Drugs in COVID-19 Clinical Trials: Predicting Transporter-Mediated Drug-Drug Interactions Using In Vitro Assays and Real-World Data

Sook Wah Yee1,†, Bianca Vora1,†, Tomiko Oskotsky2, Ling Zou1, Sebastian Jakobsen1, Osatohanmwen J. Enogieru1, Megan L. Koleske1, Idit Kosti2, Mattias Rödin1, Marina Sirota2 and Kathleen M. Giacomini1,*

Numerous drugs are currently under accelerated clinical investigation for the treatment of coronavirus disease 2019 (COVID-19); however, well-established safety and efficacy data for these drugs are limited. The goal of this study was to predict the potential of 25 small molecule drugs in clinical trials for COVID-19 to cause clinically relevant drug-drug interactions (DDIs), which could lead to potential adverse drug reactions (ADRs) with the use of concomitant medications. We focused on 11 transporters, which are targets for DDIs. In vitro potency studies in membrane vesicles or HEK293 cells expressing the transporters coupled with DDI risk assessment methods revealed that 20 of the 25 drugs met the criteria from regulatory authorities to trigger consideration of a DDI clinical trial. Analyses of real-world data from electronic health records, including a database representing nearly 120,000 patients with COVID-19, were consistent with several of the drugs causing transporter-mediated DDIs (e.g., sildenafil, chloroquine, and hydroxychloroquine). This study suggests that patients with COVID-19, who are often older and on various concomitant medications, should be carefully monitored for ADRs. Future clinical studies are needed to determine whether the drugs that are predicted to inhibit transporters at clinically relevant concentrations, actually result in DDIs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✓ As the coronavirus disease 2019 (COVID-19) pandemic continues to plague the world, approved drugs and new molecular entities are being evaluated at an unprecedented pace. Patients diagnosed with COVID-19 may be increasingly vulnerable to incur significant drug-drug interactions (DDIs), especially older patients who are more susceptible to COVID-19-related morbidities and in whom polypharmacy is most common. Although there have been a few studies of DDIs, caused by individual drugs in clinical trials for COVID-19, there has been no large scale study evaluating the potential of many drugs in clinical trials for COVID-19 to cause a clinical DDI.

WHAT QUESTION DID THIS STUDY ADDRESS?
✓ In this study, we conducted extensive in vitro experiments aimed at predicting the potential for 25 small molecule drugs in clinical trials for COVID-19 to cause transporter-mediated DDIs and used real-world data to provide preliminary support of our in vitro findings.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
✓ This study resulted in three major findings. First, many of the drugs tested, which are in clinical trials for COVID-19, inhibited transporters in cellular assays, with certain transporters being sensitive to inhibition by multiple drugs. Second, the majority of the drugs are predicted to cause at least one clinical DDI; that is, the concentrations of these drugs that inhibited the transporters in cellular assays were equal to or greater than the drug levels known to result in clinical DDIs. Finally, real-world data from the electronic health records are consistent with our predictions of transporter-mediated DDIs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
✓ This study highlights that drugs used for COVID-19 have the potential to cause transporter-mediated DDIs. More recent drugs used for COVID-19 need to be assessed. Our study suggests that patients with COVID-19, who are often older and on various concomitant medications, should be carefully monitored for known adverse drug reactions.

1Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA; 2Bakar Computational Health Sciences Institute, University of California San Francisco, San Francisco, California, USA. *Correspondence: Kathleen M. Giacomini (kathy.giacomini@ucsf.edu)
†Co-first authors; both authors contributed equally to this work.
Received December 15, 2020; accepted March 14, 2021. doi:10.1002/cpt.2236
Adverse drug reactions are often a result of drug-drug interactions (DDIs), especially in patients for whom polypharmacy is common. It is estimated that the prevalence of clinically relevant DDIs is about 50% in those taking 5, and almost 100% in those taking 10 medications.1,2 DDIs can influence drug efficacy and toxicity by affecting pharmacokinetics through the inhibition or induction of drug metabolizing enzymes and transporters in the intestines, liver, and kidneys.3,4

As the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to plague the world, approved drugs and new molecular entities are being evaluated at an unprecedented pace. Patients diagnosed with COVID-19 may be increasingly vulnerable to incur a significant DDI, especially older patients who are more susceptible to comorbidities associated with COVID-19 and in whom pre-existing multimorbidity and polypharmacy5 are most common.

Membrane transporters are important targets for DDIs as they play critical roles in the absorption, distribution, and elimination of drugs and nutrients.5 Recently, the US Food and Drug Administration (FDA) released two guidances for drug developers, which include recommendations for conducting in vitro and clinical studies of transporter-mediated DDIs. Further, they provided a list of substrates and/or inhibitors for characterizing interactions mediated by nine membrane transporters: two efflux (P-gp and BCRP) and seven influx (OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2).4 These transporters not only play an important role in the disposition of drugs but also endogenous metabolites, such as creatinine (OCT2, MATE1, and MATE2) and uric acid (OAT1, OAT3, and BCRP).7

In this study, we conducted extensive in vitro experiments aimed at predicting the potential for 25 small molecule drugs in clinical trials for COVID-19 to cause transporter-mediated DDIs (Figure 1). More specifically, we (1) performed in vitro studies to determine the inhibition potential of the 25 drugs against 11 membrane transporters, and (2) predicted the likelihood for these drugs to cause a clinical transporter-mediated DDI using literature reported plasma concentrations and criteria suggested by the FDA.8 Finally, using electronic health records (EHRs), we demonstrated that the levels of endogenous compounds that are known substrates of specific transporters are significantly elevated in individuals on the drugs that are predicted inhibitors of the transporters. Overall, these findings suggest that individuals with COVID-19 who may be prescribed these medications are at risk for transporter-mediated DDIs.

**METHODS**

**Selection of COVID-19 drugs used in clinical trials**

The following databases were searched between March 17 and April 1, 2020, to select drugs being evaluated in clinical trials for COVID-19: clinicaltrials.gov, DRUGBANK, and IUPHAR/BPS Guide to Pharmacology.8 Twenty-five small molecule drugs, which were in clinical trials as of April 1, 2020, were selected for studying transporter-mediated DDIs.

**Cell lines used for inhibition studies**

Transient cells were used for determining the transporter inhibition at one concentration, 100 μM, unless mentioned otherwise. HEK293 Flp-In cells stably overexpressing human OATP2B1,9 OCT1,10 OCT2,11 OAT1,12 OAT3,13 MATE1,14 and MATE215 were used for determining the inhibition potentials, inhibitor activity measurements to estimate half-maximum inhibitory concentrations (IC\(_{50}\)) values, of selected drugs (see next section). See Supplementary Information for more information, including methods to establish transient cells expressing OATP1B1, OATP1B3, OATP2B1, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2 in HEK293 Flp-In cells.

**Transporter inhibition studies**

Twenty-five COVID-19 drugs were screened against 11 transporters at a concentration of 100 μM, except for azithromycin (50 μM), baricitinib (50 μM), and tetratride (10 μM) due to solubility. The substrate used for each transporter is listed in Table S1. For OATP1B1, OATP1B3, OATP2B1, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2, drugs were screened in cells transiently overexpressing each of the transporters. For P-gp and BCRP, membrane vesicles were used and the vesicular transport assays were performed as reported previously16 with modifications. See Supplementary Information for detailed methods.

**Prediction of transporter-mediated inhibition**

The DDI potential for each drug was evaluated in accordance to the 2020 FDA Drug-Drug Interaction Guidance4 by calculating the ratio of predicted clinically relevant drug concentration (I) to IC\(_{50}\) (I/IC\(_{50}\)). See Supplementary Information for description on the formulas and cutoff values used to predict in vivo DDI potential. Clinical pharmacokinetic characteristics (such as peak plasma concentration (C\(_{\text{max}}\)), plasma protein binding percentage, and f\(_{\text{up}}\)) were collected from PubMed and FDA-approved labeling (Drugs@FDA; Table S2). If no information was available, fraction unbound (f\(_{\text{up}}\)) was determined by Quintara Discovery (Hayward, CA), except for piclidenoson, where the f\(_{\text{up}}\) was not estimated because it was not predicted to result in transporter-mediated inhibition even when assuming f\(_{\text{up}}\) to be 1. R\(_{\text{i}}\) were estimated to be 0.6 for acidic drug and 1 for all others. The highest possible single dose, and respective C\(_{\text{max}}\) value, was used for all calculations. If a C\(_{\text{max}}\) value following the highest possible single dose was not available, the C\(_{\text{max}}\) was scaled linearly to fit the dose.

**Electronic health record analyses**

Two EHR databases were used to extract information about patient medication use as well as perform real-world data analyses, University of California – San Francisco (UCSF) Research Data Browser and Cerner’s Real World COVID-19 Database.

The UCSF Research Data Browser with UCSF patient data from 1982 to September 2020 was utilized to search for patients (both inpatients and outpatients) who had at least one laboratory test value reported for (1) serum/plasma uric acid, (2) triglyceride, (3) LDL cholesterol, (4) total cholesterol, or (5) bilirubin. For each analysis, patients were divided into the “on” or “off” drug group depending on their medication prescriptions for sildenafil, ritonavir, darunavir, and/or lopinavir. See Supplementary Information for detailed methods.

