

A novel concept for treatment and vaccination against Covid-19 with an inhaled chitosan-coated DNA vaccine encoding a secreted spike protein portion

Abstract

A novel concept in DNA vaccine design is the creation of an inhaled DNA plasmid construct containing a portion of the coronavirus spike protein for treatment and vaccination. The secretion of a spike protein portion will function as a competitive antagonist by interfering with the binding of coronavirus to the angiotensin-converting enzyme 2 (ACE2) receptor. The secreted protein binding to the ACE2 receptor provides a unique mechanism of action for treatment to all strains of coronavirus in naïve patients, by blocking the ACE2 receptor site. An inhaled plasmid DNA vaccine replicates the route of lung infection taken by coronavirus with transfected cells secreting spike protein portions to induce immunity. Unlike most DNA vaccines with intracellular antigen presentation through MHC I, the current vaccine relies on the secreted proteins presentation through MHC II as well as MHC I to induce immunity. Lung specific production of vaccine particles by inhaled plasmid DNA is appealing since it may have limited systemic side effects, and may induce both humoral and cytotoxic immunity. Finally, the ease and ability to rapidly produce this plasmid construct makes this an ideal solution for managing the emerging threat of coronavirus.

1 | INTRODUCTION

COVID-19 is a disease which causes a contagious acute respiratory infection in humans by the coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Symptoms of infection vary from asymptomatic to critically ill with acute respiratory distress syndrome.¹ Cases of coronavirus infection have now spread to almost every country, with currently no effective treatment or vaccine available. This highlights the urgent need for the development of an effective treatment and/or vaccine.

The genome of the novel SARS-CoV-2 was first released in late December 2019, and since then a great deal of work has been done

identifying the gene involved in infection. The genome of the novel SARS-CoV-2 virus encodes structural proteins that make up the outer layer which include; the spike, envelope, and membrane proteins.² The key feature of SARS-CoV-2 is the surface spike protein, which plays a critical role in infection by mediating high specificity viral attachment to the host cell surface ACE2 receptor allowing for viral entry.³ These findings of the identified spike proteins in the SARS-CoV-2 receptor binding domain and ACE2 region may provide useful information for treatment or vaccine development.^{1,3}

Various forms of vaccines have been used to fight against bacterial and viral disease. Plasmid DNA vaccines are of great interest, since they provide the ability to be rapidly constructed and manufactured. It was first shown that plasmid DNA could protect mice from influenza challenge in the 1990s.⁴ The challenge with plasmid DNA is the optimal delivery of DNA into the desired cells for the expression of proteins needed to generate an immune response.⁵ In this paper, a series of inhaled plasmid DNA vaccine constructs containing various forms of the coronavirus spike protein sequence may provide potential treatment and vaccine options as revealed by Yu et al (Figure 1).⁶ Plasmid DNA-produced spike proteins will function to induce immunity as a vaccine candidate (Figure 2A) and work as a competitive antagonist against coronavirus host cell attachment (Figure 2B).

2 | DISCUSSION

DNA vaccination has emerged over the years as a novel approach for the prevention of bacterial and viral diseases. In recent years DNA vaccination has made considerable progress with the development of many DNA vaccines entering into human clinical trials, most notably Zika virus phase 2 and HPV infection phase 1 trials.⁷⁻⁹ The DNA vaccine for Zika virus known as VRC5283 was shown to be safe and well tolerated resulting in 100% of the participants generating humoral and cellular responses.^{7,8} VRC5283 is a plasmid DNA constructed with Zika virus genes prM, E(envelope) and a Japanese encephalitis virus coding sequence to improve secretion of the Zika vaccine particle.^{7,8} With the phase 1 success of VRC5283, phase 2 trials have now been initiated as National Clinical Trial (NCT) 03110770.⁷ In human papillomavirus (HPV) vaccination with DNA

vaccines GX-188E and VGX-3100 it has been shown that both are safe with no serious adverse events in phase 1 trials.⁹ GX-188E is a plasmid fusion protein of HPV 16/18 E6/E7 linked to FMS-like tyrosine kinase 3 ligand and VGX-3100. A mixture of two plasmids encoding HPV 16/18 E6/E7, both vaccines elicited cytotoxic lymphocyte immune response.⁹

