

# An oral live attenuated vaccine strategy against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019-nCoV)

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## Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019-nCoV) infection has become a pandemic called COVID-19. The virus binds to angiotensin converting enzyme 2 (ACE2) and TMPRSS2 which are abundantly expressed on various human cells including lung epithelial cells and intestinal cells and the virus can infect these cells. Currently no specific treatments or vaccines are available for this disease. A per oral live attenuated vaccine can be a good strategy in SARS-CoV-2 infection because the attenuated virus initially infects the gut, stimulates the mucosa associated immune system sparing the respiratory system during the initial immune response. The live virus can also spread in the community boosting herd immunity.

## Keywords

Oral Live Attenuated Vaccine, Severe Acute Respiratory Syndrome Coronavirus 2/SARS-CoV-2/2019-nCoV, Angiotensin Converting Enzyme-2 (ACE2), Gut Infection, Proximal and Distal Enterocytes, Herd Immunity

## Overview and background

COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), a virus, which is spreading across the globe. Most of the infected people show only mild symptoms, however, some progress to viral pneumonia, multi-organ failure and death. SARS-CoV-2 is mainly transmitted through droplets (resulting from cough, sneezing etc.) and fomites. The only proven preventive measure to reduce person-to-person transmission of COVID-19 is social distancing. There are no proven therapy or vaccination available for the disease. Spike (S) proteins of coronaviruses, including SARS-CoV-2 interacts with cellular receptors on their target host cells (Li et al. 2003). It is interesting to note that SARS-CoV-2 infects the cells in the gastrointestinal tract (Liang et al. 2020) and diarrhea is an important (but often ignored) symptom in COVID-19. Here we propose to make use of the entero-trophic property of SARS-CoV-2 in making an Oral Live Attenuated Vaccine for COVID-19 disease. We propose that the attenuated live virus will initially infect the gut, stimulates the mucosa associated immune system whereby sparing the respiratory system during the initial immune response and the damage caused by the immune response. The live attenuated virus can spread in the community. This silent spread will possibly boost the herd immunity and break the transmission chain.

## Objectives

To develop an oral live attenuated vaccine for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019-nCoV) Disease.

## Impact

Severe Acute Respiratory Syndrome Coronavirus 2 infection has become a pandemic called COVID-19. Several thousands of people are expected to die from this disease this year (Verity et al. 2020). Currently there is no vaccine for COVID-19. Several countries imposed "lockdown"-shops, schools, universities, transportation systems, industries were shut down. The crude oil prices and stock market crashed (Rae 2020). According to United Nations, the world economy could shrink by almost 1% in 2020 due to COVID-19 pandemic (The Associated Press 2020). The pandemic also caused political tension among different countries, especially between the United States and China (Cassidy 2020).

## The Concept and Strategy

Severe Acute Respiratory Syndrome Coronavirus 2 infection has become a pandemic called Coronavirus disease 2019 (COVID-19). Protein modeling experiments on the spike protein of the virus suggested that SARS-CoV-2 has sufficient affinity to the angiotensin converting enzyme 2 (ACE2) and TMPRSS2 receptors on human cells to use them as a mechanism of cell entry. Later it was experimentally demonstrated that ACE2 could act as the receptor for SARS-CoV-2 (Zhang et al. 2020). Diarrhea is one of the early symptoms in

a significant population of COVID-19 patients (Liang et al. 2020). The expression profiles of ACE2 in various human tissues found that ACE2 was highly expressed in the human small intestine (proximal and distal enterocytes) while the RNA level of ACE2 was quite low in lung tissues from healthy donors (Jia et al. 2005, Liang et al. 2020). Immune response against SARS-CoV-2 is believed to be effective in man. So far, no significant cases of confirmed re-infection are reported. Few cases of "re-infection" are very likely the result of false reporting (false negatives). It is important to make sure that a patient had truly recovered from COVID-19 before he is reported "negative" and disease free (Alizargar 2020). A recent study on monkeys also confirms the view that the first infection provides a useful immunity (Bao et al. 2020). We propose a live attenuated SARS-CoV-2 vaccine which would be administered orally so that it will initially infect intestinal cells, temporarily colonize gut and generate immunity. The danger of infecting lungs directly and the immediate risk of lung infection and inflammation can be avoided. An attenuated vaccine is a vaccine made by mutating the virulence of a pathogen through various methods, for example through multiple passages in culture, but keeping the virus live (Jang and Seong 2012). The virus becomes harmless or less virulent but capable to generate an immune response. A live attenuated vaccine is used for influenza vaccine in the form of a nasal spray (Smith et al. 2012). However, for SARS-CoV-2 an oral route could be better considering the abundance of ACE2 in gastrointestinal tract. Besides the mucosa-associated lymphoid tissue (MALT), a diffuse system of lymphoid tissue found in various submucosal membrane sites of the body, such as the gastrointestinal tract, would help in achieving a strong immune response against the virus (Czerkinsky and Holmgren 2010). Compared to intramuscular injections, peroral administration is easy and cost effective.

