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A Dna-nanoparticle-based Antigen Presenting Platform For The Development Of Broadly Protective Vaccines Against Acutely Infectious Viruses

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Broadly-protective vaccines are the most efficient way to confer protection against emerging and re-emerging viral pathogen challenges. The ideal vaccine technology platform should be able to pivot rapidly as new challenges emerge, elicit robust and specific immune response with minimal dosing, display heterogeneous antigens, not contribute to vaccine-related adverse events, be amenable to scale up and field-deployment. Our DNA nanoparticle (DNA-NP) platform technology assembled using the DNA origami technique, provides the advantage of exquisite control of homogeneous and heterogeneous antigen organization on the particle. This offers an immediate advantage in terms of creating multivalent vaccines with stoichiometrically controlled antigenic representation. Our objective is to develop a new generation of broadly protective DNA-NP vaccines against acutely infectious RNA viruses (eg: Venezuelan- and Eastern Equine Encephalitis Viruses [VEEV, EEEV]) that pose challenges to both the warfighter and the civilian population.

We have successfully developed a DNA-NP-based vaccine candidate against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). We used the receptor binding domain (RBD) of SARS-CoV-2 as the immunogen and reconstituted a trimer of RBD via attachment of RBD-Fc to a protein G bearing three Fc-binding sites. The protein G was further modified with a peptide nucleic acid strand via maleimide chemistry for efficient hybridization to complementary single stranded DNA overhangs. We designed a pentagonal bipyramid DNA-NPs with 10 overhangs evenly spaced in one face and validated accessibility of the antigens with surface plasmon resonance. The other face of the pentagonal bipyramid was decorated with CpGs directly hybridized on complementary overhangs. We demonstrated the proper folding and stability of the DNA-NPs in physiological conditions (80% stability after 10 hours) and validated the conjugation of the antigens. We have also demonstrated that conjugation of protein antigens to the DNA-NPs increased their stability in presence of serum (>70% stability after 10 hours in 20% serum). We performed in vivo immunization studies to quantify neutralizing antibody titer which demonstrated highly neutralizing antibodies production. Our viral challenge study using the Italy strain of SARS-CoV-2 in the K18-ACE2 mouse model employed 1 ug of immunogen and CpG per animal and resulted in 100% survival of immunized animals.

We have expanded our studies to include VEEV as a target pathogen and have developed DNA-NPs that include the E2 envelope glycoprotein. The VEEV-E2-DNA-NP has been synthesized and ongoing studies are focused on measuring immunogenicity following a prime:boost challenge. Current outcomes show that the VEEV-E2-DNA-NP appears to be well tolerated in vivo with no apparent adverse events. To develop a diversity of DNA-NPs against alphaviruses, we are also exploring E1 and E2 peptide-based NPs for VEEV and EEEV.

We envision that the versatility of the DNA-NPs platform will allow us to pivot rapidly to develop vaccines against emerging and re-emerging viral pathogens with short response times. The utility of this platform to develop broadly protective vaccines by precisely controlling antigen representation in the nanoscale will align perfectly with DTRA's mission of developing countermeasures to protect the warfighter.

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