A major obstacle in DNA vaccine development is low immunogenicity.¹⁰ Administration of naked DNA vaccines is usually inefficient, since its negative charge prevents it from crossing cell membranes because of charge repulsion with negative charge phospholipids.¹⁰ An important development in DNA vaccination is the improvement in the delivery with nanoparticles of chitosan. Chitosan nanoparticles have provided an effective means for prevention of DNA vaccine degradation while the cationic nature enables binding to the negative charge of DNA.¹⁰ Chitosan has many favourable features for DNA vaccine delivery to the mucosal surface such as mucoadhesion, highly soluble, inert and non-immunogenic.¹⁰ Since mucosal surfaces are a major site for a majority of pathogens that infect humans,¹⁰ DNA vaccination at the mucosal surfaces with the use of chitosan is highly desirable. This will allow for effective delivery of DNA vaccine to mucosal surfaces replicating the site of infection of respiratory viruses. The inhaled or intranasal immunization with chitosan nanoparticles may provide better protection since strong immune responses occur at the site of vaccination with the induction

of mucosal and systemic immunity. It has been shown that intranasal administration of plasmid DNA encoding nucleocapsid Sars-Cov loaded into chitosan nanoparticles produced high levels of IgA and IgG in mice.¹¹

Improvement in vector design with the NanoplasmidTM allows for antibiotic free selection, an FDA recommendation for DNA vaccines. The smaller size of the NanoplasmidTM also provides for increased gene size insertion as well as an increasing transfection efficiency. The insertional gene for this vector is a combination of the coronavirus spike protein and/or a secretion tag such as Japanese encephalitis virus (JEV) which will provide protective immunity by the ability of the plasmid DNA construct to direct synthesis of secreted proteins by host cells.^{7,8} A series of six plasmid DNA constructs based on the study by Yu et al, are shown in Figure 1.⁶ Three DNA constructs are the S1/S2 without the transmembrane (TM) and C-terminal (CT) domains, S1 and receptor binding domain (RBD) with trimerization tag. The other three DNA constructs contain the JEV TM and CT domains added to S1/S2, S1 and RBD. These DNA constructs can be tested for protein secretion quantity, and effectiveness of blocking coronavirus infection. Other commonly used fusion partners include the constant domain IgG (the Fc region), maltose-binding protein (MBP), small ubiquitin-like modifier (SUMO), and human serum albumin (HSA) tag.¹¹ Cells transfected by the DNA construct will also secrete a portion of the spike protein as a competitive antagonist. It

