Drug repurposing for COVID-19

Introduction

Coronaviridae is a family of viruses that is capable of infecting a variety of animals and causes respiratory, gastrointestinal, hepatic, and neurological syndromes that range from mild to severe. Severe acute respiratory syndrome (SARS) was first attributed to two zoonotic pathogenic coronaviruses in the years of 2002 and 2012, along with Middle East respiratory coronavirus (MERS-CoV), in humans[1,2]. Coronaviruses are single-stranded positive-sense RNA viruses with a genome size of about 30 kb that encodes a number of structural proteins, being one of the largest RNA viruses known to date[2,3].

In December of 2019 there were reports of an outbreak of pneumonia in Wuhan, China, with an unknown cause. This led to the identification of the causative agent, named SARS-CoV-2, and it rapidly spread to 66 countries in less than 4 months, leading to the World Health Organization (WHO) declaring it a pandemic on January 30th, 2020[2]. Continuous studies of this novel virus identified angiotensin-converting enzyme 2 (ACE2) as the main receptor for SARS-CoV-2, and because it initiates the infection process, the interaction between the viral spike protein and ACE2, on the host cell membrane, is of great interest[3]. Additionally, other targets of interest would be the inhibition of the replication machinery, RNA synthesis, assembly proteins of the viral particle and the proteolytic pathway, being that targeting of all these converges to the same goal, which is to eliminate or diminish viral infection and the consequences that derive from it[1].

According to the WHO reports' from April 5th to May 4th, 2022, there have been 29,3 million new cases reported and 96 000 people died of SARS-CoV-2 infections across the globe. Although a vaccine has been available since December 2021, which resulted in infection and death numbers showing decreasing trends, the rise of new SARS-CoV-2 variants of concern have rendered the previous vaccines less effective, making more people susceptible to infection, which can become severe. Therefore, the repurposing of drugs could aid the fight against SARS-CoV-2 providing an additional therapeutics approach to those individuals that develop severe symptoms and require hospitalisation[4,5].

Drugs that target distinct stages of the viral infection and propagation are relevant in order to attempt to optimise the outcome by inhibiting infection, ameliorating symptoms, and inhibiting viral transmission. Several drugs have demonstrated some type of efficacy in accomplishing these goals, but more efforts are necessary to obtain success and improve patient outcomes. Therefore, the use of drugs that have already been approved for certain medical conditions and the discovery of their application for other purposes is of immense value in the medical field, since it allows fast-tracking of the approval process, because the drug has already been assessed for its safety in humans and in terms of dosages[1,3]. Besides that, the creation of a *de novo* drug has an estimated cost of 1,5 billion dollars, while drug repurposing has a well-below cost of 300 million dollars[3].

While employing such repurposed medications alone may not provide a major therapeutic advantage, carefully mixed cocktails, as was the case with HIV in the 1990s, could be quite beneficial[6]. The question today is which combination will be effectively beneficial, being this the objective of this project, in regard to SARS-CoV-2.

Project proposal

In this project proposal, we plan to identify multiple drug candidates and drug combinations through *in silico* studies resorting to different databases and docking softwares. Following this, we plan to evaluate the most promising drug combinations *in vitro* using two cell lines. Lastly, we will conduct *in vivo* studies using hACE2 mice to assess the effectiveness and side effects of these drugs during SARS-CoV-2 infection (Figure 1). In Figure 2, we present a chronological plan for this project.

In order to achieve the goal of this study, we propose that *in silico* studies should be the starting point, to determine and select possible drug candidates, based on their interaction between themselves and with SARS-CoV-2 targets. For this, a literature review will be done, followed by databases and platforms such as Schrödinger Maestro[7] and SwissDrugDesign[8] (drug-target interaction), DrugBank[9] (information on the drug and side effects), IBM Micromedex[10] (drug-drug interaction and IV compatibility), DDInter[11] (drug-drug interaction) research and modelling. To obtain robust results, we will use Schrödinger Maestro and SwissDrugDesign to do molecular docking, and IBM Micromedex and DDInter to determine drug-drug interactions.

