Potential antiviral options against SARS-CoV-2 infection

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Abstract

As of May 2020, the number of people infected with SARS-CoV-2 continues to skyrocket, with more than 4,5 million cases worldwide. Both the World Health Organization (WHO) and United Nations (UN) has highlighted the need for better control of SARS-CoV-2 infections. Developing novel virus-specific vaccines, monoclonal antibodies and antiviral drugs against SARS-CoV-2 can be time-consuming and costly. Convalescent plasma and safe-in-man broad-spectrum antivirals (BSAAs) are readily available treatment options. Here we developed a neutralization assay using SARS-CoV-2 virus and monkey Vero-E6 cells. We identified most potent sera from recovered patients for treatment of SARS-CoV-2-infected patients. We also screened 136 safe-in-man broad-spectrum antivirals against SARS-CoV-2 infection in Vero-E6 cells and identified antiviral activity of nelfinavir, salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine. We found that combinations of virus-directed drug nelfinavir with host-directed drugs, such as salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine exhibit synergistic effect against SARS-CoV-2 in vitro. Finally, we developed a website to disseminate the knowledge on available and emerging treatments of COVID-19.

Introduction

Every year, emerging and re-emerging viruses such as SARS-CoV-2, SARS-CoV, MERS-CoV, Zika virus (ZIKV), Ebola virus (EBOV), and Rift Valley fever virus (RVFV) surface from natural reservoirs to infect people (Howard and Fletcher, 2012; WHO, 2015). As of May 2020, the number of people infected with SARS-CoV-2 continues to skyrocket, with more than 4,5 million cases worldwide.

Coronaviruses (CoV) are a broad family of viruses, which include species such as SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1. CoVs are composed of single-stranded positive-sense RNA, lipid envelope and several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N). HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 are usually associated with mild, self-limiting upper respiratory tract infections like the common cold. By contrast, SARS-CoV-2, MERS-CoV or SARS-CoV infections can lead to serious disease and death. In light of the current global pandemic, both the World Health Organization (WHO) and United Nations (UN) has highlighted the need for better control of SARS-CoV-2 infections.

Developing novel virus-specific vaccines, monoclonal antibodies and antiviral drugs against SARS-CoV-2 can be time-consuming and costly (Bekerman and Einav, 2015; Check Hayden, 2018; De Clercq and Li, 2016; Debing et al., 2015; Jaishankar et al., 2018; Schor and Einav, 2018; Yu et al., 2013; Yuan et

al., 2019). Convalescent plasma and safe-in-man broad-spectrum antivirals (BSAAs) are readily available treatment option.

Human convalescent plasma is collected from recently recovered individuals, and is used to transfer passive antibody immunity to those who have recently been infected or have yet to be exposed to the virus. However, the reliability of diagnostic assays, as well as the quantity and neutralizing capacity of antibodies in the plasma should be considered. Both the WHO and European Centre for Disease Prevention and Control (ECDC) have recommended the evaluation of convalescent plasma for prevention and treatment of COVID-19 disease in controlled trials (WHO/HEO/R&D Blueprint (nCoV)/2020.1) (Casadevall and Pirofski, 2020). Indeed, several trials have shown that convalescent plasma reduces viral load and is safe for the treatment of patients with COVID-19 (Duan et al., 2020; Shen et al., 2020). Interestingly, the studies also revealed variability in specific antibody production that was related to the degree of symptomatic disease. Typically, IgM and IgG antibodies develop between 6–15 days after disease onset, with the seroprevalence increasing to near 100% within 3 weeks. Some recovered patients did not have high titers of neutralizing antibodies. This may suggest that antibody levels decline with time and/or that only a subset of patients produces high-titer responses. It is also possible that non-neutralizing antibodies and cellular responses not connected to antibody production are contributing to protection and recovery, as described for other viral diseases (Gunn et al., 2018; Jenks et al., 2019; van Erp et al., 2019).

BSAAs are small molecules that inhibit human viruses belonging to 2 or more viral families, and that have passed the first phase of clinical trials. We have recently reviewed and summarized the information on dozens of safe-in-man BSAAs in the freely accessible database (https://drugvirus.info/)(Andersen et al., 2020b; Andersen et al., 2019; Ianevski et al., 2018). Forty-six of these agents inhibited SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HCoV-NL63 and/or HCoV-HKU1. Additionally, clinical investigations have started recently into the effectiveness of BSAAs such as lopinavir, ritonavir, remdesivir, hydroxychloroquine and arbidol against COVID-19 (NCT04252664; NCT0425487; NCT04255017; NCT04261517, NCT04260594). Such BSAAs represent a promising source of drug candidates for SARS-CoV-2 prophylaxis and treatment.

Here, we report isolation of several SARS-CoV-2 strains from COVID-19 patients in Norway. We showed that UVC and high temperatures destroyed SARS-CoV-2, establishing a rationale and methodology for safe work in the laboratory. We developed a neutralization assay using SARS-CoV-2 virus and monkey Vero-E6 cells and identified most potent sera from recovered patients for treatment of SARS-CoV-2-infected patients. We also screened 136 safe-in-man broad-spectrum antivirals and identified anti-SARS-CoV-2 activity of salinomycin, nelfinavir, amodiaquine, obatoclax, emetine and homoharringtonine in vitro. We further showed that combinations of virus-directed drug nelfinavir and host-directed drugs, such as salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine act synergistically. Finally, we developed a website summarizing scientific and clinical information regarding available and emerging anti-SARS-CoV-2 options.

