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Progress and pitfalls of a year of drug repurposing screens against COVID-19[☆]

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Near the end of 2019, a new betacoronavirus started to efficiently transmit between humans, resulting in the current COVID-19 pandemic. Unprecedented worldwide efforts were made to identify and repurpose antiviral therapeutics from collections of approved drugs and known bioactive compounds. Typical pitfalls of this approach (promiscuous/cytotoxic compounds leading to false positives), combined with bypassing antiviral drug development parameters due to urgency have resulted in often disappointing outcomes. A flood of publications, press-releases, and media posts, created confusion in the general public and sometime mobilized precious resources for clinical trials with minimal prospect of success. Breakthroughs have been made, not in the laboratory but in the clinic, resulting from the empiric identification of mitigators of clinical signs such as the discovery of improved disease management through immunomodulators. This opinion piece will aim to capture some of the lessons that we believe the COVID-19 pandemic has taught about drug repurposing screens.

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Introduction

In December 2019, a wave of viral pneumonia caused by a novel betacoronavirus was recorded in Wuhan, China, that expanded rapidly to pandemic level [1]. The ensuing coronavirus disease of 2019 (COVID-19) has since spread to most parts of the world and has been responsible for over 100 million cases and two million deaths worldwide

as of January 2021. To fill the anticipated gap of 1–2 years between the emergence of a new viral disease and build-up of vaccine-induced herd immunity, major efforts have been made to identify readily applicable therapeutics for the treatment of COVID-19 through the repurpose screening of approved drugs and collections of known bioactive molecules. Compared with a timeframe of several years to identify and develop new drug candidates *de novo*, this approach promises to reduce screening to a very manageable panel of only a few thousand chemical entities for most of which clinical or preclinical data and safety profiles are available from other indications. In the context of the COVID-19 pandemic, substantial insight into the molecular biology and pathogenesis of the etiologic agent, SARS-CoV-2, was rapidly gained. Combined with previous knowledge of related betacoronaviruses such as the original SARS-CoV and Middle-Eastern Respiratory Syndrome (MERS) coronavirus, therapeutic strategies have pursued a dual goal: interfering with virus replication and improving disease management through symptom therapy. We will discuss the results of these efforts and some lessons learned for the design of drug repurposing campaigns against newly emerging viral challenges.

Screening of compound libraries

In order to quickly identify chemical compounds that may interfere with SARS-CoV-2 replication and are readily applicable or can be rapidly advanced into the clinic, the most pragmatic approach is to test small molecule libraries of approved drugs or known bioactives for which preclinical and/or clinical performance profiles are already available. Looking at small-molecule drugs only, this constitutes an encouragingly narrow collection; for instance, the U.S. Food and Drug Administration (FDA) has approved less than 2000 distinct molecular entities to date [2,3]. These can be assayed even in manual medium-throughput settings in only a few days and are also amenable to testing in more advanced, and more informative systems such as primary human tissue models [4**].

Core collections are available pre-plated from different commercial sources such as, the Prestwick library of 1520 compounds [5,6] or the Library of 1280 Pharmacologically Active Compounds (LOPAC1280) [7]. Other sets available to academia free-of-charge include the NCATS Pharmaceutical Collection (~3000 compounds

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approved from several national agencies) [8] and collections that include investigational new drugs and subsets of bioactive preclinical compounds, such as the ReFRAME library (slightly larger at ~12 000 compounds) [9] and the Pandemic Response-Boxes and COVID-19-Boxes (400 and 160 compounds, respectively). A product of recent antiviral discovery efforts, the latter collections are distributed on a case-by-case basis to avoid redundant screens and make screening and counterscreening results available online (i.e. ReFRAMEdb, PubChem, COVID19 open data portal). Although these initiatives fostered the rapid generation and distribution of performance information, one needs to keep in mind that collections of known bioactives largely comprise the failed developmental candidates of earlier drug development campaigns against unrelated indications. There is no clear rationale why these synthetically often heavily experienced and ultimately abandoned chemotypes should be expected to fare better against a novel

indication such as SARS-CoV-2 than against the original developmental target.

A significant portion of known bioactive molecules and even of some approved drugs is made up of promiscuous, frequent-hitter chemotypes [10,11] that predictably emerged in many screens but are undevelopable. The motivation to rapidly identify potential inhibitors led to several of these compounds being reported as hits against COVID-19, straining wasting time and resources [11]. As it quickly turned out, the true mechanism of action of a large number of ‘antiviral candidates’ were, quite obvious, cytotoxic or, less obvious, cytostatic effects (Table 1).