In all analyses comparing patient groups, patients were matched by covariates, including age and sex, using the MatchIt package17 in R software to be comparable in both groups.
**ARTICLE**

(a) Select drugs in clinical trials for COVID-19

Screen 25 drugs against 11 transporters

Perform IC_{50} studies

Collect pharmacokinetic characteristics for 25 drugs

Use FDA criteria to identify drugs that may cause clinical DDI

Use real world data to obtain evidence that drugs interact in vivo with the transporter

25 drugs* - see Table 1 and Table S1

(b) Inhibit viral replication

- Azithromycin
- Darunavir
- Favipiravir
- Lopinavir
- Oseltamivir
- Remdesivir
- Ribavirin
- Ritonavir
- Tetrandrine
- Triazavirin

Inhibit viral entry

- Camostat
- Chloroquine
- Hydroxychloroquine
- Losartan
- Umifenovir (Arbidol)

Anti-inflammatory

- Baricitinib
- Colchicine
- Fingolimod
- Leflunomide
- Piclidinonson
- Prazosin
- Ruxolitinib
- Sildenafil
- Thalidomide
- Tofacitinib

*Total=25
Figure 1 Overall study approach to assess the risks for transporter-mediated drug-drug interactions (DDIs) of 25 drugs in clinical trials to treat patients with coronavirus disease 2019 (COVID-19). (a) Multiple approaches were used in this study, starting with in vitro assays to determine transporter inhibition (1–3), followed by applying predictive methods to evaluate the potential for DDIs (4–5), and leveraging real-world data from electronic health records (6) to validate drug-transporter interactions clinically. (b, c) Chemical structures of 25 drugs, which include 10 drugs that inhibit viral replication, 5 drugs that inhibit viral entry, and 10 anti-inflammatory drugs. FDA, US Food and Drug Administration; IC$_{50}$, inhibitor activity measurements to estimate half-maximum inhibitory concentrations.

The Cerner COVID-19 database was also utilized to search for the number of patients who have prescriptions for drugs that are known substrates or inhibitors of the transporters, P-gp, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, MATE1, MATE2, OAT1, and OAT3 (Table S8). See Supplementary Information for detailed methods.

Statistical analyses
Two-sample two-sided Mann–Whitney U tests with continuity correction were performed to compare “on” and “off” drug groups. Among patients without elevated “Pre” creatinine levels, enrichment of elevated “Post” creatinine levels for those on HCQ or CQ in comparison to the matched control group was calculated by $\chi^2$ test with Yates correction. The ggplot2 was used to plot the data in R software (version 3.4.0).

RESULTS
In vitro studies determine inhibition potencies of 25 drugs used in clinical trials for COVID-19
Among the 25 drugs screened, 15 were antimicrobial agents (10 that inhibit viral replication and 5 that inhibit viral entry) and 10 were anti-inflammatory drugs (Figure 1). Eleven or more compounds reduced transport activity of P-gp, OATP2B1, OATP1B1, OATP1B3, OCT1, MATE1, MATE2, and OAT3 by $>50\%$ (Table S1). Although there can be differences and variability across different laboratories when reporting inhibition potencies (IC$_{50}$), our experimentally determined IC$_{50}$ values (Table 1) were in agreement (i.e., within 10-fold) with published data (Table S4).

Substrate-dependent inhibition of OATP1B1 and OATP1B3.
We compared differences in potency of inhibition of various drugs using estradiol 17$\beta$-glucuronide (EG) and estrone-3-sulfate (ES) or cholecystokinin (CCK) as probe substrates for OATP1B1 and OATP1B3, respectively. In general, inhibition potencies of the drugs tested were lower when $[^3]$H-EG was used as the probe substrate in comparison to $[^3]$H-ES as the probe substrate (Table 1), as also reported by Izumi et al.$^{18}$ Darunavir, losartan, remdesivir, and ritonavir all were estimated to inhibit $[^3]$H-EG at concentrations one-tenth (or lower) than those that inhibited $[^3]$H-ES; that is, potency differences of the inhibitors for the two probes were $>10$-fold. Differences in inhibition potency of compounds were not as stark when $[^3]$H-EG and $[^3]$H-CCK were used as probe substrates for OATP1B3 (Table 1). Seven drugs had potency differences within 2-fold; however, remdesivir showed a 14-fold lower IC$_{50}$ with $[^3]$H-EG as a substrate compared with $[^3]$H-CCK, whereas darunavir was 5-fold more potent in inhibiting the uptake of $[^3]$H-CCK in comparison to that of $[^3]$H-EG (Table 1).

Similarity and differences in potencies between transporters of close homology.
In general, experimental IC$_{50}$ values for OATP2B1, OATP1B1, and OATP1B3 were significantly correlated for the 10 drugs where IC$_{50}$ values were experimentally determined (Spearman correlation coefficient, $r$, ranges from 0.74 to 0.82, $P < 0.02$). Triazavirin is the only drug that was selective for OATP2B1 (IC$_{50}$ = 17 ± 4 $\mu$M), showing no inhibition of OATP1B1 and OATP1B3 at 100 $\mu$M. For MATE1 and MATE2, 9 drugs (out of 15) had IC$_{50}$ values within 5-fold of each other; however, 3 drugs (ritonavir, remdesivir, and tofacitinib) had IC$_{50}$ values that were $>25$-fold different. In contrast, larger differences in IC$_{50}$ values were observed when comparing the 2 organic cation transporters, OCT1 and OCT2, or the two organic anion transporters, OAT1 and OAT3 (Table 1). Camostat, CQ, colchicine, darunavir, HCQ, prazosin, remdesivir, ritonavir, and umifenovir inhibited OCT1 ≥10-fold more potently relative to OCT2, when comparing predicted or actual IC$_{50}$ values. Similarly, baricitinib$^{19}$ leflunomide, piclidenoson, remdesivir, ruxolitinib, and sildenafil inhibited OAT3 ≥10-fold more potently relative to OAT1 when comparing predicted or actual IC$_{50}$ values.

Clinically relevant transporter-mediated drug-drug interactions are predicted for 20 drugs
Using the FDA guidance for evaluating transporter-mediated drug interactions, a total of 61 potentially clinically relevant drug-transporter interactions were identified (Table 2, Table S5, Figure 2). Twenty of the 25 drugs screened were predicted to inhibit at least one of the studied transporters at clinical concentrations. Ritonavir, umifenovir, darunavir, and lopinavir were the most promiscuous clinical inhibitors, with each compound predicted to inhibit at least five transporters at clinically achievable drug levels. In contrast, baricitinib, colchicine, fingolimod, piclidenoson, and prazosin were not predicted to cause any transporter mediated DDIs. Intestinal and hepatic transporters appeared to be more easily inhibited compared with renal transporters, reflecting higher drug concentrations and exposure in the intestines and liver compared with the kidneys. Additionally, intestinal P-gp appeared to be inhibitable by 15 of the 25 drugs predicted to inhibit the transporter at estimated intestinal concentrations (Figure 2).

Since the first whitepaper by the International Transporter Consortium (ITC) was published,6 many drug labels include information on whether a drug is a substrate or inhibitor of certain transporters. For the drugs in this study that were approved prior to 2010 ($n = 14$), many clinically relevant transporter interactions were predicted. In particular, 40 interactions were
Table 1 Summary table showing the inhibition potencies of drugs (as IC\textsubscript{50} in µM) in COVID-19 clinical trials against transporters that are mediators of DDIs

| COVID-19 drug | Major intestinal transporters, Pgp, BCRP, and OATP2B1 |
|---------------|-----------------------------------------------------|
|               | Pgp  | BCRP | OATP2B1 |
| Azithromycin  | 18   | > 50 | > 50    |
| Baricitinib   | > 50 | > 50 | > 50    |
| Camostat      | 35   | > 100| > 100   |
| Chloroquine   | 20   | > 100| > 100   |
| Colchicine    | 42   | > 100| > 100   |
| Darunavir     | 16   | > 100| 30.6 ± 7.7 |
| Favipiravir   | 55   | > 100| > 100   |
| Fingolimod    | 89   | > 100| > 100   |
| Hydroxychloroquine | 51.8 ± 20.6 | > 100 | > 100 |
| Leflunomide   | > 100| 4.53\textsuperscript{a} | 81.9 ± 36.1 |
| Lopinavir     | 1.7\textsuperscript{a} | 7.66\textsuperscript{a} | 0.72\textsuperscript{a} |
| Losartan      | > 100| 4.8 ± 1.1 | 2.5 ± 0.7 |
| Oseltamivir   | 44   | > 100| > 100   |
| Piclidenoson  | 50   | > 100| 12.3 ± 4.7 |
| Prazosin      | 70.7\textsuperscript{a} | > 100| > 100   |
| Remdesivir    | 14   | 25 ± 6.0 | 3.5 ± 0.4 |
| Ribavirin     | 47   | > 100| > 100   |
| Ritonavir     | 36, 0.24\textsuperscript{a} | 19.5 ± 6.6\textsuperscript{a} | 3.7 ± 1.1 |
| Ruxolitinib   | > 100| > 100| 17.4 ± 8.6 |
| Sildenafil    | 16   | 3.1 ± 2.5 | 39.0 ± 12.8 |
| Tetrandrine   | 3.8 ± 1.1 | > 10 | > 10 |
| Thalidomide   | 65   | > 100| > 100   |
| Tofacitinib   | > 100| > 100| > 100   |
| Triazavirin   | 72   | > 100| 17.4 ± 4.0 |
| Umifenovir (Arbidol) | 16.0 ± 2.0 | > 100 | 3.5 ± 1.2 |