Materials and methods

Patient samples

We selected subjects among hospitalized infected patients. Hospitalization was determined by whether a patient was able to manage symptoms effectively at home, according to local guidelines. ICU admittance was evaluated consistently with the WHO interim guidance on “Clinical management of severe acute respiratory infection when COVID-19 is suspected” (WHO/2019-nCoV/clinical/2020.4). We collected nasopharyngeal swabs (NPS) and blood samples from patients during and after infection. The patients gave their informed consent through the koronastudien.no website (clinical trial: NCT04320732; REK: 124170). For ICU patients, consent for sample collection was received after treatment or from relatives. For children, consent for sample collection was received from their parents. Blood samples from recovered COVID-19 patients were collected from hospitalized and outpatient individuals with mild

symptoms. Donors were recruited through information at the blood collection centres websites and through national media. All patients were treated in accordance with good clinical practice, following study protocols.

Cell cultures

Human telomerase reverse transcriptase-immortalized retinal pigment epithelial RPE, lung adenocarcinoma A427 and epithelial colorectal adenocarcinoma Caco-2 cells were grown in DMEM-F12 medium supplemented with 100 U/mL penicillin/streptomycin (Pen/Strep), 2 mM L-glutamine, 10% FBS, and 0.25% sodium bicarbonate (Sigma-Aldrich, St. Louis, USA). Human large-cell lung carcinoma NCI-H460 cells were grown in RPMI medium supplied with 10% FBS and Pen-Strep. Human adenocarcinoma alveolar basal epithelial A549, human embryonic kidney HEK-293 cells and kidney epithelial cells extracted from an African green monkey (Vero-E6) were grown in DMEM medium supplemented with 10% FBS and Pen-Strep. The cell lines were maintained at 37°C with 5% CO2.

Virus isolation

The SARS-CoV-2 strains were isolated and propagated in a Biological Safety Level 3 (BSL-3) facility. 200 ul of nasopharyngeal swabs (NPS) in universal transport medium were diluted 5 times in culture medium (DMEM) supplemented with 0.2% bovine serum albumin (BSA), 0.6 µg/mL penicillin, 60 µg/mL streptomycin, and 2 mM L-glutamine and inoculated into Vero E6 cells. After 4 days of incubation, the media were collected, and the viruses were passaged once again in Vero E6 cells. After 3 days a clear CPE was detected, and virus culture was harvested. Virus concentration was determined by RT-qPCR and plaque assays.

Virus detection and quantification

Viral RNA was extracted using the NTNU_MAG_V2 protocol, a modified version of the BOMB-protocol (Oberacker et al., 2019). 2,5 or 5 µL of the eluate were analysed by RT-qPCR using CFX96 Real-Time Thermal Cycler (Bio-Rad, Hercules, California, USA) as described elsewhere (Corman et al., 2020) with some modifications. A 20 µl reaction contained 10 µl of QScript XLT One-Step RT-qPCR ToughMix (2x) (Quanta BioSciences, USA), 1 µl of each primer and probe with final concentration of primers 0,6 µM and probe 0,25 µM. 2 µl of molecular grade water. Thermal cycling was performed at 50 °C for 10 min for reverse transcription, followed by 95 °C for 1 min and then 40 cycles of 95 °C for 3 s, 60 °C for 30 s.

For testing the production of infectious virions, we titered the viruses as described in our previous studies (Bosl et al., 2019a; Bulanova et al., 2017; Ianevski et al., 2018). In summary, media from the viral culture were serially diluted from 10^{-2} to 10^{-7} in serum-free DMEM media containing 0.2% bovine serum albumin. The dilutions were applied to a monolayer of Vero-E6 cells in 6 or 24-well plates. After one hour, cells were overlaid with virus growth medium containing 1% carboxymethyl cellulose and incubated for 96 h. The cells were fixed and stained with crystal violet dye, and the plaques were calculated in each well, expressed as plaque-forming units per mL (pfu/mL).

UVC- and thermostability assays

The virus (moi 0.1) was aliquoted in Eppendorf tubes and incubated at -80, -20, 4, 20, 37, and 50°C for 48 h or at 96°C for 10 min. Alternatively, the virus was aliquoted in wells of 96 well plate without a lid. The virus was exposed to UVC (λ = 254 nm) in a biosafety cabinet. The thermo- and UVC-stability of viral RNA was analysed by RT-qPCR. Subsequently, Vero-E6 cells were infected with the virus for 72 h,

and cell viability was measured using a CellTiter-Glo assay (Promega, Madison, USA). Luminescence was read using a plate reader.

Virus neutralization assay

Approximately 4×10^4 Vero-E6 cells were seeded per well in 96-well plates. The cells were grown for 24 h in DMEM medium supplemented with 10% FBS, 100 μg/mL streptomycin and 100 IU/mL penicillin (Pen/Strep). Plasma samples were prepared in 3-fold dilutions at 7 different concentrations starting from 1 mg/mL in the virus growth medium (VGM) containing 0.2% BSA, PenStrep in DMEM. The virus (Trondheim/S5/2020) was added to the samples to achieve an moi of 0.1 and incubated for 1h at 37C. No plasma was added to the control wells. The Vero-E6 Cells were incubated for 72 h with VGM. After the incubation period, the medium was removed and a CellTiter-Glo assay was performed to measure viability.

