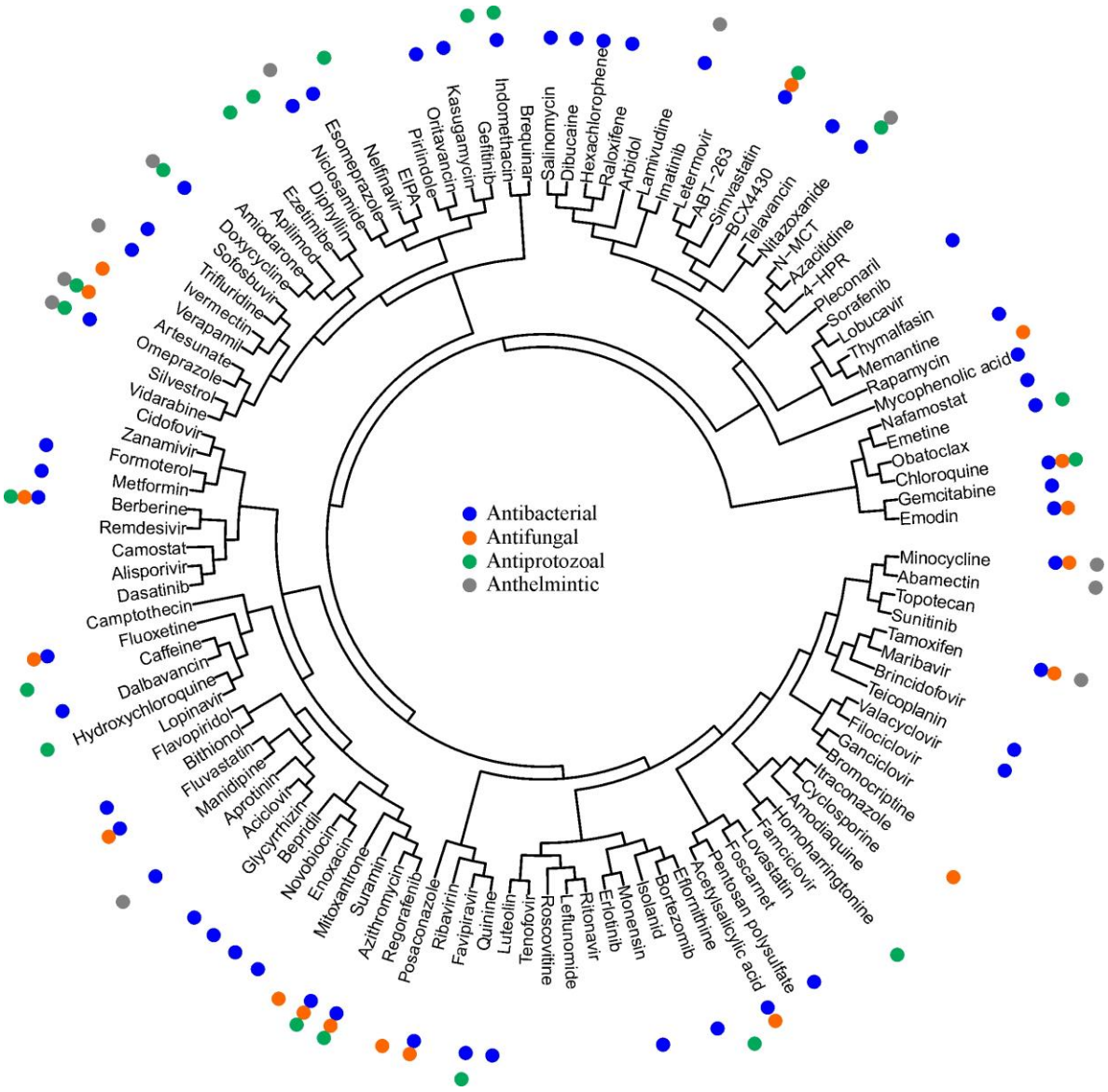


**Figure 6**. Structure-activity relationship of safe-in-man broad-spectrum antiviral agents.

**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.

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

## References

1. Disease, G. B. D., 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, G. B. D. & 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, C. R. & Fletcher, N. F. (2012) Emerging virus diseases: can we ever expect the unexpected?, *Emerg Microbes Infect.* **1**, e46.
5. Marston, H. D., Folkers, G. K., Morens, D. M. & Fauci, A. S. (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, P. I., Merits, A., Bjoras, M. & Kainov, D. (2019) Expanding the activity spectrum of antiviral agents, *Drug Discov Today.* **24**, 1224-1228.
10. Bosl, K., Ianevski, A., Than, T. T., Andersen, P. I., Kuivanen, S., Teppor, M., Zusinaite, E., Dumpis, U., Vitkauskiene, A., Cox, R. J., Kallio-Kokko, H., Bergqvist, A., Tenson, T., Merits, A., Oksenych, V., Bjoras, M., Anthonsen, M., Shum, D., Kaarbo, M., Vapalahti, O., Windisch, M. P., Superti-Furga, G., Snijder, B., Kainov, D. & Kandasamy, R. K. (2019) Common Nodes of Virus–Host Interaction Revealed Through an Integrated Network Analysis, *Front Immunology.* **Accepted**.
11. Nishimura, Y. & Hara, H. (2018) Editorial: Drug Repositioning: Current Advances and Future Perspectives, *Front Pharmacol.* **9**, 1068.
12. Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., 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.
13. 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**.
14. Zheng, W., Sun, W. & Simeonov, A. (2018) Drug repurposing screens and synergistic drug-combinations for infectious diseases, *Br J Pharmacol.* **175**, 181-191.
15. Meneghini, K. A. & Hamasaki, D. I. (1967) The electroretinogram of the iguana and Tokay gecko, *Vision Res.* **7**, 243-51.
16. 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**.
17. Bulanova, D., Ianevski, A., Bugai, A., Akimov, Y., Kuivanen, S., Paavilainen, H., Kakkola, L., Nandania, J., Turunen, L., Ohman, T., Ala-Hongisto, H., Pesonen, H. M., Kuisma, M. S., Honkimaa, A., Walton, E. L., Oksenych, V., Lorey, M. B., Guschin, D., Shim, J., Kim, J., Than, T. T., Chang, S.

