

Nitro Biosciences: Enhancing immune response via an expanded genetic code

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Abstract

Novel modalities of vaccine will be required to address the current and future public health concerns we face. Many infectious diseases lack clinically approved vaccines causing immense burden to the health care system both domestically and abroad. More concerning, the prevalence of antimicrobial resistance (AMR) is anticipated to rise over the coming decades and limit our tools to treat these infections. There is thus an urgent need to develop vaccinations to overcome these rising gaps in treatment and prevent infections moving forward. At Nitro Biosciences, we are developing a platform to create next-generation vaccines for diseases currently lacking clinically approved products. By harnessing an expanded genetic code, we can precisely modify antigens to enhance their immunogenicity, enabling a broadening of the scope of antigens to target in vaccine development and enhancing the potential to create efficacious vaccines where other efforts have failed.

The growing public health need for next generation vaccines

Microbial infections acutely impact those across the globe with underdeveloped or compromised immune systems, particularly children and the elderly. Despite this known risk to public health, many bacterial pathogens such as *E. coli*, *Shigella*, and *C. difficile* lack clinically approved vaccines. Most concerning, common methods of treatment for microbial infections are becoming less potent. In 2019, antimicrobial resistance was directly attributed to over 1 million annual deaths and over 250,000 deaths of children under 5¹. Here in the United States, more than 2.8 million infections of antibiotic resistant bacteria occur annually with 48,000 associated deaths². Urgent innovations are needed, as this burden is only expected to continue to grow over the next few decades and is predicted to result in 10 million annual deaths by 2050³. Given the current rate of antibiotic

discovery, we will need to improve measures to prevent infection, namely vaccines, to limit future burden.

Nitro Biosciences: Next-generation vaccines enabled via nitration

Recruitment of the immune system to target disease-associated antigens has been a powerful tool to both prevent and cure disease. However, for several diseases, the natural immune response toward the most conserved antigens is quite weak. For several bacterial species, many of the antigens prevalent across serotypes of the disease have evolved to evade immune detection and are weakly immunogenic. If these antigens were delivered in the manner of a traditional vaccine, the response can be poor to nonexistent, leading to a lack of protection or therapeutic efficacy.

Through our platform at Nitro Biosciences, we aim to amplify immune recognition toward these weakly immunogenic targets. Through a process known as genetic code expansion, we can selectively introduce an immunogenic (nitrated) residue within the sequence of protein antigens. The introduction of this nitrated residue within antigens can stimulate a stronger immune response⁴⁻⁹. Immunization with nitrated versions of antigens which normally are either weakly immunogenic or well tolerated modifies presentation to the immune system. The nitrated antigen contains epitopes absent from the weak wild-type antigen, which then triggers a strong CD4⁺ helper T cell based immune response. This stimulates the production of antibodies including cross-reactive antibodies which bind to unmodified variants of the antigen. While immunization with unmodified antigens in previously reported cases resulted in little to no immune response, immunization with nitrated variants was capable of breaking tolerance in previous tested studies.

Building upon this technology, we are developing improved methods for nitrated antigen delivery. By rewiring bacterial metabolism, we have created a bacterial strain that can autonomously produce nitrated forms of any user-defined recombinant antigen¹⁰. Our goal is to use these live attenuated bacteria as nitrated antigen producers and delivery vehicles to transport antigens directly to regions of infection or disease. Through this strategy, we intend to overcome two major limitations of current vaccination strategies – the lack of localized immune response and the inability to target cross-serotype or more generalized antigens.

Previous recent attempts to create vaccines for microbial infections using traditional means have had limited success, despite often robust markets. Notably, in the case of *C. difficile*, several attempts at traditional protein toxoid vaccination failed due to lack of efficacy, despite significant investment and a sizable predicted market (estimated \$1B market¹¹ and 500K annual patient size¹²). Investment in next-generation vaccine modalities to solve challenges in preventative medicine for infectious disease will be required to address broader public health concerns.

The team developing this next generation vaccine modality is composed of founders Dr. Aditya Kunjapur, Assistant Professor of Chemical and Biomolecular Engineering at the University of Delaware, and Dr. Neil Butler. Together, we developed the foundational technology core at the University of Delaware, leading to the incorporation of Nitro Biosciences to translate the potential of this technology as a tool for future vaccines. Since incorporating, the team has raised over \$200,000 in non-dilutive funds. These include mechanisms in Delaware, such as the UD Blue Hen Proof of Concept program and Delaware Biotechnology Institute Entrepreneurial Proof of Concept program, as well as nationally through the NSF I-Corps program and the 2021 Langer Prize in Innovation and Entrepreneurial Excellence from the American Institute of Chemical Engineers (awarded to Dr. Kunjapur).

Without novel methods for prevention or treatment of infection, the predicted burden from antimicrobial resistance (10 million annual deaths by 2050) may come to fruition. Through our next-generation vaccine platform, we aim to reduce this risk and create vaccines to prevent infections across disease states.

References

1. Murray, C. J. L. *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* **399**, 629–655 (2022).
2. CDC. *Antibiotic Resistance Threats in the United States*. (2019).
3. Coque, T. M. *et al.* *Bracing for Superbugs: Strengthening environmental action in the One Health response to antimicrobial resistance*. (2023).

4. Grünewald, J. *et al.* Immunochemical termination of self-tolerance. *Proc Natl Acad Sci U S A* **105**, 11276–11280 (2008).
5. Grünewald, J. *et al.* Mechanistic studies of the immunochemical termination of self-tolerance with unnatural amino acids. *Proc Natl Acad Sci U S A* **106**, 4337–4342 (2009).
6. Gauba, V. *et al.* Loss of CD4 T-cell-dependent tolerance to proteins with modified amino acids. *Proceedings of the National Academy of Sciences* **108**, 12821–12826 (2011).
7. Tian, H. *et al.* Nitrated T helper cell epitopes enhance the immunogenicity of HER2 vaccine and induce anti-tumor immunity. *Cancer Lett* **430**, 79–87 (2018).
8. Tian, H. *et al.* PDL1-targeted vaccine exhibits potent antitumor activity by simultaneously blocking PD1/PDL1 pathway and activating PDL1-specific immune responses. *Cancer Lett* **476**, 170–182 (2020).
9. Li, F. *et al.* A new vaccine targeting RANKL, prepared by incorporation of an unnatural Amino acid into RANKL, prevents OVX-induced bone loss in mice. *Biochem Biophys Res Commun* **499**, 648–654 (2018).
10. Butler, N. D., Sen, S., Brown, L. B., Lin, M. & Kunjapur, A. M. A platform for distributed production of synthetic nitrated proteins in live bacteria. *Nat Chem Biol* **19**, 911–920 (2023).
11. Coherent Market Insights. *Clostridium Vaccine Market Analysis*. (2022).
12. Nearly half a million Americans suffered from *Clostridium difficile* infections in a single year. *Centers for Disease Control and Prevention, Atlanta, GA* (2017).