OPEN ACCESS Freely available online

Journal of Antivirals & Antiretrovirals

Editorial

Appraisal of Promising Repurposed Drug Candidates for the Treatment of SARS-CoV-2 Infection and COVID-19

Nils Von Hentig[1,2,3,4*]

[1]*Sachsenhäuser Praxis für Allgemeinmedizin, Frankfurt am Main, Germany;* [2]*HIV Center, Internal Medicine II, Klinikum der Goethe Universität, Frankfurt am Main; Germany;* [3]*Institute of Clinical Pharmacology, Klinikum der Goethe Universität, Frankfurt am Main, Germany;* [4]*Head of the Section of Clinical Pharmacology in the German AIDS Society, Germany*

ABSTRACT

Since November 2019 the severe acute respiratory syndrome (SARS) or coronavirus disease 2019 (Covid 19) caused by the new human corona virus (HCoV), is spreading around the world, causing a major loss of lives.

Keywords: Covid 19

INTRODUCTION

HCoV is a single stranded RNA virus which is part of the ß-coronavirus family (like SARS 2002 and MERS 2012). The high prevalence of hospitalization and mortality, in addition to the lack of vaccines and therapeutics forces scientists and clinicians around the world to evaluate new therapeutic options. One strategy is the repositioning of already known drugs, which were approved drugs for other indications [1].

In this case targets for research would be the receptor used by SARS to enter the cells to be infected, ACE2, the RNA polymerase or virus protease, needed for replication and embedding of new viruses in the host cells. Thus, entry inhibitors, RNA polymerase inhibitors and protease inhibitors seem to be valuable targets of research.

The Clinical trials.gov Webpage currently lists 469 trials related to the treatment of SARS-CoV-2 (14.04.2020) of which 12 is found to be completed. Only one small Chinese study with 30 patients investigated the treatment of Covid-19 with hydroxychloroquine and was published in February 2020. Taken together with a small number of further studies not listed in the CTG webpage, the current evidence of drug treatment of Covid-19 is scarce. So a huge number of studies are planned or already started.

This editorial will introduce a selection of trials with already approved drugs which are repurposed during the SARS-CoV-2 pandemic worldwide.

Principles of treatment

Nucleoside analogues have been already studied as potential therapeutic options against SARS infection. In general these drugs are taken as prodrugs and have to be phosphorylated in the target cells order to be active false components used by RNA polymerase leading to the abruption of viral RNA replication. This principle has been used as part of antiretroviral therapies since decades (REF).

A number of in-silico and in-vitro studies evaluated the binding of drugs to RNA polymerase and protease [2-5].

The proteinase processing polyprotein and virus maturation is called main protease (Mpro) and has already been evaluated as an approach towards SARS treatment [6]. We also know from HIV treatment, that HIV-1 protease inhibitors are highly able to deactivate Mpro by inactivating the active center of the protease being nominated as potential drug against SARS infection.

Therefore studies have been designed to evaluate the molecular interaction of HIV-1 protease inhibitors with Mpro.

***Corresponding author:** Nils Von Hentig, Praxis für Allgemeinmedizin / HIV-Schwerpunkt Ziegelhüttenweg 1-3, 60598 Frankfurt am Main, Germany; E-mail: Hentig@em.uni-frankfurt.de

Received date: April 18, 2020; **Accepted date:** April 23, 2020; **Published date:** April 30, 2020

Citation: Hentig NV (2020) Appraisal of Promising Repurposed Drug Candidates for the Treatment of SARS-COV-2 Infection and COVID-19. J Antivir Antiretrovir. 12:195. DOI: 10.35248/1948-5964.20.12.195

Dayer et al. have tested the inhibitory potency of HIV-1 protease inhibitors to coronavirus protease and found the inhibitory potency represented by the similarity of the tested drugs to the certain binding site of Mpro of LPV>RTV>APV>TPV>SQV>ATV>DRV>NFV>IDV, so that in this case LPV was the most powerful inhibitor of coronavirus protease. The inhibitory potency of Lopinavir in a molecular dynamic simulation was represented by the binding site similarity of 66.67%, whereas the second-generation PI Darunavir/Cobicistat revealed a binding site similarity of <30%.

Nukleos(t)ide polymerase inhibitors

Sofosbuvir, Remdesivir and Tenofovir-DF are nucleos(t)ide analogues and thus prodrugs, which are 2-3x phosphorylated in the target cells in order to become active false components used by RNA polymerase leading to the abruption of viral RNA replication. Sofosbuvir is a uridine-analogue, Remdesivir is an adenosine-analogue and tenfovir-disoprxylfumarate is a thymidine-analogue. Most of the chain-terminating drugs are nucleoside-analogues missing a functional 3'OH-group (e.g. Tenofovir, Aciclovir or Azidothymidin). Sofosbuvir is an exemption, because it is modified at the 2`OH-group for better antiviral activity against HCV. All of these can cause a premature chain termination by steric inhibition of the viral polymerase [7,8].

Remdesivir was found to be active against Ebola, although the results were not as encouraging as expected [9].

Analyses of patients (n=66, US, EU and China) who received Remdesivir in a compassionate use program showed, that Remdesivir standard dosing can improve the pulmonary status, help clear the virus and decrease fever in patients with severe Covid-19, especially those who had an O2-saturation <94% and who were oxygenated [10].