In addition to these conventional direct repurposing tests, some very innovative new screens were developed [4**,5,7,12] and applied to the bioactives collections for proof-of-concept. Unfortunately, this exercise in some cases only showcased the ‘least bad hits’ as *bona fide*

Table 1

Examples of approved drugs with claimed anti-SARS-CoV-2 activity that are confounded by a very low (≤ 10) selectivity index

Hit candidate	EC ₅₀	CC ₅₀	Selectivity index	Drug class
2-deoxy-D-glucose	9.1 mM [12]	9.1 mM [12]	1	Hexokinase inhibitor
Amodiaquine dihydrochloride dihydrate,	4.9 μM [6]	34.4 μM [6]	7	Antimalarial
Amodiaquine hydrochloride	5.6 μM [6]	>38.6 μM [6]	>6.8	Antimalarial
Auranofin	1.4 μM [82]	5.7 μM [82]	4.1	Antirheumatic agent
Baloxavir acid	>100 μM [83]	85.9 μM [83]	<1	IAV Endonuclease inhibitor
Carmofur	24.3 μM [84]	133.4 μM [84]	5.5	Antineoplastic
Chloroquine (phosphate)	4.7 μM [62*] 46.8 μM [6]	>50 μM [62*,6] >100 μM [4**]	>10 [62*] >1 [6]	Antimalarial
	3.9 μM [4**] 30 μM [32]	45 μM [32]	>25.6 [4**] 1.5 [32]	
Chlorpromazine hydrochloride	4 μM [6]	11.9 μM [6] 48.9 μM [83]	2.9	Antipsychotic
Cycloheximide	0.17 μM [12] 0.5 μM [NCATS OpenData Portal – SARS-CoV-2 cytopathic effect]	1.7 μM [ReFRAMEdb] 0.8 μM [NCATS OpenData Portal – SARS-CoV-2 cytopathic effect]	10 [ReFRAMEdb] 1.6 [NCATS OpenData Portal – SARS-CoV-2 cytopathic effect]	Protein synthesis inhibitor
Emetine	0.47 μM [12]	0.8 μM [ReFRAMEdb]	1.7	Protein synthesis inhibitor
Enisamium	1 mM [85]	10 mM [86]	10	Putative RNA polymerases inhibitor
Hydroxychloroquine	11.2 μM [6]	>50 μM [6]	>4.5	Antimalarial
Imatinib mesylate	5.3 μM [6] 4.9 μM [4**]	>30.7 μM [6] >37.3 μM [4**]	>5.8 [6] 7.6 [4**]	Tyrosine kinase inhibitor
Ivermectin	2.2 μM [60] 1.7 μM [62*]	2.2 μM [62*]	1–1.3	Anthelmintics
Lopinavir	26.6 μM [83]	49.75 μM [83]	1.9 [83]	HIV aspartic protease inhibitor
Mefloquine	8.1 μM [6]	18.5 μM [6]	2.3	Antimalarial
Nitazoxanide	1.0 μM [62*]	3.3 μM [62*]	3.3	Antiprotozoal
NMS873	25 nM [12]	25 nM [12]	1	ATPase inhibitor
Pladienolide	7 nM [12]	7 nM [12]	1	Spliceosome inhibitor
Quinacrine dihydrochloride	2.8 μM [4**]	22 μM [4**]	7.8	IMPDH inhibitor
Ribavirin	70 μM [12]	70 μM [12]	1	IMPDH inhibitor
Ritonavir	48.9 μM [83]	>100 μM [83]	>2 [83]	HIV aspartic protease inhibitor

Table 2

Examples of repurposed or redirected drugs that have progressed to clinical trials

