



Improving and addressing lymphocytes response against SARS-CoV-2 and its variants with intralveolar BCG administration

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Abstract

Bacillus Calmette Guerin administered by the aerosol route may locally elicit and increase T1 lymphocyte response in pulmonary alveoli, which are the main target of SARS-2-CoV-2 and its variants, thus preventing and anticipating the virus attack. This may lead to a decrease of the most severe forms of the disease considered as an acute autoimmune virus induced disease. The lymphocyte response may start to work before and regardless of anti-CoVid vaccination.

Introduction

Since November 2019 a pandemic infection due to the SARS-CoV-2 virus is still expanding in the world through human-to-human transmission. It affects the respiratory tract, mainly the lungs, but in its most serious forms can also impact other major organs such as kidney, intestine, heart and brain. This infection has the following characteristics:

- a) It is rapidly contagious via airborne droplets containing viral particles from the mouth and nose through the larynx and the bronchi.
- b) It has various effects, which may range from infection without clinical manifestations to light or moderate pulmonary symptoms with spontaneous recovery, to rapidly evolving severe forms with serious respiratory insufficiency requiring forced oxygenation, with multi-organ failure and death.¹ The characteristics of CoVid-19 pneumonia are different from those of bacterial pneumonia.^{2,3}
- c) The virus may undergo rapid changes and mutations, which are likely to increase its contagiousness^{4,5} and consequent lethality.
- d) Severe forms are more frequent in older patients or patients with pre-existing comorbidities, such as diabetes and arterial hypertension.⁶
- e) In severe forms the amount of CD4 and CD8 lymphocytes has been significantly reduced in comparison with lighter forms and this has been attributed to an immunological impairment whose defense might be overcome by the virus.^{7,8}
- f) In the progression of the disease from "mild" to "severe" forms, a "cytokine storm reaction", "leukocyte changes" and vascular intracoagulation⁹ occur and the presence of viral particles has been demonstrated in the bronchial mucosa or in the alveoli.¹⁰ It has been hypothesized, however, that global BCG immunization may significantly impact the progression of the SARS-CoV-2 pandemic.¹¹

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g) BCG vaccination may induce a "heterologous" effect on "trained innate immunity", which may be recalled through non-specific BCG-immunostimulation.^{12,13} The administration of various kinds of vaccines for the production of IgM and IgG serum antibodies against CoVid-19 has begun. These vaccinations should be given to a large part of the world population as soon as possible, but the process is slow and complex and various trials are underway.^{14,15} There is no certainty that these vaccines will be effective against some mutated or varied forms of the virus and on the duration of their immunization over time.

Hypothesis

We suppose that CoVid-19 is a relevant immune-pathogenesis disease mainly involving the lungs, which can be more or less mild: local intra-alveolar administration of aerosolized BCG, in advance in a population at risk may alert and stimulate in a non-specific way the "trained native immunity" promoting the recruiting of T lymphocytes and directing them in advance towards the alveoli, which are the major targets of the virus. This may reduce the progression to more severe forms of the disease and the spread of the infection.

The action of aerosolized BCG would consist in directing the response of T lymphocytes, especially CD4+ and CD8+, which are crucial in eliciting a specific and adequate immune response and producing a long-term immunological memory,¹⁶ preventing the virus's action in the alveoli, which tends to dodge these defenses.¹⁷ This may occur prior to, or concurrently or regardless of the vaccine becoming available, which could be months away and be subject to a certain degree of uncertainty.¹⁸

This hypothesis is different as it gives more importance to no or weak lymphocyte response in severe forms with respect of the action of the virus on the alveoli, because lymphopenia prevents CSR and not the contrary. Viral lung injuries may be the result of weak, late or no action by T-lymphocytes whose components are reduced and in which the absolute numbers of T lymphocytes, CD4+ T cells, and CD8+ T cells decreased in nearly all the patients, and were markedly lower in severe cases (294.0, 177.5, and 89.0×106/L, respectively) than moderate cases (640.5, 381.5, and 254.0×106/L, respectively). It was thought that SARS-CoV-2 infection may affect primarily T lymphocytes, particularly CD4+ and CD8+ T cells, resulting in a decrease in numbers as well as IFN- γ production by CD4+ T cells. These potential immunological markers may be of importance because of their correlation with disease severity in COVID-19.¹⁹ Viral lung injuries may be caused by weak, late or no action by T-lymphocytes action rather than by the aggression of the virus.²⁰