## **Severe Acute Respiratory Syndrome Coronavirus 2**

The SARS-CoV-2 was first discovered in Wuhan, China. The disease (COVID-19) spread rapidly and killed about 54,000 people as on 3 April 2020 (Berlinger et al. 2020). It causes a disease known as Coronavirus disease 2019 (COVID-19) which has predominantly respiratory symptoms. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as the 2019 novel coronavirus (2019-nCoV), is a positive-sense single-stranded RNA virus (Riou and Althaus 2020). SARS-CoV-2 has been sequenced and the phylogenetic analysis suggested that the virus might have originated from bats (Andersen et al. 2020). SARS-CoV-2 spreads among humans and has an R0 of 2-2.5 (meaning that each new person spreads the disease to about 2 to 2.5 people). The World Health Organization (WHO) has declared the ongoing pandemic of COVID-19 International Public Health Emergency. A few vaccine trials are ongoing (Table 1), however, no vaccines are currently available for COVID-19. To our knowledge none of the clinical trials are using an oral live attenuated vaccine strategy. No specific therapeutics are currently available, and current management is limited to travel restrictions, patient isolation, and supportive medical care for amelioration of symptoms. However, recently there are some studies reporting the effectiveness of hydroxychloroquine and Remdesivir (Liu et al. 2020).

Table 1.

Ongoing nCoV-19 vaccine trials.

Study title	Method/Aim	Location
Safety and Immunity of COVID-19 aAPC Vaccine	COVID-19 minigenes engineered based on multiple viral genes, using an efficient lentiviral vector system (NHP/ TYF) to express viral proteins and immune modulatory genes to modify artificial antigen presenting cells (aAPC) and to activate T cells. In this study, the safety and immune reactivity of this aAPC vaccine will be investigated.	Shenzhen Geno-Immune Medical Institute
Immunity and Safety of COVID-19 Synthetic Minigene Vaccine	COVID-19 minigenes engineered based on multiple viral genes, using an efficient lentiviral vector system (NHP/ TYF) to express viral proteins and immune modulatory genes to modify dendritic cells (DCs) and to activate T cells.	Shenzhen Geno-Immune Medical Institute
Reducing Health Care Workers Absenteeism in Covid-19 Pandemic Through BCG Vaccine	The risks of BCG vaccination are considered low. The objective of this trial is to evaluate the beneficial effects of BCG vaccination through a lower work absenteeism rate of HCW and/or a mitigated clinical course of Covid-19 infection.	UMC Utrecht, Radboud University
Safety and Immunogenicity Study of 2019-nCoV Vaccine (mRNA-1273) for Prophylaxis SARS-CoV-2 Infection	mRNA-1273 is a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized spike (S) protein of SARS-CoV-2.	Emory Vaccine Center - The Hope Clinic Decatur, Georgia, US National Institutes of Health, Kaiser Permanente
BCG Vaccination to Protect Healthcare Workers Against COVID-19	Subjects will be randomized to receive a single dose of BCG vaccine, or no BCG vaccine. Participants will be followed-up for 12 months to identify COVID-19 infection.	Royal Children's Hospital Melbourne, Victoria, Australia
A Phase I Clinical Trial in 18-60 Adults	To understand the safety, reactogenicity and immunogenicity of Recombinant Novel Coronavirus Vaccine (Adenovirus Type 5 Vector). Intramuscular (IM) injection of experimental vaccine	Hubei Provincial Center for Disease Control and Prevention Wuhan, Hubei, China
A Study of a Candidate COVID-19 Vaccine (COV001)	A phase I/II single-blinded, randomised, placebo controlled, multi-centre study to determine efficacy, safety and immunogenicity of the candidate Coronavirus Disease (COVID-19) vaccine ChAdOx1 nCoV-19 in UK healthy adult volunteers aged 18-55 years. The vaccine will be administered intramuscularly (IM).	NIHR WTCRF University Hospital Southampton NHS Foundation Trust Imperial College University of Oxford
Reference: <a href="https://clinicaltrials.gov/ct2/results?cond=Covid+vaccine&amp;term=&amp;cntry=&amp;state=&amp;city=&amp;dist=Accessed">https://clinicaltrials.gov/ct2/results?cond=Covid+vaccine&amp;term=&amp;cntry=&amp;state=&amp;city=&amp;dist=Accessed</a> on 5 April 2020, 20:20 IST A detailed list is available at: <a href="https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1">https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1</a>		