Candidate drug	Sponsor	Phase	Outcome	Trial ID	Reference
AT-527	Hoffmann-La Roche	Phase 2	Completion date: Feb/May, 2021	NCT04709835,	
	Atea			NCT04396106	
Baricitinib	Pharmaceuticals, Inc. Eli Lilly and Company	Phase 3	Completion date: June 2021	NCT04421027	
Baricitinib plus remdesivir	National Institute of Allergy and Infectious Diseases (NIAID)	Phase 3	Reduce recovery time	NCT04401579	[79]
Chloroquine/ hydroxychloroquine	Multiple	Phase 3	Not effective	'Solidarity' clinical trial, NCT04501952	[17**] https://www.covid19treatmentguidelines.nih.gov/tables/table-2b/
CD24Fc	Oncolmmune, Inc.	Phase 3	Completion date: October 2020	NCT04317040	
Dexamethasone	University of Oxford	Phase 2/3	Low 28-day mortality	NCT04381936 (Recovery Trial)	[87]
Emtricitabine and tenofovir	Universidad Nacional de Colombia	Phase 2/3	Completion date: May 2021	NCT04359095	
Favipiravir	University of Pecs	Phase 3	Completion date: June 2021	NCT04600999	
Interferon β -1a	Synairgen Research Ltd.	Phase 2	Better recovery	NCT04385095	[81]
Ivermectin	Clinica Universidad de Navarra, Universidad de Navarra	Phase 2	Reduce viral loads in mild COVID-19	NCT04390022	[88] https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/ivermectin/table-ivermectin-clinical-data/
Leflunomide					City of Hope Medical Center
Lopinavir/ritonavir	University of Oxford	Phase 2/3	Not effective	NCT04381936 (Recovery Trial)	[89]
Molnupiravir (MK-4482)	Merck & Co.	Phase 2/3	Completion date: December, 2021	NCT04575584, NCT04575597	
PTC299	PTC Therapeutics	Phase 2/3	Completion date: July 2021	NCT04439071	
Remdesivir	Gilead Sciences	Phase 3	Not effective, Completion date: April 2021	'Solidarity' clinical trial, NCT04501952	[17**]
Ruxolitinib	Novartis Pharmaceuticals	Phase 3	Did not meet endpoint	NCT04362137	https://www.novartis.com/news/media-releases/novartis-provides-update-ruxcovid-study-ruxolitinib-hospitalized-patients-covid-19
Sarilumab	Sanofi	Phase 3	Did not meet endpoint	NCT04327388	https://www.sanofi.com/en/media-room/press-releases/2020/2020-09-01-07-00-00
Siltuximab	EusaPharma (UK) Limited	Phase 3	Completion date: June, 2022	NCT04616586	
Sofosbuvir (plus Ledipasvir)	Almaza Military Fever Hospital	Phase 3	Completion date: July, 2020	NCT04530422	
Tocilizumab	University of Oxford	Phase 2/3	Improved survival	NCT04381936 (Recovery Trial)	[76]

therapeutic candidates, for instance even claiming a specific antiviral effect of cycloheximide [12]. To date, a broad panel of compounds has been selected based on repurposing screens or previously reported anti-coronavirus potential for clinical testing against COVID-19. Based on their general mechanism of action, this set can be subdivided into direct-acting and host-directed antivirals

(Table 2), and we will consider their strengths and challenges individually.

Direct-acting antivirals

Of all stages of the SARS-CoV-2 life cycle, processes associated with viral entry and replication in particular appear to be readily druggable (Figure 1). Repurposed