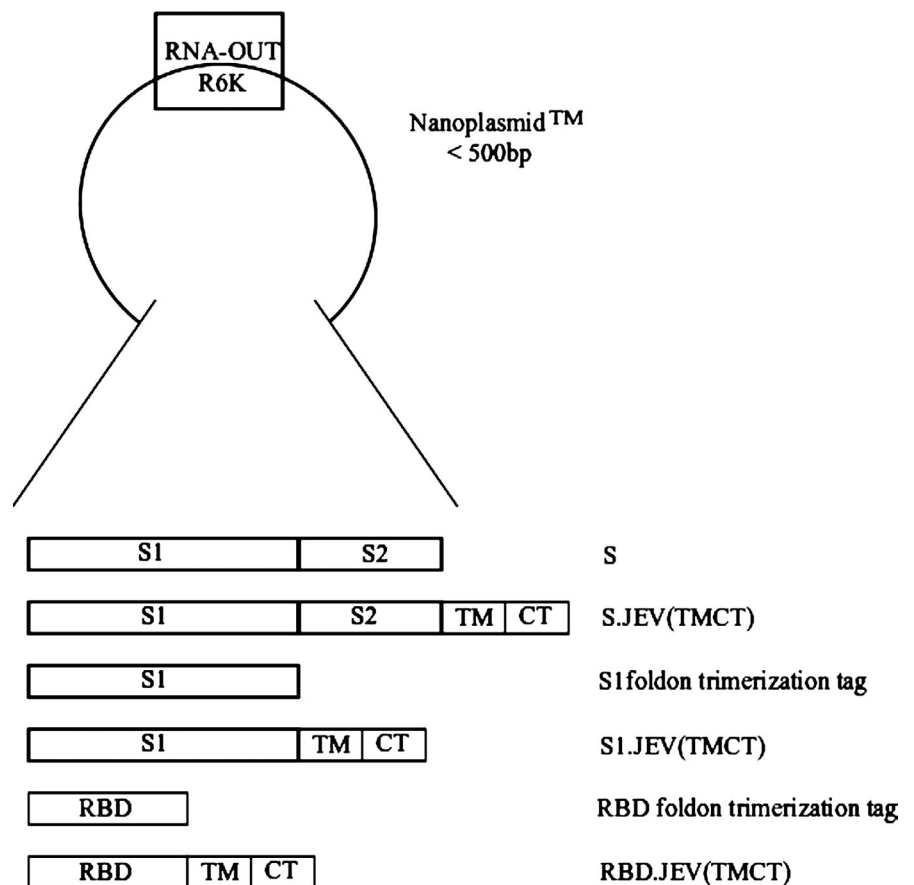


FIGURE 1 Vector construct for DNA vaccination using the NanoplasmidTM platform with S1 coronavirus spike protein portion with or without secretion-enhancing tag

has been shown that the S1 protein fragment of the S1 protein binds with high efficiency to the ACE2 receptor revealing the potential of a portion of the spike protein to work as a competitive antagonist once secreted.¹²

The mechanism of action of DNA vaccination with these inhaled chitosan DNA constructs involves encoding antigen(s) that are transfected into either antigen presenting cells (APC) or somatic cells. In one scenario intracellular processing of plasmid DNA leads to vaccine derived endogenous peptide presentation on MHC I molecules.^{9,13,14} This antigen is expressed to the cell surface with MHC I and presented to cytotoxic CD8 + T cells stimulating cell mediated immunity.⁹ In the other scenario antigens of exogenous origin or secreted protein from plasmid DNA are loaded on MHC II resulting in activation of helper CD4⁺ T cells which in turn contribute to B cell priming to yield a humoral immune response.⁹ In mucosal vaccination, the plasmid DNA antigen is secreted into the extracellular space where M cells participate in antigen uptake and transport them to the extracellular space.⁹ At this point the antigen is released to APC cells for presentation to immune cells.⁹ These antigenic proteins expressed and secreted by plasmid DNA vaccines access both the exogenous and endogenous pathways in the activation of both humoral and cellular mediated immune responses.¹⁵

There are studies that show a patient's immune response resulting from delivery of a vaccine to the lung may equal or exceeds the

immune response created by vaccine injection.¹⁶ It has been proposed that a mucosal immune response may be created via particles of the vaccine material depositing on the upper respiratory tract.¹⁷ A systemic immune response may be created via particles of the vaccine material depositing into the deep lung.¹⁷ In a study by Weaver there were significant differences in antibody and T-cell response with intramuscular (IM) immunized mice compared to inhaled immunization.^{8,15} However, there was no significant difference between IM and inhaled immunized mice response when challenged with lethal influenza virus amounts.¹⁵ These results suggest that vaccine efficacy of IM and inhaled vaccines are almost identical, and inhaled vaccination may be an adequate route of administration even though antibody and T-cell responses are low.

In this paper the primary use of an inhaled plasmid DNA vaccine containing the coronavirus spike protein DNA sequence is for treatment. The small chitosan plasmid DNA size of approximately 138 nm¹⁸ can reach deep into the lung, similar to that of the coronavirus with a size of 125 nm.³ Once in the lower respiratory tract or alveolar region of the lung deposited plasmid DNA will be taken up and expression of coronavirus spike proteins by host cells such as pneumocytes will occur. This will create a competitive antagonistic environment whereby coronavirus will have few options available for binding to the ACE2 receptor. Thus, the expression of coronavirus spike proteins provides a protective barrier against coronavirus

