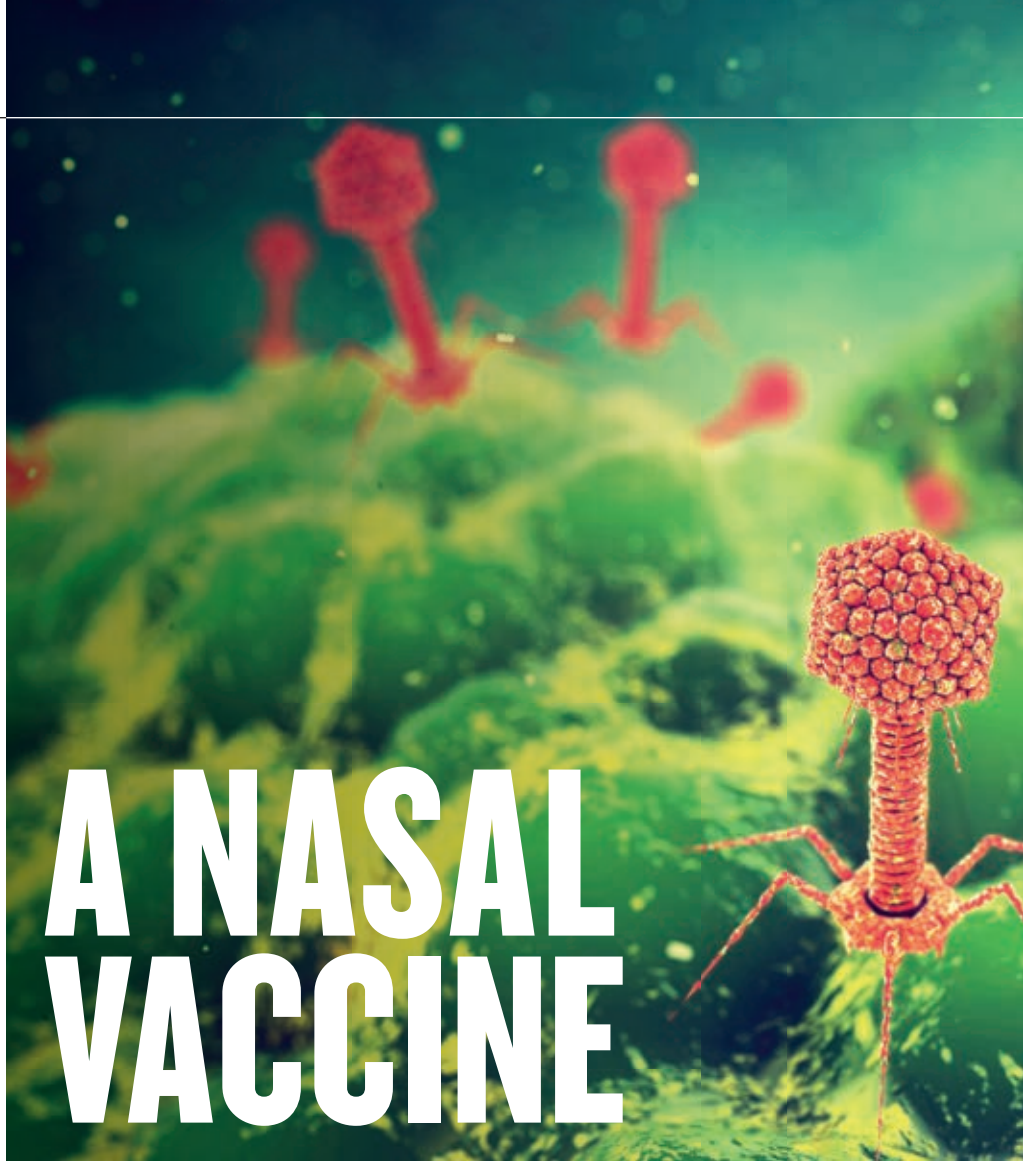


A NASAL VACCINE

Researchers have found the first non-infectious needle-free nasal vaccine to be effective against COVID-19. Senior study co-author **Dr Venigalla B Rao** explains how it works.

In March 2020, a research team of biologists and immunologists in the US submitted a grant proposal to the National Institutes of Health to develop a COVID-19 vaccine that uses a bacteriophage T4 virus-like particle (VLP) platform. The researchers had already published papers on anthrax and plague vaccines that use the bacteriophage T4 platform, and had submitted a grant proposal two months earlier to develop a "universal" influenza vaccine – which was rejected.

"We were making a shift to make this platform more universal by using CRISPR engineering – to be able to produce vaccines rapidly against any emerging or pandemic pathogen," says Dr Venigalla B Rao, Professor in Biology at The Catholic University of America in Washington DC and senior study co-author. "Our thought process and a template had already been in place in late 2019, and this proposal was submitted at the time COVID-19 was emerging in Wuhan and the US."