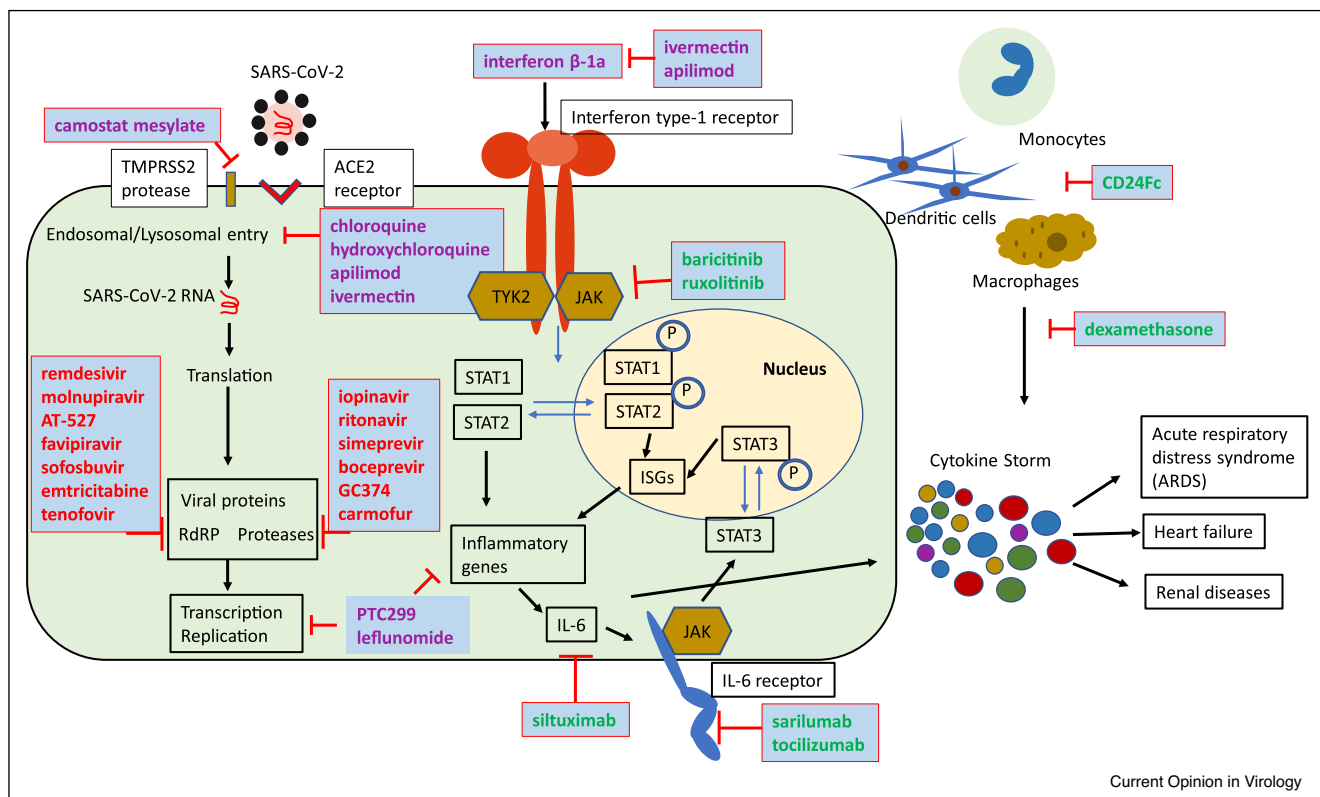
drug candidates that interfere with the entry machinery predominantly belong to the host-directed antiviral group. However, SARS-CoV-2 replication critically relies on viral protease and RNA-dependent RNA polymerase (RdRP) activities that were considered attractive targets for repurposing of drugs that, for instance, successfully block the equivalent functions in the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) replication cycles.

The main SARS-CoV-2 protease Mpro in particular has been the target of repeated repurposing attempts including testing of HCV serine protease inhibitors (i.e. simeprevir and boceprevir [13,14]) and HIV aspartic protease inhibitors (i.e. lopinavir/ritonavir [14–16,17**,18]). SARS-CoV-2 Mpro is a cysteine protease, however, and these inhibitors designed against different protease classes returned at best marginal anti-coronavirus activity in cell culture and failed in clinical trials. An exception could be the serine protease inhibitor GC376, which has demonstrated *in vitro* activity against SARS-CoV-2 [14,19] and

showed efficacy *in vivo* against a feline coronavirus [20,21]. The compound appears promising, but has yet to enter clinical development.

The viral RdRP complex has greater promise to serve as a valid target for existing drugs or advanced clinical candidates, based on its relatively high degree of conservation [22] and a tendency of nucleoside analog polymerase inhibitors for a broadened antiviral indication spectrum. It is therefore not surprising that repurposing attempts favored competitive substrate-analogs over allosteric polymerase inhibitors. Coronaviruses can be a challenging target for nucleoside analog inhibitors, however, due to a 3'-to-5' exoribonuclease proofreading activity that is unusual among RNA viruses but can mediate resistance, for instance, to the nucleoside analog drug ribavirin [23–25]. Nevertheless, the only direct-acting inhibitor that has received emergency approval in the United States in the first year of the pandemic for clinical use against severe COVID-19 has been the broad-spectrum nucleoside analog remdesivir [26].

Figure 1



Schematic of different stages of the SARS-CoV-2 cellular replication cycle that have been subjected to drug targeting attempts. Examples of approved drugs and advanced experimental therapeutics that have been tested are shown. Red: direct-acting antivirals; purple: host-targeted antivirals; green: immune-modulators for improved disease management.