| COVID-19 drug | Major liver transporters, OATP1B1, OATP1B3, and OCT1 |
|---------------|-----------------------------------------------------|
|               | OATP1B1 (ES) | OATP1B1 (EG) | OATP1B3 (CCK) | OATP1B3 (EG) | OCT1 |
| Azithromycin  | > 50         | > 50         | > 50         | > 50         | > 50 |
| Baricitinib   | > 50         | 30           | > 50         | 47           | 22.9 ± 18.0 |
| Camostat      | > 100        | > 100        | 90           | > 100        | 20.3 ± 21.3 |
| Chloroquine   | > 100        | > 100        | > 100        | > 100        | 10.7 ± 10.4 |
| Colchicine    | > 100        | 42           | > 100        | > 100        | 29.7 ± 38.6 |
| Darunavir     | 82.5 ± 21.0  | 6.2 ± 1.4    | 7.6 ± 1.3    | 42.9 ± 12.9  | 6.0 ± 6.6 |
| Favipiravir   | > 100        | > 100        | > 100        | > 100        | > 100 |
| Fingolimod    | > 100        | > 100        | > 100        | > 100        | > 100 |
| Hydroxychloroquine | > 100     | 33.3 ± 4.9   | 21.2 ± 2.0   | > 100        | > 100 |
| Leflunomide   | > 100        | 33.3 ± 4.9   | 21.2 ± 2.0   | > 100        | > 100 |
| Lopinavir     | 0.6 ± 0.1    | 0.3 ± 0      | 4.2 ± 0.6    | 2.7 ± 1.7    | > 100 |
| Losartan      | 26.3 ± 14.8  | 1.4 ± 0.5    | 4.0 ± 1.0    | 1.8 ± 2.1    | > 100 |
| Oseltamivir   | > 100        | > 100        | > 100        | > 100        | > 100 |
| Piclidenoson  | 17.0 ± 0.1   | 6.3 ± 0.5    | 12.9 ± 5.0   | 9.0 ± 3.9    | 16.5 ± 10.9 |
| Prazosin      | 78.9 ± 22.8  | 47.1 ± 6.2   | 36.7 ± 2.8   | 40.6 ± 4.8   | 1.8 ± 2.0 |
| Remdesivir    | 36.1 ± 24.4  | 1.4 ± 0.02   | 5.5 ± 1.2    | 0.4 ± 0.04   | 10.1 ± 0.01 |

(Continued)
Table 1 (Continued)

COVID-19 drug | OATP1B1 (ES) | OATP1B1 (EG) | OATP1B3 (CCK) | OATP1B3 (EG) | OCT1
--- | --- | --- | --- | --- | ---
Ribavirin | > 100 | > 100 | > 100 | > 100 | > 100
Ritonavir | 18.7 ± 1.7 | 0.6 ± 0.15 | 1.6 ± 0.6 | 0.7 ± 0.2 | 3.8 ± 0.1
Ruxolitinib | 47.9 ± 3.6 | 12.7 ± 1.4 | 19.1 ± 2.9 | 23.9 ± 11.8 | 9.7 ± 4.3
Sildenafil | 13.3 ± 2.2 | 3.0 ± 0.8 | 12.8 ± 0.04 | 20.7 ± 1.4 | 19.8 ± 6.4
Tetrandrine | > 10 | > 10 | > 10 | > 10 | 8.6
Thalidomide | > 100 | > 100 | > 100 | > 100 | > 100
Tofacitinib | > 100 | 39 | > 100 | > 100 | 41.4 ± 13.0
Triazavirin | > 100 | > 100 | > 100 | > 100 | > 100
Umifenovir (Arbidol) | 17.5 ± 11.9 | 5.1 ± 0.6 | > 100 | 6.5 ± 1.7 | 1.2 ± 0.1

COVID-19 drug | OCT2 | MATE1 | MATE2 | OAT1 | OAT3
--- | --- | --- | --- | --- | ---
Azithromycin | > 50 | > 50 | > 50 | > 50 | > 50
Baricitinib | 48 | 36.0 ± 24.0 | 6.7 ± 5.6 | > 50 | 12.7 ± 5.0
Camostat | > 100 | 3.4 ± 2.9 | 2.0 ± 1.3 | > 100 | > 100
Chloroquine | > 100 | 0.8 ± 0.8 | 0.7 ± 0.2 | > 100 | > 100
Colchicine | > 100 | > 100 | > 100 | 69 | > 100
Darunavir | > 100 | 43.8 ± 14.4 | 30.7 ± 20.2 | 95 | 37.9 ± 9.5
Favipiravir | > 100 | > 100 | > 100 | 52 | 84.2 ± 19.5
Fingolimod | > 100 | > 100 | > 100 | 64 | > 100
Hydroxychloroquine | > 100 | 1.9 ± 0.3 | 0.8 ± 0.1 | > 100 | > 100
Leflunomide | > 100 | 9.5 ± 5.5 | 12.6 ± 4.0 | > 100 | 41.4 ± 13.0
Lopinavir | > 100 | 22.5 ± 4.0 | 25.1 ± 9.6 | > 100 | > 100
Losartan | > 100 | > 100 | > 100 | 12 | 1.6 ± 0.1
Oseltamivir | > 100 | > 100 | > 100 | 90 | > 100
Piclidenoson | 40.7 ± 9.4 | 29.2 ± 19.6 | 15.2 ± 6.7 | > 100 | 8.7 ± 0.9
Prazosin | > 100 | 0.5 ± 0.2 | 2.4 ± 0.9 | > 100 | 29.8 ± 13.0
Remdesivir | > 100 | 0.4 ± 0.1 | 15.2 ± 9.7 | > 100 | 14.0 ± 1.7
Ribavirin | > 100 | > 100 | > 100 | 99 | > 100
Ritonavir | > 100 | 0.5 ± 0.3 | 12.6 ± 8.2 | 89 | 99
Ruxolitinib | 10.7 ± 3.7 | 0.7 ± 0.01 | 3.7 ± 2.3 | > 100 | 6.1 ± 0.6
Sildenafil | 68.0 ± 20.1 | 2.4 ± 0.1 | 14.4 ± 1.3 | > 100 | 20.5 ± 3.1
Tetrandrine | > 10 | 1.2 ± 0.2 | > 10 | > 10 | > 10
Thalidomide | > 100 | > 100 | > 100 | > 100 | 54.6 ± 2.4
Tofacitinib | > 100 | 1.0 ± 0.5 | 68.5 ± 6.2 | > 100 | 51.6 ± 7.0
Triazavirin | > 100 | > 100 | > 100 | 4.1 ± 0.7 | 2.3 ± 0.4
Umifenovir (Arbidol) | 14.9 ± 4.9 | 0.7 ± 0.2 | 4.1 ± 1.6 | > 100 | 58.6 ± 21.9

Inhibition potencies of drugs in COVID-19 clinical trials against transporters that are mediators of DDIs. Inhibition potencies are expressed as mean ± SD (µM) IC_{50} values for each transporter based on experimental data (see Supplementary Information). Values shown are from at least two independent experiments. When only a single value is shown without an SD, the value represents a predicted IC_{50}. Experimental IC_{50} values are shown as mean ± SD. For OATP1B1, OATP1B3, OCT1, OCT2, MATE1, and MATE2, experimentally IC_{50} are reported (even if IC_{50} values were available in the literature).

*For P-gp, BCRP, OATP2B1, OAT1, and OAT3, literature IC_{50} values are reported.
Predicted IC_{50} values were determined from one experiment and this is denoted in the table as a mean value (with no SD). Predicted IC_{50} (prIC_{50}) was calculated using the equation: V = V_0/(1 + [I]/prIC_{50}), where V and V_0 are the activity with and without inhibitor, respectively, and I is the inhibitor concentration. IC_{50} values were not estimated for drugs that showed no transporter inhibition or were predicted to have an IC_{50} value greater than the screening concentration. This is noted with a greater than value sign (>).

CCK, cholecystokinin; COVID-19, coronavirus disease 2019; DDI, drug-drug interaction; EG, estradiol 17B-glucuronide; ES, estrone-3-sulfate; IC_{50}, inhibitor activity measurements to estimate half-maximum inhibitory concentrations.

**Experimentally determine IC_{50} (µM) or from references**

- <= 5
- >5-20
- >20-40
- >40-60
- >60-100
- Greater than the concentration screened

Inhibition potencies of drugs in COVID-19 clinical trials against transporters that are mediators of DDIs. Inhibition potencies are expressed as mean ± SD (µM) IC_{50} values for each transporter based on experimental data (see Supplementary Information). Values shown are from at least two independent experiments. When only a single value is shown without an SD, the value represents a predicted IC_{50}. Experimental IC_{50} values are shown as mean ± SD. For OATP1B1, OATP1B3, OCT1, OCT2, MATE1, and MATE2, experimentally IC_{50} are reported (even if IC_{50} values were available in the literature).

