JANUARY 2023 Volume 2, Issue 1

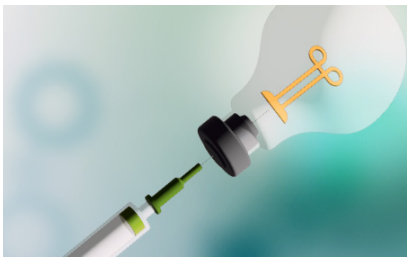
VACCINE INSIGHTS

SPOTLIGHT ON:
Preclinical research & next-generation vaccine platforms

GUEST EDITOR:
Isis Kanevsky, Pfizer

VACCINE INSIGHTS

Volume 2, Issue 1



Preclinical research & next-generation vaccine platforms

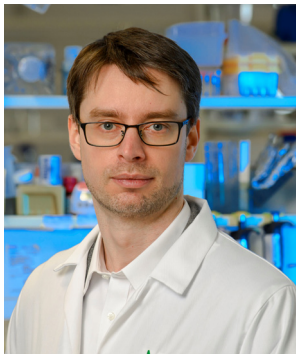
VACCINEINSIGHTS

PRECLINICAL RESEARCH &
NEXT-GENERATION VACCINE PLATFORMS

SPOTLIGHT

Exploring the potential of circular RNA

Piotr S Kowalski



"Circular RNA has the potential to expand the toolbox of therapeutic RNAs and address some of the limitations of current RNA vaccines and therapeutics."

VIEWPOINT

Vaccine Insights 2023; 2(1), 5–8

DOI: 10.18609/vac.2023.002

The success of the mRNA COVID vaccines has boosted confidence in the safety and potential efficacy of mRNA as a therapeutic molecule. In the pharmaceutical industry, as well as the public eye, these molecules are seen as a potential therapeutic of the future. However, challenges with mRNA vaccines remain; in particular, high costs and limited storage stability. Could circular RNA offer a potential solution?

As the leader of a research group focused on developing novel, clinically relevant delivery technologies for nucleic acid-based therapies, I saw firsthand how the COVID pandemic accelerated manufacturing and scale-up processes and regulatory frameworks for mRNA-based drugs. The pandemic catalyzed academic and industry research and development (R&D) activities in the mRNA field, as well as in the lipid nanoparticle (LNP) technology used to deliver the vaccines. However, there is still huge scope for improvement.

CHALLENGES OF RNA

mRNA technology is not entirely new – it has been in R&D for around 20 years – so thankfully a critical mass of research was available and could be applied to develop mRNA vaccines for COVID. However, the urgency of the pandemic did not allow sufficient time to optimize every aspect of the vaccine.

Specifically, there remains room to improve delivery and better understand the important role of the LNP in vaccine efficacy. Developing more efficient biomaterials and nanocarriers that can help reduce the dose or minimize potential adverse effects of vaccine administration would be highly beneficial.

Various modes of delivery have also been considered. There has been a lot of interest in nasal or inhaled formulations, with vaccines that act on the upper respiratory tract to induce mucosal immunity and better prevent infection.

Another important area for improvement lies in the shelf life and storage of vaccines, especially relevant to address accessibility in countries that lack infrastructure for maintaining cold-chain storage.

Finally, mRNA drugs are not cheap to make. The use of more sustainable and affordable manufacturing methods and a better supply of necessary reagents would help make RNA-based drugs more accessible.

CIRCULAR RNAS

Circular RNAs are a new class of non-coding RNAs, normally characterized by a closed-loop structure. They are produced through an alternative form of splicing, known as back-splicing, and have been shown to possess unique roles and structural features. The majority of circular RNAs are conserved amongst species, with tissue-specific expression patterns, and are involved in the control of gene expression and protein function, although their biological role is still being explored. Circular RNAs have been implicated in several human diseases, including neurological disorders, cancer, diabetes, chronic inflammation, and cardiovascular diseases, hinting at their potential for therapeutic applications.

Recent reports have shown that circular RNA can also play a role in the regulation of innate immune responses, for example by suppressing the activation of protein kinases. In addition, circular RNAs that contain sequences allowing for translation initiation or are modified with N6-methyladenosine have the potential to translate into peptides, though their biological role is still not well understood.

While very little translational research has been carried out to date, circular RNA could have advantages compared with linear RNA in a number of areas. Their circular shape means that they lack the free 5′ and 3′ ends found in mRNAs that are the target of exonuclease-mediated degradation, making them significantly more stable and extending their half-life.