Y., Hukkanen, V., Kulesskiy, E., Marjomaki, V. S., Julkunen, I., Nyman, T. A., Matikainen, S., Saarela, J. S., Sane, F., Hober, D., Gabriel, G., De Brabander, J. K., Martikainen, M., Windisch, M. P., Min, J. Y., Bruzzone, R., Aittokallio, T., Vaha-Koskela, M., Vapalahti, O., Pulk, A., Velagapudi, V. & Kainov, D. E. (2017) Antiviral Properties of Chemical Inhibitors of Cellular Anti-Apoptotic Bcl-2 Proteins, *Viruses.* **9**.

18. 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, S. A., Dumpis, U., Vitkauskiene, A., Ohrmalm, C., Bondeson, K., Bergqvist, A., Aittokallio, T., Cox, R. J., 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.
19. Muller, K. H., Spoden, G. A., Scheffer, K. D., Brunnhofer, R., De Brabander, J. K., Maier, M. E., Florin, L. & Muller, C. P. (2014) Inhibition by cellular vacuolar ATPase impairs human papillomavirus uncoating and infection, *Antimicrob Agents Chemother.* **58**, 2905-11.
20. Sarzotti-Kelsoe, M., Bailer, R. T., Turk, E., Lin, C. L., Bilska, M., Greene, K. M., Gao, H., Todd, C. A., Ozaki, D. A., Seaman, M. S., Mascola, J. R. & Montefiori, D. C. (2014) Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1, *J Immunol Methods.* **409**, 131-46.
21. 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, *Journal of virological methods.* **233**, 56-61.
22. 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.
23. Lee, M., Yang, J., Jo, E., Lee, J. Y., Kim, H. Y., Bartenschlager, R., Shin, E. C., Bae, Y. S. & Windisch, M. P. (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.
24. 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.
25. de Graaf, M., Herfst, S., Schrauwen, E. J., van den Hoogen, B. G., Osterhaus, A. D. & Fouchier, R. A. (2007) An improved plaque reduction virus neutralization assay for human metapneumovirus, *J Virol Methods.* **143**, 169-74.
26. 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.
27. Jupille, H. J., Oko, L., Stoermer, K. A., Heise, M. T., Mahalingam, S., Gunn, B. M. & Morrison, T. E. (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.
28. Konig, A., Yang, J., Jo, E., Park, K. H. P., Kim, H., Than, T. T., Song, X., Qi, X., Dai, X., Park, S., Shum, D., Ryu, W. S., Kim, J. H., Yoon, S. K., Park, J. Y., Ahn, S. H., Han, K. H., Gerlich, W. H. & Windisch, M. P. (2019) Efficient long-term amplification of hepatitis B virus isolates after infection of slow proliferating HepG2-NTCP cells, *J Hepatol.* **71**, 289-300.