Remdesivir is now tested in trials worldwide. The two biggest trials, Solidarity and Discovery, initiated by the WHO and the French ANSM plan to enroll thousands of patients worldwide, of which 3200 will be included in countries of the European Union under the guidance of the French National Agency for Medicines and Health Products Safety (ANSM). The SOLIDARITY trial is an open randomized adaptive controlled trial, where patients are either assigned to receive Remdesivir intravenously 100 mg daily for the duration of the hospitalization and up to 10 days total course, including a loading dose of 200 mg at inclusion or Hydroxychloroquine will be given orally (in the ICU in gastrointestinal tubes) with 800 mg loading dose followed by 400 mg every day for a total of 10 days, plus standard of care treatment (SOC) in each arm [11].

The manufacturer of Remdesivir, Gilead Sciences Inc., also announced the initiation of two Phase 3 clinical studies in March 2020 to evaluate the safety and efficacy of Remdesivir in adults diagnosed with COVID-19. This randomized, open-label, multicenter studies will enroll approximately 1,000 patients at medical centers primarily across Asian countries, as well as other countries globally with high numbers of diagnosed cases. The studies will assess two dosing durations of Remdesivir, administered intravenously.

ClinicalTrials.gov lists a total number of 10 trials with Remdesivir against SARS-CoV-2 infection. (https://clinicaltrials.gov/ct2/results?cond=COVID19&term=remdesivir&cntry=&state=&city=&dist=&Search=Search)

In Italy it is planned to start a multi-center, randomized, double-blind, placebo-controlled (1:1) clinical study to explore the efficacy and safety of Favipiravir in the treatment of adult subjects with COVID-19-moderate type in July 2020. Subjects within 10 days of COVID-19 onset will be screened, and be randomized as early as possible within 24 hours following screen success. It is planned to randomize 100 subjects in a 1:1 ratio. Subjects in the test group will receive supportive care recommended in the current guidelines + Favipiravir, and subjects in the control group will receive supportive care recommended in the current guidelines + placebo control; the efficacy and safety of Favipiravir versus the placebo in the treatment of COVID-19-moderate type will be compared [12].

A Chinese multicenter interventional trial evaluates the combination of Favipiravir combined with tocilizumab. 150 participants will be assigned to receive either the combination of favipiravir or favipiravir or tocilizumab alone, each plus SOC.

Favipiravir will be given 1600 mg BID on the 1st day and 600 mg BID from day 2 to 7, p.o.

The first dose of tocilizumab is 4 ~ 8 mg/kg as i.v. infusion and the recommended dose is 400 mg. For fever patients, an additional application (the same dose as before) is given if there is still fever within 24 hours after the first dose and the interval between two medications ≥ 12 hours. The maximum of cumulative number is two, and the maximum single dose is up to 800 mg [13].

Finally, an 8-armed study with planned 320 participants will test various combinations of HIV-1 protease inhibitors, Oseltamivir, Favipiravir and Hydroxychloroquine for the treatment Covid-19 (THDMS-COVID-19) in Bangkok, Thailand. Further information is provided on https://clinicaltrials.gov/ct2/show/NCT04303299?term=favipiravir&cond=COVID19&draw=2&rank=3.

There are no current studies listed for Sofosbuvir in ClinicalTrials.gov.

Four Trials are listed for oseltamivir, a drug against influenza infection, inhibiting the neuraminidase and thus the viral spreading after infection of a patient. They do neither eliminate the virus nor are they able to substantially shorten the course of flu disease in patients. Oseltamivir is considered to be helpful in case of a pandemic in order to decrease the viral spread in society [14,15]. However, an effect against SARS-CoV-2 has never been proven in vitro or in vivo. (https://clinicaltrials.gov/ct2/results?cond=COVID19&term=oseltamivir&cntry=&state=&city=&dist=&Search=Search)

Ribavirin was tested in vitro 2004 against SARS-CoV and revealed a limited effect on the elimination of SARS-CoV-1, however the authors concluded that from their pk-data it must be expected that effective concentrations in humans will not be reached by approved human doses [16].

Side effects/tolerability of nucleosides

In general the nucleoside analogues can affect the bone marrow and blood cell count, causing hemolytic anemia, decreased hemoglobin and thrombocytopenia. Tenofovir-DF can also decrease bone density and alter kidney function with increased creatinine values. Proteinuria is a symptom of a possible Fanconi-syndrome. Nucleoside analogues also may cause mitochondrial toxicity with peripheral subcutaneous fat loss. However, these side effects usually occur after a long-term intake of NAs, in short periods of treatment such as against SARS-CoV these side effects only play a minor role. The foremost side effects in the first weeks of treatment are nausea, dizziness, headache and diarrhea.

HIV-protease inhibitors

As there is a similarity in the nucleotide sequence of SARS-CoV to SARS-CoV-2 of 79% and to MERS-CoV of 51.8%, and protease is a key enzyme for the replication of coronaviruses, there are data from previous studies in these earlier endemics, which are translated now into therapy concepts for SARS-CoV-2 and Covid-19.

Lopinavir was one of four FDA-approved drugs, which were revealed contradictory results and the question occurs why this could be [2,4,17-19]. The EC50 of Lopinavir was 8 μM which is usually reached by BID dosing of Lopinavir/Ritonavir 400/100mg in vivo, if the drug concentrations in plasma are measured as entire concentration; however the unbound fraction of Lopinavir is much lower in plasma due to ist protein binding capacity of 98%-99% [17,20].