My work in the lab of Daniel Anderson at MIT has shown that it is possible to make synthetic circular RNAs that retain similar properties to linear mRNA but are more stable inside cells. We also discovered that those circular RNAs can be less immunogenic compared to linear RNAs since their unique shape helps avoid recognition by toll-like receptors [1]. Circular mRNAs are also potentially more streamlined and sustainable to

DOI: 10.18609/vac.2023.002

manufacture, as they do not rely on a 5′ cap and 3′ poly-A tail, reducing the enzymatic reactions and reagents required.

We have found that circular mRNAs can achieve higher protein production in mice, having more than a double half-life compared to linear mRNAs. This needs to be studied further in larger animal models, but it is promising for therapeutic applications [2].

One recent study demonstrated the potential of using circular RNA for developing COVID vaccines. A circular RNA encoding a spike protein was shown to protect and boost memory against SARS-CoV-2 in mice and rhesus macaques [3]. The vaccine induced potent humoral and cellular immune responses and outperformed a linear mRNA vaccine in producing neutralizing antibodies. This interesting dataset showcases the promising future of cicRNAs in vaccine development.

LOOKING AHEAD

Circular RNA has the potential to expand the toolbox of therapeutic RNAs and address some of the limitations of current RNA vaccines and therapeutics, however, research on circular RNA-based drugs is in the very early stages. There are concerns that if circular RNA is not properly purified, it could induce undesirable immune responses. As the mechanism of circular RNA translation is not yet well understood, the breadth of its potential in therapeutic applications is still unknown. Most basic research on circular RNAs has been focused on understanding the native role of circular RNAs while translational research has not yet been pursued.

In my group, we aim to harness the unique features of synthetic circular RNAs for research and translation, to unlock the therapeutic potential of this new class of RNA molecules.

BIOGRAPHY

PIOTR KOWALSKI is an Associate Professor in advanced therapies at the School of Pharmacy, University College Cork, and a Funded Investigator at the APC Microbiome Ireland. He earned his PhD in 2014 from the University of Groningen (the Netherlands) which focused on the development of lipid-based systems for tissue selective delivery of siRNA. He received his postdoctoral training at the Koch Institute for Integrative Cancer Research at the Massachusetts Institute of Technology in the laboratories of Professor Daniel Anderson and Prof Robert Langer. His multidisciplinary research focused on engineering novel biomaterials to enable the delivery of messenger RNAs to treat inflammatory diseases, cancer, and diabetes. Dr Kowalski's work resulted in a number of high-impact publications, several patents on RNA delivery technologies, and the creation of a US-based biotech startup (Orna therapeutics). His research at UCC is centered on developing Advanced Therapy Medicinal Products, in particular, novel clinically relevant drug delivery technologies for parental and non-parental applications, to facilitate effective nucleic acid-based therapies aimed at high medical need diseases that lack effective treatment. Dr Kowalski has recently won a prestigious European Research Council Starting grant to develop a new class of circular RNA therapeutics. Currently, his group investigates the therapeutic potential of RNA molecules, including short interfering RNAs, messenger RNAs and circular RNAs, to treat diseases such as sepsis, inflammatory bowel disease, and cancer and develops methods to deliver these RNA-based drugs to diseased cells.

REFERENCES

1. Wesselhoeft RA, Kowalski PS, Anderson DG. Engineering circular RNA for potent and stable translation in eukaryotic cells. *Nat. Commun.* 2018; 9(1), 2629.
2. Wesselhoeft RA, Kowalski PS, Parker-Hale FC *et al*. RNA circularization diminishes immunogenicity and can extend translation duration *in vivo*. *Mol. Cell.* 2019; 74(3), 508–520.e4.
3. Qu L, Yi Z, Shen Y *et al*. Circular RNA vaccines against SARS-CoV-2 and emerging variants. *Cell* 2022; 185(10), 1728–1744.e16.

AFFILIATION

Piotr S Kowalski
Associate Professor and Principal Investigator,
the School of Pharmacy, University College Cork, Ireland
and
Funded investigator,
the APC Microbiome Ireland

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Kowalski PS discloses his group that is working on Circular RNA research is funded by the European Re-search Council Starting Grant (101041424 — CIRCLE). He also discloses that he has patents is-sued/planned (PCT/US2019/035531). Kowalski P also discloses he is a scientific co-founder and share-holder of Orna Therapeutics, LLC.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Jan 18 2023; **Publication date:** Jan 24 2023

DOI: 10.18609/vac.2023.002

INTERVIEW

DNA vaccines: the story so far… & the next chapter

Charlotte Barker Editor, *Vaccine Insights*, talks to **Michele Kutzler**, Associate Dean for Faculty, Professor of Medicine and Microbiology & Immunology, Drexel University College of Medicine

