# A VACCINE AGAINST COVID-19 THAT STRONGLY INDUCES THREE BRANCHES OF IMMUNITY

## Team

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IMMvention Therapeutix- procurement for microparticles
Duke University or NIAID Rocky Mountain Laboratories-testing site for ferrets

The pandemic caused by SARS-2-CoV is causing the historic loss of human lives and unprecedented economic disruption in the world, and North Carolina is not exempted from this tragedy. It is widely accepted that an effective vaccine is needed to combat this disease. However, a successful vaccine is not guaranteed. A prime example is the human immunodeficiency virus (HIV) which causes AIDS. HIV was discovered almost 4 decades ago, yet an efficient vaccine against the virus is still elusive. Another example is the dengue viral vaccine, which elicits responses that are undesirable. For example, the typical B cell response that is induced by a vaccine causes the production of antibody, which typically can bind to the virus and neutralize its infection. However, in Dengue infection, the antibody also causes a reaction called antibody-dependent enhancement (ADE) of disease, wherein these antibodies actually increase disease severity. It is generally thought that the induction of a separate branch of the immune response, those caused by T lymphocytes that can both kill the virus and help B cells to generate a vigorous antibody response is needed. The latter in particular is thought to favor viral neutralization which would neutralize the virus, over ADE which would enhance the infection.

Numerous candidate vaccines for SARS-2-CoV are progressing rapidly, thus at issue is whether another candidate vaccine is needed. However many of the platforms used have yet produce a single successful vaccine for any infectious disease. This includes at least two of the frontline vaccines, both of which involve RNA that code for viral spike (S) protein. For these to work, the RNA are expected to enter the appropriate cells in the body and translate into the S antigen to elicit both strong and appropriate immune responses that can effectively kill off the virus. The latter is particularly important as there are reports that SARS, a closely related family member of SARS-2-CoV, can induce ADE. In addition, the existing focus of most candidate vaccines remains the elicitation of a B cell response while T cell activation is not a focus. Finally, a major challenge for a vaccine to contain COVID-19 is the need for effective immunity in the elderly that represents one of the most vulnerable populations. A problem in the elderly is that they typically have a weak immune system, hence a booster is needed. A solution involves the third branch of the immune response, called innate immunity, which is a strong booster of both T and B cell response. Innate immunity can be induced by Pathogen-associated Molecular Patterns (PAMPs). PAMPs are small molecules from viruses, bacteria or fungus that can induce a strong innate immune response to augment T and B cell immunity against an infection. The successful use of PAMPs in the elderly is illustrated in the shingles vaccine, Shingrix, which contains PAMPs delivered in liposomes. It achieves >97% efficacy in the 50-69 year old group and >91% efficacy in the >70 group. This contrasts with the less successful shingles vaccine Zostavas, which is comprised of a live attenuated virus and achieves 50% protection in people over 60.

The overarching purpose of this proposal is to produce a strong anti-SARS-2-CoV vaccine by optimizing all three branches of the immune system: B cell antibody response, T lymphocytes and innate immunity. This involves the specific design of two components of a vaccine: (1) an antigen to

activate specific T and B cell responses, and (2) PAMPs which serve as the adjuvant to enhance the innate immune response:

1. Antigen: We propose to use a fusion protein that consists of (a) the SARS-2-CoV S protein, which is used in almost all of the candidate vaccines and is known to activate a B cell response, and (b) several viral peptides that are predicted to bind and activate T cells. This combination is expected to generate strong T and B cell responses. As an example of the success of this approach, we have used a fusion of a weak Universal Flu antigen with a flu T cell antigen, and produced strong immunity that eliminated influenza virus in infected animals.

2. Adjuvant: We propose to use a microparticle that specifically goes into antigen presenting immune cells (APC) which can activate T cells. APCs include macrophages and dendritic cells and are major components of innate immunity. Our microparticles can preferentially enter APCs and effectively deliver PAMPs into the lysosome to further activate innate immunity. In the past, we have used a non-toxic microparticles to deliver several PAMPs, including a cyclic-diGMP-AMP (cGAMP), poly(I:C) or imiquimod. All three are safely used in the clinic and can increase the functions of APC, T and B cells. These microparticles increased the delivery of PAMPs into APC and amplified the immune response. For example, we have found that cGAMP in microparticles enhanced antibody responses to an influenza antigen by $10^5$-fold and induced a significant T cell response.

Finally, it is important to note that the success of the above antigen-adjuvant combination has already shown in a candidate vaccine for influenza virus. In a recently submitted paper, we showed in both mice and ferrets that a single dose of a flu antigen with cGAMP-containg microparticles is more effective than a combination of the same flu antigen with the top adjuvant, MF-59-like Addavax. Importantly, our vaccine shortened the time for viral clearance, reduced the viral load, and produced durable immunity that was measured one year after vaccination. All of these outcomes are highly desirable features for a successful SARS-2-CoV vaccine.