The drug is a phosphoramidate prodrug of the monophosphate form of GS-441524, a 1'-CN modified adenosine nucleoside. Originally developed against HCV and subsequently unsuccessfully repurposed against Ebola virus, anti-SARS-CoV-2 activity of remdesivir in cell culture was discovered at the onset of the pandemic and the drug has become standard-of-care since [27–30]. Despite its success, the therapeutic impact of remdesivir is compromised by a requirement for intravenous administration and high tissue exposure *in vivo* in the liver, which was favorable for its original HCV indication but is less desirable for use against SARS-CoV-2 [26]. The lack of oral bioavailability restricts the recipient pool to hospitalized patients presenting with complicated disease, although greater therapeutic impact could without doubt be achieved by initiating treatment immediately after a positive test for SARS-CoV-2, ideally even before the development of clinical signs.

Repurposing attempts of orally available nucleoside analog drugs such as sofosbuvir (HCV) [31,32], emtricitabine and tenofovir (HIV) [32], or conditionally approved favipiravir (influenza virus) [31–35] failed *in vitro* and/or in the clinic, highlighting that nucleoside analogs have heightened potential for a broadened indication spectrum, but cross-antiviral activity cannot be taken for granted. However, novel orally available nucleoside analogs that were in earlier stages of formal development showed cross-activity against betacoronaviruses and have entered clinical trials since the onset of the pandemic. AT-527, a 2'-fluoro-2'-methyl guanosine analog prodrug, is currently being evaluated to treat HCV and SARS-CoV-2 infections. It has returned promising results in differentiated human airway primary cells and shown favorable pharmacokinetic profile after oral delivery to human and non-human primates [36]. Likewise, an orally available prodrug of the cytidine analog β -D-N⁴-hydroxycytidine, EIDD-2801 (molnupiravir) that was in development against influenza viruses [37**], was found to be orally efficacious against SARS-CoV-2 in the ferret infection model, reducing both virus burden and direct contact transmission rates [38**]. Phase I clinical trials demonstrated that molnupiravir is well tolerated and reaches antiviral plasma levels in humans that exceed efficacious concentrations in ferrets [37**,39]. Efficacy of the drug, which is currently in advanced Phase II/III clinical trials, was furthermore confirmed in mouse and hamster models of SARS-CoV-2 [40–42].

An inherent problem of broad-spectrum nucleoside analogs is their recognition and incorporation by host polymerases, which can result in undesirable off-target effects. Because of a lack of proofreading activity of host cell mitochondrial RNA polymerases, mitochondrial toxicity is frequently observed [43] and must be carefully monitored during development. Despite these potential liabilities, broad-spectrum nucleoside analogs have emerged in the pandemic as the antiviral drug class with

perhaps the highest potential to provide a first line defense against a newly emerged pandemic pathogen.

Host-directed antivirals

The idea of repurposing host-directed drugs for antiviral therapy has experienced a renaissance in the past two decades, based on the promise that host-directed antivirals may combine a broad antiviral indication spectrum with a heightened barrier against the emergence of viral resistance. Screening campaigns for host-directed antivirals typically deliver an abundance of seemingly attractive antiviral 'hits', many of which come from the cancer therapeutics and metabolic disease drugs subgroups [11]. Two decades of repurposing attempts of these drug classes against changing viral targets (i.e. SARS-CoV, pandemic 2009 influenza virus, Ebola virus, Zika virus, SARS-CoV-2) have unfortunately highlighted some fundamental limitations to their use for the treatment of acute RNA virus infections. Major liabilities include severe cytotoxic and/or cytostatic effects that are often the basis for *in vitro* 'antiviral activity' but can be counterproductive when trying to control a viral infection *in vivo*, side effects unacceptable for an antiviral indication, and pharmacokinetic properties and/or tissue exposure levels that do not meet those required for antiviral activity.

An additional potential caveat of this approach that was less appreciated pre-pandemic but amplified by COVID-19 arises from flaws in screening protocol design and assay interpretation, which can lead to the selection of host targets with little physiological relevance in the context of a natural infection. A prominent example of this problem are the anti-malaria and anti-rheumatoid arthritis therapeutics chloroquine and hydroxychloroquine. These drugs are polypharmacological, but one mechanism of action is interference with host cell lysosomal activity and autophagy. As one may anticipate, chloroquine showed a clear, albeit moderate, anti-SARS-CoV-2 *in vitro*, using as a host system the VeroE6 cell line that is derived from African green monkey kidney tissue [27,44]. Based on this minimal cell culture-derived evidence, the drug has since been tested against COVID-19 in a large number of clinical trials, which have consistently demonstrated that it lacks efficacy [45–48]. Despite this overwhelming clinical information, significant public attention was focused for months on a claimed benefit supposedly revealed in poorly designed clinical tests that were not conducted in compliance with accepted proper scientific standards [49,50].