MICHELE KUTZLER is a Professor of Medicine and Microbiology and Immunology at Drexel University College of Medicine. After completing a PhD in microbiology and immunology at Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA, and a post-doctoral research fellowship in gene therapy and vaccines at Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, she joined Drexel University College of Medicine in the Division of Infectious Diseases & HIV Medicine. Dr Kutzler works to develop nucleic acid-based prophylactic vaccine strategies against pathogens including Human Immunodeficiency Virus, the bacterium *Clostridioides difficile*, and more recently, SARS-CoV2. Her expertise is in the use of nucleic acid-based antigenic platforms and molecular immunoadjuvant systems to boost immune durability and enhance the quality of immune responses to vaccines, particularly in the elderly.

Vaccine Insights 2023; 2(1), 15–19

DOI: 10.18609/vac.2023.004

Michele Kutzler discusses her career in vaccine research, the roadblocks that have, until recently, kept DNA vaccines out of the clinic, and why she believes a happy ending is in sight for the platform.

Q How did you get involved in research on vaccines?

MK: I'm motivated by being a part of innovative work that could ultimately improve patients' lives. I had an interest in the field of immunology from the time of my undergraduate studies into my PhD training which was focused on examining how human immunodeficiency virus (HIV) interacts with host cells and the role chemokines and their receptors play in controlling susceptibility to infection when drugs of abuse like heroin are present. After my PhD, I joined the laboratory of David Weiner, who is considered the founder of DNA vaccines, and spent seven years in his University of Pennsylvania laboratory working on a DNA vaccine for prevention of HIV. Beyond the science, I learned a lot about the huge impact that vaccines can have on decreasing transmission, and how academic researchers can partner with industry to move discoveries from bench to bedside. Philadelphia is a great location for innovative academic-industry partnerships.

The early 2000s were an exciting time for vaccine research at the University of Pennsylvania – while I was working in the budding field of DNA vaccines with David Weiner, Drew Weismann was doing his ground-breaking work on mRNA vaccines in his laboratory close by. Fast forward to today, and it's amazing to see the progress that has been made with nucleic acid-based vaccines.

Q What is your focus now?

MK: HIV targets CD4 positive T cells, 80% of which are found in the gastrointestinal tract, so at the University of Pennsylvania, I worked on targeting immunity to mucosal sites using adjuvants. When started my own laboratory at Drexel University, I wanted to apply that work to other mucosal pathogens and became very interested in *Clostridioides difficile*, a toxin producing bacteria that causes illness after use of antibiotic medications and most commonly affects older adults in hospitals or in long-term care facilities. *C. difficile* carries a high risk for severe disease and mortality in elderly patients. It is a disease that requires a mucosal antibody response against the toxins produced by the bacteria, so the mucosal-targeting technology I had been working on was a great fit. This has been an exciting new area of research for my laboratory, and we have been successful in developing a DNA vaccine based on *C. difficile* toxins [1]. Now, we continue to develop next-generation vaccines; in particular, formulations that are more immunogenic in the elderly.

Q What are the advantages of DNA vaccines?

MK: It has been documented that DNA vaccines are able to stimulate both B- and T-cell responses, have improved thermostability, and have the relative ease of design and large-scale manufacture. With emerging infections across the globe, we were always cognizant of being able to manufacture with reduced development costs and risks, and the DNA platform addresses many of these goals. In particular, it is very stable and so it has less extreme temperature requirements than mRNA vaccines. Furthermore, like

DOI: 10.18609/vac.2023.004

mRNA, it can be rapidly designed and manufactured so you can design antigens to match emerging viruses.

Q Why has only one DNA vaccine (ZyCoV-D) made it onto the market so far?

MK: The slow uptake of DNA vaccines is predominantly due to delivery requirements. The difference between mRNA and DNA vaccines is that the DNA vaccine antigen has to be delivered through the plasma membrane to the nucleus so that it can be transcribed into RNA. To help shuttle the antigen into the nucleus requires enhanced delivery methods such as lipid nanoparticles, electroporation, jet injectors and gene guns..Gaining US Food and Drug Administration approval for delivery devices will be an important step toward gaining clinical approval for more DNA vaccines.

Early studies with DNA vaccines also showed poor immune responses, but with the help of improved delivery methods, antigen design, and inclusion of molecular adjuvants in formulations, we have advanced well beyond those first vaccines. Newer DNA vaccines are demonstrating immunogenicity and durability levels similar to those seen with adenoviral vector vaccines.

Q What adjuvants are being used to boost immunogenicity?