In a retrospective cohort study, n=33 adults with laboratory-confirmed COVID-19 without Invasive ventilation, were given either oral Arbidol or LPV/r in the combination group (n=16) or oral LPV/r only in the monotherapy group (n=17) for 5-21 days. The primary endpoint was a negative conversion rate of coronavirus from the date of COVID-19 diagnosis (day7, day14), and it was assessed whether the pneumonia was progressing or improving by chest CT (day7). Baseline clinical, laboratory, and chest CT characteristics were similar between groups. The SARS-CoV-2 could not be detected for 12 (75%) of 16 patients' nasopharyngeal specimens in the combination group after 7 days, compared with 6 (35%) of 17 in the monotherapy group ($p<0.05$). After 14 days, 15 (94%) of 16 and 9 (52.9%) of 17, respectively, SARS-CoV-2 could not be detected ($p<0.05$). The chest CT scans were improving for 11 (69%) of 16 patients in the combination group after seven days, compared with 5 (29%) of 17 in the monotherapy group ($p<0.05$) [21].

In a randomized, open-label trial involving hospitalized adult patients with confirmed SARS-CoV-2 infection, and an oxygen saturation (SaO_2) of 94% or less, patients were randomly assigned in a 1:1 ratio to receive either Lopinavir-ritonavir (400 mg and 100 mg, respectively) twice a day for 14 days, in addition to standard care, or standard care alone. The primary end point was the time to clinical improvement, defined as the time from randomization to either an improvement of two points on a seven-category ordinal scale or discharge from the hospital, whichever came first. A total of 199 patients with laboratory-confirmed SARS-CoV-2 infection and Covid-19 underwent randomization; 99 were assigned to the Lopinavir-ritonavir group, and 100 to the standard-care group. Treatment with Lopinavir-ritonavir was not associated with a difference from standard care in the time to clinical improvement (hazard ratio for clinical improvement, 1.24; 95% confidence interval [CI], 0.90 to 1.72). Mortality at 28 days was similar in the Lopinavir-ritonavir group and the standard-care group (19.2% *vs.* 25.0%; difference, -5.8 percentage points; 95% CI,-17.3 to 5.7). The percentages of patients with detectable viral RNA at various time points were similar. In a modified intention-to-treat analysis, Lopinavir-ritonavir led to a median time to clinical improvement that was shorter by 1 day than that observed with standard care (hazard ratio, 1.39; 95% CI, 1.00 to 1.91). Gastrointestinal adverse events were more common in the Lopinavir-ritonavir group, but serious adverse events were more common in the standard-care group. Lopinavir-ritonavir treatment was stopped early in 13 patients (13.8%) because of adverse events [22].

Lopinavir/r was also tested in human cell lines and a mouse model against MERS-CoV and revealed lesser therapeutic effects than previously reported. Antiviral activity (EC_{50}) in a human lung cell line at concentrations of 8.5 μM was low. LPV/r did not stop weight loss or improve hemorrhage, but LPV/r improved pulmonary function in vivo, measured by accessing the FEV1 [18].

Side effects/tolerability of HIV-protease inhibitors

The side effects of HIV-PI are well known and have been extensively studies over the past two decades. The most limiting side effects occur during long-term treatment and include especially lipodystrophy and cardiovascular side effects, which can be life threatening [23]. But one has to take into account that treatment against Covid-19 will be of very limited duration, so that most probably only short-term side effects will occur, especially headache, dizziness and diarrhea, well known from the early days of HIV-1 treatment.

Antibiotics/Antimalarial

The effects of hydroxychloroquine and Chloroquine are different from the above mentioned drug classes. Antimalarial drugs have been found to be effective in treatment of SARS-1 and MERs infections in the early 2000s. Not only that they have the capacity to inhibit SARS-CoV entry into epithelial cells, or influence viral budding, they furthermore display certain anti-inflammatory effects positively influencing the clinical course of Covid 19.

SARS-CoV-2 uses the ACE2-receptor, which is expressed on human cell surface, most importantly pulmonary epithelial cells, kidneys and myocardium [24,25].

Budding of the SARS-CoV occurs in the Golgi apparatus and results in the incorporation of the envelope spike glycoprotein into the virion. Angiotensin-converting enzyme-2 (ACE2) has been identified as a functional cellular receptor of SARS-CoV spike glycoprotein [25-29].

Chloroquine is a weak base that increases the pH of acidic vesicles. When added extracellularly, the non-protonated portion of chloroquine enters the cell, where it becomes protonated and concentrated in acidic, low-pH organelles, such as endosomes, Golgi vesicles, and lysosomes.

Chloroquine has been widely used to treat human diseases, such as malaria, amoebiasis, HIV, and autoimmune diseases [30].

Preinfection chloroquine treatment reduces cellular SARS-CoV infection

In order to investigate the effect of chloroquine on SARS-CoV infection, cell-lines (permissive Vero E6 cells) were pretreated with various concentrations of chloroquine (0.1-10 μM) for 20-24 h prior to virus infection [1]. Cells were then infected with SARS-CoV, and virus antigens were visualized by indirect immunofluorescence. Microscopic examination of the control cells (untreated) revealed extensive SARS-CoV-specific immunostaining of the monolayer. A dose-dependent decrease in virus antigen-positive cells was observed starting at 0.1 μM chloroquine, and concentrations of 10 μM completely abolished SARS-CoV infection. Pretreatment with 0.1, 1, and 10 μM chloroquine reduced infectivity by 28%, 53%, and 100%, respectively [29].