29. Fischer, C., Torres, M. C., Patel, P., Moreira-Soto, A., Gould, E. A., Charrel, R. N., de Lamballerie, X., Nogueira, R. M. R., Sequeira, P. C., Rodrigues, C. D. S., Kümmerer, B. M., Drosten, C., Landt, O., Bispo de Filippis, A. M. & Drexler, J. F. (2017) Lineage-Specific Real-Time RT-PCR for Yellow Fever Virus Outbreak Surveillance, Brazil, *Emerging infectious diseases.* **23**, 1867-71.
30. 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.
31. Landry, M. L. (1990) Nucleic acid hybridization in viral diagnosis, *Clinical Biochemistry.* **23**, 267-277.
32. 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 research.* **186**, 20-31.
33. Perez, J. T., Garcia-Sastre, A. & Manicassamy, B. (2013) Insertion of a GFP reporter gene in influenza virus, *Current protocols in microbiology.* **Chapter 15**, Unit 15G.4.
34. Sashital, D. G. (2018) Pathogen detection in the CRISPR-Cas era, *Genome Med.* **10**, 32-32.
35. Zhou, L., Peng, R., Zhang, R. & Li, J. (2018) The applications of CRISPR/Cas system in molecular detection, *J Cell Mol Med.* **22**, 5807-5815.
36. Carter, M. & Shieh, J. (2015) Chapter 14 - Cell Culture Techniques in *Guide to Research Techniques in Neuroscience (Second Edition)* (Carter, M. & Shieh, J., eds) pp. 295-310, Academic Press, San Diego.
37. Postnikova, E., Cong, Y., DeWald, L. E., Dyall, J., Yu, S., Hart, B. J., Zhou, H., Gross, R., Logue, J., Cai, Y., Deiuliis, N., Michelotti, J., Honko, A. N., Bennett, R. S., Holbrook, M. R., Olinger, G. G., Hensley, L. E. & Jahrling, P. B. (2018) Testing therapeutics in cell-based assays: Factors that influence the apparent potency of drugs, *PLoS One.* **13**, e0194880.
38. Denisova, O. V., 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, R. M., De Brabander, J. K., Saelens, X. & Kainov, D. E. (2012) Obatoclax, saliphenylhalamide, and gemcitabine inhibit influenza a virus infection, *J Biol Chem.* **287**, 35324-32.
39. 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.
40. Alves, M. P., Vielle, N. J., Thiel, V. & Pfaender, S. (2018) Research Models and Tools for the Identification of Antivirals and Therapeutics against Zika Virus Infection, *Viruses.* **10**.
41. Fink, S. L., Vojtech, L., Wagoner, J., Slivinski, N. S. J., Jackson, K. J., Wang, R., Khadka, S., Luthra, P., Basler, C. F. & Polyak, S. J. (2018) The Antiviral Drug Arbidol Inhibits Zika Virus, *Sci Rep.* **8**, 8989.
42. Robinson, C. L., Chong, A. C. N., Ashbrook, A. W., Jeng, G., Jin, J., Chen, H., Tang, E. I., Martin, L. A., Kim, R. S., Kenyon, R. M., Do, E., Luna, J. M., Saeed, M., Zeltser, L., Ralph, H., Dudley, V. L., Goldstein, M., Rice, C. M., Cheng, C. Y., Seandel, M. & Chen, S. (2018) Male germ cells support long-term propagation of Zika virus, *Nat Commun.* **9**, 2090.
43. Rausch, K., Hackett, B. A., Weinbren, N. L., Reeder, S. M., Sadovsky, Y., Hunter, C. A., Schultz, D. C., Coyne, C. B. & Cherry, S. (2017) Screening Bioactives Reveals Nanchangmycin as a Broad Spectrum Antiviral Active against Zika Virus, *Cell Rep.* **18**, 804-815.