Drug test

We have previously published a library of 136 safe-in-man BSAAs (Ianevski et al., 2018). Table S1 lists these compounds, their suppliers and catalogue numbers. To obtain 10 mM stock solutions, compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany) or milli-Q water. The solutions were stored at −80 °C until use.

Approximately 4×10^4 Vero-E6 cells were seeded per well in 96-well plates. The cells were grown for 24h in DMEM medium supplemented with 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin (Pen/Strep). The medium was replaced with VGM containing 0.2% bovine serum albumin (BSA), PenStrep in DMEM. The compounds were added to the cells in 3-fold dilutions at 8 different concentrations starting from 30 μM. No compounds were added to the control wells. The cells were mock- or virus (Trondheim/E9/2020)-infected at an moi of 0.1. After 72 h of infection, the medium was removed from the cells and a CellTiter-Glo assay was performed to measure viability.

Drug and serum sensitivity quantification

The half-maximal cytotoxic concentration (CC_{50}) for each compound was calculated based on viability/death curves obtained on mock-infected cells after non-linear regression analysis with a variable slope using GraphPad Prism software version 7.0a. The half-maximal effective concentrations (EC_{50}) were calculated based on the analysis of the viability of infected cells by fitting drug dose–response curves using four-parameter (*4PL*) logistic function *f(x)*: $f(x) = A_{min} + \frac{A_{max}-A_{min}}{1+(\frac{x}{m})^{\lambda}}$, where *f(x)* is a response value at dose *x*, A_{min} and A_{max} are the upper and lower asymptotes (minimal and maximal drug effects), *m* is the dose that produces the half-maximal effect (EC_{50} or CC_{50}), and λ is the steepness (slope) of the curve. A relative effectiveness of the drug was defined as selectivity index (*SI* = CC_{50}/EC_{50}). The threshold of SI used to differentiate between active and inactive compounds was set to 3.

Area under dose-response curve *AUC* was quantified as $AUC = \int_{x_{min}}^{x_{max}} f(x)\,dx$, using numerical integration implemented in MESS R package, where x_{max} and x_{min} are the maximal and minimal measured doses. Serum sensitivity score (*SSS*) was quantified as a normalized version of standard *AUC* (with the baseline noise subtracted, and normalization of maximal response at the highest concentration often corresponding to off-target toxicity) as $SSS = \frac{AUC-t(x_{max}-x_{min})}{(100-t)(x_{max}-x_{min})\log_{10} Amin}$, where activity threshold *t* equals 10%.

Drug combination test and synergy calculations

Cells were treated with different concentrations of a combination of two BSAAs. After 72 h, cell viability was measured using a CellTiter-Glo assay. To test whether the drug combinations act synergistically, the observed responses were compared with expected combination responses. The expected responses were calculated based on ZIP reference model using SynergyFinder web-application, version 2 (Ianevski et al., 2017).

ELISA assays

Protein concentrations in serum samples were quantified using NanoDrop. We measured the IgG and IgM in human serum using Epitope Diagnostics enzyme linked immunosorbent assays (ELISA) according to manufacturer specifications (Epitope Diagnostics, San Diego, CA). Background-corrected OD values were divided by the cutoff to generate signal-to-cutoff (S/CO) ratios. Samples with S/CO values greater than 1.0 were considered positive. The Pearson correlation coefficients were calculated by means of stats R package, with the significance determined using Student's t test.

https://sars-coronavirus-2.info/ website

We reviewed the current landscape of available diagnostic tools, as well as emerging treatment and prophylactic options for the SARS-CoV-2 pandemic, and have summarized the information in a database that can be freely accessed at https://sars-coronavirus-2.info. The information in the database was obtained from PubMed, clinicaltrials.gov, DrugBank, DrugCentral, Chinese Clinical Trials Register (ChiCTR), and EU Clinical Trials Register databases (Kim et al., 2019a; Ursu et al., 2019; Wishart et al., 2018) as well as other public sources. The website was developed with PHP v7 technology using D3.js v5 (https://d3js.org/) for visualization. The COVID-19 statistics is automatically exported from COVID-19 Data Repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (https://github.com/CSSEGISandData/COVID-19).

Results

Isolation of SARS-CoV-2 from patients with COVID-19

We isolated 7 SARS-CoV-2 strains from 22 NPS samples of COVID-19 patients using Vero-E6 cells. The RT-qPCR cycle threshold (Ct) values for the viruses were 18-20 in NPS samples and 13-15 after propagation of the viruses in Vero-E6 cells (Fig. S1a,b). We tested 7 cell lines and found that Vero-E6 was the most susceptible for virus-mediated cell death and virus amplification (Fig. S1c,d).

Effect of UVC and temperature on virus stability and infectivity

The virus was incubated at different temperatures for 48 h or exposed to UVC radiation for different time periods. The resulting virus preparations were analysed by RT-qPCR and used to infect Vero-E6 cells. Virus incubation at 37°C for 48 h or UVC exposure for 10 sec destabilized the virus and rescued infected cells from virus-mediated death (Fig. S2).

Sera from patients recovered from COVID-19 contain antibodies which neutralize SARS-CoV-2

We evaluated neutralization capacity of 7 serum samples obtained from patients recovered from COVID-19. We also used sera from patients recovered from endemic coronavirus infections and from healthy blood donors as controls. Five samples from patients recovered from COVID-19 had serum sensitivity score (SSS) > 5 and rescued >50% cells from virus-mediated death at 1 mg/mL concentration (Fig. 1a,b).