Postinfection chloroquine treatment prevented the spread of SARS-CoV infection

In order to investigate the antiviral properties of chloroquine on SARS-CoV after the initiation of infection, Vero E6 cells were infected with the virus and fresh medium supplemented with various concentrations of chloroquine was added immediately after virus adsorption. Infected cells were incubated for an additional 16-18 h, after which the presence of virus antigens was analyzed by indirect immunofluorescence analysis. When chloroquine was added after the initiation of infection, there was a dramatic dose-dependent decrease in the number of virus antigen-positive cells. As little as 0.1-1 μM chloroquine reduced the infection by 50% and up to 90-94% inhibition was observed with 33-100 μM concentrations. At concentrations of chloroquine in excess of 1 μM, only a small number of individual cells were initially infected, and the spread of the infection to adjacent cells was all but eliminated. The C50 was reached at a chloroquine concentration of 4.4 ± 1.0 μM and the addition of chloroquine can effectively reduce the establishment of infection and spread of SARS-CoV if the drug is added immediately following virus adsorption [29].

Chloroquine effects interaction between ACE2 and SARS-CoV-2

The authors investigated the terminal glycosylation status of ACE2 receptor when the cells were treated with chloroquine and observed a stepwise increase in the electrophoretic mobility of ACE2 with increasing concentrations of chloroquine. At 25 μM chloroquine, the faster electrophoretic mobility of the Golgi-modified form of ACE2 was clearly evident. Hence, chloroquine impaired the terminal glycosylation of ACE2. Although ACE2 was expressed in similar quantities at the cell surface, the variations in its glycosylation status might render the ACE2-SARS-CoV interaction less efficient and inhibit virus entry when the cells are treated with chloroquine [29,31].

Still, the mechanisms of action of chloroquine on SARS-CoV are not fully understood. Immunoprecipitation results of ACE2 demonstrated that effective anti-SARS-CoV concentrations of chloroquine impaired the terminal glycosylation of ACE2. However, the flow cytometry data demonstrated that there were no significant differences in the cell surface expression of ACE2 in cells treated with chloroquine. In the case of chloroquine treatment prior to infection, the impairment of terminal glycosylation of ACE2 may result in reduced binding affinities between ACE2 and SARS-CoV spike protein. When chloroquine or NH4Cl are added after infection, these agents can rapidly raise the pH and subvert on-going fusion events between virus and endosomes, thus inhibiting the infection.

Clinical trials with Hydroxychloroquine (HCLQ) or Chloroquine (CLQ)

Up to date, one study in Shanghai, China, has been completed, evaluating the treatment of moderately ill Covid-19 patients with Hydroxychloroquine: subjects received either hydroxychloroquine 400 mg per day for 5 days (n=15), or standard of care (SOC, n=15). The primary outcome measures were the virological clearance rate of throat swabs, sputum, or lower respiratory tract secretions at day 3, or mortality at day 14. On day 7, COVID-19 nucleic acid of throat swabs was negative in 13 (86.7%) cases in the HCQ group and 14 (93.3%) cases in the control group (P>0.05). The median duration from hospitalization to virus nucleic acid negative conservation was 4 (1-9) days in HCQ group, which is comparable to that in the control group[2 (1-4) days,(U=83.5, P>0.05)]. The median time for body temperature normalization in HCQ group was 1day (0-2) after hospitalization, which was also comparable to that in the control group. Radiological progression was shown on CT images in 5 cases (33.3%) of the HCQ group and 7 cases (46.7%) of the control group, and all patients showed improvement in follow-up examination. Four cases (26.7%) of the HCQ group and 3 cases (20%) of the control group had transient diarrhea and abnormal liver function (P>0.05). The authors conclude that the prognosis of common COVID-19 patients is good. However, the results are neither valid nor very encouraging for hydroxychloroquine [32].

Side effects of HCLQ or CLQ

A 200 mg oral dose of hydroxychloroquine has a half-life of 537 hours or 22.4 days in blood, and 2963 hours or 123.5 days in plasma. Patients experiencing an overdose may present with headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions, hypokalemia, rhythm and conduction disorders including QT prolongation, torsade de pointes, ventricular tachycardia, and ventricular fibrillation [33,34]. (FDA Approved Drug Products: Hydroxychloroquine Oral Tablets) This may progress to sudden respiratory and cardiac arrest. Another concern is irreversible retina damage, when taken over a longer period. Although these side effects are rare, the result can be dramatic [35]. The use of the anti-malarial drug chloroquine to treat COVID-19 patients has recently been halted at several Swedish hospitals due to reported side effects such as cramps and loss of peripheral vision.

PrEP Trials

Since HIV-PrEP-trials could prove the effect and benefit of a prophylactic intake of HIV-1 nucleoside reverse transcriptase inhibitors in patients with risk for HIV-infection, it seems to be logic evaluating the same principle in SARS-CoV-2 infection, as the active drugs is identical.

The Mahidol University Unit/Bangkok - Oxford conducts started a worldwide trial for the prevention of SARS-CoV-2 infection in medical staff. The idea is to randomly assign 40.000 persons around the world, especially in resource poor areas of Asia and Africa to either standard dose of HCLQ/CLQ or placebo over 3 months in order to evaluate the prophylactic properties of such regimen. The investigators chose Chloroquine because of its worldwide availability also in poor countries.

Another study started in Spain in March evaluating the short-time prophylactic intake of a combination of DRV/COBI + HCLQ. The rationale for the study is that a short course of a drug (DRV/COBI for 7 days, HCLQ for 4 days) might be able to prevent disease or lessen its impact in nursing home residents, and household contacts of COVID-19 patients who have already been exposed to the virus (n=199). But potential side effects of chloroquine and hydroxychloroquine, including heart arrhythmia, are a concern. Furthermore both drugs interfere with each other using the same CYP3A4 isoenzyme in hepatic metabolism. Cobicistat is a strong inhibitor of CYP3A4, while Hydroxychloroquine is N-dealkylated by CYP3A4 to the active metabolite desethylhydroxychloroquine, as well as the inactive metabolites desethylchloroquine and bidesethylchloroquine [36,37]. Desethylhydroxychloroquine is the major metabolite.