*For P-gp, BCRP, OATP2B1, OAT1, and OAT3, literature IC_{50} values are reported.
Predicted IC_{50} values were determined from one experiment and this is denoted in the table as a mean value (with no SD). Predicted IC_{50} (prIC_{50}) was calculated using the equation: V = V_0/(1 + [I]/prIC_{50}), where V and V_0 are the activity with and without inhibitor, respectively, and I is the inhibitor concentration. IC_{50} values were not estimated for drugs that showed no transporter inhibition or were predicted to have an IC_{50} value greater than the screening concentration. This is noted with a greater than value sign (>).

CCK, cholecystokinin; COVID-19, coronavirus disease 2019; DDI, drug-drug interaction; EG, estradiol 17B-glucuronide; ES, estrone-3-sulfate; IC_{50}, inhibitor activity measurements to estimate half-maximum inhibitory concentrations.

**Experimentally determine IC_{50} (µM) or from references**

- <= 5
- >5-20
- >20-40
- >40-60
- >60-100
- Greater than the concentration screened

Inhibition potencies of drugs in COVID-19 clinical trials against transporters that are mediators of DDIs. Inhibition potencies are expressed as mean ± SD (µM) IC_{50} values for each transporter based on experimental data (see Supplementary Information). Values shown are from at least two independent experiments. When only a single value is shown without an SD, the value represents a predicted IC_{50}. Experimental IC_{50} values are shown as mean ± SD. For OATP1B1, OATP1B3, OCT1, OCT2, MATE1, and MATE2, experimentally IC_{50} are reported (even if IC_{50} values were available in the literature).

*For P-gp, BCRP, OATP2B1, OAT1, and OAT3, literature IC_{50} values are reported.
Predicted IC_{50} values were determined from one experiment and this is denoted in the table as a mean value (with no SD). Predicted IC_{50} (prIC_{50}) was calculated using the equation: V = V_0/(1 + [I]/prIC_{50}), where V and V_0 are the activity with and without inhibitor, respectively, and I is the inhibitor concentration. IC_{50} values were not estimated for drugs that showed no transporter inhibition or were predicted to have an IC_{50} value greater than the screening concentration. This is noted with a greater than value sign (>).

CCK, cholecystokinin; COVID-19, coronavirus disease 2019; DDI, drug-drug interaction; EG, estradiol 17B-glucuronide; ES, estrone-3-sulfate; IC_{50}, inhibitor activity measurements to estimate half-maximum inhibitory concentrations.
### Table 2 Summary of the prediction of drugs in clinical trials for COVID-19 to cause a transporter-mediated DDI

| COVID-19 drug          | FDA approval date | Dose, mg | No. of transporters at major organ sites inhibited at clinical concentrations | \(I_{\text{EC}50}/IC_{50}\) |
|------------------------|------------------|---------|--------------------------------------------------------------------------------|---------------------------|
|                        |                  |         |                                                                                 | P-gp  | BCRP | OATP2B1 |
| Azithromycin           | 1991             | 2,000   | 1                                                                              |      |      |         |
| Baricitinib            | 2018             | 4       | 0                                                                              |      |      |         |
| Camostat               | Not approved     | 400     | 1                                                                              |      |      |         |
| Chloroquine            | 1949             | 1,000   | 4                                                                              |      |      |         |
| Colchicine             | 1961             | 1.5     | 0                                                                              |      |      |         |
| Darunavir              | 2006             | 800     | 5                                                                              |      |      |         |
| Favipiravir            | Not approved     | 2,400   | 3                                                                              |      |      |         |
| Fingolimod             | 2010             | 0.5     | 0                                                                              |      |      |         |
| Hydroxychloroquine     | 1955             | 800     | 4                                                                              |      |      |         |
| Leflunomide            | 1998             | 100     | 2                                                                              |      |      |         |
| Lopinavir              | 2000             | 800     | 5                                                                              |      |      |         |
| Losartan               | 1995             | 150     | 4                                                                              |      |      |         |
| Oseltamivir            | 1999             | 300     | 1                                                                              |      |      |         |
| Piclidenoson           | Not approved     | 2       | 0                                                                              |      |      |         |
| Prazosin               | 1976             | 10      | 0                                                                              |      |      |         |
| Remdesivir             | 2020             | 200     | 4                                                                              |      |      |         |
| Ribavirin              | 1998             | 1,200   | 2                                                                              |      |      |         |
| Ritonavir              | 2000             | 600     | 7                                                                              |      |      |         |
| Ruxolitinib            | 2011             | 25      | 1                                                                              |      |      |         |
| Sildenafil             | 1998             | 100     | 4                                                                              |      |      |         |
| Tetrandrine            | Not approved     | 60      | 1                                                                              |      |      |         |
| Thalidomide            | 1998             | 400     | 1                                                                              |      |      |         |
| Tofacitinib            | 2012             | 10      | 1                                                                              |      |      |         |
| Triazavirin            | Not approved     | 250     | 4                                                                              |      |      |         |
| Umifenovir             | Not approved     | 200     | 6                                                                              |      |      |         |

\(I_{\text{EC}50}/IC_{50}\) for transporters at major organ sites inhibited at clinical concentrations:

| COVID-19 drug          | OATP1B1 | OATP1B3 | OCT1 |
|------------------------|---------|---------|------|
| Azithromycin           | ND      | ND      | ND   |
| Baricitinib            | 0.01    | 0.01    | 0.01 |
| Camostat               | NC      | NC      | NC   |
| Chloroquine            | ND      | ND      | 0.88 |
| Colchicine             | 0.004   | ND      | 0.005|
| Darunavir              | 1.36    | 0.2     | 1.4  |
| Favipiravir            | ND      | ND      | ND   |
| Fingolimod             | ND      | ND      | ND   |
| Hydroxychloroquine     | ND      | ND      | 0.46 |
| Leflunomide            | NC      | NC      | NC   |
| Lopinavir              | 13.2    | 1.46    | ND   |
| Losartan               | 0.34    | 0.26    | ND   |
| Oseltamivir            | ND      | ND      | ND   |
| Piclidenoson           | 0.05    | 0.03    | 0.02 |
| Prazosin               | 0.002   | 0.002   | 0.04 |
| Remdesivir             | 0.78    | 2.72    | 0.11 |
| Ribavirin              | ND      | ND      | ND   |

(Continued)
Five drugs approved during or after 2010 were predicted to have limited potential to inhibit these transporters in vivo, with the exception of remdesivir (approved in October 2020). Remdesivir inhibited four transporters at clinically relevant concentrations (Table 2). Interestingly, for the 6 drugs that have not been approved by the FDA (Table 4),

| COVID-19 drug | OATP1B1 | OATP1B3 | OCT1 | Tabelle 2 (Continued)  |
|---------------|---------|---------|------|------------------------|
| Ritonavir     | 12.7    | 11.2    | 2.03 |                        |
| Ruxolitinib   | 0.01    | 0.008   | 0.02 |                        |
| Sildenafil    | 0.1     | 0.01    | 0.01 |                        |
| Tetrandrine   | ND      | ND      | ND   |                        |
| Thalidomide   | ND      | ND      | ND   |                        |
| Tolafitinib   | 0.03    | ND      | 0.03 |                        |
| Triazavirin   | ND      | ND      | ND   |                        |
| Umifenovir    | 0.93    | 0.73    | 3.93 |

| COVID-19 drug | OCT2 | MATE1 | MATE2-K | OAT1 | OAT3 |
|---------------|------|-------|---------|------|------|
| Azithromycin  | ND   | ND    | ND      | ND   | ND   |
| Baricitinib   | 0.001| 0.002 | 0.01    | ND   | 0.005|
| Camostat      | NC   | NC    | NC      | NC   | NC   |
| Chloroquine   | ND   | 0.46  | 0.5     | ND   | ND   |
| Colchicine    | ND   | ND    | ND < 0.001 | ND   | ND   |
| Darunavir     | ND   | 0.01  | 0.02    | 0.007| 0.02 |
| Favipiravir   | ND   | ND    | ND      | 5.2  | 3.21 |
| Fingolimod    | ND   | ND    | ND < 0.001 | ND   | ND   |
| Hydroxychloroquine | 0.64 | 1.57 | ND      | ND   | ND   |
| Leflunomide   | NC   | NC    | NC      | NC   | NC   |
| Lopinavir     | ND   | 0.02  | 0.01    | ND   | ND   |
| Losartan      | ND   | ND    | ND 0.003a | 0.02a| 0.02 |
| Oseltamivir   | ND   | ND    | ND 0.002 | ND   | ND   |
| Picidenoson   | 0.001a | 0.001a | 0.003a | ND   | 0.005a|
| Prazosin      | ND   | 0.01  | 0.002   | ND   | 0.001c |
| Remdesivir    | ND   | 2.48  | 0.05    | ND   | 0.08 |
| Ribavirin     | ND   | ND    | ND      | 0.11 | ND   |
| Ritonavir     | ND   | 0.66  | 0.02    | 0.02a| 0.003|
| Ruxolitinib   | 0.003| 0.04  | 0.01    | ND   | 0.005|
| Sildenafil    | 0.001| 0.02  | 0.003   | ND   | 0.002|
| Tetrandrine   | ND   | 0.01  | ND      | ND   | ND   |
| Thalidomide   | ND   | ND    | ND      | ND   | 0.08 |
| Tolafitinib   | ND   | 0.12  | 0.002   | 0.001| 0.002|
| Triazavirin   | ND   | ND    | ND 0.26 | 0.45 |       |
| Umifenovir    | 0.006| 0.13  | 0.02    | ND   | 0.002|

Predictions are expressed as estimated clinical concentration relative to in vitro inhibition potency. IC_{50}, for each organ (intestines, liver, and kidneys) and their respective transporters. For OATP1B1 and OATP1B3 DDI prediction, the IC_{50} values using estradiol glucuronide as substrates were used. Bolded values meet FDA criteria to consider a clinical DDI study.