In hindsight, the molecular basis for the clinical failure of chloroquine is inherent in its interference with the cellular endocytotic pathway, since it prevents the cathepsin-mediated maturation of SARS-CoV-2 spike (S) protein. However, SARS-CoV-2 entry depends on cathepsin activity only in cells such as VeroE6, which lack the host serine

protease TMPRSS2 [51^{••},52[•]], but is of limited relevance *in vivo*. Accordingly, chloroquine lacks anti-SARS-CoV-2 activity in engineered VeroE6 cells expressing TMPRSS2 [51^{••},52[•]] and is equally inactive in any naturally TMPRSS2-positive cell line [51^{••},52[•]], differentiated primary human bronchial epithelial cells [53[•]], hamsters [34], ferrets [54] and non-human primates [53[•],55]. The claimed anti-SARS-CoV-2 activity of chloroquine is emblematic for overinterpretation of a single assay that — in an attempt to accelerate the drug discovery process — was not subjected to a rigorous counterscreen of biological relevance in informative systems such as, disease-relevant primary cells. As a consequence, precious resources were directed to unproductive clinical trials in a time of need, trial participants were needlessly subjected to a conceptually flawed therapeutic approach, and people could even be harmed by the side effects of an ineffective drug.

As an interesting counterpoint to the chloroquine failure, camostat mesylate blocks TMPRSS2 and was therefore missed as an anti-SARS-CoV-2 candidate in screens conducted on VeroE6 cells. However, the compound showed antiviral activity when tested against SARS-CoV-2 in TMPRSS2 expressing cells including human airway organoids [51^{••},56], and has justifiably been advanced to clinical testing [57,58].

Whereas chloroquine appears to have run its course in the second year of the pandemic, a controversy surrounding another anti-parasitic drug, ivermectin, is still playing out. Ivermectin has exquisite, nanomolar potency against its original indication [59^{••}]. Standing at the center of debate is a very basic dose-response virus inhibition assay that was again conducted on VeroE6 cells and returned modest anti-SARS-CoV-2 activity in the low micromolar range [60]. Similar antiviral effects of ivermectin have been previously reported for a range of other viruses [61]. However, two basic parameters of antiviral drug development are overlooked in these studies: i) the allegedly 'active' antiviral concentration of ivermectin is roughly equivalent to the known cytotoxic concentration in many cell lines, including Vero cells [61,62[•]], which confounds interpretation of a specific antiviral effect; and ii) human pharmacokinetic data for ivermectin reveal that this antiviral (and thus, cytotoxic) concentration is not reached in human plasma, which also explains the excellent safety profile of the drug when used as approved [59^{••},63,64].

Without doubt, the availability of clinical and advanced preclinical data typically comprising pharmacokinetics, pharmacodynamics, tissue distribution, and tolerability in different species including humans is a fundamental advantage of drug repurposing over *de novo* development. However, this advantage becomes tangible only if the available knowledge is applied to a critical evaluation of whether a seemingly exciting antiviral effect observed in cell culture has any therapeutic relevance. Ideally, this

assessment should be carried out before human trials for the new indication are considered.

In addition to cytotoxic compounds, drugs and bioactives targeting host metabolic pathways have also been rediscovered in screening campaigns against SARS-CoV-2. Many of these have an impressive literature history of *in vitro* inhibitory activity against numerous viral targets. A prime example for this group and the challenges associated with their antiviral use is the large and structurally diverse family of cellular dihydroorotate dehydrogenase (DHODH) inhibitors that has been discovered over the years. These compounds act in cytostatic and antiviral manners through interference with the *de novo* pyrimidine biosynthesis pathway [65]. Representatives include the drug leflunomide, approved to treat rheumatoid arthritis, and the experimental inhibitor PTC299 [66–69]. DHODH blockers are known for exquisite broad-spectrum antiviral activity in cell culture [68,70,71]. *In vivo*, they can reduce expression of inflammatory cytokines, but universally lack antiviral activity because of the pyrimidine salvage pathway that is available to cells in a living organism but insignificant in cell culture [65]. Despite this well understood 'deal-breaker' that prevents *in vivo* efficacy, DHODH inhibitors were advanced to clinical testing against SARS-CoV-2 [66–69]. Again, these trials bound resources and burdened participants despite better knowledge and without much scientifically grounded prospect of success.