MK: There are a lot of interesting chemokines and cytokines; for example, interleukin (IL)-12 and IL-15 cytokines play important roles in boosting T cell responses and the function of antigen-presenting cells, and chemokines CCL27 and CCL28 can also drive mucosal immunity when co-delivered with an antigen [2-6]. Another exciting adjuvant we are developing at Drexel is adenosine deaminase-1 (ADA-1), an enzyme that is expressed at high levels in lymphoid tissues and functions to eliminate a toxic molecule called deoxyadenosine, which is generated when DNA is broken down thus helps to maintain immune function. We have found that when combined with vaccine antigens, it enhances quality and quantity of T cell responses in the lymph node and in turn helps to boost the durability of antibody responses to the vaccine [7,8]. Individuals who do not express ADA-1 have severe combined immunodeficiency (SCID), so it's clear that the enzyme is critical to effective immune function and can be harnessed as a vaccine adjuvant.

Q You co-authored a 2008 paper asking whether DNA vaccines were ready for primetime [9]: have things changed since then?

MK: That article was updated recently by my colleague Dr Ebony Gary, and I agree with her view that primetime is now [10]! The newer synthetic DNA vaccines that have recently advanced to clinical studies have an impressive degree of immune potency and tolerability, and there have been improvements in DNA delivery such as jet and gene gun delivery, and advanced electroporation techniques that are well tolerated. These studies have shown that you can induce robust humoral and cellular immunity in humans.

"In the future, I envision DNA vaccines being deployed in low- and middle-income countries that lack effective cold chain storage, against emerging infectious diseases, such as Ebola, Zika, COVID, and others."

Research teams [11,12] have also moved DNA vaccines into the therapeutic space, using the DNA platform to deliver antibodies. It is an exciting move forward for DNA vaccines.

In the future, I envision DNA vaccines being deployed in low- and middle-income countries that lack effective cold chain storage, against emerging infectious diseases, such as Ebola, Zika, COVID, and others. Additionally, I see this technology used in the cancer immune therapy space, for cancers caused by viruses such as human papillomavirus, hepatitis B virus, and hepatitis C virus.

Q What's next for your research?

MK: Our laboratory is interested in continuing our work to develop immune adjuvants that boost immunogenicity and durability to vaccines in the elderly. In addition, we are developing DNA delivery of anti-toxin antibodies as a treatment for *C. difficile*. Our goal is to use the DNA vaccine platform to deliver monoclonal antibodies against bacterial toxins, to help during the acute disease phase, especially in the elderly, who have a hard time mounting an initial immune response. Once recovered, a patient can then receive a vaccine that encodes for toxin-based antigens in combination with adjuvants such as ADA-1 to boost immune memory to *C. difficile*, thus decreasing morbidity and mortality and decreasing rates of disease recurrence.

REFERENCES

1. Baliban S, Michael A, Shammassian B *et al.* An optimized, synthetic DNA vaccine encoding the toxin A and toxin B receptor binding domains of Clostridium difficile induces protective antibody responses in vivo. Infect. Immun. 2014; 82(10), 4080–4091.
2. Gary EN, Kathuria N, Makurumidze G *et al.* CCR10 expression is required for the adjuvant activity of the mucosal chemokine CCL28 when delivered in the context of an HIV-1 Env DNA vaccine. *Vaccine* 2020; 38(11), 2626–2635.
3. Kutzler MA, Wise MC, Hutnick NA *et al.* Chemokine-adjuvanted electroporated DNA vaccine induces substantial protection from simian immunodeficiency virus vaginal challenge. *Mucosal Immunol.* 2016; 9(1), 13–23.
4. Kutzler MA, Kraynyak KA, Nagle SJ *et al.* Plasmids encoding the mucosal chemokines CCL27 and CCL28 are effective adjuvants in eliciting antigen-specific immunity *in vivo*. *Gene Ther.* 2010; 17(1), 72–82.
5. Aldon Y, Kratochvil S, Shattock RJ, McKay PF. Chemokine-Adjuvanted Plasmid DNA Induces Homing of Antigen-Specific and Non-Antigen-Specific B and T Cells to the Intestinal and Genital Mucosae. *J. Immunol.* 2020; 204(4), 903–913.
6. Tregoning JS, Buffa V, Oszmiana A, Klein K, Walters AA, Shattock RJ. A "prime-pull" vaccine strategy has a modest effect on local and systemic antibody responses

DOI: 10.18609/vac.2023.004

to HIV gp140 in mice. *PLoS One* 2013; 19, 8(11), e80559.