(Disclosure: IMMvention is a company cofounded by Drs. Ting, Bachelder and Ainslie)

**IMPACT TO THE STATE** (300 word limit)

- *Description of the problem or challenge being addressed and how the problem impacts those in the state of North Carolina*
- *Describe how the proposed research will provide impactful solutions to the described problem to help the state of North Carolina*

COVID-19, a disease caused by SARS-CoV-2 infection represents an historic public health challenge in North Carolina. It has also caused economic devastation, including a near 15% unemployment rate. A primary strategy for the planned control of SARS-CoV-2 is successful vaccination. Although numerous vaccine strategies are being tested, many of the strategies may not yield appropriate and strong immunity. A successful anti-SARS-CoV-2 vaccine must show efficacy in the elderly due to their vulnerability to COVID-19 and their weakened immunity. Furthermore, a weak vaccine may produce low levels of antibody that lack virus neutralization, but instead may cause antibody-dependent enhancement of the disease.

An impactful solution for an anti-SARS-CoV-2 vaccine is one that causes a vigorous immune response activating all branches of the immune system: antibody response, T lymphocyte activation and innate immune promotion. Although many platforms of vaccines are being tested, it is widely accepted that a subunit vaccine is the safest. It is comprised of an antigen and an adjuvant that amplifies the immune response. While almost all candidate COVID-19 vaccines use the Spike (S) protein as the primary antigen, we propose a chimeric antigen comprised of the S protein plus two T cell epitopes predicted based on HLA binding, ranked affinity and prevalence in the human population. This adjuvant is formulated of polymeric microparticles encapsulating a cyclic-dinucleotide that kick-starts an interferon response to augment T, B and innate immunity. This adjuvant has been tested for a flu vaccine, and is retooled here for a SARS-CoV-2 vaccine. We show that this new adjuvant is superior to a top adjuvant MF59 by increasing antibody response logarithmically and inducing T cell activation. It also possesses ideal features for vaccine incorporation, including intramuscular delivery, good safety profile, easy sterilization with □-radiation and durable protection over a year in ferrets.

**MILESTONES** (300 word limit)
*Description of what will be accomplished and what can be delivered by August 31, 2020, and by Dec. 31, 2020. The start date will be June 1, 2020.*

**August 31, 2020 Milestones**

The major milestones are:

(1) Selection of the T cell activating peptides from SARS-CoV-2 that elicit an antigen-specific T cell response as measured by interferon-gamma production;
(2) Production of antigenic epitope of intact S protein as evaluated by binding to anti-S antibodies;
(3) Construction of expression vector encoding chimeric antigens of S protein fused to the T cell epitopes identified in (1);
(4) Establishment of neutralizing antibody assay for the immunogens;
(5) Establishment of the T cell ELISPOT activation assay; and
(6) Incorporation of the top immunogen in Acetated dextran microparticles.

**December 31, 2020 Milestones**

The major milestones are:

1) Immunization of mice with the S protein;
2) Immunization of mice with the chimeric protein;
3) Repeat of (1- 2) with the microparticle encapsulated cGAMP adjuvant versus a top adjuvant, MF59;
4) ) Comparison of these vaccine candidates for the induction of anti-S B cell antibody titer;
5) ) Comparison of these vaccine candidates for the induction of T cell activation; and
6) ) Comparison of Balb/c versus C57BL/6 mice to assure that different genetic backgrounds will yield successful immunization.

**BUDGET JUSTIFICATION** (200 word limit)
*Funds are limited. We encourage all teams to revisit their budget and determine if it can be reduced.*

Total Budget= $342,411

**Personnel**
$118,911

**Key Personnel** (We will lower costs by keeping PI efforts to a minimum):

Jenny Ting, Ph.D., PI (1% effort, over six months it is listed as 0.5%) plan and oversee the research.

Benjamin Vincent, M.D., Investigator (1% effort) will supervise the selection of peptides.

Eric M. Bachelder, Ph.D., Investigator (1% effort) and Kristy M. Ainslie, Ph.D., Investigator (1% effort) will optimize microparticle incorporation of S antigens.

Mark Heise, Ph.D., Investigator (1% effort) will supervise the BSL3 virus work. (0.5%)

**Research Staff**

Adam Sandor, Ph.D., Postdoctoral Research Associate, (100% effort, over six months it is 50%); Ting lab; immunization)

Kaylee Gentry, M.S. (100% effort over6.0 calendar months, Vincent lab; peptide selection and immunization)

Sharon Taft-Benz, Ph.D., Research Associate (50% effort over 6.0 calendar months, Heise lab; virology)

Cole Batty, Graduate Research Assistant, (100% effort over 6.0 calendar months, Ainslie lab; microparticle with S protein)

**Supplies**
$100,000

Mice (300 Balb/c and 300 C57BL/6) - $20,000
Serology - $10,000
Tissue Culture - $10,000
Plasticware - $15,000
Anti-S ELISA - $20,000
Microparticle + cGAMP - $25,000

**Tuition**
$6,500

**Other Costs**
$117,000

Mouse housing - $35,000

Flow Cytometry – $5,000
Dishwashing - $5,000
Plasmid construct, antigen production, UNC protein core - $30,000
BSL3 testing of vaccine by Heise - $40,000