COVID-19, coronavirus disease 2019; DDI, drug-drug interaction; FDA, US Food and Drug Administration; IC_{50}, inhibitor activity measurements to estimate half-maximum inhibitory concentrations; NA, not applicable; NC, not calculated due to missing C_{max} values; ND, not determined due to IC_{50} being above the screening concentration.

| Protein binding not reported, so f_u,p assumed to be 1. Remdesivir is intravenously administered. For liver transporters DDI prediction, C_{u,max}/IC_{50} was used. |
| Using IC_{50} value from literature. |
| I_{u,in,max} = Predicted drug concentration in the intestine; I_{u,in,max} = Predicted drug concentration in the liver inlet; C_{u,max} = Maximum plasma drug concentration. |
Figure 2 Results of predictions of 25 drugs in coronavirus disease 2019 (COVID-19) clinical trials to cause in vivo transporter-mediated drug-drug interactions (DDIs). Predictions are based on in vitro inhibition potency data and are expressed as the clinical drug concentration (e.g., intestinal, portal vein, or systemic unbound concentration) relative to the in vitro inhibitor activity measurements to estimate half-maximum inhibitory concentrations (IC50) value for each transporter. Drugs that are predicted to cause in vivo transporter-mediated DDIs in the intestines, liver, and kidneys are shown in brown, red, and yellow circles, respectively. Drugs that do not inhibit the transporter at clinically relevant concentrations are shown as grey circle. Drugs that inhibit the transporters at IC50 greater than the maximum concentration tested (100 µM for all, except azithromycin and baricitinib at 50 µM and tetrandrine at 10 µM), then the in vivo transporter-mediated DDI could not be determined accurately (white circle). For OATP1B1 and OATP1B3, the DDI risk prediction shown were from data using estradiol glucuronide as substrates. See Table 2 and Table S5 for the predicted risk for DDI values. FDA, US Food and Drug Administration.
15 potentially relevant drug-transporter interactions were identified. For example, umifenovir and triazavirin each were predicted to cause 6 and 4 clinically relevant drug-transporter interactions, respectively. Camostat and leflunomide are rapidly converted to their active metabolites, thus the IC\textsubscript{50} for the active metabolites are required for determining the I/IC\textsubscript{50} calculations for hepatic and renal transporters. Although we took a conservative approach, and assumed that the f\textsubscript{u,p} was 1 for piclidenoson, as the information was not available, the drug was not predicted to cause a DDI for any of the transporters (Figure 2, Table 2, Table S5).

**Electronic health record analyses complement in vitro findings on clinically relevant transporter-mediated DDIs**

To investigate the clinical relevance of the inhibitors identified in vitro, we mined the EHR (electronic health record) database from the UCSF Research Data Browser (\(n = 2,888,884\) total patients from the general population). Specifically, we compared specific laboratory values in patients prescribed commonly used drugs (i.e., sildenafil, darunavir, ritonavir, and lopinavir) to laboratory values in patients not prescribed the respective drug. Endogenous biomarkers chosen and compared for each of these analyses were driven by literature-based evidence (Table S6).

To assess whether sildenafil, which was predicted to inhibit BCRP at clinical concentrations, actually inhibited the transporter in patients, we used uric acid levels from the UCSF database as a biomarker of BCRP activity; higher uric acid levels have been previously associated with reduced BCRP activity. Patients prescribed sildenafil had statistically significant higher uric acid levels (average: 6.84 mg/dL vs. 5.94 mg/dL) compared with age-matched and sex-matched controls not prescribed sildenafil (\(P \text{ value} < 2.2 \times 10^{-16}\), \(n = 636 \text{ "on" drug}, n = 3,180 \text{ "off" drug}\), consistent with inhibition of BCRP (Figure 3, Table 3). Additional sensitivity analyses, including (1) a maximum separation date of 1 year between the first medication order start date and laboratory collection date, (2) limiting our analysis to patients diagnosed with pulmonary hypertension, and (3) further filtering to only include laboratory values taken on or after initial diagnosis start date, (4) only including sildenafil (i.e., excluding Viagra and Revatio) medication orders with a dose greater than 25 mg in the medication name, and (5) excluding male patients, showed a significant difference between the 2 groups, where patients “on” drug had statistically significant higher uric acid levels compared with patients “off” drug (see \(P\) values in Table 3). Furthermore, to test the sensitivity of these analyses and selection of controls, multiple iterations were performed for each analysis where the ratio used to sex-match and age-match the two groups was varied; in every iteration, the “on” drug group had significantly higher uric acid levels compared to the “off” drug group (Table S7).

To assess the potential of ritonavir and darunavir to inhibit OCT1 at clinical concentrations, we used pharmacodynamic end points using data from the UCSF database. That is, reduction in the function of OCT1 has been associated with higher lipid levels.\(^10\) Triglyceride, LDL cholesterol, and total cholesterol levels were significantly increased in patients with HIV prescribed ritonavir compared with age-matched and sex-matched patients with HIV not prescribed ritonavir, when comparing laboratory values taken on or after initial HIV diagnosis start.

**Figure 3** Endogenous levels of transporter biomarkers in patients prescribed drugs that are predicted to cause a transporter-mediated drug-drug interaction. Levels of each biomarker were obtained from patient electronic health records. Boxplots compare (a) levels of uric acid, a biomarker of BCRP activity, in patients prescribed sildenafil versus patients not prescribed sildenafil (\(P \text{ value} < 2.2 \times 10^{-16}\)) and (b–d) levels of triglycerides, LDL cholesterol, and total cholesterol, biomarkers of OCT1 activity, in patients with HIV prescribed ritonavir vs. patients with HIV not prescribed ritonavir (\(P\) value: \(7.8 \times 10^{-12}\), 0.0033, \(3.1 \times 10^{-12}\), respectively). Figure 3b is plotted on a log scale. [Colour figure can be viewed at wileyonlinelibrary.com]
Table 3 Summary table of EHR analyses comparing endogenous biomarkers in patients prescribed predicted clinical inhibitors of transporters vs. patients not prescribed predicted clinical inhibitors

| Analysis                                                                 | Total patients                  | Matched patients                  | Mann-Whitney-Wilcoxon P value |
|--------------------------------------------------------------------------|---------------------------------|-----------------------------------|------------------------------|
|                                                                          | On drug (N) Off drug (N)        | Ratio On drug (N) Off drug (N)    | Average SUA On/Off drug (mg/dL) | Median SUA On/Off drug (mg/dL) |
|                                                                          | 636 Off drug (N) 53,808         | 1:5 636 Off drug (N) 3,180        | 6.84/5.94                    | 6.6/5.7                      | <2.2E-16          |
| 1) Criteria: exclude laboratory values taken < 1 year after first medication order start date | 319 Off drug (N) 53,808         | 1:5 319 Off drug (N) 1,595        | 6.97/5.91                    | 6.7/5.7                      | 2.2E-13           |
| 2) Criteria: exclude patients without a diagnosis of pulmonary hypertension | 175 Off drug (N) 1,483          | 1:5 175 Off drug (N) 875          | 7.35/6.19                    | 6.9/5.6                      | 6.1E-07           |
| 3) Criteria: exclude laboratory values taken before diagnosis of pulmonary hypertension | 152 Off drug (N) 1,017          | 1:5 152 Off drug (N) 760          | 7.41/6.31                    | 7.2/5.7                      | 6.1E-06           |
| 4) Criteria: only include Sildenafil medication orders with dose > 25 mg in medication name | 183 Off drug (N) 53,808         | 1:5 183 Off drug (N) 915          | 6.86/6.1                     | 6.8/5.9                      | 1.2E-08           |
| 5) Criteria: exclude male patients                                       | 76 Off drug (N) 27,659          | 1:5 76 Off drug (N) 380          | 7.29/5.04                    | 7/4.6                       | 2.9E-11           |

| Ritonavir                                                                | On drug (N) Off drug (N)        | Ratio On drug (N) Off drug (N)    | Average On/Off drug (mg/dL) | Median On/Off drug (mg/dL) | Mann-Whitney-Wilcoxon P value |
|--------------------------------------------------------------------------|---------------------------------|-----------------------------------|------------------------------|---------------------------|------------------------------|
| Triglyceride                                                             | 782 Off drug (N) 1,249          | 1:1 782 Off drug (N) 782          | 252/162                      | 156/124                    | 7.8E-12                      |
| LDL cholesterol                                                          | 719 Off drug (N) 1,058          | 1:1 719 Off drug (N) 719          | 106/99.5                     | 101/96                     | 0.0033                       |
| Total cholesterol                                                        | 803 Off drug (N) 1,410          | 1:1 803 Off drug (N) 803          | 190/168                      | 182/165                    | 3.1E-13                      |
| Darunavir                                                                | Triglyceride                    | 386 Off drug (N) 1,407            | 1:2 386 Off drug (N) 772     | 180/160                    | 137/118                      | 0.0022                       |
|                                                                            | LDL cholesterol                 | 357 Off drug (N) 1,234            | 1:2 357 Off drug (N) 714     | 105/98.7                   | 100/95                       | 0.0077                       |
|                                                                            | Total cholesterol               | 364 Off drug (N) 1,572            | 1:2 364 Off drug (N) 728     | 183/170                    | 180/166                      | 5.18E-06                    |
| Ritonavir and/or Lopinavir                                               | Total bilirubin                 | 1,089 Off drug (N) 1,697          | 1:1 1,089 Off drug (N) 1,089 | 1.39/0.91                  | 0.9/0.7                      | <2.2E-16                    |