Medication evaluated in clinical trials

Table 1: Repurposing of drugs for the treatment of SARS-CoV-2 infection and Covid-19.

Substance	Drug class	Status
Sofosbuvir	Nucleotide Polymerase inhibitor	EU-approval 01/2014 against HCV-infection, SOC
Lopinavir/Ritonavir	HIV-Proteaseinhibitor	EU-approval 1999 against HIV-infection, stand-by ART
Remdesivir	Nukleoside Analogue (Polymerase inhibitor)	EU/German Approval 04/2020 against Covid-19 in severe cases
Favipiravir	Nukleoside Analogue (Polymerase inhibitor)	Japan-Approval against SARS
Tenofovir-Disoproxylfumarate/ Emtricitabine	Nukleoside Analogue (Polymerase inhibitor)	EU-approval 2004 against HIV-infection
Ribavirin	Nukleoside Analogue (Polymerase inhibitor)	EU-approval against HCV-infection, out of use
Chloroquine	Antimalarial	Approval against Malaria, out of use in the EU
Hydroxychloroquine	Antimalarial	Switzerland-Approval against Malaria 1998

Table 2: Past Clinical Trials.

Substance	Regimen	Status
Remdesivir	RDV, n=66, case reports	Compassionate Use [10]
Favipiravir	Favipiravir vs. LPV/r, n=33	Small RCT [38]
Ribavirin	RBV, n=x	Case reports in SARS [39-42]
Lopinavir/r	LPV/r, n=x	Small uncontrolled retrospective trials in SARS-CoV and MERS, case reports in SARS-CoV [21,41,43-45]
Chloroquine	CLQ, n=x	Case reports [46]
Hydroxychloroquine	HCLQ vs. Placebo, n=30	Small RoT [47]
Azithromycin	AZM vs. Placebo, n=30	Small RoT [47]

Table 3: A selection of current clinical trials.

Trial name	Regimen	Status
Solidarity	RDV vs. CLQ/HCLQ vs. LPV/r vs. LPV/r + ß-IF Worldwide outside EU, xx Patients, 03/2020-03/2021	Recruiting
Discovery	EU Solidarity Amendment, 3200 patients in EU + 800 patients in F. 03/2020-03/2021	Recruiting
Vico	Vietnam, Chloroquine vs. placebo, RCT, n=250 https://clinicaltrials.gov/ct2/show/NCT04328493	Recruiting
No covid-19	Norway, SOC+Chloroquine vs. SOC, open-label randomized, n=202 https://clinicaltrials.gov/ct2/show/NCT04316377	Recruiting
Gilead	U.S.A., Remdesivir in two doses; n=1000, RCT Phase III	Recruiting
Germany	Remdesivir, n=1000, dose 1.day 100mg BID, day 2-7 100mg QD n=600 mild- moderate conditions, n=400 severe Covid-19	Recruiting

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7105280/pdf/rpsp-44-e40.pdf

Table 4: Suggested Combinations.

Substance	Drug Class	Probable Use
Remdesivir + LPV/r	NPI + PI	Favorable, scarce data, monitor DDI of LPV/r
Remdesivir + HCLQ	NPI + Antimalarial	Possible, monitor HCLQ side effects
Truvada + LPV/r	NRTI + PI	Possible, moderate side effects, monitor DDI of LPV/r
Favipiravir + LPV/r	NPI + PI	Favorable, scarce but encouraging data, monitor DDI of LPV/r
DRV/COBI + HCLQ	PI + Antimalarial	Unfavorable due to DDI

Table 5: Pre-Exposition (PrEP) trials.

Substance	Drug Class	Status
Thailand/Worldwide(a)	HCLQ/CLQ vs. Placebo; n=40.000	Recruiting, medical staff
Spain (a)	HCLQ vs. Placebo; n=440	High-Risk Healthcare Workers Unicentric, Double-Blinded RCT
EPICOS Spain (a)	HCLQ vs. TVD vs. HCLQ+TVD vs. Placebo, n=4000	Healthcare workers, RCT
HCQ4COV19 Spain (a,b)	DRV/COBI + HCLQ vs Placebo, n=3040	Recruiting
South Korea (a)	LPV/r as PEP (MERS-CoV patients)	Uncontrolled trial in medical staff, S.K. 2003

(a) Source https://clinicaltrials.gov/ (b) The combination of DRV/COBI and HCLQ ist not really reasonable due to the expected DDIs

Drug-drug interactions

Poizot-Martin et al. simulated the interaction potential of DAAs with an antiretroviral therapy (ART) in 2500 HIV/HCV co-infected patients of the French Dat´AIDS cohort during the years 2012-2015: 97.1% of patients received a cART with NRTIs, in 43.6% of cases combined with a boosted PI, in 17.3% with NNRTI, 15.4% with INI and 23.7% of different combinations of these.

The University of Liverpool Drug Interaction Database also evaluated contraindications and potential drug-drug interactions between DAAs and Antiretrovirals. It was stated that the least contraindications/interactions were found with Sofosbuvir (0.2%/0%) [48,49].

The interaction potential of ritonavir-boosted HIV-protease inhibitors unfortunately is quite high, so that it is a challenge to combine LPV/r with other currently taken drugs in multimorbid elderly patients, who are the most vulnerable to an infection with coronavirus.