7. Gary E, O'Connor M, Chakhtoura M *et al*. Adenosine deaminase-1 enhances germinal center formation and functional antibody responses to HIV-1 Envelope DNA and protein vaccines. *Vaccine*. 2020; 38(22), 3821–3831.

8. Cusimano GM, Gary EN, Bell MR *et al*. Improved Durability to SARS-CoV-2 Vaccine Immunity following Coimmunization with Molecular Adjuvant Adenosine Deaminase-1. *J Immunol.* 2022; 209(1), 118–127.

9. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? *Nat. Rev. Genet.* 2008; 9(10), 776–788.

10. Gary EN, Weiner DB. DNA vaccines: prime time is now. *Curr. Opin. Immunol.* 2020; 65, 21–27.

11. Parzych EM, Du J, Ali AR, Schultheis et al. DNA-delivered antibody cocktail exhibits improved pharmacokinetics and confers prophylactic protection against SARS-CoV-2. *Nat. Commun.* 2022; 13(1), 5886.

12. Patel A, Bah MA, Weiner DB. In Vivo Delivery of Nucleic Acid-Encoded Monoclonal Antibodies. *BioDrugs*. 2020; 34(3), 273–293.

AFFILIATION

Michele Kutzler, PhD
Associate Dean for Faculty, Professor of Medicine and Microbiology & Immunology, Drexel University College of Medicine

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Kutzler M discloses she has sponsored research agreement from Pfizer, Inc. She has royalities from Inovio Pharmaceuticals, Inc. for patents on DNA Vaccines and molecular adjuvants. She has a patent on 'Compositions and methods employing adenosine deaminase-1 (ada-1) as an adjuvant'.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an interview with Michele Kutzler carried out on Jan 18 2023.

Interview held: Jan 18 2023; **Revised manuscript received:** Feb 20 2023; **Publication date:** Feb 22 2023

INTERVIEW

(Plasmid) launching a new vaccine platform

Charlotte Barker, Editor, *Vaccine Insights*, talks to **Kai Dallmeier,** Associate Professor, KU Leuven, and **Hanne Callewaert,** CEO, Astrivax

KAI DALLMEIER, PhD, is Associate Professor of Virology at the University of Leuven (KU Leuven), Belgium, and leads the Molecular Vaccinology & Vaccine Discovery (MVVD) group at the KU Leuven Rega Institute. In a multidisciplinary approach and using the live-attenuated yellow fever vaccine as platform, he and his team develop vaccines for emerging infections (such as Zika, Ebola and COVID-19) as well as therapeutic vaccines (for instance for chronic hepatitis B). Thermostable and easy-to-manufacture plasmid-launched vaccines aim to tackle vaccine shortages and unmet public health needs faced particularly by people living in LMIC. For deeper mechanistic insight into virus replication and virus-induced disease, this translational work on vaccines is complemented by the study of viral infections and pathogenesis in a range of cell culture and animal models.

HANNE CALLEWAERT is co-founder and CEO of Astrivax. She has global industry experience in large pharmaceutical companies such as GSK Vaccine, as well as smaller biotechs. Before co-founding Astrivax, she was Entrepreneur in residence at KU Leuven and COO at ophthalmic drug developer Oxurion. Hanne has strong experience in development of commercial-, late- or early-stage vaccines, monoclonal antibodies, peptides, and small molecules. She blends regulatory affairs, drug, and vaccine development expertise with business and corporate development., with a proven track record of building and leading diverse teams.

Vaccine Insights 2023; 2(1), 9–13

DOI: 10.18609/vac.2023.003

A new vaccine platform, plasmid-launched live attenuated vaccine (PLLAV), hopes to combine the efficacy of a live attenuated vaccine with the thermostable properties of a DNA plasmid. To find out more, we caught up with Kai Dallmeier, one of the original inventors of the technology, and Hanne Callewaert, CEO of Astrivax, the spin-off company commercializing the platform.

Q For readers who might not be aware of the technology, what is a plasmid-launched live attenuated vaccine (PLLAV) in a nutshell?

KD: The PLLAV platform is a modified *E. coli* bacterial plasmid that incorporates the genome of a live-attenuated flavivirus vaccine. The plasmid is taken up into cell nuclei and translated into a live attenuated virus, which then replicates as usual. The mechanism of action is that of a live vaccine, but because it is made of DNA, it is more thermostable.

Q Can you describe the work leading to the development of this technology?