This absent or delayed action would facilitate the action of the virus whose interaction with the CD147-spike protein²¹ on the ACE-2 receptor is essential for SARS-CoV-2's entry into the lung cells.²²

Infections of alveolar macrophage by SARS-CoV-2 might be drivers of the "cytokine storm", which might result in damages in pulmonary tissues, heart and lung, and lead to the failure of multiple organs²³ with the consequent CID with changes in circulating leukocyte subsets and cytokine secretion, particularly IL-6, IL-1 β , IL-10, TNF, GM-CSF, IP-10 (IFN-induced protein 10), IL-17, MCP-3, and IL-1ra. coagulation and vessels impairments, cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection.²⁴

A. BCG against other virus and pathogens

According to our hypothesis, lung and multi-organ injuries from Covid-19 could be prevented by stimulating lymphocyte response to BCG before virus attack as it has been observed against metapneumovirus in humans²⁵ and against respiratory syncytial virus in mice.²⁶ A non-specific cellular immunity induced by BCG may facilitate, even with SARS-CoV-2 (which has the same respiratory gate and pulmonary target) at the same time, an effective recruitment of Th1 lymphocytes directed against the virus,²⁷ which is capable of inducing a strong proinflammatory cytokine response blocking the production of type I and III IFNs to SARS-CoV-2. It has been seen that treatment of airway cultures with immune molecules type I or type III interferon (IFN) is able to inhibit SARS-CoV-2 infection and represents a potential antiviral treatment for COVID-19 patients.²⁸

It has been demonstrated that BCG, besides having been used for decades against tuberculosis,^{29,30} has been successfully used as "carrier" to promote a non-specific immune response, effective also against other bacteria, parasites, and viral pathogens, like *Bordetella pertussis*,³¹ HIV,³² *Plasmodium falciparum*³³ and capable of enhancing protection against other respiratory viruses.³⁴ Similarly BCG may be used also to promote an immune response against Sars-2-CoV-2, which has the same gate and the same target of other pulmonary viruses.³⁴

B. Local intralveolar contact of BCG

This recognition and stimulation could be more effective and faster if induced locally by aerosolized BCG as it can happen with pulmonary tuberculosis.³⁵ In the light of the importance of T lymphocytes decrease, particularly CD4+ and CD8+ cells and their correlation with severity of Covid-19,¹⁹ aerosol BCG given in strict contact with alveolus may act as a prime boost capable of eliciting a "trained immunity" and a T-lymphocytes increase, which may prevent contagion or infection but also help to decrease the severity of the disease. The second boost may be represented by subsequent alveolar/virus combination, which may find a more effective and prompt response by BCG pre-prepared T lymphocytes. Indeed what may happen in severe forms of the disease is the fact that the virus is not attacked so quickly and effectively to prevent a sequence of the most serious pathological events, mainly pulmonary, of Covid-19 disease.

C. Covid-19 and autoimmune diseases

The idea came from the similarities observed in moderate and serious forms of Covid-19 and pulmonary tuberculosis, considered an autoimmune disease on BCG vaccination is effective,^{36,37} and other autoimmune diseases (AID). An AID occurs when the immune system attacks self-molecules as a result of a breakdown of immunologic tolerance to autoreactive immune cells. Autoimmune diseases include insulin-dependent diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, thyroiditis, and multiple sclerosis.³⁸ Our hypothesis considers Covid-19 as a virus induced acute multi-organ autoimmune disease similar to other autoimmune diseases and pulmonary tuberculosis, where CD4 T cells have a dual role, both are required for host protection as each of these cell types also has potential for mediating tissue damage.³⁹