Our neutralization test of 32 samples (Table S2) showed a moderate positive correlation with IgG ($r=0.59$, $p < 0.001$) and IgM ($r=0.43$, $p = 0.01$) s/co values obtained using commercial ELISA kits that recognize

SARS-CoV-2 N protein (Fig. 1c,d). The correlation between the IgG and IgM ELISAs results was only r=0.28, p=0.11.

Repurposing safe-in-man BSAAs

Through previous literature review, we identified 136 safe-in-man BSAAs and made a database to bring this information into the public domain (https://drugvirus.info/). Recently, we have expanded on the spectrum of activities for some of these BSAAs (Andersen et al., 2020a; Andersen et al., 2019; Bosl et al., 2019b; Ianevski et al., 2019a; Ianevski et al., 2018). Some of these agents could be repositioned for treatment of SARS-CoV-2 infection.

We tested 136 BSAAs against SARS-CoV-2 in VERO-E6 cells. Remdesivir was included as a positive control (Yin et al., 2020). Seven different concentrations of the compounds were added to virus-infected cells. Cell viability was measured after 72 h to determine compound efficiency. After the initial screening, we identified apilimod, emetine, amodiaquine, obatoclax, homoharringtonine, salinomycin, arbidol, posaconazole and nelfinavir as compounds that rescued virus-infected cells from death (AUC>285; Table S1). AUC for remdesivir was 290. Interestingly, several groups of compounds possessed structure-activity relationship (Fig. 2a).

We repeated the experiment with hit compounds monitoring their toxicity and efficacy. We confirmed antiviral activity of emetine, amodiaquine, obatoclax, homoharringtonine, salinomycin, and nelfinavir (Fig 2b, c). Importantly, amodiaquine had superior SI over its analogues, chloroquine, hydroxychloroquine and quinacrine (Fig. S3). Thus, we identified and validated anti-SARS-CoV-2 activities for 6 BSAAs in Vero-E6 cell culture.

BSAA combinations

To test for potential synergism among the validated hits, we treated cells with varying concentrations of a two-drug combination and monitored cell viability (Fig. 3a,b). The observed drug combination responses were compared with expected combination responses calculated by means of zero interaction potency (ZIP) model (Ianevski et al., 2020; Ianevski et al., 2017). We found that combinations of nelfinavir with salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine were synergistic (Fig. 3c), Most Synergistic Area (MSA) scores > 7. Moreover, nelfinavir-amodiaquine combination was effective against different SARS-CoV-2 strains. Thus, we identified promising synergistic combinations against SARS-CoV-2 infections.

Sars-coronavirus-2.info website summarizes emerging antiviral options

To rapidly respond to the COVID-19 outbreak, we developed a freely accessible website summarizing novel anti-SARS-CoV-2 developments, including the tracking of therapeutic/antiviral drug development, development of vaccines, and currently approved diagnostic options around the globe.

The ‘Treatment’ section of the website summarizes 542 in-progress and completed clinical trials that test the efficacy of therapeutic agents to treat COVID-19 or complications that arise from COVID-19. These trials include over 192 unique therapeutic agents, in varying combinations and applications. Importantly, we list 47 clinical trials that are already completed or are projected to be completed by the end of May 2020. Of note among this list are trials of remdesivir, favipiravir, lopinavir/ritonavir, hydroxychloroquine, dipyridamole, and interferons alpha and beta, which all have phase 3 or 4 clinical trials projected to be completed by the end of May 2020.

The 'Prevention' section summarizes 23 current vaccine trials taking place around the globe. Although vaccine development lags considerably behind drug development, several repurposed vaccine options have also emerged. This includes trials of the cross-reactivity of the MMR (Measles, Mumps, Rubella) vaccine, as well as several trials of the BCG vaccine among high-risk populations, such as health care workers.

Finally, the 'Testing' section of the website provides a summary of 377 currently available laboratory-based and point-of-care diagnostic tests that are approved for clinical diagnosis in at least one country.

The website also includes predictions of experimental and approved drugs effective against SARS-CoV-2, as well as provides a summary of information about the coronavirus pandemic. The website allows interactive exploration of the data with built-in feedback and is available in several languages. The website is updated as soon as novel anti-SARS-CoV-2 options emerge, or the statuses of existing ones are updated.

Discussion

Here, we reported the isolation of 7 SARS-CoV-2 strains from samples of patients with COVID-19. We showed that >10 sec of UVC radiation or 48 h incubation at 37 °C neutralized SARS-CoV-2, establishing a rationale and methodology for safe work in the laboratory. These results are consistent with previous studies showing that physical factors destabilize SARS-CoV-2 and other viruses (Cadnum et al., 2020; Demongeot et al., 2020; Ianevski et al., 2019b; Sobral et al., 2020).

Neutralization tests are crucial tools for assessments of previous SARS-CoV-2 exposure and potential immunity (Duan et al., 2020; Shen et al., 2020). We have developed such a test and assessed the neutralization capacity of serum samples from patients recovered from SARS-CoV-2 infections, patients with endemic coronavirus infections and healthy blood donors. Results from our test positively correlated with those from commercial ELISA assays. However, the correlation between the IgG and IgM ELISAs results was only $r=0.28$, $p=0.11$. The difference could be associated with time of sample collection, production of immunoglobulins, or sensitivity that can be attributed to the technique and the antigen in use (for example, IgM is the first immunoglobulin to be produced in response to an antigen and can be detected during early onset of disease, whereas IgG is maintained in the body after initial exposure for long term response and can be detected after the infection has passed). Thus, combination of neutralization test and ELISA assays allowed us to identify the most potent sera from recovered patients for treatment of SARS-CoV-2-infected patients.