KD: The first spark of the idea came in the early 2010s, when we were working on flaviviruses for drug development purposes. As a safe and easy-to-work-with surrogate for dangerous flaviviruses such as the Dengue virus, we used the attenuated vaccine strain of the yellow fever virus (YF17D) in our experiments. At one point, we needed to make genetic variants of the virus to characterize drug activity, and while using the available reverse genetics system for that purpose I immediately felt that there was much space for improvement. Our new BAC (bacterial artificial chromosome) based system was eventually so compact and efficient at launching the virus in culture, that we decided to test it in animals. We found that the virus thus produced could not be distinguished from the original YF17D vaccine strain.

Around the time of these experiments, I saw a press release about an outbreak of yellow fever in a refugee camp in South Sudan. The press release explained that because the current live attenuated vaccine is not thermostable, it would take months to set up a cold chain and get vaccines to the region to tackle the outbreak. Immediately, I thought that our technology, now known as PLLAV, would be an elegant technical solution to allow a fantastic existing vaccine to reach people faster and save more lives.

> "The PLLAV platform is a modified *E. coli* bacterial plasmid that incorporates the genome of a live-attenuated flavivirus vaccine."
>
> **- Kai Dallmeier**

Q At what stage was the spin-off company, Astrivax, launched?

KD: Of course, we are an academic research lab, so there was a limit to how

DOI: 10.18609/vac.2023.003

far we could progress toward the clinic. We were able to show that PLLAV works in several step-up animal models, but we obviously do not have the knowledge nor the capacity to produce vaccines for clinical trials and commercialize the technology.

HC: The technology is at an academically mature level, and now Astrivax is looking at how to bring this to the clinic and ultimately onto the market, which is a very different mindset.

KD: We still work closely together and provide research capacity to the company, but the driving seat is with Hanne and her team.

Q How does PLLAV compare with other vaccine platforms?

HC: We see our technology as being complementary to other vaccine platforms. Every platform has its pros and cons, and the challenge is to use the right platform for the indication and population you are targeting. Where our platform has advantages over classical DNA vaccines is that the immune response generated by our vaccine is much more potent and polyfunctional, because we produce a live attenuated virus from our plasmid.

Compared with a live attenuated virus vaccine for yellow fever, the biological activity of our vaccine is the same, but there are advantages in manufacturing and stability. Yellow fever vaccines are still being produced in fertilized chicken egg embryos, which are difficult to scale and suffer regular supply disruptions. Our vaccine is much easier to produce via bioprocessing and as it is more stable than a live attenuated virus, it may be able to skip parts of the cold chain.

We believe PLLAV combines some of the key advantages of both DNA and live attenuated vaccines. Of course, it has some challenges too. For example, like most live attenuated vaccines, it may not necessarily be suitable for immuno-compromised subjects.

Q One of the main issues with DNA vaccines has been transfection into the nucleus: is that true for PLLAV?

HC: The mechanism is somewhat different. Classical DNA vaccines need high abundance in order to produce sufficient antigens to trigger an immune response whereas, with our platform, only a few live attenuated viruses need to be produced, as they will rapidly self-amplify. We are currently investigating the best delivery mechanisms.

KD: I can say that we are quite confident that we don't need the complex, expensive delivery devices or formulations that have proved a major limiting factor for DNA vaccines.

Q What are the main targets for commercializing PLLAV?

KD: The YF17D vaccine is considered one of the most efficacious current vaccines because it can trigger strong and varied immune responses, which may last for almost a lifetime. Replicating these responses but with an easier production process and no need for a cold chain could solve important real-world problems. However, PLLAV is also a platform technology, and we are not restricted to vaccines for yellow fever. We can use YF17D as a viral vector and add a range of antigens. These antigens benefit from the immunological environment generated by YF17D. For instance, the current rabies vaccines are complex to produce, suffer from supply chain issues, and while excellent in provoking an antibody response, they fail to induce long-lasting cellular immunity. By adding the current rabies vaccines antigen into the backbone of YF17D in our plasmid, we can induce a richer immune response, which we think will be longer-lasting. This vaccine would protect against both rabies and yellow fever, which would be valuable for travelers or residents in areas where both viruses are endemic.

> "The plasmid is 'plug and play,' so we can easily add and replace antigens. There is a limitation to the size that can be integrated, but this platform could certainly be valuable in a pandemic preparedness setting..."
>
> **– Hanne Callewaert**

HC: We are focusing on both prophylactic and therapeutic targets. On the prophylactic side, we naturally chose yellow fever as the lead indication as YF17D is the basis of the platform, and the second indication is yellow fever plus rabies, as Kai has described. For both diseases, there is a vaccine available, which means that there is a correlate of protection established for those antigens, which will help us validate our findings in the clinic.