Disease management

Whereas repurposing attempts of host-directed drugs with the goal of blocking virus replication have largely not succeeded in the COVID-19 pandemic, improving management of severe disease through an empirical evaluation of the effect of immune-modulators in the clinic has been successful. Unlike antiviral therapeutics that are most effective when treatment is initiated at the earliest stage of infection, the highly dynamic nature of acute viral disease mandates careful timing of immune-modulating treatment to limit risks of a compromised initial host antiviral response and secondary nosocomial infections as a result of impaired immune function. Early characterization of COVID-19 in hospitalized patients revealed a biphasic pattern of disease progression, starting with a period of relatively moderate clinical signs that could advance at a second stage to acute respiratory distress syndrome (ARDS), systemic inflammatory responses, and a 'cytokine storm' [72,73]. These hallmarks of severe COVID-19 triggered repurposing attempts of different classes of immunoactive drugs. To date, the low-cost corticosteroid dexamethasone is still one of the most effective treatments of severe COVID-19 symptoms identified. Treatment reduced patient mortality in the phase 3 ReCOVERY study [74^{••}], confirming a role of late-stage hyperinflammation in case fatalities.

Table 3

Collection of some online resources for COVID-19 antiviral research

Resource	URL
WHO – COVID-19 Clinical trials compilation	https://www.who.int/clinical-trials-registry-platform
clinicaltrials.org – COVID-19 Clinical trials compilation	https://clinicaltrials.gov/ct2/results?cond=COVID-19
NIH – SARS-CoV2 Antiviral Therapeutics Summit	https://videocast.nih.gov/watch=38975
NIH – COVID-19 treatment guidelines	https://www.covid19treatmentguidelines.nih.gov/
NCATS – Preclinical Research Toolbox	https://ncats.nih.gov/expertise/preclinical
NCATS – COVID19 open data portal	https://opendata.ncats.nih.gov/covid19/
PubChem – open database	https://pubchemdocs.ncbi.nlm.nih.gov/covid-19
ReFRAME – Compound collection and open database	https://reframedb.org/
COVID box – Compound collection	https://www.mmv.org/mmv-open/covid-box
Pandemic Response Box – Compound collection	https://www.mmv.org/mmv-open/pandemic-response-box
FDA – Guidance for Industry – Antiviral Product Development	https://www.fda.gov/media/71223/download
Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) initiative	https://covid19.nih.gov/nih-strategic-response-covid-19/about-activ
SOLIDARITY trial	https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments
RECOVERY trial	https://www.recoverytrial.net/
High performance Computing Consortium (HPC)	https://covid19-hpc-consortium.org/
WHO – Access to COVID-19 Tools (ACT)	https://www.who.int/initiatives/act-accelerator
Corona Accelerated R&D in Europe (CARE)	https://cordis.europa.eu/project/id/101005077

Although the COVID-19-associated ‘cytokine storm’ has not yet been fully characterized, interleukin-6 (IL-6) is known as a major driver of hyperinflammatory responses. Potent inhibitors of IL-6 and its receptor such as siltuximab and tocilizumab showed mild benefits in phase 3 clinical trials [75–77]. Targeting downstream Janus kinase (JAK) signaling pathways with baricitinib in combination with remdesivir furthermore showed clear benefit compared to remdesivir with placebo in reducing severe illness [78,79], granting this combination treatment emergency use approval by the FDA in November 2020.

The fusion protein CD24Fc provides protection against simian immunodeficiency virus induced pneumonia in rhesus monkeys by fortifying the immune checkpoint CD24–Siglec 10 interaction to reduce inflammation [80]. Repurposing this approach against COVID-19 appeared also effective, since preliminary evidence reported a 50% reduction in mortality and respiratory failures in a still active phase 3 study. By comparison, antiviral interferon β -1a had little to none effect on hospitalized patients in the large Solidarity trial [17**]. However, patients improved more rapidly when an aerosolized form of interferon β -1a was administered at an earlier disease stage [81]. Current standard-of-care and ongoing trials have thus consistently confirmed the importance of immunomodulators as crucial therapeutic tools to mitigate severe COVID-19.