Hepatic metabolism

Cytochrome oxidases (CYP) are involved in human drug metabolism, especially during the intestinal absorption and hepatic first-pass metabolism. Around 60% of all drugs are metabolized *via* CYP-isoenzymes. Of these ~40% *via* CYP3A, 25% *via* CYP2C, 18% *via* CYP1A and another 18% *via* other CYPs. The most important human cytochrome oxidases are CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. Other important metabolizing hepatic enzymes are transferases, e.g. UDP-Glucuronyltransferase (UGT) and N-Acetyltransferase (NAT).

Regarding the complexity and number of potential DDis between, especially LPV/r and co-medication, the author refers to the package inserts and EMA-INN. Also the University of Liverpool drug-drug interaction webpage gives a very comprehensive update regarding these DDis.

Transmembrane transporters

P-Glykoprotein (ABCB1): On their way into target cells drugs have to overcome different cellular barriers. This can happen by passive diffusion but in many cases also be means of active transport proteins. Most familiar is P-glycoprotein (P-gp), called ABCB1 transporter in new nomenclature, a transmembrane glycoprotein efflux transporter, encoded by the MDR1 gene. This membrane-located ATP-dependent transport mechanism is used by human cells to discharge toxins or drugs identified as toxins.

In humans P-gp is located in excretory tissues like the intestine, liver and kidney, but also in pancreas, heart and brain or e.g. tumor cells. The overexpression in tumor cells e.g. is one reason for the decreased response to cytostatics, known as multidrug resistance. The spectrum of substrates of P-gp is broad and includes drugs, food constitutes, environmental toxins, hormones, amino acids, sugar or peptides, i.e. molecules from 400-2000 Da.

P-gp can be either inhibited or induced, whereas substrates of P-gp often are also substrates of CYP3A4 (Table 6). Obviously, expression and activity of both are regulated via the same pathways. Thus, the differentiation of effects, caused by each are complicated.

A number of drugs, discussed in this editorial are substrates and/or inhibitors of P-gp (Figure 1).

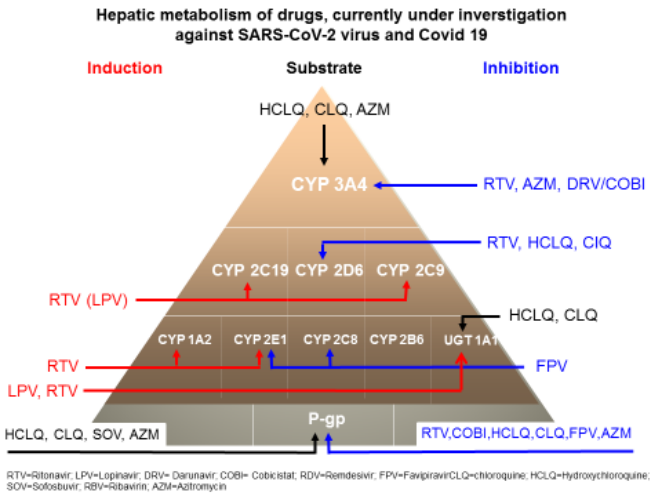

Figure 1: Drug-drug interaction potential regarding cytochrome oxidases and cellular transporters of currently evaluated drugs against SARS-CoV-2 infection.

DISCUSSION

The number of ongoing studies regarding the treatment of COVID-19 is impressive: Clinical Trials. Gov lists 468 studies worldwide (14.04.2020), but current evidence for the treatment of COVID 19 is quite low.

There are reasonable arguments from in-vitro and drug modelling studies as well as small open trials or case reports supporting the repurposing of already available drugs and possible combinations of such. However, some turn out to cause more problems in real life than possibly expected. In Sweden e.g. recently all studies with hydroxychloroquine were stopped due to the accumulation of severe side effects in patients. (International Press)

Table 4. Pharmacological characteristics of currently tested drugs against SARS-CoV-2.

Generic name	Drug class	Molecular weight	Plasma protein-binding	Metabolism	Cellular Transport	Additional Information
Sofosbuvir [50-55]	Nukleoside Analogue Polymerase inhibitor Active metabolite	529.5 g/mol	61-65%	Cathepsin A (CatA), (CES1) und (Hint1). UMP/CMP/NDP-Kinasen	P-gp Substrate, BCRP	Prodrug; Intracellular Phosphorylation to Uridintriphosphat-Analogue
Remdesivir	Nucleotide Analogue Polymerase inhibitor Active metabolite	602.6	n.a.	Phosphorylation	P-gp- and OATP1B1 Substrate	Prodrug; Intracellular Phosphorylation [56] IC t1/2 = 20h
Favipiravir	Nukleoside Analogue Polymerase inhibitor Active metabolite	157.1	n.a.	Phosphorylation via Hypoxanthine-guanine phosphoribosyltransferase P-gp, SLC22- inhibitor	P-gp Substrate+Inhibitor	Prodrug; Intracellular [57] Phosphorylation to Guaninmono/triphosphat-Analogon
Tenofovir	Nucleotide Analogue Polymerase inhibitor Active metabolite	287.2	7.20%	Phosphorylation by Nucleoside Diphosphate Kinase A+B and Adenosinkinase	P-gp Substrate	Prodrug; Intracellular Phosphorylation to Adenosintriphosphate-Analogon
Ribavirin	Nukleoside Analogue Polymerase inhibitor Active metabolite	244.2	none	Phosphorylation via Adenosin-Kinase	SLC-Substrate	Prodrug; Intracellular Phosphorylation to Adenosintriphosphate-Analogon
Oseltamivir	Neuraminidase inhibitor	312.4	42%	CES1	ABCC4, SLC15A1	Prodrug: CES1 → Oseltamivir-Carboxylate
Lopinavir/r	Proteaseinhibitor (Mpro)	628,8 g/mol	98-99%	CYP3A4 Substrate+Inhibitor (CYP2D6)	P-gp Substrate	Dosing BID
Darunavir/c	Proteaseinhibitor (Mpro)	547.8	95%	CYP3A4 Substrate+Inhibitor	P-gp Substrate	Dosing QD 800mg or BID 600mg