44. Barrows, N. J., Campos, R. K., Powell, S. T., Prasanth, K. R., Schott-Lerner, G., Soto-Acosta, R., Galarza-Munoz, G., McGrath, E. L., Urrabaz-Garza, R., Gao, J., Wu, P., Menon, R., Saade, G., Fernandez-Salas, I., Rossi, S. L., Vasilakis, N., Routh, A., Bradrick, S. S. & Garcia-Blanco, M. A. (2016) A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection, *Cell Host Microbe.* **20**, 259-70.

45. Lee, M. N., Ye, C., Villani, A. C., Raj, T., Li, W., Eisenhaure, T. M., Imboywa, S. H., Chipendo, P. I., Ran, F. A., Slowikowski, K., Ward, L. D., Raddassi, K., McCabe, C., Lee, M. H., Frohlich, I. Y., Hafler, D. A., Kellis, M., Raychaudhuri, S., Zhang, F., Stranger, B. E., Benoist, C. O., De Jager, P. L., Regev, A. & Hacohen, N. (2014) Common genetic variants modulate pathogen-sensing responses in human dendritic cells, *Science.* **343**, 1246980.

46. Zhang, Y. H., Zhao, Y., Li, N., Peng, Y. C., Giannoulatou, E., Jin, R. H., Yan, H. P., Wu, H., Liu, J. H., Liu, N., Wang, D. Y., Shu, Y. L., Ho, L. P., 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.

47. Zhou, T., Tan, L., Cederquist, G. Y., Fan, Y., Hartley, B. J., Mukherjee, S., Tomishima, M., Brennand, K. J., Zhang, Q., Schwartz, R. E., 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.

48. Lanko, K., Eggermont, K., Patel, A., Kaptein, S., Delang, L., Verfaillie, C. M. & 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.

49. 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**.

50. Simonin, Y., Erkilic, N., Damodar, K., Cle, M., Desmetz, C., Bollore, K., Taleb, M., Torriano, S., Barthelemy, J., Dubois, G., Lajoix, A. D., 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.

51. Ferreira, A. C., Reis, P. A., de Freitas, C. S., Sacramento, C. Q., Villas Boas Hoelz, L., Bastos, M. M., 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, H. C., Boechat, N., Rehen, S. K., Bruning, K., Bozza, F. A., Bozza, P. T. & Souza, T. M. L. (2019) Beyond Members of the Flaviviridae Family, Sofosbuvir Also Inhibits Chikungunya Virus Replication, *Antimicrob Agents Chemother.* **63**.

52. Xia, Y., Carpentier, A., Cheng, X., Block, P. D., Zhao, Y., Zhang, Z., Protzer, U. & Liang, T. J. (2017) Human stem cell-derived hepatocytes as a model for hepatitis B virus infection, spreading and virus-host interactions, *J Hepatol.* **66**, 494-503.

53. Yin, Y., Chen, S., Hakim, M. S., Wang, W., Xu, L., Dang, W., Qu, C., Verhaar, A. P., Su, J., Fuhler, G. M., Peppelenbosch, M. P. & Pan, Q. (2018) 6-Thioguanine inhibits rotavirus replication through suppression of Rac1 GDP/GTP cycling, *Antiviral Res.* **156**, 92-101.

54. Watanabe, M., Buth, J. E., Vishlaghi, N., de la Torre-Ubieta, L., Taxidis, J., Khakh, B. S., Coppola, G., Pearson, C. A., Yamauchi, K., Gong, D., Dai, X., Damoiseaux, R., Aliyari, R., Liebscher, S.,

Schenke-Layland, K., Caneda, C., Huang, E. J., Zhang, Y., Cheng, G., Geschwind, D. H., Golshani, P., Sun, R. & Novitch, B. G. (2017) Self-Organized Cerebral Organoids with Human-Specific Features Predict Effective Drugs to Combat Zika Virus Infection, *Cell Rep.* **21**, 517-532.