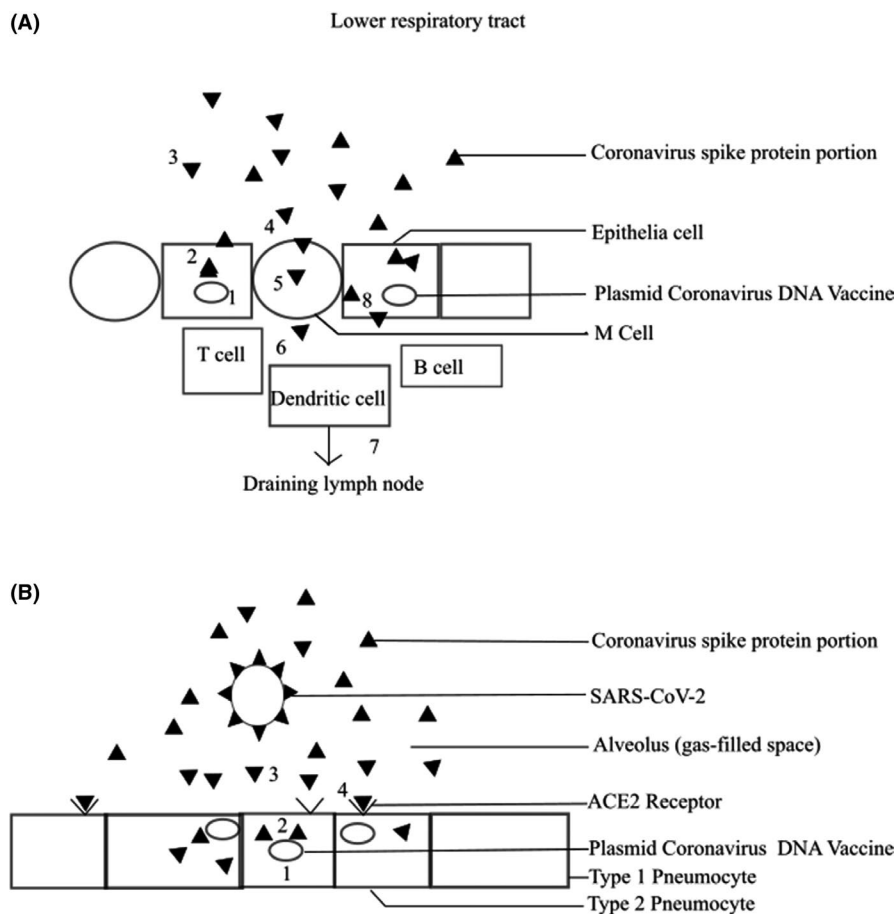


FIGURE 2 A, DNA vaccine induction of humoral and cellular mucosal immune response. 1 transfection of epithelial cell 2 expression of S1 protein, 3 secretion of S1 protein, 4 S1 protein taken up by M cells, 5 transported to immune cells with 6 uptake by dendritic cells and MHC II antigen presentation to T-cells with subsequent draining into lymph node stimulating humoral response. 8. MHC I presentation stimulated cytotoxic lymphocyte response. B, DNA vaccine treatment with secreted S1 spike protein acting as a competitive antagonist by interfering with the coronavirus binding to ACE2 receptors. 1 transfected cell 2. expression of S1 protein, 3 secretion of the S1 protein, and 4 ACE2 receptor blockade by S1 protein preventing SARS-CoV-2 from binding to ACE2 receptor

infection of type 2 pneumocytes. A major concern with using an inhaled chitosan DNA vaccine as a treatment is obtaining sufficient production of secretion protein to compete with the coronavirus. Currently, there are few studies available using a DNA vaccine by the respiratory route for treatment. A study by Kodama et al, reveals the practicality of this route by using the luciferase gene to show gene expression in lung tissue by fluorescence intensity.¹⁹ This study revealed that high levels of inhaled DNA vaccine expression can occur in lung tissue as seen by the increase in fluorescence in lung samples, and in microscopic lung sections.¹⁹ These results indicate that high levels of protein expression may be generated by inhaled DNA for competitive antagonism against coronavirus. A large dose of inhaled chitosan DNA construct or multiple doses can easily be given when symptoms first appear when there is a lower viral load early in infection. In comparison to drug treatment approaches, inhaled DNA treatment has many advantages, with a prolonged half-life, site-specific action, no metabolic concerns, and also ease of manufacture, administration and storage.

Another major concern with coronavirus vaccine development is the harmful inflammatory response known as antibody-dependent enhancement in which non-neutralizing antibodies bind to coronavirus and enhance entry into cells.²⁰ A recent study by Yu et al has shown protective immunity in rhesus monkeys with little side effects of DNA vaccination by using various forms of the coronavirus spike protein.⁶ The DNA vaccine constructs in the article by Yu et al, reveals that both secreted and non-secreted proteins were able to provide protective immunity.⁶

The mechanism of action of the inhaled DNA construct may also work for treatment and vaccination of other respiratory viruses such as influenza where a HA expressed protein prevents binding of any strain of influenza. In addition, this novel method of treatment may be used for delivering DNA encoding proteins to other tissues where secretion of proteins into the extracellular space may provide a therapeutic benefit. A DNA vaccine encoding secretion of interferon beta protein to locally transfected tissue could be inhaled for delivery to lungs or oral delivery to the gastrointestinal tract.