Drug repurposing, also called repositioning, is a strategy for generating additional value from an existing drug by targeting disease other than that for which it was originally intended (Nishimura and Hara, 2018; Pushpakom et al., 2019). 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. Therefore, repositioning of drugs to Covid-19 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 (Ianevski et al., 2019a; Pizzorno et al., 2019; Zheng et al., 2018).

We tested 136 safe-in-man BSAAs against SARS-CoV-2 in cell culture. We identified salinomycin, obatoclax, amodiaquine, nelfinavir, emetine, and homoharringtonine as anti-SARS-Cov-2 drug candidates. Nelfinavir (Viracept) is an orally bioavailable inhibitor of human immunodeficiency virus HIV-1. It targets HIV protease and is widely prescribed in combination with HIV reverse transcriptase

inhibitors for the treatment of HIV infection (Zhang et al., 2001). Molecular docking studies predicted nelfinavir binding site on SARS-CoV-2 protease (Mothay and Ramesh, 2020). Nelfinavir could also inhibit cell fusion caused by the SARS-CoV-2 S glycoprotein (https://doi.org/10.1101/2020.04.06.026476) (Musarrat et al., 2020). It also inhibits CHIKV, DENV, HCV, HSV-1, and SARS infections (https://doi.org/10.1039/C5RA14469H) (Kalu et al., 2014; Toma et al., 2009; Yamamoto et al., 2004). These studies warrant further investigations of the potential of nelfinavir alone or in combination with other drugs to inhibit SARS-CoV-2 infections.

Salinomycin is antibiotic, which is active against Gram-positive bacteria. It also inhibits FLUAV, RSV and CMV infections (Kapoor et al., 2013; Norris et al., 2018). Salinomycin was proposed to disrupt endosomal acidification and to block entry of the viruses into cells (Jang et al., 2018). This supports further development of salinomycin as an anti-SARS-CoV-2 drug candidate (Jeon et al., 2020).

Amodiaquine is a medication used to treat malaria. It also showed broad-spectrum antiviral activity. It inhibited ZIKV, DENV, HCV, MERS-CoV, SARS-CoV, SARS-CoV-2, RRV, SINV, WNV, YFV, EBOV, LASV, RABV, VSV and HSV-1 viruses (https://doi.org/10.1101/2020.03.25.008482) (Boonyasuppayakorn et al., 2014; Dyall et al., 2014; Hulseberg et al., 2019; Mazzon et al., 2019; Sakurai et al., 2018; Zhou et al., 2017). It could be pursued as a potential anti-SARS-CoV-2 drug candidate. Importantly, amodiaquine showed more potent antiviral activity than its analogues chloroquine and hydroxychloroquine, both of which are currently being seriously investigated in clinical trials as a potential anti-SARS-CoV-2 drug.

Obatoclax was originally developed as an anticancer agent. Several phase II clinical trials were completed that investigated the use of obatoclax in the treatment of leukemia, lymphoma, myelofibrosis, and mastocytosis. In addition, obatoclax showed antiviral activity against FLUAV, ZIKV, WNV, YFV, SINV, JUNV, LASV, HSV-2, EV1, HMPV, RVFV and LCMV in vitro (Andersen et al., 2019; Denisova et al., 2012; Kim et al., 2019b; Kuivanen et al., 2017; Varghese et al., 2017). It was shown that obatoclax inhibited viral endocytic uptake by targeting cellular Mcl-1 protein (Denisova et al., 2012). It may be also pursued as a potential anti-SARS-CoV-2 drug candidate.

Emetine is an antiprotozoal drug. It is also used to induce vomiting. In addition, it possessed antiviral effects against ZIKV, EBOV, RABV, CMV, HCoV-OC43, HSV-2, EV1, HMPV, RVF, FLUAV, HIV-1 and SARS-CoV-2 (Andersen et al., 2019; Chaves Valadao et al., 2015; Choy et al., 2020; MacGibeny et al., 2018; Mukhopadhyay et al., 2016; Shen et al., 2019; Yang et al., 2018) (https://doi.org/10.1101/2020.03.25.008482). Emetine was proposed to inhibit viral polymerases, though it could have some other targets (Khandelwal et al., 2017). Emetine may represent anti-SARS-CoV-2 drug candidate.

Homoharringtonine is an anticancer drug which is indicated for treatment of chronic myeloid leukaemia. It also possesses antiviral activities against HBV, MERS-CoV, HSV-1, EV1, VZV and SARS-CoV-2 (Andersen et al., 2019; Cao et al., 2015; Choy et al., 2020; Dong et al., 2018; Kim and Song, 2019; Romero et al., 2007). Homoharringtonine binds to the 80S ribosome and inhibits viral protein synthesis by interfering with chain elongation (Dong et al., 2018). It may also represent a potent anti-SARS-CoV-2 drug candidate.

Our results uncover several existing BSAAs that could be re-positioned to SARS-CoV-2 infections. Because PK/PD and toxicology studies have been performed on these compounds, they can be used against the COVID-19 epidemic with much more efficiency than newly developed antiviral compounds. This information could be used to initiate efficacy studies in vivo, saving time and resources.