On the therapeutic side, we are moving forward with a therapeutic hepatitis B vaccine. There is no functional cure for chronic hepatitis B, which is a difficult chronic infection to tackle; there are more than 250 million chronic carriers globally and more than a million deaths from the resulting liver cirrhosis or carcinomas each year. The immune response profile that we can generate with our vaccine is similar to that in the very few patients who see a spontaneous cure from chronic Hepatitis B. There are a lot of new prophylactic and therapeutic indications that can be targeted with this technology.

Q Could the platform be used for pandemic response vaccines?

HC: Absolutely, yes. The plasmid is 'plug and play,' so we can easily add and replace antigens. There is a limitation to the size that can be integrated, but this platform could certainly be valuable in a pandemic preparedness setting, with the advantages of being thermostable and relatively easy to produce.

DOI: 10.18609/vac.2023.003

KD: When the COVID pandemic started, we stopped all other programs in the lab and started work on a COVID program; within a few weeks we had several candidates we could test in animals for efficacy and, while others were faster to the market on this occasion, we feel this showcases the potential.

AFFILIATIONS

Kai Dallmeier, PhD
Associate Professor,
KU Leuven

Hanne Callewaert
CEO,
Astrivax

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Dallmeier K discloses he has grants/contracts from FWO/FRNS Excellence of Science (No. 40007527), Internal Funds KU Leuven (IDN/20/011), Internal Funds KU Leuven (C24M/19/006), Internal Funds KU Leuven (C3/19/057), Bill and Melinda Gates Foundation (OPP1195179) and EU H2020/ Transvac2 (TNA1710-01). He receives consulting fees from AstriVax (contract pending). He has patents issued and is listed as inventor on several patents related to PLLAV technology and use of YF17D as vaccine vector. Lastly, he has stocks/stock options in AstriVax BV. Callewaert H discloses that she has stock options in AstriVax.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an interview with Kai Dallmeier and Hanne Callewaert carried out on Jan 17 2023.

Interview held: Jan 17 2023; **Revised manuscript received:** Feb 7 2023; **Publication date:** Feb 20 2023

VACCINEINSIGHTS

PRECLINICAL RESEARCH &
NEXT-GENERATION VACCINE PLATFORMS

SPOTLIGHT

Exploring original antigenic sin as the basis for *S. aureus* vaccine failures

George Y Liu

"The implication of the orginal antigenic sin concept for vaccine development is the need for a fundamental shift in working platform and a more complex way of rethinking how successful vaccines can be developed."

VIEWPOINT

Vaccine Insights 2023; 2(1), 1–4

DOI: 10.18609/vac/2023.001

Original antigenic sin (OAS) is a term coined by Thomas Francis in 1960 to describe the recall of memory response to a primary influenza infection by subsequent influenza infections or vaccines [1]. The concept is used to explain why host responses to influenza infections or vaccines are suboptimal following antigenic drift associated with seasonal

influenza. Since first hypothesized, OAS has successfully explained human adaptive responses to other pathogens, mostly RNA viruses. For example, OAS describes how the recall of human response to one dengue virus serotype leads to cross-reactivity to a second serotype that inefficiently protects against the second virus or makes the infection worse [2]. OAS has been reported with Respiratory Syncytial Virus (RSV), SARS-1 and SARS-CoV-2, Human Immunodeficiency Virus (HIV), and pertussis.

S. aureus is a major pathogen that has been described by the Centers for Disease Control as a 'threat' because of the pathogen's impact on infectious disease burden and antibiotic resistance propagation [3]. Over the past decades, approximately 30 clinical vaccine trials have been conducted to control the spread of *S. aureus*, but none have been successful as defined by their endpoints [4,5]. The underlying reasons for the failures have been abundantly debated, but there has been no clear consensus. It has been noted by several investigators that up to 50% of human infants are colonized or infected with *S. aureus* in the first few months of life [6]. Yet despite abundant production of serum anti-*S. aureus* antibodies, humans are not robustly protected from *S. aureus* reinfections [7]. This observation could be consistent with OAS, wherein the recall of non-protective memory responses drives suboptimal staphylococcal vaccine response [8]. By comparison, the same vaccines are likely successful in pre-clinical settings because they are evaluated in mice naïve to human *S. aureus*. Simulating the failed vaccine trial targeting staphylococcal iron-regulated surface determinant protein B (IsdB) [9], we directly tested the OAS hypothesis and showed that naïve mice mount a highly protective antibody response to IsdB vaccination, but mice previously infected with *S. aureus* are not protected by the same IsdB vaccine [10]. Instead of priming for a protective response, pre-infected mice preferentially recalled the non-protective anti-IsdB memory humoral responses. We showed that the IsdB findings applied to at least two other cell wall-anchored vaccine antigens, Fhud2 and MntC. OAS, therefore, provides a new framework to reassess why *S. aureus* vaccines have failed.