55. Li, C., Deng, Y. Q., Wang, S., Ma, F., Aliyari, R., Huang, X. Y., Zhang, N. N., Watanabe, M., Dong, H. L., Liu, P., Li, X. F., Ye, Q., Tian, M., Hong, S., Fan, J., Zhao, H., Li, L., Vishlaghi, N., Buth, J. E., Au, C., Liu, Y., Lu, N., Du, P., Qin, F. X., Zhang, B., Gong, D., Dai, X., Sun, R., Novitch, B. G., Xu, Z., Qin, C. F. & Cheng, G. (2017) 25-Hydroxycholesterol Protects Host against Zika Virus Infection and Its Associated Microcephaly in a Mouse Model, *Immunity.* **46**, 446-456.
56. Sacramento, C. Q., de Melo, G. R., de Freitas, C. S., Rocha, N., Hoelz, L. V., Miranda, M., Fintelman-Rodrigues, N., Marttorelli, A., Ferreira, A. C., Barbosa-Lima, G., Abrantes, J. L., Vieira, Y. R., Bastos, M. M., de Mello Volotao, E., Nunes, E. P., Tschoeke, D. A., Leomil, L., Loiola, E. C., Trindade, P., Rehen, S. K., Bozza, F. A., Bozza, P. T., Boechat, N., Thompson, F. L., de Filippis, A. M., Bruning, K. & Souza, T. M. (2017) The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication, *Sci Rep.* **7**, 40920.
57. Xu, M., Lee, E. M., Wen, Z., Cheng, Y., Huang, W. K., Qian, X., Tcw, J., Kouznetsova, J., Ogden, S. C., Hammack, C., Jacob, F., Nguyen, H. N., Itkin, M., Hanna, C., Shinn, P., Allen, C., Michael, S. G., Simeonov, A., Huang, W., Christian, K. M., Goate, A., Brennand, K. J., Huang, R., Xia, M., Ming, G. L., 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.
58. Yin, Y., Wang, Y., Dang, W., Xu, L., Su, J., Zhou, X., Wang, W., Felczak, K., van der Laan, L. J., Pankiewicz, K. W., van der Eijk, A. A., Bijvelds, M., Sprengers, D., de Jonge, H., Koopmans, M. P., Metselaar, H. J., Peppelenbosch, M. P. & Pan, Q. (2016) Mycophenolic acid potently inhibits rotavirus infection with a high barrier to resistance development, *Antiviral Res.* **133**, 41-9.
59. Yin, Y., Bijvelds, M., Dang, W., Xu, L., van der Eijk, A. A., Knipping, K., Tuysuz, N., Dekkers, J. F., Wang, Y., de Jonge, J., Sprengers, D., van der Laan, L. J., Beekman, J. M., Ten Berge, D., Metselaar, H. J., de Jonge, H., Koopmans, M. P., Peppelenbosch, M. P. & Pan, Q. (2015) Modeling rotavirus infection and antiviral therapy using primary intestinal organoids, *Antiviral Res.* **123**, 120-31.
60. Morrison, T. E. & Diamond, M. S. (2017) Animal Models of Zika Virus Infection, Pathogenesis, and Immunity, *J Virol.* **91**.
61. Haese, N. N., Broeckel, R. M., Hawman, D. W., Heise, M. T., Morrison, T. E. & Streblow, D. N. (2016) Animal Models of Chikungunya Virus Infection and Disease, *J Infect Dis.* **214**, S482-S487.
62. Taylor, G. (2017) Animal models of respiratory syncytial virus infection, *Vaccine.* **35**, 469-480.
63. Thangavel, R. R. & Bouvier, N. M. (2014) Animal models for influenza virus pathogenesis, transmission, and immunology, *J Immunol Methods.* **410**, 60-79.
64. Louz, D., Bergmans, H. E., Loos, B. P. & Hoeben, R. C. (2013) Animal models in virus research: their utility and limitations, *Crit Rev Microbiol.* **39**, 325-61.
65. Alabaster V. & In Vivo Pharmacology Training Group (2002) The fall and rise of in vivo pharmacology, *Trends in pharmacological sciences.* **23**, 13-8.
66. Rizk, M. L., Zou, L., Savic, R. M. & Dooley, K. E. (2017) Importance of Drug Pharmacokinetics at the Site of Action, *Clin Transl Sci.* **10**, 133-142.
67. Parasuraman, S. (2011) Toxicological screening, *J Pharmacol Pharmacother.* **2**, 74-79.