The proposal was accepted and the research team worked through the entire lockdown period. By November 2020, they found that the phage T4-COVID-19 vaccine they constructed was effective in mice, showing complete protection.

The team went on to test its effectiveness as a needle-free vaccine. Two doses of the T4-COVID-19 vaccine were administered intranasally in mice, 21 days apart. The vaccine induced robust mucosal immunity as well as strong systemic humoral and cellular immune responses. The results were published in *mBio*, the flagship journal of the American Society of Microbiology.

The vaccine also neutralised multiple COVID-19 variants, including the mouse-adapted SARS-CoV-2 MA10 strain, the ancestral WA-1/2020 strain, and the most lethal Delta variant in mouse models. The vaccine did not affect the gut microbiota, and the immune responses were much stronger in intranasally vaccinated mice than those injected with the same vaccine.

"In eight months since conceiving the vaccine design, we have developed the recombinant phages and tested them to determine which one would actually work. By the time the mRNA vaccines came along, we had demonstrated that we had a vaccine using our platform," Rao adds. While the vaccine needs to be tested on non-human primates, as well as humans, previous research using the same platform for an anthrax vaccine reproduced the same findings – complete protection – in rhesus macaques as mice. "Our vaccine has the promise to prevent or greatly minimise person-to-person transmission of COVID-19," Rao adds.

Protection

While having a "tremendous impact on preventing severe infections, hospitalisations, and deaths", mRNA and adenovirus-based vaccines do not induce mucosal immunity or prevent transmission, Rao explains. "The Omicron variants are now spreading like wildfire, despite the fact that 60%–70% of the US population are vaccinated and boosted. That's the same all over the world. These breakthrough COVID-19 infections will continue and the emergence of a more lethal and transmissible variant remains a possibility."

The mucosal immunity offered by the T4 vaccine means that the COVID-19 virus may not be able to enter through the respiratory pathways. Even when transmission occurs

it may not lead to full-blown infection or long COVID because the well-developed immunity in the respiratory airways might limit the viral load.

The T4 vaccine appears to provide sterilising immunity too. It might limit shedding of the infectious virus that can be transmitted to another individual. The virus could not be detected in the lungs of the mice even though they were challenged with lethal doses of COVID-19.

Unlike most of the nasal vaccines under development, which use an infectious human virus, the T4 vaccine is non-infectious, free of adjuvants (chemicals used to stimulate immune responses), and stable at room temperature.

"Mucosal vaccines in general are not as robust as injected vaccines in generating systematic immune responses, such as neutralising antibodies," Rao says. "Surprisingly, the nasal T4-COVID-19 vaccine stimulated greater systemic immune responses, including neutralising antibodies and T cell responses than the injected intramuscular vaccine. This is even better because the nasal vaccine has both breadth and strength of immune responses."

Future applications

The platform that has been created is near universal, Rao says. "At the time we were developing the vaccine design template, we had also developed the CRISPR engineering strategy. It was a convergence of different technologies that led to the development of this universal platform. To my knowledge, it's one of the most flexible and powerful vaccine design platforms out there."

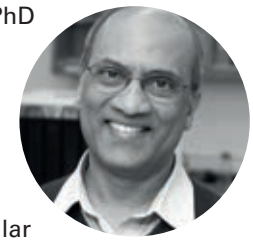
Because the platform uses a bacteriophage that can be grown in bacteria, it can be cost-effectively manufactured. "If the manufacturing process is in place, it could be produced in compatible levels of timeframes and urgency as the mRNA vaccines, but it is expected to be more potent because we are including antigens directly into the vaccine," Rao adds.

But substantial funding is required if the vaccine is to be tested to establish effectiveness in humans, and that is hard to secure. "In my opinion, this is a powerful platform that we should invest in. It nicely complements the mRNA- and adenovirus-based platforms in our preparedness for pandemics and the pay-off would be tremendous" Rao says.

He has spent more than 40 years researching bacteriophages, including a period working with Professor Michael Rossman, the physicist and structural biologist who discovered the structure of the common cold virus. "We've learned a lot about bacteriophage structure, mechanisms, and so on. That's been our primary motivation and inspiration – we accumulated enough basic knowledge that we started thinking about how to harness this knowledge for applications."

Rao is working on a new paper on novel genetic therapies and human cells using bacteriophage T4. "This could potentially be a breakthrough," he says. "All we do is observe nature closely for what it is and what it is teaching us, then solutions will come up." TBMS

VENIGALLA B. RAO



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- ✔ **2001 – 2019** – Chair, Biology, Centre for Advanced Training in Cell and Molecular Biology
- ✔ **2021** – Founded the Bacteriophage Medical Research Centre, The Catholic University of America, and currently serving as Founding Director
- ✔ **2021** – elected Fellow, American Society of Microbiology
- ✔ **2021** – elected Fellow, National Academy of Inventors.

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