Other autoimmune diseases are virus-associated because in some instances, immune regulatory mechanisms may falter, culminating in the breakdown of self-tolerance, resulting in immune-mediated attack directed against both viral and self-antigens. During this response, cytotoxic autoantibodies, stimulatory autoantibodies, blocking autoantibodies, or cell-mediated autoimmunity may be observed. It seems then likely that some infectious factors, either viral or bacterial, may change the perception of self recognition of the organ self antigens by the same immune system.⁴⁰ For a long time, viruses have been shown to modify the clinical picture of several autoimmune diseases, including type 1 diabetes (T1D), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), herpetic stromal keratitis (HSK), celiac disease (CD), and multiple sclerosis (MS). Best examples of viral infections that have been proposed to modulate the induction and development of autoimmune diseases are enteric virus infections such as coxsackie B virus (CVB) and rotavirus, as well as influenza A viruses (IAV), and herpesviruses. Other viruses that have been studied in this context include measles, mumps and rubella.⁴¹ On the other hand, the appearance of autoinflammatory/autoimmune phenomena in patients with COVID-19 calls attention for the development of new strategies for the management of life-threatening conditions in critically ill patients. Antiphospholipid syndrome, autoimmune cytopenia, Guillain-Barré syndrome and Kawasaki disease have each been reported in patients with COVID-19.⁴² The knowledge about SARS-CoV-2 disease pathogenesis and immune response is a cornerstone to develop rationale-based clinical therapeutic strategies. In addition to that, several studies have recently suggested that immune dysregulation and hyperinflammatory response induced by SARS-CoV-2 are more involved in disease severity than the virus itself.⁴³ As a result, the use of autoimmune disease drugs can be useful in treating COVID-19.⁴⁴

D. BCG can prime an immune response in AID and cancer

The idea evolved considering analogies between reverse im-

mune reaction with BCG in cancer and AID by stimulating trained immunity. The innate immune system can mount resistance to reinfection, a phenomenon termed trained immunity or innate immune memory. During trained immunity, innate immune cells display gene- or locus-specific changes in their chromatin profiles induced by a previous stimulation. These changes, however, allow increased response to re-stimulation of the cells through both the same and different PRRs.^{45,46} A large number of murine and human studies taken together suggest that innate immune responses have the capacity to be "primed" or "trained," by a live vaccine like BCG and to mount a more robust response to secondary non-related stimuli after being initially primed (or trained) by a challenge of BCG.⁴⁷ Such reprogramming causes a changed immune response to secondary stimuli. These include conditions characterized by excessive trained immunity, such as inflammatory and autoimmune disorders, allergies and cardiovascular disease and conditions driven by defective trained immunity, such as cancer.⁴⁸ Thereby in order to exert a new type of immunological memory upon re-infection, the non-specific protective effect against Covid-19 offered by BCG through induced trained immunity has been recently discussed.^{49,50} In Table 1 the types of cancers treated with intralesional BCG are summarized according to their priorities.

The Proposed Mechanism

- Our hypothesis is that local stimulation of the alveoli with BCG aerosol, as for TB (35) may trigger an early reaction by T1 lymphocytes against the same target of the virus, increasing the non-specific pulmonary defense before the viral attack. The alveolus would represent, in fact, the same target of the BCG, of the virus and of the response of CD4 and CD8 T1 lymphocytes. Pneumonia from COVID might be related to immune system dysregulation with possible production of autoantibody and hyper-triggered response rather than viral infection consequence (3).
- The decrease of blood lymphocytes is higher in severe forms and lower in less severe forms. Lymphocytes in severe forms are alerted too late and will not act in time (16-19-20). The virus may have blinded, bypassed or anticipated the immune response because it is unknown and acts quickly in the alveoli, which are the targets of the disease.¹⁷
- BCG may act in preventing not only tuberculosis (29) but also, in an unspecific manner, other bacterial and viral diseases (31-32-33-34) and since an inverse correlation between vaccinations with BCG and COVID has been observed,⁵¹ its use has been suggested.⁴⁹⁻⁵⁰
- Local administration of BCG in some human tumors (cancer of the ovary, bladder, melanoma)⁵²⁻⁵⁶ results in complete remission of the tumor probably altering antigen presentation and

"reversing" the lymphocyte reaction against the new tumor/ BCG antigen combination, no longer recognized as "self" by the "trained immunity" that first did not attack it. The use of

BCG with intradermal injection of extracts of the same human tumors in patients presensitized only with intradermal BCG was effective only on 2/14 patients.⁵⁷

Table 1: Types of malignancies with priority BCG treatment, references and main results reported.