Following this, the obtained candidates from the *in silico* studies, will be evaluated in cell lines of HeLa ACE2 and HEK293/ACE2 to determine the best combination of drugs to advance to the next stage, which is *in vivo*. The VEROE6 cell line will be used to replicate the SARS-CoV-2 virus for this study. The controls would be cells without infection with and without treatment, and cells with infection. The treatments would be composed of paired drug combinations, and determination of drug ratios (50:50; 60:40; 70:30). To access these results, molecular markers related to SARS-CoV-2, such as RNA-dependent RNA polymerases (RdRp), 3C-like protease (3CLpro), spike protein (S glycoprotein), nucleocapsid proteins, RNA molecule of SARS-CoV-2, membrane and envelop proteins, will be determined through qPCR. Additionally, ImageStream will be used to evaluate cellular viability, through the use of fluorescein diacetate, and antibodies labelled with fluorochromes against SARS-CoV-2 spike protein. Lastly, statistical analysis will be conducted to determine their significance.

The two drug pairs that show highest efficacy, in the previous studies, will be selected to be applied *in vivo* in mice expressing hACE2. Four mice, two males and two females, for each different age group will be used: juveniles (5 weeks); mature adults (3-6 months); middle-aged adults (10-14 months) and old (18-24 months). These mice will be fed a standard diet and water *ad libitum* and will be infected using intranasal inoculation. Regarding control groups, these are composed of non-infected mice without treatment. In terms of treatment, the selected drug pairs will be administrated on day 1, 3 and 8, post-infection. Additionally, mice's weight will be monitored daily. All mice will be sacrificed 10 days post-infection, and the lungs and blood samples will be collected for analysis. To assess the results, qPCR of lung samples to detect SARS-CoV-2 using the previously described molecular markers, as well as an ELISA assay of the serum of each mouse will be done to evaluate antibody production. Fluorescent microscopy applied to the lungs will be conducted to visualise histopathology slides of the tissue, stained with haematoxylin and eosin, and the same antibodies against SARS-CoV-2 used in the *in vitro* studies.