68. Smee, D. F. & Barnard, D. L. (2013) Methods for evaluation of antiviral efficacy against influenza virus infections in animal models, *Methods in molecular biology (Clifton, NJ).* **1030**, 407-25.
69. Oh, D. Y. & Hurt, A. C. (2016) Using the Ferret as an Animal Model for Investigating Influenza Antiviral Effectiveness, *Frontiers in Microbiology.* **7**.
70. Barré-Sinoussi, F. & Montagutelli, X. (2015) Animal models are essential to biological research: issues and perspectives, *Future Sci OA.* **1**, FSO63-FSO63.
71. Shanks, N., Greek, R. & Greek, J. (2009) Are animal models predictive for humans?, *Philos Ethics Humanit Med.* **4**, 2-2.
72. U.S Food and Drug Administration (2018) The Drug Development Process: Step 3 in
73. Umscheid, C. A., Margolis, D. J. & Grossman, C. E. (2011) Key concepts of clinical trials: a narrative review, *Postgrad Med.* **123**, 194-204.
74. Ursu, O., Holmes, J., Bologa, C. G., Yang, J. J., Mathias, S. L., Stathias, V., Nguyen, D. T., Schurer, S. & Oprea, T. (2019) DrugCentral 2018: an update, *Nucleic Acids Res.* **47**, D963-D970.
75. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J. & Bolton, E. E. (2019) PubChem 2019 update: improved access to chemical data, *Nucleic Acids Res.* **47**, D1102-D1109.
76. Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., 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.
77. Pickett, B. E., Greer, D. S., Zhang, Y., Stewart, L., Zhou, L., Sun, G., Gu, Z., Kumar, S., Zaremba, S., Larsen, C. N., Jen, W., Klem, E. B. & Scheuermann, R. H. (2012) Virus pathogen database and analysis resource (ViPR): a comprehensive bioinformatics database and analysis resource for the coronavirus research community, *Viruses.* **4**, 3209-26.
78. Mazzon, M., Ortega-Prieto, A. M., Imrie, D., Luft, C., Hess, L., Czieso, S., Grove, J., Skelton, J. K., Farleigh, L., Bugert, J. J., 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**.
79. Hulseberg, C. E., Feneant, L., Szymanska-de Wijs, K. M., Kessler, N. P., Nelson, E. A., Shoemaker, C. J., Schmaljohn, C. S., Polyak, S. J. & White, J. M. (2019) Arbidol and Other Low-Molecular-Weight Drugs That Inhibit Lassa and Ebola Viruses, *J Virol.* **93**.
80. Kao, J. C., HuangFu, W. C., Tsai, T. T., Ho, M. R., Jhan, M. K., Shen, T. J., Tseng, P. C., Wang, Y. T. & Lin, C. F. (2018) The antiparasitic drug niclosamide inhibits dengue virus infection by interfering with endosomal acidification independent of mTOR, *PLoS Negl Trop Dis.* **12**, e0006715.
81. Cairns, D. M., Boorgu, D., Levin, M. & Kaplan, D. L. (2018) Niclosamide rescues microcephaly in a humanized in vivo model of Zika infection using human induced neural stem cells, *Biol Open.* **7**.
82. 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.
83. Wang, Y. M., Lu, J. W., Lin, C. C., Chin, Y. F., Wu, T. Y., Lin, L. I., Lai, Z. Z., Kuo, S. C. & Ho, Y. J. (2016) Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission, *Antiviral Res.* **135**, 81-90.

84. 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.

85. Wu, C. J., Jan, J. T., Chen, C. M., Hsieh, H. P., Hwang, D. R., Liu, H. W., Liu, C. Y., Huang, H. W., Chen, S. C., Hong, C. F., Lin, R. K., Chao, Y. S. & Hsu, J. T. (2004) Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide, *Antimicrob Agents Chemother.* **48**, 2693-6.

86. Kuivanen, S., Bespalov, M. M., Nandania, J., Ianevski, A., Velagapudi, V., De Brabander, J. K., Kainov, D. E. & 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.