Chloroquine	Antimalaria	319.9	n.a.	CYP2C8, 3A4, 1A1, 2D6 (Inhibitor)	P-gp Inhibitor	[1,2] t1/2 = 30-60d [58]
H-chloroquine	Antimalaria	335.9	~50% S-enantiomer 64% L-enantiomer 37%	CYP3A4 → Desethylhydroxychloroquine (active metabolite)	P-gp Inhibitor	[1,2] Prodrug activated via CYP3A4 t1/2 = 44-50d [59]
Azithromycin	Antibiotics	749	7-51% dose-dependent	CYP3A4 Substrate	P-gp Substrate+Inhibitor	[2]

Source: https://www.drugbank.ca/; DA = Dalton; 1 do not combine with PI [2] monitor QTc-prolongation; IC = Intracellular; t1/2 = half-life n.a.=not available, n.k.= not known

Still, a number of questions remain if all current efforts are appraised:

No head-to-head studies of promising medications, such as Remdesivir vs. TDF/FTC + X

No combination therapy studies, such as Remdesivir + X or TDF/FTC + X or LPV/r + X. Combination therapies are well established as treatment options against HBV, HCV, HIV, etc.

The vast majority of the multicenter studies focusing on Remdesivir/Hydroxychloroquine only, not other promising nucleosides/PIs. But: Remdesivir is manufactured by one Company, price and availability are currently unknown.

No studies of such a combination as LPV/r + TDF/FTC, which are worldwide available at reasonable prices, TDF/FTC even as generic drug.

No PrEP-Studies with TDF/FTC, well established PrEP against HIV-Infection at a cost of less than 40 E/month? The drug modelling studies revealed a slightly less favorable binding property of TDF/FTC vs. Remdesivir, which may be as well outweighed by the advantages of a much cheaper price and the availability as generic drug, produced in many countries of the world.

The repurposing of drugs in case of a pandemic is more than reasonable, but it needs internationally concerted policies regarding science, production and supply of drugs. These are a social and federal responsibilities, a question of independent science and marketing independent supply and general access to the best available treatment. This cannot not be committed to either the manufacturer's side or single academic research units.

All together we can do better.

REFERENCES

1. Martinez MA. Compounds with therapeutic potential against novel respiratory 2019 coronavirus. Antimicrob Agents Chemother. 2020.
2. Chen F, Chan KH, Jiang Y, Kao RY, Lu HT, Fan KW, et al. In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. J Clin Virol. 2004,31:69-75.
3. Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 Protease against COVID-19. J Biomol Struct Dyn. 2020:1-7.
4. Nukoolkarn V, Lee VS, Malaisree M, Aruksakulwong O, Hannongbua S. Molecular dynamic simulations analysis of ritonavir and lopinavir as SARS-CoV 3CL(pro) inhibitors. J Theor Biol. 2008; 254:861-867.
5. Totura AL, Bavari S. Broad-spectrum coronavirus antiviral drug discovery. Expert Opin Drug Discov. 2019;14:397-412.
6. Rabaan AA, Alahmed SH, Bazzi AM, Alhani HM. A review of candidate therapies for Middle East respiratory syndrome from a molecular perspective. J Med Microbiol. 2017; 66:1261-1274.
7. Elfiky AA. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. Life Sci. 2020; 117592.
8. Liu Y, Chen H, Tang K, Guo Y. Clinical manifestations and outcome of SARS-CoV-2 infection during pregnancy. J Infect. 2020.
9. Mulangu S, Dodd LE, Davey RT Jr, Tshiani Mbaya O, Proschan M, Mukadi D, et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019; 381:2293-2303.
10. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med. 2020.
11. WHO. SOLIDARITY. Clinical Trials.gov. 2020.
12. Rizzardini G. Clinical Study To Evaluate The Performance And Safety Of Favipiravir in COVID-19. Clinical Trials.gov. 2020.
13. Wang G, Shanghai Uo. Favipiravir Combined With Tocilizumab in the Treatment of Corona Virus Disease 2019. 2020.
14. Jefferson T, Demicheli V, Rivetti D, Jones M, Di Pietrantonj C, Rivetti A. Antivirals for influenza in healthy adults: Systematic review. Lancet 2006; 367:303-313.
15. Jefferson TO, Demicheli V, Di Pietrantonj C, Jones M, Rivetti D. Neuraminidase inhibitors for preventing and treating influenza in healthy adults. Cochrane Database Syst Rev. 2006:CD001265.
16. Tan EL, Ooi EE, Lin CY, Tan HC, Ling AE, Lim B, et al. Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. Emerg Infect Dis. 2004; 10:581-586.
17. De-Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother. 2014; 58:4875-4884.
18. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020; 11:222.
19. Chan K, Lai S, Chu C, al. e. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: A multicentre retrospective matched cohort study. Hong Kong Med J. 2003; 9:399-406.
20. Von Hentig N. Lopinavir/ritonavir: appraisal of its use in HIV therapy. Drugs Today (Barc). 2007; 43:221-247.
21. Deng L, Li C, Zeng Q, Liu X, Li X, Zhang H, et al. Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: A retrospective cohort study. J Infect. 2020.
22. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N

Engl J Med. 2020.