Combination therapies became a standard for the treatment of HIV and HCV infections. BSAA combinations could result in better efficacy and decreased toxicity for the treatment of SARS-CoV-2 infections. We found that combinations of virus-directed nelfinavir with host directed salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine were synergistic. This finding and previous studies suggest that synergy is achieved when virus-directed and host-directed antivirals target the same stage of virus replication cycle, whereas combinations of host-directed antivirals are usually toxic for the cells (Andersen et al., 2019; Andreani et al., 2020; Fu et al., 2016; Kuivanen et al., 2017) (https://doi.org/10.1101/2020.04.14.039925).

Our future goal is to complete preclinical studies and translate our findings into trials in patients. The most effective and tolerable BSAAs or their combinations will have a global impact, improving protection of the general population from emerging and re-emerging viral infections or co-infections, the rapid management of drug-resistant strains. Our bigger ambition is to assemble a toolbox of BSAAs for the treatment of emerging and re-emerging viral infections. This toolbox can be offered to the WHO as a means for the fast identification of safe and effective antiviral options.

We have summarized the information about the status of currently available and emerging anti-SARS-CoV-2 options in the freely accessible website (https://sars-coronavirus-2.info). The website allows interactive exploration of the data with built-in feedback and is available in several languages. The website is updated as soon as novel anti-SARS-CoV-2 options emerge, or the statuses of existing ones are updated.

In conclusion, sera from recovered patients, BSAAs and other available and emerging treatments could have a pivotal role in the battle against COVID-19 and other emerging and re-emerging viral diseases. Further development of these options could save time and resources which are required for the development of alternatives - virus-specific drugs and vaccines. They could have a global impact by decreasing morbidity and mortality, maximizing the number of healthy life years, improving the quality of life of infected patients, and decreasing the costs of patient care curtailing to the impact of the current SARS-CoV-2 pandemic as well as future viral outbreaks.