## Intestinal Cells are rich in ACE2 which is a SARS-CoV-2 Receptor

The virus enters human cells by binding to angiotensin converting enzyme 2 (ACE2) receptors. This was predicted using computer modeling (Zhang et al. 2020). SARS-CoV spike protein has a strong binding affinity to human ACE2, based on biochemical interaction studies and crystal structure analysis. The glutamine amino acid residue at position 394 in the SARS-CoV-2 receptor-binding domain corresponding to residue 479 in SARS-CoV, will interact with the critical lysine at position 31 human ACE2 receptor (Li 2005, Wan et al. 2020, Zhang et al. 2020). It is interesting to note that diarrhea is an important symptom of COVID-19. This can be explained by the richness of ACE2 expression in proximal and distal enterocytes and the virus, as expected, infects the gut epithelial cells (Liang et al. 2020).

## Oral Live attenuated Vaccine for COVID-19

A vaccine is expected to be effective for COVID-19 because the virus, Corona Virus, is a large and complex virus compared to other RNA virus, possesses several essential and conserved domains and a recent study reported that reinfection could not occur in SARS-CoV-2 infected rhesus macaques (Bao et al. 2020). Live vaccines contain weakened/attenuated form of the pathogenic organism (SARS-CoV-2) that causes a disease. Since the pathogens used in live vaccines causes an infection which is similar to natural infection, a strong and lasting immune response develops against the pathogen but without causing the actual disease (Minor 2015).

We could infect and serially passage SARS-CoV-2 in Vero cells [ATCC-1586] (ATCC 2020), (a cell line from African Green Monkey (*Chlorocebus* sp.) which used for the production of several attenuated and inactivated viral vaccines), expressing ACE2 the SARS-CoV-2 receptor. Vero cells unlike other cell lines, do not secrete interferon  $\alpha/\beta$  when infected by a virus (Ammerman et al. 2008). SARS-CoV-2 can infect Vero cells expressing ACE2. Virus will undergo mutations and selections in culture with each passage especially at suboptimal conditions. These mutations would make the virus less virulent and pathogenic. The serially passaged virus would eventually be allowed to infect human intestinal cell line such as Hs 1.Int /HuTu 80/ Caco-2 after experimentally verifying the expression of ACE2, infectivity and viral replication (ATCC 2020, Jang and Seong 2012). Alternatively, a genetic reassortment between attenuated donor Corona virus strain and SARS-CoV-2 can be achieved (Jang and Seong 2012). This strategy has the potential to provide an extended coverage to heterologous infections which is a possibility with RNA viruses along the course of the epidemic due to inherent mutability and generation of hybrid viruses by genetic mixing with other wild viruses (Jang and Seong 2012, Andersen et al. 2020).

The live attenuated virus would be perorally administered. The virus would infect the intestinal cells, multiply and is expected to provide a long-lasting immunity. The advantages of these live vaccines are "community spread" and faster development of herd immunity and the ease of administration. The live vaccine is potentially transmissible, speeding the herd immunity in the community (Bull et al. 2018, Nuismer et al. 2019). Live vaccine

contains the live virus although attenuated. Theoretically, it is possible that through genetic variability and spontaneous changes in the genetic material the virus can revert to its wild strain. Therefore, some live vaccines are administered only in healthy people ages 5 to 49 with no comorbidities. It may be noted that 70-90% of the infected and tested positive people recovers from COVID-19 without the need for hospitalization. We know that most of the mortality occurs in older or aged, diabetic and immunocompromised patients and those with cardiovascular diseases (Messner 2020). These groups would not be eligible for a live vaccine. However, it maybe noted that, if 40-60% of the population becomes infected with SARS-CoV-2, herd immunity develops sufficiently to break and block the transmission chain which is a major advantage of this "live attenuated virus approach" (Horton 2020).

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## **Hosting institution**

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## **Ethics and security**

This work is being presented as a hypothesis and therefore Institutional Ethical Committee Approval is required.

## **Author contributions**

Sanal Madhusudana Girija: Conceived the idea and drafted the manuscript.

Ravi Chandra Dubey: Commented on/reviewed the mauscript.

## **Conflicts of interest**

None of the authors have any conflict of interests to declare.

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