Sildenafil is a predicted clinical inhibitor of BCRP; ritonavir and darunavir are predicted to inhibit OCT1; ritonavir and lopinavir are predicted to inhibit OATP1B1 and OATP1B3. EHR, electronic health record; LDL, low-density lipoproteins; SUA, serum uric acid.
date, consistent with inhibition of OCT1 (Figure 3, Table 3). A similar, significant increase was seen in patients with HIV prescribed darunavir (Table 3). The percentage of patients with at least one statin prescription was higher in the “on” drug group or comparable between both groups for all ritonavir and darunavir analyses when comparing prescriptions to drugs (classified as antihyperlipidemic-HMG-CoA reductase inhibitors (statins)) with a medication order start date within 1 year before the laboratory collection date (Table S3).

Patients with HIV prescribed ritonavir and/or lopinavir had significantly higher total bilirubin levels compared to patients with HIV not prescribed ritonavir and/or lopinavir, when comparing laboratory values taken on or after initial HIV diagnosis start date, consistent with inhibition of OATP1B1 and OATP1B3 (Table 3).

Serum creatinine levels are determined by glomerular filtration rate and its active transport in renal proximal tubules by transporters, including MATE1.21 Thus, we used the change in creatinine level over time in patients prescribed HCQ and CQ, which are predicted to inhibit MATE1, to determine whether these drugs may actually inhibit this transporter clinically (Table 3). We used the Cerner COVID-19 database (n = 117,496 total patients with COVID-19) to identify patients prescribed HCQ and CQ (Table 4, Figure S1). The Cerner database contains data about medication order status (e.g., “complete,” and “incomplete”) that informs about medication administration as well as timestamps for laboratory values and medications that provide a temporal relationship between events of interest, and thus used for this analysis instead of the UCSF Research Data Browser.

In the analysis of patients with COVID-19 in the Cerner database who have “pre” creatinine levels within the upper limit of normal range, the “on drug” cohort had a significantly higher prevalence of “post” creatinine levels elevated above the normal range than the “off drug” control cohort matched using a propensity score, which included age, sex, race, ethnicity, outcome (death), and medication indication (systemic lupus erythematosus, discoid lupus, and rheumatoid arthritis, and malaria), with a 1:2 ratio (14.23% vs. 8.37%. Chi-square test, P value = 4.6 × 10⁻⁴, Table 4, Table S3). Creatinine elevations are consistent with inhibition of MATEs.

**DISCUSSION**

In the present study, we determined the inhibition of 25 drugs (18 approved drugs, 6 investigational drugs, and 1 recently approved) in COVID-19 clinical trials against 11 transporters and evaluated their potential to cause transporter-mediated DDIs. Patients with COVID-19 are often older and are taking multiple medications, many of which are substrates of transporters and thus, subject to transporter-mediated DDI. For example, furosemide (OAT1 and OAT3 substrate), atorvastatin (OATP1B1 and OATP1B3 substrate), and morphine (OCT1 substrate) are known substrates of transporters and are commonly used in patients with COVID-19 to treat co-existing conditions (Table S8).

This study resulted in three major findings. First, many of the drugs tested, which are in clinical trials for COVID-19, inhibited transporters in cellular assays with certain transporters being sensitive to inhibition by multiple drugs. Second, most of the drugs (20 of 25) were predicted to cause at least one clinical DDI; that is, the concentrations of these drugs that inhibited the transporters in cellular assays were equal to or greater than drug levels known to result in clinical DDIs. Finally, real-world data from the EHR were consistent with our predictions of transporter-mediated DDIs. In particular, recorded levels of certain solutes (such as creatinine and uric acid), which are endogenous substrates of particular transporters, were significantly elevated in individuals taking drugs predicted to inhibit the transporters clinically, in comparison to matched subjects not taking the drugs (Table 3, Table 4). Below we discuss each of these findings.

| Analysis | Number of patients with creatinine levels above normal level | Total Creatinine above normal level | χ² | P value |
|----------|-------------------------------------------------------------|------------------------------------|-----|---------|
| Main     | on HCQ/CQ                                                   | 90                                 | 584 | 15.41%  | 5.07     | 0.024    |
| Main     | Control                                                     | 134                                | 1168| 11.47%  |          |          |
| 1        | On HCQ/CQ                                                   | 74                                 | 520 | 14.23%  | 12.26    | 4.6E-04  |
| 1        | Control                                                     | 87                                 | 1040|          | 8.37%    |          |

In the main analysis, patients were matched by age, sex, race, ethnicity, and outcome (mortality). In analysis 1, patients with chronic kidney disease were excluded and patients were matched by age, sex, race, ethnicity, outcome (mortality), and medication indication. Chi-squared tests were performed to compare the percent of patients who have creatinine levels within the upper limit of normal range in the “on” drug group and the control (“off”) drug group.

HCQ, chloroquine; EHR, electronic health record; HCQ, hydroxychloroquine.

Woman’s normal creatinine levels = 1.1 mg/dL, Man’s normal creatinine levels = 1.2 mg/dL.

| Table 4 Table of EHR analyses comparing serum creatinine levels in patients prescribed HCQ and CQ vs. patients not prescribed HCQ and CQ (control) |
|-------------------------------------------------------------|------------------------------------|-----|---------|
| Analysis | Number of patients with creatinine levels above normal level | Total Creatinine above normal level | χ² | P value |
|----------|-------------------------------------------------------------|------------------------------------|-----|---------|
| Main     | on HCQ/CQ                                                   | 90                                 | 584 | 15.41%  | 5.07     | 0.024    |
| Main     | Control                                                     | 134                                | 1168| 11.47%  |          |          |
| 1        | On HCQ/CQ                                                   | 74                                 | 520 | 14.23%  | 12.26    | 4.6E-04  |
| 1        | Control                                                     | 87                                 | 1040|          | 8.37%    |          |

In Table 4, we discuss each of the findings.
Drugs in clinical trials for COVID-19 inhibited membrane transporters that are targets for clinical DDIs

Seventeen of the 25 drugs tested in this study have been reported to be substrates or inhibitors of 1 or more of the 11 membrane transporters that are targets for DDIs (Table S1); however, none of them have been assessed against all 11 transporters in a single study. There were 8 drugs where no information was available about their interactions with any of the 11 transporters, including: (1) 3 drugs (HCQ, ribavirin, and thalidomide), which were approved before 2000 and have no information about transporter inhibition reported in their FDA approved labels, their product inserts, or the literature; (2) 2 drugs (fingolimod and ruxolitinib), which were approved after 2010 but have limited information about their IC₅₀ values in their labels and product inserts; and (3) 3 drugs (piclididenoson, triazavirin, and umefenovir), which have not been approved. Examination of the data suggest that hepatic uptake transporters, including OATP1B1, OATP1B3, and OCT1, are subject to inhibition by multiple drugs in clinical trials for COVID-19. These hepatic transporters are known to interact with structurally diverse molecules from a range of pharmacologic classes and play critical roles in xenobiotic detoxification.²²,²³ OATP2B1, also in the liver and intestines, similarly interacts with multiple drugs. In the kidneys, MATE1 and MATE2 were inhibited by several drugs (Figure 2, Table 2). These transporters are known to interact with many drugs,²⁴ and clinically relevant DDIs have been reported between inhibitors of MATE1, such as cimetidine and the commonly prescribed antidiabetic drug, metformin.²⁵ In contrast, the renal transporters OCT2 and OCT1 and the intestinal efflux transporter BCRP, were not subject to inhibition by multiple drugs (Table 1, Figure 2). The majority of the drugs on the market that inhibit OCT2 are more potent inhibitors of MATE1 and MATE2 at clinically relevant concentrations, such as pyrimethamine and trimethoprim.²⁶ OCT1, which is responsible for the renal secretion of many acidic drugs, such as tenofovir, is inhibited by a few prescription drugs, such as probe-necid, but is considered a less promiscuous paralog than OCT3, which interacts with a more diverse array of drugs and their metabolites.¹³ The fact that BCRP was not inhibited by most of the drugs tested (Table 1) is interesting. BCRP has an endogenous role in the elimination of uric acid (Table S6), and drugs that inhibit this transporter may increase risk for hyperuricemia and gout; whereas drugs that inhibit other uric acid transporters, such as URAT1, may decrease uric acid levels.