87. Kakkola, L., Denisova, O. V., Tynell, J., Viiliainen, J., Ysenbaert, T., Matos, R. C., Nagaraj, A., Ohman, T., Kuivanen, S., Paavilainen, H., Feng, L., Yadav, B., Julkunen, I., Vapalahti, O., Hukkanen, V., Stenman, J., Aittokallio, T., Verschuren, E. W., Ojala, P. M., Nyman, T., Saelens, X., Dzeyk, K. & Kainov, D. E. (2013) Anticancer compound ABT-263 accelerates apoptosis in virus-infected cells and imbalances cytokine production and lowers survival rates of infected mice, *Cell Death Dis.* **4**, e742.

88. Shim, J. M., Kim, J., Tenson, T., Min, J. Y. & Kainov, D. E. (2017) Influenza Virus Infection, Interferon Response, Viral Counter-Response, and Apoptosis, *Viruses.* **9**.

89. Sheahan, T. P., Sims, A. C., Graham, R. L., Menachery, V. D., Gralinski, L. E., Case, J. B., Leist, S. R., Pyrc, K., Feng, J. Y., Trantcheva, I., Bannister, R., Park, Y., Babusis, D., Clarke, M. O., Mackman, R. L., Spahn, J. E., Palmiotti, C. A., Siegel, D., Ray, A. S., Cihlar, T., Jordan, R., Denison, M. R. & Baric, R. S. (2017) Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses, *Science translational medicine.* **9**.

90. Patil, S. A., Patil, V., Patil, R., Beaman, K. & Patil, S. A. (2017) Identification of Novel 5,6-Dimethoxyindan-1-one Derivatives as Antiviral Agents, *Medicinal chemistry (Shariqah (United Arab Emirates)).* **13**, 787-795.

91. Muller, K. H., Kainov, D. E., El Bakkouri, K., Saelens, X., De Brabander, J. K., Kittel, C., Samm, E. & Muller, C. P. (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.

92. Cheng, Y. S., Williamson, P. R. & Zheng, W. (2019) Improving therapy of severe infections through drug repurposing of synergistic combinations, *Curr Opin Pharmacol.* **48**, 92-98.

93. Fu, Y., Gaelings, L., Soderholm, S., Belanov, S., Nandania, J., Nyman, T. A., Matikainen, S., Anders, S., Velagapudi, V. & Kainov, D. E. (2016) JNJ872 inhibits influenza A virus replication without altering cellular antiviral responses, *Antiviral Res.* **133**, 23-31.

94. Moreno, S., Perno, C. F., Mallon, P. W., Behrens, G., Corbeau, P., Routy, J. P. & Darcis, G. (2019) Two-drug vs. three-drug combinations for HIV-1: Do we have enough data to make the switch?, *HIV Med.* **20 Suppl 4**, 2-12.

95. Isakov, V., Paduta, D., Viani, R. M., Enejosa, J. V., Pasechnikov, V., Znoyko, O., Ogurtsov, P., Bogomolov, P. O., Maevskaya, M. V., Chen, X. & Shulman, N. S. (2018) Ombitasvir/paritaprevir/ritonavir+dasabuvir+ribavirin for chronic hepatitis C virus genotype 1b-infected cirrhotics (TURQUOISE-IV), *Eur J Gastroenterol Hepatol.* **30**, 1073-1076.

96. De Clercq, E. (2019) Fifty Years in Search of Selective Antiviral Drugs, *J Med Chem.* **62**, 7322-7339.

 doi:10.20944/preprints201910.0144.v1



97. Foucquier, J. & Guedj, M. (2015) Analysis of drug combinations: current methodological landscape, *Pharmacology research & perspectives.* **3**, e00149.

98. Schor, S. & Einav, S. (2018) Combating Intracellular Pathogens with Repurposed Host-Targeted Drugs, *ACS Infect Dis.* **4**, 88-92.

99. Mandell, L. A., Wunderink, R. G., Anzueto, A., Bartlett, J. G., Campbell, G. D., Dean, N. C., Dowell, S. F., File, T. M., Jr., Musher, D. M., Niederman, M. S., Torres, A., Whitney, C. G., 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.

100. Montoya, M. C. & Krysan, D. J. (2018) Repurposing Estrogen Receptor Antagonists for the Treatment of Infectious Disease, *MBio.* **9**.