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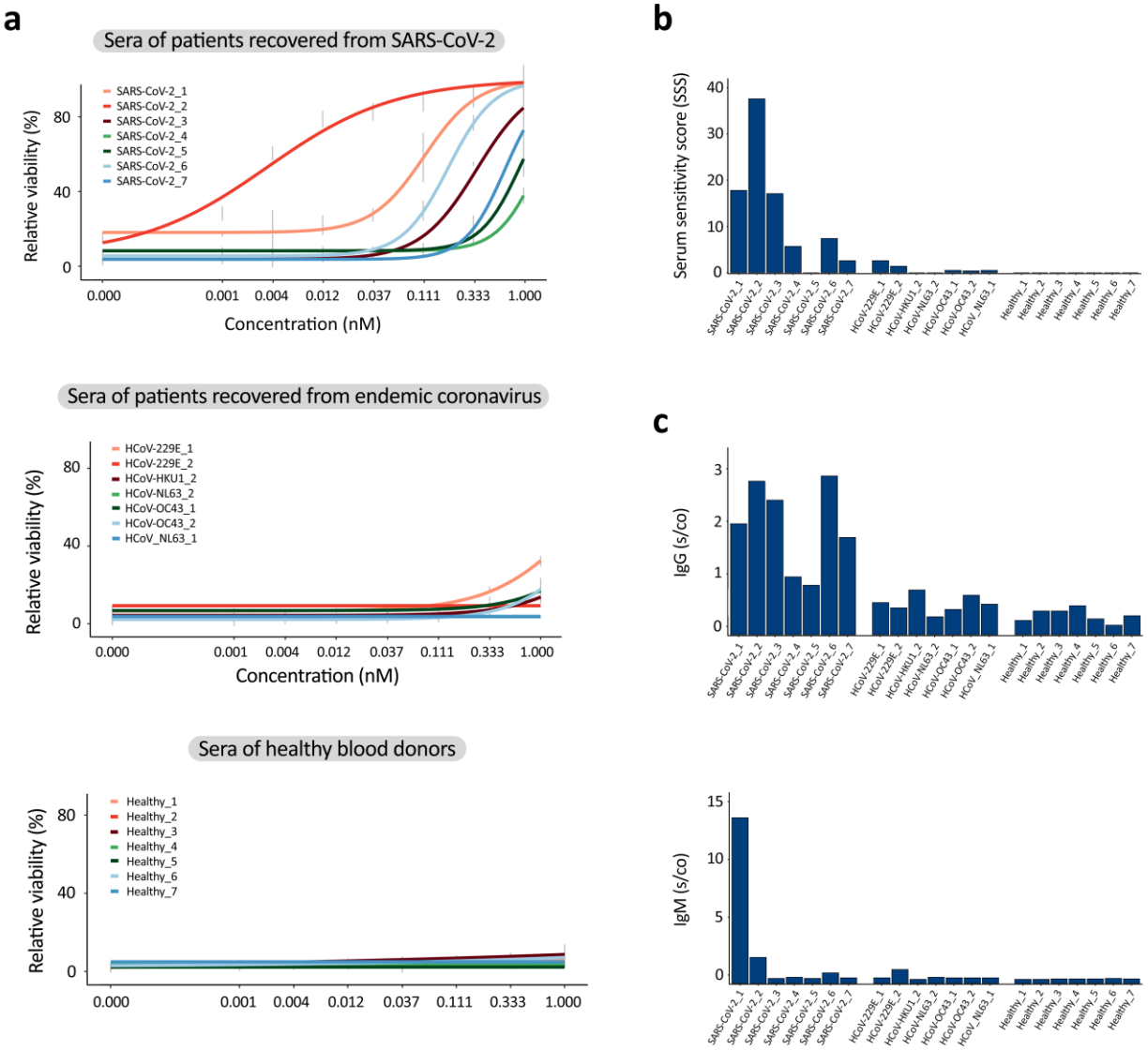
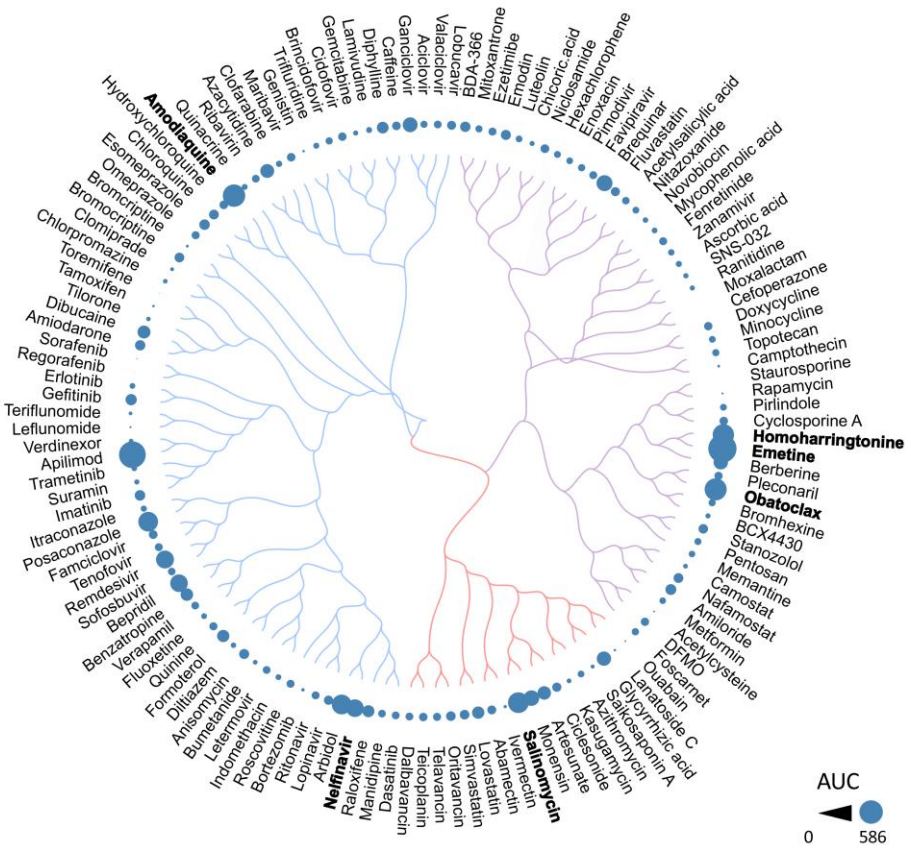
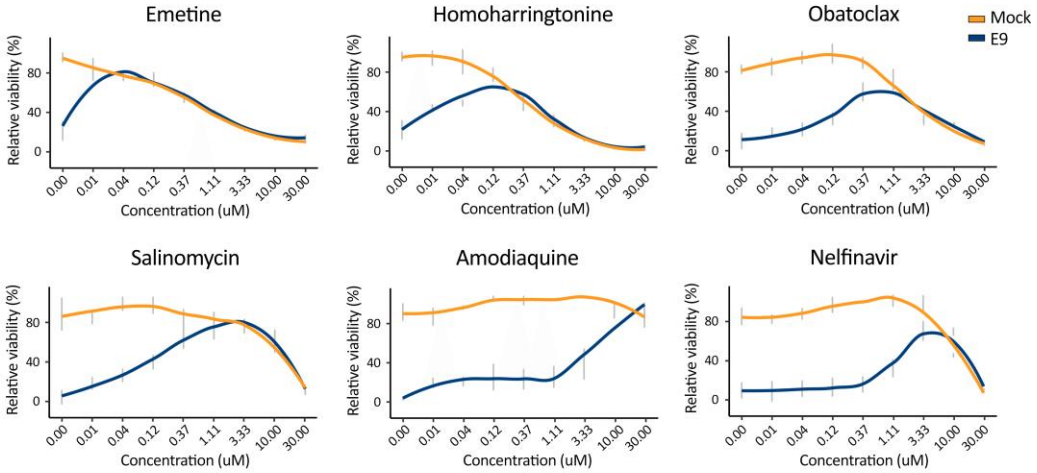


Figure 1. Sera from patients recovered from COVID-19 neutralize SARS-CoV-2 strain and prevent virus-mediated death of Vero-E6 cells. (**a**) Trondheim/E9/2020 strain (moi 0,1) was incubated with indicated concentrations of sera obtained from 7 patients recovered from COVID-19, 7 patients recovered from endemic coronavirus infections and 7 healthy blood donors. The mixtures were added to Vero-E6 cells. Cell viability was measured after 72 h. Mean ± SD, n = 3. (**b**) Serum sensitivity scores (SSS) were calculated based on curves in (a). (**c,d**) The IgG and IgM levels were analyzed in the sera of these patients using commercial Elisa kits.

