Proposal for supportive/preventive treatment of COVID19 using siRNA to suppress expression of ACE2 receptors and vaccines based phage-display technology developed by epitopeRX

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Abstract

This paper proposes a combined prophylasis and treatment for COVID-19 with two main parts consisting of epitopeRX phage-display vaccine and treatment of COVID-19 with ACE2 suppression via native, cell-level siRNA silencing technology.

Authors have reviewed the evolving literature and have proposed solutions which are driven by a combination of trends and observations that are emerging. They anticipate further advances in precision of tools (e.g., use of PCR and other sequencing technology) used to more definitively characterize the viral evolution as well as refinement of vaccine candidates through animal and clinical trials.

Given the COVID-19 pandemic, the authors recognize the importance of continuing to uncover potential treatments through an extensive examination of existing literature, research and generation of novel insights regarding potential treatments.

Introduction

The current pandemic outbreak of SARS-CoV-2 that originated in Wuhan, China poses a great risk in terms of straining healthcare systems in affected areas and causing substantial damage to the population.

While mortality rate is usually in focus, what makes the situation even worse is potentially high number of convalescents with permanent disabilities.

Those disabilities, most likely deficiencies in pulmonary functions, can be expected given relatively (in comparison to seasonal flu) high ratio of severe viral pneumonia.

Therefore, it's crucial to develop means to prevent spread of the virus in the first place, but also reduce incidence of severe cases among already infected. We propose multi-part solution, addressing the above issues.

Viral mechanism

As per Xu, X., Chen, P., Wang, J. et al. [1] the virus uses predominantly ACE2 cell receptors as its point of entry attaching to them thanks to its spike protein. That protein is nearly identical in all SARS/SARS-like viruses, though small differences in SARS-CoV-2 version result in stronger binding force. That property may explain higher virulence of SARS-CoV-2 when compared to related viruses.

Additionally, as per Diao et al. [2] the virus manages to significantly reduce populations of immune $CD4^+$ and $CD8^+$ cells leading to increased spread inside host's body and decreased survivability.

As per Wang et al. [3] the virus binds also to CD147 receptor.

Proposal

The SARS-CoV-2 binds to the ACE2 receptors predominantly in the lungs (however other organs with high expression of it are also affected). Given that fact, decreasing availability of those receptors should result in limiting virus' ability to invade cells and effectively attenuate a SARS-CoV-2 infection.

ACE2 receptor is responsible for mediating angiotensin II hormone that is a part of renin-angiotensin system regulating blood pressure. Consequently, observed distribution of COVID-19 cases in population reflects a pattern of blood pressure variability in population. Also, most frequent co-morbidities resulting in higher susceptibility to infection and development of severe viral pneumonia - hypertension, diabetes, cardiovascular problems - are mostly related to blood pressure regulatory mechanism [4].

Regulating blood pressure is a common practice in treating hypertension and thus drugs used for this purpose are studied for their potential use in treating COVID-19. However, their metabolic pathways and side effects can limit their application in hypertensive patent populations.

Our proposal of addressing COVID-19 disease can be split into three parts, listed below.

Silencing ACE2

As mentioned above, RBD of the spike protein evolved to bind specifically ACE2 receptors and is very effective in doing so. As it was shown by Haga et al. [5] in case of very similar spike's RBD of SARS-CoV (the close relative of SARS-CoV-2 that caused an outbreak in 2002/2003): "Experiments using deletion mutants of ACE2 revealed that the cytoplasmic tail of ACE2 is re-

quired for shedding". Further in that research authors note that "siRNAs of TACE and ACE2 blocked viral infection".

Therefore, silencing ACE2 impairs virus' ability to infect cells. Can siRNA be delivered efficiently? Apparently, yes. Alam et al. [6] cite several successful attempts at delivering siRNA using nanocarriers in treatment of hypertension. Techniques presented in detail in Koenig et al.[7] and Nolte et al.[8] show that use of siRNA provides a tool to efficiently shape responses of renin-angiotensin system.