Type	Description	Authors	References	Notes
Leukemia	BCG after the graft of the tumor	Mathé G. et al.	Boll Ist Sieroter Milan. 1971 Jul-Aug;50(4):251-9.	Improvement and increase of survival in all children
Ovarian cancer	Intraparenchymal tumor BCG injection, without any surgery or chemotherapy at inoperable stage 3-4	Carretti N. et al. (3 with intralesional BCG, 16 with i.d. stimulation)	Atti del 56° Congresso Nazionale della Società Italiana di Ostetricia e Ginecologia (Padua, 3-5 October 1974) p465-468	1 complete recovery for 10 years;
				1 remission with 1 year survival.
				1 death after 5 months.
				No answer in 16 i.d. treated
Bladder tumor	Superficial, non invasive	Morales A. et al	J Urol. 1976; 116: 180-183	Recommended by FDA in 1990 as standard of care
Lung Cancer postop. follow up report	intrapleural BCG :5 years follow up reports	Bakker et al	Cancer Immune Immunother. 1986	56 p. treated: no survival benefits at 2 and 5 years
Lung Cancer non small cells lung cancer post surgical stage I	intrapleural BCG	Macchiarini P et al.	Anticancer Res. 1989	17 p.: no significant improvement at 5 years
Intraperitoneal	I.P. alpha 1 interferon Corynebacterium parvum and IL-2	Foon KA, Fanning; J Bast et al.	Cancer Research 43,1395-14011983	Limited activity
			Semin Surg Onc 1990;6(6):364-8	Prohibitive Toxicity Limited experience
Ovarian Carcinoma	Autologous T lymphoid in vitro activated. by i.p. route	Canevari S. et al	JNCI.Oct 1995,87, p1463-1469	23 treated patients. Complete response in 3 patients (26,23,18 months)
Stages III-IV				
Colon cancer stage II	Surgery + Oncovax (BCG)	CA Uyl-de Groot et al	Vaccine, 2005 Mar 18;23 (17-18):2379-87	Statistically improves 5 y. survival
Melanoma	Advanced melanoma	Stewart JH and EA Levine	Expert.Rev of Antic.Ther. Vol.11,	Statistically improves survival compared to patients not treated with BCG
			2011 p1671-1676	
Ovarian, Gastric cancer. Regional immunotherapy	TILs, peripheral blood mononuclear cells	Zeeltman M. et al.	Am.J.Surg. 2016 (Oct)	Increase of survival in the regional administration of cell-based clinical trials over the last 5 years.
Systemic review of 5 years				

Importance of the Hypothesis

The importance of the hypothesis is to decrease or cancel the evolution towards the most serious pulmonary forms of Covid-19 by increasing T1 lymphocytes response through local administration of aerosolized BCG given in advance to patients at risk of infec-

tion. We believe that changing antigen presentation of pulmonary alveoli locally with administration of BCG aerosol could stimulate and direct trained immunity against the ensuing action of SARS-CoV-2 on the organ targeted by the disease, as observed in human tumors^{57,58} by intraparenchymal use of BCG.⁵² This procedure may be taken into account with regard to elderly patients, patients with

heart diseases, diabetes, cancer, immunodepression and all other groups of people at higher risk.

Potential Implications of the Hypothesis

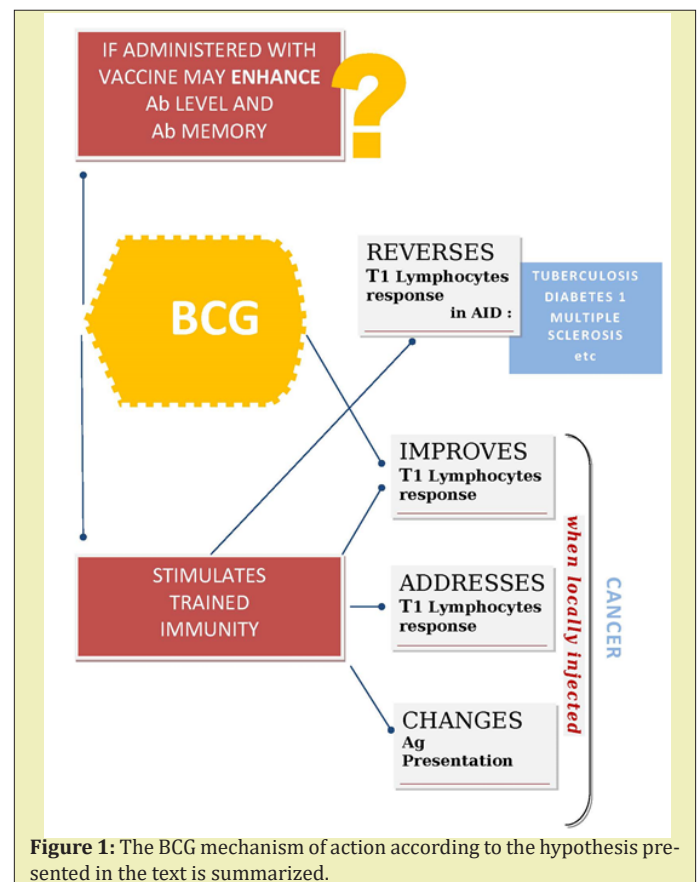
Our hypothesis may have, at least, the following potential implications:

- It could reduce the progression from mild to serious forms of COVID-19 correlated with the detection of a low amount of lymphocytes (1-7) whose defense may be "overcome" by the virus (8) increasing the presence of T lymphocytes (12) and interferon (9-28).
- It highlights the importance of stimulating a response of the native trained immunity using BCG (12-46-47-48) against COVID-19 (49-50) delivered locally by aerosol (35) and directed against the alveoli of the lungs, primary target of the virus infection.¹⁰
- This response is faster than a vaccine, which requires more than 30 days to develop the presence of IgG antiviral antibodies, and prevents the virus from dodging guest's defenses.¹⁷
- In fact, the duration of immunity of current vaccines is not well known.
- It does not depend on genetic variants of the virus^{4,59,60} and on changes of the CD-147-spike protein which may increase contagion, infectivity and lethality.⁵
- BCG (formed by inactivated tubercle bacilli) may be administered along with the vaccines, acting as an adjuvant (as happens with Freund's complete adjuvant) to increase the level of specific serum antibodies, the duration of their immunization over time (58) and, perhaps, having a favourable effect against some virus variants.
- The response to BCG could be tested in advance and, if reduced—as in elderly patients,³⁸⁻⁶² cancer patients,⁶³ cardiac patients (heart diseases are present in 8-12% of CoVid cases),⁶⁴ patients with vascular and atherosclerotic diseases,⁶⁵ diabetic patients, who are the ones with the worst prognosis in case of severe forms of CoVid-19,⁶⁶ and others—, can be solicited.⁵⁷
- The administration is simpler as it can be delivered via aerosol. This route of immunization offers not only the scientific advantage of delivering the vaccine directly to the respiratory mucosa, but also practical and logistical advantages. Being less expensive, it does not require complex structures and medical and nursing organization and it is easier to implement.³⁵
- The result may be subsequently monitored and tested through dermal tests or tests that determine lymphocyte response such as CD4/CD8 ratio: in fact, a renewed interest has emerged

about the usefulness of CD4/CD8 ratio as a strong marker of immune activation and immune senescence.⁶⁷

It anticipates the virus action on AM of Sars-2-Covid characterized by fast contagion, progression and worsening of conditions, which require equally quick responses from the body's immune system.

Figure 1 we propose the use of local intralveolar BCG given by aerosol route in at-risk Covid-19 subjects with the aim of soliciting, preventing and addressing a "trained immunity" response to possibly neoformed virus-MA Antigen.



Discussion

The first idea and attempt to use BCG intralveolarly first used in a solid human tumor in 1973 may be considered still relevant,⁵² also in the light of successive developments of cancer immunotherapy with immune checkpoint blockade therapy.⁶⁸ It is one of the most successful intralveolar biotherapies for cancer in use and has been employed in ovarian cancer,⁵²⁻⁵⁴ especially to treat non-muscle-invasive bladder cancer in which it has represented the "golden therapy" for more than 30 years,⁵⁵ and in metastatic melanoma therapy.⁵⁶ The mechanism of its therapeutic effect is still under investigation. Available evidence suggests that tumor cells (including ovarian cancer or bladder cancer cells themselves) and cells of the immune system both have crucial roles in the therapeutic an-

titumor effect of BCG. The possible involvement in bladder cancer cells as in other tumors includes attachment and internalization of BCG, secretion of cytokines and chemokines, and presentation of BCG and/or cancer cell antigens to cells of the immune system. It is thought that immune system cell subsets that have potential roles in BCG therapy include CD4(+) and CD8(+) lymphocytes, natural killer cells, granulocytes, macrophages, and dendritic cells.⁵⁵ However what seems crucial in all the circumstances in which this kind of immunotherapy approach of cancers has been used, is the strict interaction that is created between BCG and tumor cell.⁵²⁻⁵⁵