Most of the drugs (20 of 25) tested were predicted to cause at least one transporter-mediated clinical DDI

A surprising finding of our study was that many of the drugs in clinical trials for COVID-19 had the potential to cause at least one transporter-mediated DDI. In particular, 20 of the 25 drugs screened were predicted to inhibit at least 1 of the 11 transporters at clinically achievable concentrations (Table 2). Ten of the 20 drugs have supporting information in the literature suggesting that they cause DDIs in humans or mice or have reported adverse events that are consistent with transporter inhibition (Table S6). The finding that the drugs may cause DDIs mediated by one or more transporters is consistent with the notion that these transporters work together with drug metabolizing enzymes to detoxify a plethora of xenobiotics, including environmental toxins, and exogenous chemical and prescription drugs. Thus, the transporters and enzymes interact with structurally diverse molecules and are subject to inhibition interactions. For example, azithromycin, a known in vivo P-gp inhibitor, increases plasma levels of the non-sedating antihistamine, fexofenadine, and the anticoagulant, ximelagatran, both of which are substrates of P-gp.²⁷,²⁸ The anti-viral combination drugs, lopinavir/ritonavir or lopinavir/daranavir, are known in vivo inhibitors of OATP1B1, OATP1B3, and P-gp. As such, they are associated with increase plasma levels of rosuvastatin, which is a substrate of OATP1B1/B3 and BCRP,²⁹,³⁰ and fexofenadine, which is a P-gp substrate.³¹ The effectiveness and cardiac safety of HCQ, CQ, and azithromycin in patients with COVID-19 have been the subject of considerable discussion in the literature.³²–³⁵ One of the major adverse events of these drugs is prolongation of the QT interval. This study and others have shown that azithromycin, HCQ, and CQ are substrates and inhibitors of P-gp.²⁷,²⁸,³⁶,³⁷ Therefore, the use of azithromycin in combination with CQ or HCQ needs to be carefully assessed and monitored.

Real-world data from the EHR were consistent with our predictions of transporter-mediated DDIs

Human genetic association studies and knockout mouse studies have shown that reduced function genetic variants of BCRP, OAT1, and OAT3 are associated with higher uric acid levels.¹²,³⁸,³⁹ Similarly, genetic variants in OCT2 and MATE1 are associated with higher serum creatinine levels and, hence, reduced estimated glomerular filtration rate.⁴⁰–⁴² Finally, genetic variants of OATP1B1 and OATP1B3 are associated with increased bilirubin levels.⁴³ All of these metabolites are also substrates of the respective transporters. Additionally, reduced function polymorphisms in OCT1 and Oct1 knockout mice have been shown to have increased plasma levels of LDL cholesterol, total cholesterol, and triglycerides.⁴⁴ Because levels of uric acid, creatinine, bilirubin, and various lipids are routinely measured and recorded in the EHR, these levels may be exploited to validate predictions from in vitro transporter assays of clinically relevant DDIs. Sildenafil has been shown to increase risk of gout (Table S6), and we found that patients who were prescribed sildenafil, a potent inhibitor of BCRP, had significantly elevated serum uric acid levels relative to patients not prescribed sildenafil (Table 3, Figure 3). In our study, average serum uric acid levels in the “on” drug group ranged from 6.8–7.4 mg/dL whereas the average levels for the “off” drug group ranged from 5.0–6.3 mg/dL, across all analyses. Previous studies have reported incidents of sildenafil-induced gouty arthritis.⁴⁴,⁴⁵ In contrast, other inhibitors of phosphodiesterase 5, such as vardenafil and tadalafil, are weak inhibitors of BCRP⁴⁶ and serum uric acid levels have been shown to significantly decrease following a 1 year treatment with vardenafil.⁴⁷

In addition to exploiting uric acid as an endogenous substrate of BCRP, we used lipid levels as biomarkers of OCT1 activity. That is, patients prescribed ritonavir and/or darunavir, both of which are OCT1 inhibitors (Table 1, Table 2), had significantly higher triglyceride, LDL cholesterol, and total cholesterol levels, compared with patients not prescribed either of these drugs.

120
(Table 3, Figure 3). Increases in cholesterol and triglyceride levels are listed as possible side effects in the FDA product label for ritonavir, as well as the Warnings and Precautions section to ensure these levels are monitored before and during therapy. Although other targets of ritonavir that affect liver metabolism may account for the observed increases in lipid levels associated with these drugs, reduced OCT1 activity is consistent with the elevated lipid levels. The in vitro studies and in vivo DDI risk predictions were focused on 25 drugs, selected during the beginning of the COVID-19 shelter-in-place; therefore, other drugs which may be currently used, such as dexamethasone, were not included here. However, based on transporter inhibition studies in the literature (Table S9), it is unlikely that dexamethasone will reach the FDA criteria to cause a transporter-mediated DDI (<0.2 µM; Table S9). A limitation of transporter inhibition assays is non-specific binding of compounds to cell-culture plates and/or cells during pre-incubation. However, the pre-incubation step was not performed in our assays except for OA TP1B1 and OA TP1B3, which is recommended in the FDA guidance. The EHR analyses were limited by lack of data on how long patients were on each respective medication, patient compliance (i.e., picking up medication from pharmacy, and abiding by dosing schedule), and robust controls (i.e., other phosphodiesterase 5 and protease inhibitors), as well as difficulty in getting measurable outcomes and noisy data. As more EHR data become available for research purposes, we will be able to account for these variables and covariates as well as increase the sample size and robustness of our analysis. Importantly, the endogenous solutes and lipids measured may be elevated for other reasons beyond transporter inhibition (e.g., creatinine elevation can result from dehydration and elevation of lipids, in particular triglycerides, can be detected for hours after high-fat meals); thus, the EHR results need to be interpreted cautiously and only as supporting information. Additional studies, such as controlled randomized clinical trials of DDIs or use of validated biomarkers for transporter-mediated DDIs, need to be conducted.

CONCLUSION
This study highlights that drugs for COVID-19 have the potential to cause transporter-mediated DDIs. Our study suggests that patients with COVID-19, who are often older and on various concomitant medications, should be carefully monitored for known adverse drug reactions. However, clinical DDI studies in healthy volunteers or patients are needed to confirm these DDI predictions.

FUNDING
This publication was made possible by grant number U01FD004979/U01FD005978 from the US Food and Drug Administration (FDA), which supports the UCSF-Stanford Center of Excellence in Regulatory Sciences and Innovation. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Health and Human Services (HHS) or the FDA. B.V. is supported by an Oak Ridge Institute for Science and Education (ORISE) Fellowship and is the recipient of an Achievement Rewards for College Scientists (ARCS) Scholarship. This study was supported in part by the Program for Breakthrough Biomedical Research (PBBR) Grant.

CONFLICT OF INTEREST
The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
S.W.Y., B.V., T.T.O., L.Z., S.J., and K.M.G. wrote the manuscript. S.W.Y., B.V., and K.M.G. designed the research. S.W.Y., B.V., T.T.O., L.Z., O.J.E., and K.M.G. performed the research. S.W.Y., B.V., T.T.O., L.Z., S.J., O.J.E., M.L.K., M.R., and K.M.G. analyzed the data. I.K., M.S., and K.M.G. contributed new reagents/analytical tools.

DISCLAIMER
As Deputy Editor-in-Chief of Clinical Pharmacology and Therapeutics, Kathleen M. Giacomini was not involved in the review or decision process for this paper.

© 2021 The Authors. Clinical Pharmacology & Therapeutics © 2021 American Society for Clinical Pharmacology and Therapeutics

1. Johnell, K. & Klarin, I. The relationship between number of drugs and potential drug-drug interactions in the elderly: a study of over 600,000 elderly patients from the Swedish Prescribed Drug Register. Drug Saf. 30, 911–918 (2007).
2. Patel, P.S., Rana, D.A., Suthar, J.V., Malhotra, S.D. & Patel, V.J. A study of potential adverse drug-drug interactions among prescribed drugs in medicine outpatient department of a tertiary care teaching hospital. J. Basic Clin. Pharm. 5, 44–48 (2014).
3. Cole, S., Kerwash, E. & Andersson, A. A summary of the current drug interaction guidance from the European Medicines Agency and considerations of future updates. Drug. Metab. Pharmacokinet. 35, 2–11 (2020).
4. Sudsakorn, S., Bahadduri, P., Fretland, J. & Lu, C. 2020 FDA drug-drug interaction guidance-comparison analysis and action plan by pharmaceutical industrial scientists. Curr. Drug Metab. 21, 406–426 (2020).
5. Khezrian, M., McNeil, C.J., Murray, A.D. & Myint, P.K. An overview of prevalence, determinants and health outcomes of polypharmacy. Ther. Adv. Drug Saf. 11, 2042098620933741 (2020).
6. Giacomini, K.M. et al. Membrane transporters in drug development. Nat. Rev. Drug Discov. 9, 215–236 (2010).
7. Ouy, X. et al. Clinical probes and endogenous biomarkers as substrates for transporter drug-drug interaction evaluation: perspectives from the international transporter consortium. Clin. Pharmacol. Ther. 104, 836–864 (2018).
8. Alexander, S.P.H. et al. A rational roadmap for SARS-CoV-2/COVID-19 pharmacotherapeutic research and development: IUPHAR Review 29. Br. J. Pharmacol. 177, 4942–4966 (2020).
9. Zou, L. et al. Bacterial metabolism rescues the inhibition of intestinal drug absorption by food and drug additives. Proc. Natl. Acad. Sci. USA 117, 16009–16018 (2020).
10. Chen, E.C. et al. Discovery of competitive and noncompetitive ligands of the organic cation transporter 1 (OCT1; SLC22A1). J. Med. Chem. 60, 2685–2696 (2017).
11. Kido, Y., Mattson, P. & Giacomini, K.M. Profiling of a prescription drug library for potential renal drug-drug interactions mediated by the organic cation transporter 2. J. Med. Chem. 54, 4548–4558 (2011).
12. Zou, L. et al. Molecular mechanisms for species differences in organic anion transporter 1, OAT1: implications for renal drug toxicity. *Mol. Pharmacol.* 94, 689–699 (2018).