Given the above, we propose treatment based on above techniques aimed at designing siRNA molecules temporarily silencing expression of ACE2 receptors in order to reduce virus' infection surface to levels enabling immune system to effectively stop infection if it's pending or prevent it otherwise.

Out of carriers capable of efficient delivery, two seem promising. First, SNALP (stable nucleic acid lipid particles) liposomes. It was shown in primates [8] that they deliver siRNA effectively and that the effect persists for prolonged time (11 days).

Second – the virus itself. Inactivated virus carrying siRNA could certainly be efficient due to selective nature and certain delivery to cells that are endangered by live virus infection. Use of the viral coat and spike proteins that could encapsulate and targeted deliver the siRNA could serve two purposes – aimed delivery to the cells that are subject to COVID-19 binding as well as blocking COVID-19 binding.

Therefore, using the viral envelope would serve as transport mechanism for the siRNA. However, in subsequent subsection we show why exposing fully formed spike proteins doesn't have to be beneficial.

Vaccination

Vaccines have had enormous impact in prophylactically preventing disease spread as well as, in some cases, offering treatment. However, anti-viral vaccines can be designed using an array of different methodologies (RNA/DNA vaccines, recombinant proteins, phage display, live in-active, and other methodologies). Extensive clinical testing is required as the bar is high to 'cause no harm' in a disease-free population that the vaccines are administered to. In addition, the human immune system with cellular and humeral components is complicated and there are no means of anticipating which vaccine candidate will be efficacious and safe, or predicting a side effect profile.

The actual level of complexity of such task is shown by Huisman et al. [9] - preparation of vaccines, specifically choice of proteins that are provided to the immune system to facilitate the system's production of antibodies and/or sensitivity of T-cells to pathogens can be tricky. Providing full, unaltered versions of binding domain proteins, bears risks ranging from no or low efficiency to hypersensitivity.

It should be noted that given [3] findings exposing unaltered version of spike proteins can potentially trigger hyper-inflammation as in live virus infection.

Therefore, it's crucial to present to the immune system proteins that won't cause adverse effects. One of most promising ways to produce such proteins

is phage-display (as now studied for use in immunizing against SARS-CoV-2 by epitopeRX). The company, working with a consortium of organizations, enables screening for COVID-19 immunogenic surface proteins that can be used for vaccine production, rapid vaccine construct implementation, high scalability through fermentation, thermal stability (not requiring cold-chain transport) and low cost with a target of less than $2 (US) per vaccine dose. All of these advantages are critical for global vaccine production.

Supportive treatment - needs further evaluation

As mentioned above, SARS-CoV-2 exhibits ability to significantly reduce populations $CD4^+$ and $CD8^+$ cells.

It's therefore important to shield them from virus' influence.

While precise way that influence occurs seems not to be well researched, analogous case of impact of resveratrol on MERS virus described by Lin et al. [10] together with this observation by Cullen et al. [11] seem to suggest that CCR2 chemokine receptor is a likely viral target that alters behavior of immune cells. Fairly close relation of SARS and MERS and incidence of cytokine storms in COVID-19 support need for investigating this path.

Articles cited above provide a candidate for inhibiting drug – resveratrol. It's highly selective in inhibiting both CCR2 and CCR4 chemokine receptors, both responsible for mediation of CCL2. If proven efficient, could complement the treatment when infection is already pending and symptomatic by prohibiting overdrive inflammation, however its cytotoxic properties must be taken into consideration.

Moreover, recent identification by Wang et al. [3] of additional receptor to which SARS-CoV-2 binds as well - CD147 - suggests that monocyte reaction can be a response to a chain of immune signals triggered by the virus.

Discussion

This paper describes two means of controlling the spread of COVID-19. One involves a method for decreasing the morbidity of the infection by diminishing the ability of the virus to propagate by decreasing availability of the ACE2 receptors. In addition, we have found that the virus appears to reduce populations of the $CD4^+$ and $CD8^+$ cells which modulate the cellular immunity of the body and suggested a possible solution, though that path needs further investigation.