This DNA construct has a great deal of versatility for use as a treatment or vaccine. Many studies have proven DNA vaccines to be safe and well tolerated. The use of plasmid DNA provides an easy means of production which should provide a low cost method for delivering vaccines to the lung with some form of nebulizer or metered-dose inhaler.

There may be many cons associated with inhaled DNA vaccination such as auto-immunity and/or unseen problems with the quantity of vaccine particles produced, however, lung specific production of coronavirus spike proteins may be a safer alternative than systemic drug treatment.

Currently, plasmid DNA vaccination is only licensed for veterinary use, hopefully further testing may allow for licensing in humans.

KEYWORDS

ACE2, coronavirus, Covid-19, plasmid DNA, spike proteins, treatment, vaccine

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CONFLICT OF INTEREST

There are no conflicts of interest to report.

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REFERENCES

- Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell Mol Immunol.* 2019;2020:1-9.
- Neuman BW, Joseph JS, Saikatendu KS, et al. Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. *J Virol.* 2008;82:5279-5294.
- Fehr AR, Perlman S (eds.). Coronaviruses: An Overview of their Replication and Pathogenesis. *Methods in Molecular Biology.* New York, NY: Humana Press., 2015;1282:1-23.
- Ulmer JB, Donnelly JJ, Parker SE, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science.* 1993;259:1745-1749.
- Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines.* 2019;7:37.
- Yu J, Tostanoski LH, Peter L. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science.* 2020;369(6505):806-811.
- Gaudinski MR, Houser KV, Morabito KM, et al. Safety, tolerability, and immunogenicity of two Zika virus DNA vaccine candidates in healthy adults: randomised, open-label, phase 1 clinical trials. *Lancet.* 2017;391:552-562.
- Diamond MS, Ledgerwood JE, Pierson TC. Zika virus vaccine development: progress in the face of new challenges. *Ann Rev Med.* 2019;70(1):121-135.
- Cheng MA, Farmer E, Huang C, Lin J, Hung CF, Wu TC. Therapeutic DNA vaccines for human papillomavirus and associated diseases. *Hum Gene Ther.* 2018;29:971-996.
- Xu Y, Yuen PW, Lam JK. Intranasal DNA vaccine for protection against respiratory infectious diseases: the delivery perspectives. *Pharmaceutics.* 2014;6:378-415.
- Raghuwanshi D, Mishra V, Das D, Kaur K, Suresh MR. Dendritic cells targeted chitosan nanoparticles for nasal DNA immunization against SARS CoV nucleocapsid protein. *Mol Pharm.* 2012;9:946-956.

12. Reuten R, Nikodemus D, Oliveira MB, et al. Maltose-binding protein (MBP), a secretion-enhancing tag for mammalian protein expression systems. *PLoS One*. 2016;11:e0152386.
13. Wong SK, Li W, Moore MJ, Choe H, Farzan MA. 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *J Biol Chem*. 2004;279:3197-3201.
14. Kimura S, Mutoh M, Hisamoto M, et al. Airway M cells arise in the lower airway due to RANKL signaling and reside in the bronchiolar epithelium associated with iBALT in murine models of respiratory disease. *Front Immunol*. 2019;10:1-15.
15. Grodeland G, Mjaaland S, Roux KH, Fredriksen AB, Bogen B. DNA vaccine that targets hemagglutinin to MHC class II molecules rapidly induces antibody-mediated protection against influenza. *J Immunol*. 2013;191:3221-3231.
16. Weaver EA. Dose effects of recombinant adenovirus immunization in rodents. *Vaccines*. 2019;7:144.
17. Hellfritzsch M, Scherließ R. Mucosal vaccination via the respiratory tract. *Pharmaceutics*. 2019;11:375.
18. Huang T, Song X, Jing J, et al. Chitosan-DNA nanoparticles enhanced the immunogenicity of multivalent DNA vaccination on mice against *Trueperella pyogenes* infection. *J Nanobiotechnol*. 2018;16:8.
19. Kodama Y, Nakashima M, Nagahara T, et al. Development of a DNA Vaccine for melanoma metastasis by inhalation based on an analysis of transgene expression characteristics of naked pDNA and a ternary complex in mouse lung tissues. *Pharmaceutics*. 2020;12:540.
20. Yang ZY, Werner HC, Kong WP, et al. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. *Proc Natl Acad Sci USA*. 2005;102(3):797-801.

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