Rational Vaccinology to Protect Against Cancer and Infectious Disease Using Spherical Nucleic Acids

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Statement of Purpose: Vaccines potently activate the immune system against disease, stimulating targeted responses. Using an adjuvant (immune system activator) and an antigen (immune system target), these vaccines can drive the immune system to seek out and kill tumor cells or viral pathogens. Previously, it was thought that the composition of the vaccine solely influenced the downstream response. However, recent work in our group has demonstrated that the *presentation* of the relevant components also plays a key role and can vastly alter the resulting immune response.[1–3] We are exploring this effect in the context of the spherical nucleic acid (SNA), an emergent therapeutic architecture which consists of a dense shell of oligonucleotides radially conjugated to a nanoparticle core. This arrangement imparts these structures with enhanced properties, including increased cellular uptake and higher resistance to nuclease degradation compared to the same sequence of linear oligonucleotides, and gives them the potential to induce immune activation through toll-like receptors.

Methods: We chemically synthesize SNAs through post-liposome intercalation of immunostimulatory oligonucleotides by using hydrophobic anchors attached to the DNA. We can incorporate antigen target in the form of peptides or proteins in various locations on the SNA including but not limited to: encapsulation into the liposome core, and conjugation to a complementary DNA sequence hybridized externally to the SNA shell. Using these SNAs, we evaluate immune responses against various targets *in vitro*, *ex vivo,* and *in vivo*.

Results: The modularity of the SNA has allowed us to uncover key structure-activity relationships and arrive at highly potent immunostimulatory constructs. In this work, we explore critical structure-function relationships between vaccine design and raised immunity, and successfully: 1) reformulate and elevate prior failed prostate cancer clinical targets, 2) address tumor heterogeneity through rational design of complex vaccines for melanoma incorporating targets for multiple T cell subtypes, and 3) generate a potent and adaptable infectious disease vaccine using SARS-CoV-2 as a case study.

Firstly, we employed a particular SNA architecture designed to activate the immune system effectively and efficiently. By incorporating one of two previously discovered and clinically implemented human prostate targets (prostate specific membrane antigen and T-cell receptor γ alternate reading frame protein) into the SNA, we directly elevate antitumor responses (*i.e.,* cytokine secretion, T cell killing ability) in humanized mice and human immune cells compared to the same "free" peptide antigen in solution. Secondly, we discovered that structural placement within the SNA of two classes of peptide targets (one targeting $CD8^+$ and another targeting $CD4^+$ T cells) dramatically alters vaccine efficacy against melanoma by inducing changes at the genetic, cellular, and organismal levels. SNA structure dictated gene expression in T cells, resulting in cytokine secretion for an optimized structure and ultimately murine melanoma tumor reduction when implemented in combination with checkpoint inhibitor therapy. Thirdly, we elucidated structure-function relationships with the SNA as an antiviral vaccine specifically targeting SARS-CoV-2, which protected 100% of humanized ACE2 mice in a lethal viral challenge.

Conclusion: This work has wide implications across vaccine design. Through our studies utilizing human clinical targets, we are advancing the promise of a clinically translatable solution and show how prostate cancer immunotherapy can be greatly improved through rational design at the nanoscale. Our discoveries elucidating the impact of antigen placement within a nanoscale construct highlights how the heterogeneity of tumors can be addressed through the rational design of complex vaccines. Finally, our work developing the SNA as an antiviral vaccine underscores the ability to rapidly adapt this platform for infectious disease. Overall, these concepts are broadly applicable to the field of vaccine design and highlight that nanostructure design strategies are powerful ways to present and coordinate the processing of immunostimulatory cues to immune cells.

References:

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