a



b



c

Drug	CC_{50} (CTG), uM	EC_{50} (CTG), uM	EC_{50} (Plaque), uM	SI (CTG)	SI (Plaque)
Emetine	0.7 ± 0.4	<0.01	<0.01	>700	>700
Homohar.	0.36 ± 0.6	0.03 ± 0.02	0.06 ± 0.02	12.0	6.0
Obatoclax	2.2 ± 1.0	0.3 ± 0.1	0.5 ± 0.2	7.3	4.4
Salinomycin	13.9 ± 0.3	0.2 ± 0.1	0.4 ± 0.3	69.5	34.8
Amodiaquine	>30	4.2 ± 0.2	5.0 ± 0.7	>7.1	>6.0
Nelfinavir	9.7 ± 0.4	2.1 ± 0.6	3.1 ± 1.0	4.6	3.1

Figure 2. Anti-SARS-CoV-2 activity of safe-in man broad-spectrum antivirals in Vero-E6 cells. (**a**) Structure-antiviral activity relation of 136 BSAAs. The compounds were clustered based on their structural similarity calculated by ECPF4 fingerprints and visualized using D3 JavaScript library. The anti-SARS-CoV-2 activity of the compounds was quantified using AUC and shown as bubbles. Size of the bubbles corresponds to compounds AUCs. (**b**) Vero-E6 cells were treated with increasing concentrations of a compound and infected with Trondheim/E9/2020 strain (moi, 0.1) or mock. After 72 h the viability of the cells was determined using the CTG assay. Mean ± SD; n = 3. (**c**) Table showing half-maximal cytotoxic concentration (CC50), the half-maximal effective concentration (EC50), and selectivity indexes (SI=CC50/EC50) for selected anti-SARS-CoV-2 compounds calculated from CTG and plaque assays. Mean ± SD; n = 3.

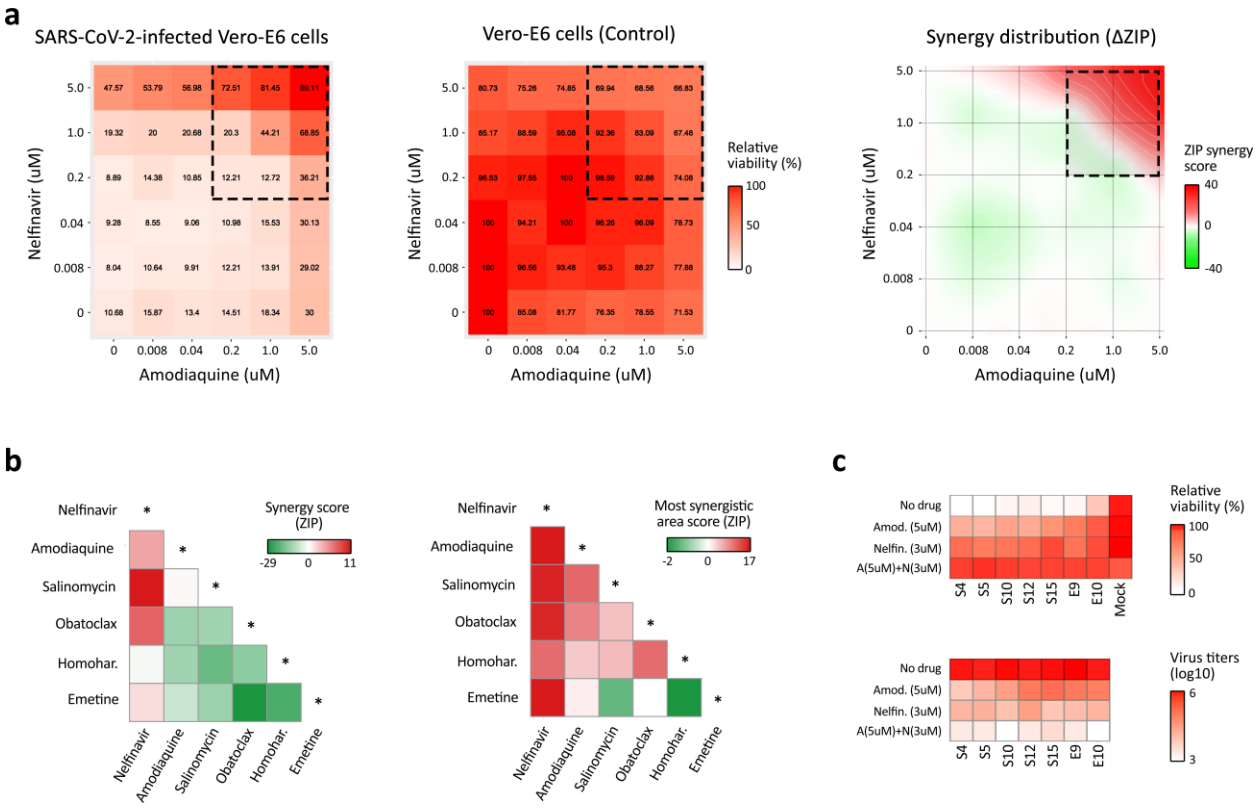


Figure 3. Effect of drug combinations on SARS-CoV-2 infection. (**a**) The representative interaction landscapes of one of the drug combinations (amodiaquine-nelfinavir) measured using CTG assay and SARS-CoV-2- and mock-infected cells (left and central panels). Synergy distribution is shown for virus-infected cells (right panel). (**b**) Synergy scores and the most synergistic area scores of 15 drug combinations. (**c**)The effect of amodiaquine-nelfinavir combination on viral replication and viability of cells infected with 7 different SARS-CoV-2 strains.