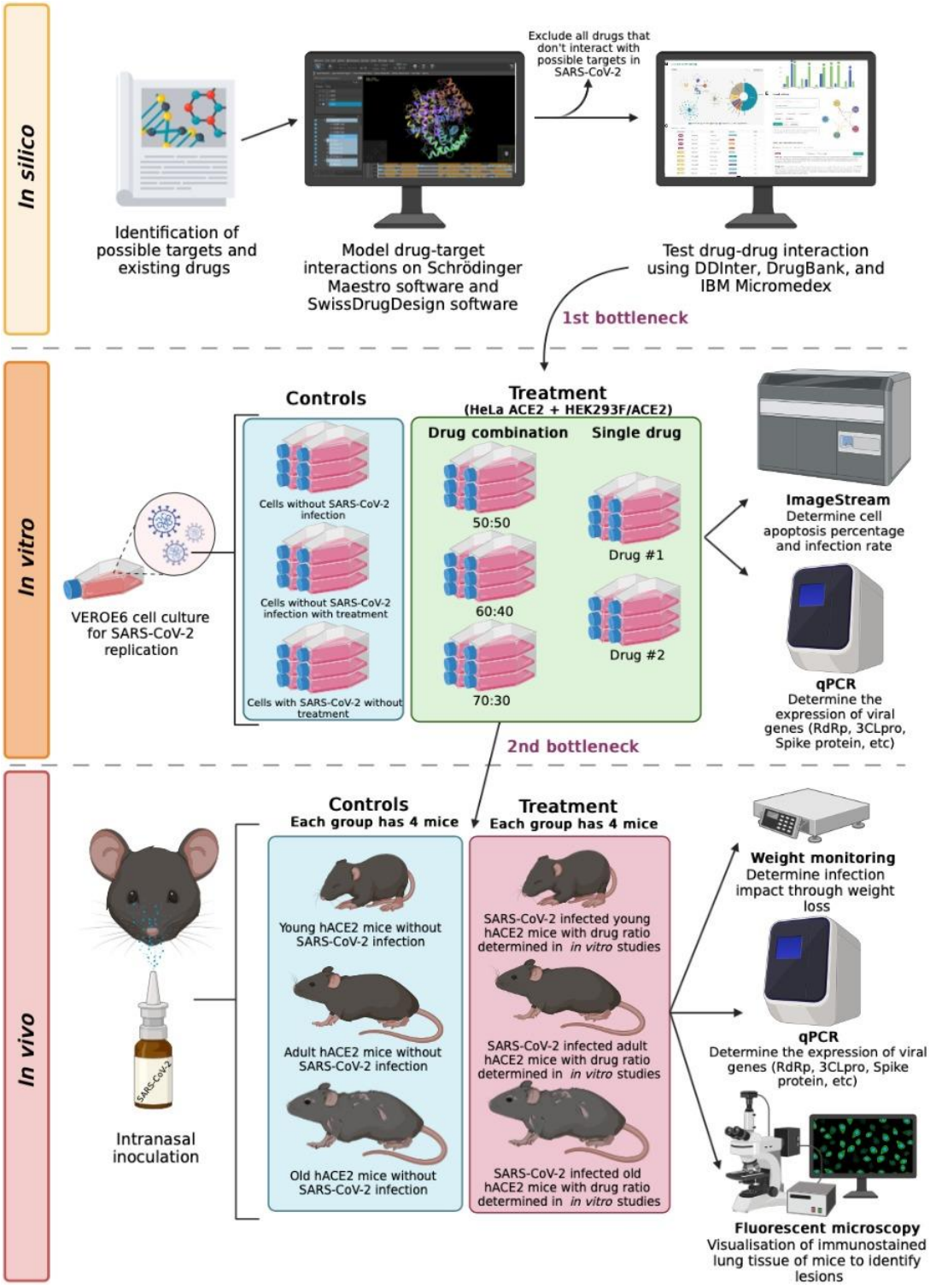

Figure 1| Schematic representation of the methodology to be applied in this study. Created with Biorender.com.

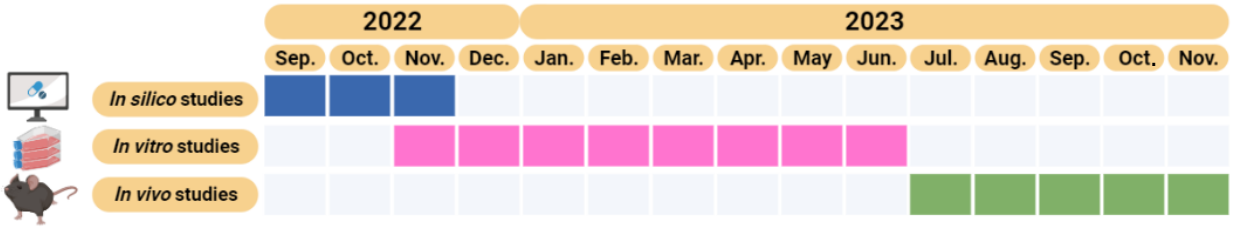

Figure 2| Chronological representation of the planning foreseen for each step. Created with Biorender.com.

Expected results

Through this experimental design, we hope to obtain two possible drug combinations, which can inhibit or diminish infection, transmission and/or COVID-19 symptoms *in vivo*. We consider that a few challenges could arise, namely during the *in silico* studies, since high volumes of data will be generated and collected, and these identified drug combinations could possibly not even work *in vitro*.

Ethics disclosure

All animal procedures will comply with the Federation of European Laboratory Animal Science Associations (FELASA) regulations.

Future perspectives

AI drug repurposing based on genomics, transcriptomics and phenomics. The effectiveness of this drug repurposing technique will be determined by whether such drugs compare favourably to virus-specific vaccines or small molecules, both of which have long been considered gold standards in modern therapeutic research.

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Methodology softwares for *in silico* studies

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