23. Law M PR, Cheng AK, Cooper DA, Carr A. Evaluation of the HIV lipodystrophy case definition in a placebo-controlled,144-week study in antiretroviral-naive adults. Antivir Ther. 2006; 1:179-186.
24. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020.
25. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003; 426:450-454.
26. Ng ML, Tan SH, See EE, Ooi EE, Ling AE. Proliferative growth of SARS coronavirus in Vero E6 cells. J Gen Virol. 2003; 84:3291-3303.
27. Ng ML, Tan SH, See EE, Ooi EE, Ling AE. Early events of SARS coronavirus infection in vero cells. J Med Virol. 2003; 71:323-331.
28. Bergeron E, Vincent MJ, Wickham L, Hamelin J, Basak A, Nichol ST, et al. Implication of proprotein convertases in the processing and spread of severe acute respiratory syndrome coronavirus. Biochem Biophys Res Commun. 2005; 326:554-563.
29. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005; 2:69.
30. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis. 2003; 3:722-727.
31. Song HC, Seo MY, Stadler K, Yoo BJ, Choo QL, Coates SR, et al. Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. J Virol. 2004;78:10328-10335.
32. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J Zhejiang Univ (Med Sci). 2020; 49.
33. Ratiopharm. Fachinformation Hydroxychloroquin (Quensyl) German. 2019.
34. Bayer. Fachinformation Chloroquine (Resochin) German. 2004.
35. Yusuf IH, Ledingham JM, MacPhie E, Lotery AJ, Galloway J. Monitoring for retinal toxicity in patients taking hydroxychloroquine and chloroquine. Rheumatology (Oxford). 2019; 58:3-4.
36. Lim HS, Im JS, Cho JY, Bae KS, Klein TA, Yeom JS, et al. Pharmacokinetics of hydroxychloroquine and its clinical implications in chemoprophylaxis against malaria caused by Plasmodium vivax. Antimicrob Agents Chemother. 2009; 53:1468-1475.
37. Collins KP, Jackson KM, Gustafson DL. Hydroxychloroquine: A Physiologically-Based Pharmacokinetic Model in the Context of Cancer-Related Autophagy Modulation. J Pharmacol Exp Ther. 2018; 365:447-459.
38. Lu CC, Chen MY, Chang YL. Potential therapeutic agents against COVID-19: What we know so far. J Chin Med Assoc. 2020.
39. Chiou HE, Liu CL, Buttrey MJ, Kuo HP, Liu HW, Kuo HT, et al. Adverse effects of ribavirin and outcome in severe acute respiratory syndrome: experience in two medical centers. Chest. 2005; 128:263-272.
40. Hon KL, Leung CW, Cheng WT, Chan PK, Chu WC, Kwan YW, et al. Clinical presentations and outcome of severe acute respiratory syndrome in children. Lancet. 2003; 361:1701-1703.
41. Tsang K, Zhong NS. SARS: pharmacotherapy. Respirology. 2003; 8:S25-30.
42. Gomersall CD, Joynt GM, Lam P, Li T, Yap F, Lam D, et al. Short-term outcome of critically ill patients with severe acute respiratory syndrome. Intensive Care Med. 2004; 30:381-387.
43. Momattin H, Al-Ali AY, Al-Tawfiq JA. A Systematic Review of therapeutic agents for the treatment of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Travel Med Infect Dis. 2019; 30:9-18.
44. Park SY, Lee JS, Son JS, Ko JH, Peck KR, Jung Y, et al. Post-exposure prophylaxis for Middle East respiratory syndrome in healthcare workers. J Hosp Infect. 2019; 101:42-46.
45. Arabi YM, Alothman A, Balkhy HH, Al-Dawood A, AlJohani S, Al Harbi S, et al. Treatment of Middle East Respiratory Syndrome with a combination of lopinavir-ritonavir and interferon-beta1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials. 2018; 19:81.
46. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020.
47. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020:105949.
48. Osinusi A, Townsend K, Kottilil S. Drug-Drug Interactions in Patients Co-infected With HCV and HIV--Reply. JAMA. 2015; 314:186-187.
49. Poizot-Martin I, Naqvi A, Obry-Roguet V, Valantin MA, Cuzin L, Billaud E, et al. Potential for Drug-Drug Interactions between Antiretrovirals and HCV Direct Acting Antivirals in a Large Cohort of HIV/HCV Coinfected Patients. PLoS One. 2015; 10:e0141164.
50. Liverpool U. Sofosbuvir PK Fact Sheet. 2015.
51. Ltd. GS. Sovaldi® Summary of Product Characteristics. 2013.
52. Ltd. GS. Sovaldi® US Prescribing Information. 2013.
53. Lawitz E, Rodriguez-Torres M, Cornpropst M, et al. The effect of hepatic impairment on the pharmacokinetics and antiviral activity of PSI-7977 in hepatitis C infected subjects treated for seven days 47th EASL, Barcelona. 2012; 1130.
54. AM. Session5. 14th International Workshop on Clinical Pharmacology of HIV Therapy 2013, Amsterdam, Netherlands.
55. Application for inclusion of Harvoni®tablets in the WHO Model List of Essential Medicines. 2014.
56. GILEAD. Remdesivir INN. EMA. 2020.
57. Smee DF, Hurst BL, Egawa H, Takahashi K, Kadota T, Furuta Y. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virus-infected cells. J Antimicrob Chemother. 2009; 64:741-746.
58. Krishna S, White NJ. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. Clin Pharmacokinet. 1996; 30:263-299.
59. Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. Br J Clin Pharmacol. 1989; 27:771-779.