13. Zou, L., Matsson, P., Stecula, A., Ngo, H.X., Zur, A.A. & Giacomini, K.M. Drug metabolites potently inhibit renal organic anion transporters, OAT1 and OAT3. *J. Pharmacol. Sci.* 110, 347–353 (2021).

14. Wittwer, M.B. et al. Discovery of potent, selective multidrug and toxin extrusion transporter 1 (MATE1, SLC47A1) inhibitors through prescription drug profiling and computational modeling. *J. Med. Chem.* 56, 781–795 (2013).

15. Morrisey, K.M., Stocker, S.L., Chen, E.C., Castro, R.A., Brett, C.M. & Giacomini, K.M. The effect of nizatidine, a MATE2K selective inhibitor, on the pharmacokinetics and pharmacodynamics of metformin in healthy volunteers. *Clin. Pharmacokinet* 55, 495–506 (2016).

16. Zou, L. et al. Interactions of oral molecular excipients with breast cancer resistance protein, BCRP. *Mol. Pharm.* 17, 748–756 (2020).

17. Ho, D., Imai, K., King, G. & Stuart, E.A. MatchIt nonparametric preprocessing for parametric causal inference. *J. Stat. Softw.* 42, 28 (2011).

18. Izumi, S. et al. Substrate-dependent inhibition of organic anion transporting polypeptide 1B1: comparative analysis with prototypical probe substrates estradiol-17β-glucuronide, estrone-3-sulfate, and sulfobromophthalein. *Drug Metab. Dispos.* 41, 1859–1866 (2013).

19. Posada, M.M. et al. Prediction of transporter-mediated drug-drug interactions for baricitinib. *Clin. Transl. Sci.* 10, 509–519 (2017).

20. Liang, X. et al. Organic cation transporter 1 (OCT1) modulates multiple cardiometabolic traits through effects on hepatic thiamine content. *PLoS Biol.* 16, e2002907 (2018).

21. Chu, X., Bleasby, K., Chan, G.H., Nunes, I. & Evers, R. The complexities of interpreting reversible elevated serum creatinine levels in drug development: does a correlation with inhibition of renal transporters exist? *Drug Metab. Dispos.* 44, 1498–1509 (2016).

22. Niemi, M., Pasanen, M.K. & Neuvonen, P.J. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol. Res.* 63, 157–181 (2011).

23. Koepsell, H. Organic cation transporters in health and disease. *Pharmacol. Res.* 72, 253–319 (2020).

24. Yonezawa, A. & Inui, K. Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxidynamics and pharmacogenomics. *Br. J. Pharmacol.* 164, 1817–1825 (2015).

25. Ito, S. et al. Competitive inhibition of the luminal efflux by multidrug and toxin extrusions, but not basolateral uptake by organic cation transporter 2, is the likely mechanism underlying the pharmacokinetic drug-drug interactions caused by cimitidine in the kidney. *J. Pharmacol. Exp. Ther.* 340, 393–403 (2012).

26. Nakada, T., Kudo, T., Kume, T., Kusuhasha, H. & Ito, K. Estimation of changes in serum creatinine and creatinine clearance caused by renal transporter inhibition in healthy subjects. *Drug Metab. Pharmacokinet* 34, 233–238 (2019).

27. Gupta, S. et al. Pharmacokinetic and safety profile of desloratadine and fexofenadine when coadministered with azithromycin: a randomized, placebo-controlled, parallel-group study. *Clin. Ther.* 23, 451–466 (2001).

28. Dorani, H. et al. Pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor ximelagatran co-administered with different classes of antibiotics in healthy volunteers. *Eur. J. Clin. Pharmacol.* 63, 571–581 (2007).

29. Kiser, J.J., Gerber, J.G., Predhomme, J.A., Wolfe, P., Flynn, D.M. & Hoody, D.W. Drug/drain interaction between lopinavir/ritonavir and rosuvastatin in healthy volunteers. *J. Acquir. Immune Defic. Syndr.* 47, 570–578 (2008).

30. Saminini, D., Desai, P.B., Samllans, L. & Fichtenbaum, C.J. Steady-state pharmacokinetic interactions of darunavir/ritonavir with lipid-lowering agent rosuvastatin. *J. Clin. Pharmacol.* 52, 922–931 (2012).

31. van Heeswijk, R.P. et al. Time-dependent interaction between lopinavir/ritonavir and fexofenadine. *J. Clin. Pharmacol.* 48, 758–767 (2008).

32. Fiolet, T., Guihur, A., Rebeaud, M.E., Mulot, M., Peifer-Smadja, N. & Mahamat-Saleh, Y. Effect of hydroxychloroquine with or without azithromycin on the mortality of coronavirus disease 2019 (COVID-19) patients: a systematic review and meta-analysis. *Clin. Microbiol. Infect.* 27, 19–27 (2021).

33. Roden, D.M., Harrington, R.A., Pappas, A. & Russo, A.M. Considerations for drug interactions on QTc interval in exploratory COVID-19 treatment. *J. Am. Coll. Cardiol.* 75, 2623–2624 (2020).

34. Nguyen, L.S. et al. Cardiovascular toxicities associated with hydroxychloroquine and azithromycin: an analysis of the World Health Organization Pharmacovigilance database. *Circulation* 142, 303–305 (2020).

35. Roden, D.M., Harrington, R.A., Pappas, A. & Russo, A.M. Considerations for drug interactions on QTc in exploratory COVID-19 treatment. *Circulation* 141, e906–e907 (2020).

36. Weiss, J., Bajakartaj-Sylejmani, G. & Haefeli, W.E. Interaction of hydroxychloroquine with pharmacokinetically important drug transporters. *Pharmaceutics* 12, 919 (2020).

37. Scherrmann, J.M. Intracellular ABCB1 as a possible mechanism to explain the synergistic effect of hydroxychloroquine-azithromycin combination in COVID-19 therapy. *AAPS J.* 22, 86 (2020).

38. Hosomi, A., Nakanishi, T., Fujita, T. & Tamai, I. Extra-renal elimination of uric acid via intestinal efflux transporter BCRP/ABC2. *PLoS One* 7, e30456 (2012).

39. Ichida, K. et al. Decreased extra-renal urate excretion is a common cause of hyperuricemia. *Nat. Commun.* 3, 764 (2012).

40. Pattaro, C. et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nat. Commun.* 7, 10023 (2016).

41. Kanai, M. et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.* 50, 390–400 (2018).

42. Morris, A.P. et al. Trans-ethnic kidney function association study reveals putative causal genes and effects on kidney-specific disease aetiologies. *Nat. Commun.* 10, 29 (2019).

43. Johnson, A.D. et al. Genome-wide association meta-analysis for total serum bilirubin levels. *Hum. Mol. Genet.* 18, 2700–2710 (2009).

44. Ali, T., Imeren, Y., Erdil, M. & Gunders, H. Gouty arthritis at interphalangeal joint of foot after sildenafil use: a case report. *Int. J. Surg. Case Rep.* 4, 11–14 (2013).

45. Chen, W.L., Chen, H.I. & Loh, C.H. Acute gouty arthritis after oral direct thrombin inhibitor ximelagatran co-administered with potent inhibitor of ABCB1/P-glycoprotein transporter. *PLoS One* 6, e19329 (2011).

46. Ding, P.R. et al. The phosphodiesterase-5 inhibitor vardenafil is a potent inhibitor of ABCB1/P-glycoprotein transporter. *PLoS One* 6, e19329 (2011).

47. Jing, Z.C. et al. Vardenafil treatment for patients with pulmonary arterial hypertension: a multicentre, open-label study. *Heart* 95, 1531–1536 (2009).

48. NORVIR. NORVIR [package insert]. North Chicago, IL: AbbVie Inc. <https://www.rxabbvie.com/pdf/NORVIR.tab_pi.pdf>.

49. Tatrai, P., et al. A systematic in vitro investigation of the inhibitor preincubation effect on multiple classes of clinically relevant transporters. *Drug Metab. Dispos.* 47, 768–778 (2019).

50. Mathialagan, S., Feng, B., Rodrigues, A.D. & Varma, M.V.S. Drug-drug interactions involving renal OCT2/MATE transporters: clinical risk assessment may require endogenous biomarker-informed approach. *Clin. Pharmacol. Ther.* https://doi.org/10.1002/cpt.2089. [e-pub ahead of print].