First and foremost, we strongly suggest the use of a vaccine construct that is agile as RNA viruses such as COVID-19 frequently mutate and hence the vaccine construct must have the capability for rapid construction to vaccinate against the potential changes that will likely occur with this pathogen. Furthermore, a low-cost vaccine enables world-wide distribution which is needed for comprehensive pandemic immunization. The vaccine also needs to be rapidly scaled to achieve the needed volume for a world-wide vaccination campaign. Lastly, it is preferable to provide a vaccine that does not require cold-chain transportation so that there is no need for refrigeration which may not be available during a pandemic as well as in certain areas lacking this

infrastructure. Ideally, the vaccine can be provided in a trans-dermal administration, to enable use in situations in which needles are not trusted or are stolen or misused.

EpitopeRX provides the technology for a bacteriophage display antigen technology that has all of the above advantages. Our concern is that many of the vaccines are based on technologies that do not have proven efficacy and also have significant challenges for scalability; while the approach that we have outlined makes use of a similar approach as recombinant vaccine production (which is one of standard methods), it also provides significant advantages including more rapid vaccine construct design, faster scalability, and means to introduce minor modifications to produce vaccines that can address future changes in the genome of SARS-CoV-2.

While efficient vaccination is an ultimate goal when it comes to extinguishing COVID-19 pandemic, ACE2 silencing is a technology that can serve both as a pre-treatment before the vaccine is introduced and a treatment of choice for all viral strains targeting ACE2 receptors. Below we list benefits and traits related to that component of proposed treatment.

Benefits:

- **Both preventive and suppressive action**
  Proposed treatment can be applied to both healthy and sick individuals with minimal risks. In healthy it would minimize chances of infection, in sick - lower risks of development of severe form.
- **Use of native, cell-level mechanism**
  Targeting mechanisms responsible for ACE2 expression directly results in greater control and predictability.
- **Agnosticism with respect to a pathogen**
  Actual pathogen repelling function is just an accompanying effect of down-regulating renin-angiotensin system. Therefore, all pathogens that target ACE2 as entry path will be affected.
- **Adjustment**
  Treatment can be adjusted based on metrics provided by commonly used markers, like plasmin. It should be noted that humans tolerate wide range blood pressure regulation and that property is leveraged by hypertension treatments.
- **Hypertension screening**
  A by-effect, a need to adjust treatment to the activity level of blood pressure regulatory system would help to identify individuals needing further evaluation in terms of hypertension or hypertension related comorbidities.
- **Versatility**
  Drugs developed to silence ACE2 receptors can further be used in treating patients with hypertension or hypertension related comorbidities even if the pandemic is extinguished.
- **Prolonged effect**
  As shown in [8] the effect can be stretched in time to several days. That factor is of particular importance in time of pandemic when minimizing

contacts frequency is important.

Traits:

- **siRNA specific problems**
  Issues like off-target activity and unwanted immune responses should be concerning. However, we believe, based on evidence a part of which is presented above, that addressing expression of ACE2 is already a well reseached subject. Also, use of proper carriers can eliminate adverse effects from immune system.
- **Choice of proper carrier**
  As already pointed out, leveraging viral envelope as a carrier can yield adverse effects that may out-weight benefits. Hence, use of synthetic, maximally inert, carrier should be investigated first.

Conclusion

This analysis is based on a rapidly evolving literature, experience in phage-display technology, and insights that we have gleaned from multi-disciplinary analysis. In addition, with more viral testing, which makes use of genomic sequencing, we will be in receipt of more specific data that we can use to formulate therapeutic and prophylactic means of intervening in the pandemic. This work will be ongoing and needs to continue to be 'crowd-sourced' to enable examination of the best therapeutic options.

Given the ongoing COVID-19 pandemic, our rational, structured, flexible and, above all fast in terms of implementation, proposal is an effort to arrest the progression of this pathogen as well as seeding novel ideas for the prevention and treatment of the pandemic both for the current and future viral strains.

Additional literature and information available upon request.

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