Conclusions

Many repurposing screens carried out in response to the COVID-19 pandemic have had very little impact because some basic principles of drug development were

overlooked or may have been considered irrelevant in the face of a global pandemic. Looking forward, we believe that avoiding known caveats in two critical areas of hit discovery specifically will help to rapidly separating distractions from viable therapeutic candidates:

i) Assay design:

- Permissive immortalized cell lines such as VeroE6 cells offer a readily available system for initial screens. However, as steps of the viral replication cycle might be fundamentally altered in these cell lines, the risk of false positives (i.e. chloroquine) and false negatives (i.e. camostat mesylate) is high. Meaningful counterscreens must be integrated into any discovery campaign that validate the physiological relevance of results (i.e. disease-relevant primary cells or organoid models [4**]).

ii) Contextualization of data:

- Basic cytotoxicity/cytostatic assays must be part of any antiviral screen, and very low SI values are a warning that the observed effect is likely off-target. Rigorous assay evaluation is critical, since especially cytostatic effects can easily be masked by improper assay design. For repurposed drugs, historic cytotoxicity data are usually plentiful in the literature and online databases (Table 3) provide a path to instant virtual counterscreens.
- Minute antiviral effects must not be overinterpreted, since virus growth is logarithmical in nature. This problem is often exaggerated by a narrow focus solely on EC_{50} (rather than more robust EC_{90} or EC_{99}) values of inhibitor candidates that do not necessarily reflect true antiviral impact.

- Known pharmaceutical parameters of repurposed drugs such as pharmacokinetics, pharmacodynamics, tissue distribution, and tolerability must be included in the evaluation of whether an antiviral effect observed in cell culture has clinical potential (i.e. ivermectin). Approved drug status should not be falsely interpreted as a guarantee of safety in humans at any concentration.

Considering these basic parameters during the COVID-19 crisis could have avoided unnecessary clinical trials that burdened trial participants unnecessarily and bound resources. This experience also raises the fundamental question of how likely drug repurposing screens are to deliver on their core promise to identify a rapid pharmacological first-line defense against a newly emerged viral pathogen.

Well conducted drug repurposing strategies have saved lives, as demonstrated by the examples of direct-acting remdesivir and the immune modulators. By contrast, repurposing searches for applicable host-directed antivirals and rushed efforts to test compendiums of known bioactives against SARS-CoV-2 were unsuccessful, as were similar earlier activities against, for instance, pandemic 2009 influenza virus, Ebola virus, MERS, and Zika virus. This disappointing outcome is not surprising, given that a large proportion of the known bioactives is made up of failed developmental candidates of yesterday's campaigns that were presumably abandoned for good reason. However, the COVID-19 pandemic has channeled the development of exciting new tools (such as the REFRAME initiative) to make comprehensive profiles of known drug properties rapidly available to investigators. This information can greatly accelerate the discovery process if there is willingness in the research community to accept that an exciting repurposing 'hit' may simply represent a cytotoxic compound unsuitable for antiviral therapy.

In our view, the greatest potential for successful repurposing against a newly emerged viral challenge has been the selective testing of approved or advanced-stage experimental drugs with known broadened-spectrum direct antiviral activity. We believe that it is no coincidence that a known broad-spectrum antiviral, remdesivir, is the only small molecule drug that has received emergency approval for anti-SARS-CoV-2 therapy to date and that two promising candidates currently in clinical trials, molnupiravir and AT-527, had likewise demonstrated broad spectrum direct acting antiviral activity before the COVID-19 pandemic struck.

COVID-19 is without doubt not the last global viral pandemic. We believe that efforts are best directed at expanding the broad-spectrum antiviral arsenal in inter-pandemic times, which requires that viable non-

pandemic indications are identified that open up a path to clinical approval of new drugs before a crisis unfolds. Whereas nucleoside analog inhibitors have high promise of meeting the broad-spectrum requirement among the small-molecule drug classes, liabilities such as off-target effects, mitochondrial toxicity, and mutagenic and/or teratogenic potential must be understood and, if possible, mitigated. The magic drug that meets the needs of all patient groups and addresses all future viral challenges may remain perpetually elusive. However, in reach may be the development of a reasonably sized subset of antiviral drugs with diverse strengths and limitations, and overlapping but not identical broadened indication spectra that could drastically shorten first-line response times to a newly emerged viral challenge, fundamentally improving the global status of pandemic preparedness.

Conflict of interest statement

Nothing declared.

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