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The challenging pathway towards the identification of SARS-CoV-2/COVID-19 therapeutics

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The development of therapeutic agents against SARS-CoV-2/COVID-19 faces numerous barriers and a multidisciplinary approach to evaluating drug efficacy and toxicity is essential. Experimental and preclinical data should be integrated into a comprehensive analysis, where drug potency, the timing of therapy initiation, drug combinations, variability in systemic and local drug exposure and short- and long-term toxicities represent fundamental factors for the rational identification of candidates and prioritization of clinical investigations. Although the identification of SARS-CoV-2 therapeutics is a priority, rigorous and transparent methodologies are crucial to ensure that accelerated research programmes result in high-quality and reproducible findings.

The SARS-CoV-2/COVID-19 pandemic is one of the biggest challenges in recent history and multiple tools to control the spread and impact of the virus are currently under investigation. Although therapeutic agents have the potential to be useful tools in ameliorating symptoms and reducing mortality in patients, the development of effective drugs against SARS-CoV-2 has multiple challenges that require careful analysis. Here we summarize some of the key pharmacological challenges related to SARS-CoV-2 therapeutics.

Successful therapies for most other viral infections have been based on the combination of multiple agents.[1] Only in a very limited number of viral infections has monotherapy been shown to effectively inhibit viral replication and a combination of therapy targeting different steps of the viral life cycle is often critical to achieve clinically relevant viral suppression.[2] Is this important in relation to SARS-CoV-2? Is there the likelihood of selecting resistance mutations? We currently have a limited understanding of mutation frequency and the possible downstream effects on antiviral resistance and infectivity.[3] The selection of viral mutations may depend on the difference between the achieved plasma exposure and the potency of the drug. We clearly need to be cautious when conducting clinical trials of monotherapy with drugs of limited potency and often unfavourable pharmacokinetic/pharmacodynamic (PK/PD) profile.

The timing of initiation of therapy represents a pivotal element in defining the therapeutic effects. How long from the beginning of infection will an antiviral approach be valid? The interval between the acquisition of the SARS-CoV-2 infection and treatment initiation will vary but, currently, the great majority of patients will be identified and receive treatment after the onset of symptoms and often when seriously ill.[4,5] Therefore it is likely that the viral load will already be elevated in numerous anatomical sites and downstream physiopathological processes already triggered. Earlier treatment may allow for a drug with partial activity to change the trajectory of the clinical disease even if the virus is not completely suppressed. However, pharmacological strategies based only on the inhibition of viral replication will likely have a limited overall effect on the clinical outcome and a broader consideration of potential therapeutic strategies is essential. The pharmacological management of the downstream cytokine storm, inflammation, coagulation and physiopathological effects on other anatomical sites should be carefully considered in parallel or even with higher priority compared with the inhibition of viral replication.[6,7]

Antiviral drug potency is routinely measured through *in vitro* assays, which have methodological limitations that should be carefully considered. These assays utilize cell media containing limited amounts of proteins compared with the physiological environment of plasma and tissues in the human body and this can result in a much higher fraction of unbound drug compared with that in plasma and tissue. Consequently, the correction of EC_{50} and EC_{90} for protein binding (protein-adjusted EC_{90}, $PAEC_{90}$) is an important step to help in interpreting experimental data. This will be particularly relevant for drugs characterized by high protein binding (such as many of the suggested agents for repurposing) and a careful evaluation of available data is necessary, as done routinely in many other disease areas (e.g. HIV).[8] Additionally, the *in vitro* potency assays are based on immortalized cell lines, which are often not derived from human cells and as such do not represent the main target for the SARS-CoV-2 replication (e.g. Vero cell lines are derived from the kidneys of the African Green Monkey).[9]

The extrapolation of EC_{50}/EC_{90} values measured in such an environment to the *in vivo* situation should be taken cautiously unless there is a detailed understanding and characterization of the correlation between *in vitro* and *in vivo* antiviral potencies. Lessons learned from other disease areas can provide a rational framework to better understand the PK/PD requirements for guiding the identification of potential treatment options and only drugs that result in plasma and tissue concentrations several-fold above the $PAEC_{90}$ for the entire dose interval are likely to represent suitable candidates. It is important that adequate drug concentrations are present in all sites of viral replication. An integration of experimental *in vitro* approaches with animal models of SARS-CoV-2 should be pursued to further refine our understanding of PK/PD relationships and consequently limit the probability of overstretching the relevance of *in vitro* data, especially for drugs with low potency (EC_{90} in the μM range). In the great majority of cases, antivirals that have found successful application in other diseases are characterized by potency in the low nM range, further suggesting caution is needed when considering clinical investigation of drugs with *in vitro* activity in the μM range.[10–14] *In vitro*, animal and clinical data can be integrated into quantitative pharmacology approaches, but it is essential that the modelling framework is based on solid foundations and assumptions in order to provide a reliable representation of PK/PD of novel agents and therefore support sustainable strategies and prioritize clinical trials.

Many patients who could potentially benefit from receiving treatments against SARS-CoV-2 will have complex physiological characteristics and, in many cases, concomitant comorbidities and comedications.[15–17] Additionally, an increasing number of studies are describing how the SARS-CoV-2 infection can cause damage to different organs and tissues including the CNS, kidneys and heart.[18–20] Therefore, a comprehensive evaluation of the safety profile of potential therapeutic agents is of paramount importance, especially when considering repurposed drugs at higher doses than for the treatment of their original indication(s) and which can be characterized by concentration-dependent toxicities (e.g. QT prolongation).[21] However, we also draw attention to the fact that known toxicities of drugs used long term, often in non-infectious disease indications, may be less relevant if used short term against viral infections. The correct management of drug–drug interactions is also an important consideration when introducing these pharmacological agents in complex patients with multiple comorbidities. To date, hundreds of clinical trials are ongoing for the investigation of repurposed and novel therapeutic agents against SARS-CoV-2 and appropriately designed randomized clinical studies represent the most appropriate strategies to fully clarify the value of selected candidates. International efforts should be focused on the optimization of clinical investigations, enhancing the quality of study design, increasing the number of patients included in studies and reducing the number of studies for each drug and/or combination to avoid unnecessary duplication. It is also important to note that drugs that have insufficient activity for treatment may still have benefit in prevention/prophylaxis.

All the above elements indicate the importance of multidisciplinary collaborative initiatives to provide a broad and effective vision to identify and meet the challenges related to developing and refining pharmacological tools against SARS-CoV-2. The input from molecular, clinical and quantitative pharmacology, immunology, toxicology, medicinal chemistry and infectious disease is vitally important to give the best outcome. In addition, although fully aware of the urgency to develop tools to mitigate the damage caused by SARS-CoV-2, we also recognize the potential of compromising quality in the pursuit of accelerating research programmes. Publication and dissemination of data based on a less-than-rigorous approach is detrimental, not only for the constructive and progressive advance of pharmacological/medical knowledge but also for the messaging to the general public as well as other communities of specialists. In this regard, peer review is essential to ensure that scientific findings are robust and of high quality so that we do not compromise the vital trust that the public has in medical and scientific experts.[22]

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