As far as COVID-19 is concerned, our hypothesis considers that BCG given by aerosol route, might work, similarly to what happens in tumors, by altering the antigen presentation of alveoli with which it comes into contact: such as a newly formed combination represented by alveolar/BCG complex may change the immune response of CD4/CD8 T1 lymphocytes which, before this challenge, recognized the alveolar target as "self". This approach may anticipate and prevent the immune reaction of the secondary contact with the virus, which has the same target of locally administered intra-alveolar BCG. This could determine an effective and robust local response by T1 lymphocytes, as it occurs with BCG aerosol in pulmonary TB35 and in tumors (ovary, melanoma, bladder) on which BCG is effective only when directly injected⁵²⁻⁵⁴ or in close contact with tumor cells,⁵⁵ plausibly changing the tumor antigen presentation. It has been seen, in fact, that the down regulation/loss of antigen presentation in cancer is a major immune escape mechanism. It allows tumor cells to become 'invisible' and avoid immune attack by antitumor T cells. On the contrary down regulation/loss of antigen presentation may also prevent direct priming of naïve T cells by tumor cells as it happens by forcing tumor cells to present their antigens turning tumor presenting antigen and immune response.⁶⁹ With regard to protection against COVID-19, our hypothesis is that the contact of BCG on the bronchial and/or alveolar mucosa (first challenge) determines a locally induced change of the "trained immunity" and of the lymphocyte component also against the virus/alveolar macrophages (AM) antigen²³ combination after coming into contact with COVID-19 (second challenge). The approach of using an adjuvant system through intranasal route to improve protective effectiveness and elicit a long-term robust immune response has recently proven effective against avian coronavirus.⁷⁰

Final considerations and conclusions

As intraparenchymal BCG is effective in treating tumors only if injected locally, in the same way we propose to solicit locally, by intralveolar administration, the trained immunity for the purpose of obtaining a higher T-lymphocyte response after a second challenge on the same target represented by the possible presence of SARS-COV-2. This response would anticipate the virus's harmful action and progression to more severe forms of the disease. BCG may work

locally priming the AM (first boost), changing antigen presentation, stimulating the "trained immunity" in a new manner and directing against this newly formed alveolar BCG modified antigen the T lymphocytes reaction if contact with Sars-2-CoV-2 occurs on the same alveolar target (second boost). The mechanism would be similar to the reaction that occurs in cancer after intralesional injection of BCG: in both cases, cancer and the pulmonary alveoli might be antigenically modified locally by BCG and, subsequently, the T lymphocyte reaction is addressed locally. Thus the use of BCG aerosol may be proposed in order to locally elicit and improve an immune-mediated CD4+ and CD8+ T-cell as well as IFN- γ production by CD4+ T lymphocytes therefore preventing the most severe forms of pulmonary damages due to COVID-19, with the idea of preventing and anticipating the virus's aggression to the lung's AM which represents its most harmful aspect for the entire sequence of the ensuing events described (CSR and CID). As a conclusion, in our opinion, the Corona virus pandemic should be addressed from an opposite perspective before resorting to vaccination, that is to strengthen in a certain population or higher risk areas (care homes for the elderly, presence of underlying diseases such as hypertension, diabetes, heart diseases chemotherapy treatments, etc) the "trained immunity" in the target organ of COVID-19, the lung, in a non-specific but "matching" manner with local administration of BCG. The strengthening and implementing of T lymphocytes' action, both locally and a specifically, directing it in advance for the protection of the real target of the disease, the lung, could also reduce the need to chase, with vaccination alone, the continuous changes of the spike protein and of the viral genoma, which can occur very rapidly.

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Conflict of interest

Authors declare that there is no conflict of interest.

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