Although OAS can help recalibrate the way we think globally about *S. aureus* vaccine failures, the concept provides only a partial understanding of the mechanisms underpinning the ineffectiveness of the vaccines or the strategies that could overcome vaccine interference. Unlike most other pathogens that elicit OAS recall responses, *S. aureus* has coexisted with humans and co-evolved various strategies to suppress adaptive immune responses that promote survival of the pathogen. The *S. aureus*-related vaccine suppression mechanism also does not require a change in antigen sequence between primary and subsequent exposures. These key differences have several important mechanistic ramifications.

First, in the presence of non-protective specific antibodies, vaccines need to generate protective antibodies that outcompete these non-protective antibodies to achieve efficacy. Antibody competition thus represents an independent and complementary mechanism of vaccine suppression to OAS. For IsdB, we have shown that protective vaccine-generated antibodies are ineffective when recipient mice are pre-infused with either natural mouse or human anti-IsdB antibodies that develop from prior infection or colonization [10]. This unexpectedly robust mechanism could explain the failure of anti-*S. aureus* monoclonal antibodies in clinical trials [4], which OAS could not.

Second, anti-IsdB antibodies generated after infection are non-neutralizing at the Fab domain (targets a non-neutralizing domain of IsdB domain) and non-opsonic at the Fc domain because of altered α2,3 sialylation [10]. How *S. aureus* induces these changes in antibody structures associated with non-protection, in the absence of a shift in antigen sequence, remains unexplained. Understanding these mechanisms likely holds the key to unraveling why all *S. aureus* vaccines have failed. For instance, if we determine that the Fc glycosylation feature is common to

DOI: 10.18609/vac/2023.001

non-protective antibodies, a common host mechanism that induces increased Fc sialylation could be a target for modulation of vaccine efficacy using adjuvants.

For investigators engaged in preclinical *S. aureus* vaccine development, transitioning from the routine use of a naïve animal model to a model that captures the human 'experience' would be a significant but needed departure to achieve vaccine success, given that the naïve mouse model has no predictive value for vaccine efficiency in human trials to date. The greater debate is what would make for an optimal platform that mimics the chronic state of human colonization/infection with *S. aureus*. We have shown that robust humoral imprints of *S. aureus* infection develop after 2–3 intraperitoneal infections, a single intravenous infection followed by antibiotic treatment (to clear the pathogen prior to vaccination), or two subcutaneous infections [10]. Nasal or gastrointestinal colonization with a human *S. aureus* strain has been insufficient to induce a robust antibody response, likely because of the suboptimal staphylococcal colonization model. Importantly, transfer of B cells alone from *S. aureus*-infected mice was sufficient to suppress IsdB vaccine efficacy in the recipient mice, corroborating the importance of OAS [10]. A complementary platform we utilized was the adoptive transfer of human anti-*S. aureus* antibodies into naïve mice followed by *S. aureus* challenge [11]. The model relies on the established finding that human and mouse IgG subclasses have similar affinity of binding to mouse Fc receptors [12], permits the evaluation of purified human anti-*S. aureus* antibodies in vivo, and could be exploited to assess the efficacy of vaccine-generated antibodies from phase I trials prior to advancing to Phase 2 or 3 testing.

In summary, we argue that framing *S. aureus* vaccine failures in the context of OAS is an important first step in understanding why staphylococcal vaccines have failed. But to fully appreciate how OAS impacts *S. aureus* vaccines, there needs to be a deeper understanding of the host–pathogen–vaccine interaction, to define what makes *S. aureus* imprints non-protective in the first place, as such insight is likely to unravel a unifying mechanism that explains why so many *S. aureus* vaccines have failed. Only with this deeper understanding could we fully harness the strategies that overcome vaccine interference. The implication of the OAS concept for vaccine development is the need for a fundamental shift in working platform and a more complex way of rethinking how successful vaccines can be developed. We anticipate that lessons learned from *S. aureus* investigations will be applicable to other pathobionts or pathogens that have also resisted traditional vaccine development.

BIOGRAPHY

GEORGE LIU is Professor and Chief of Pediatric Infectious Diseases at the University of California San Diego. He received his PhD from the University of Cambridge, UK, and his MD from the University of California, San Diego, USA. His areas of research interest include host – *S. aureus* interaction and staphylococcal vaccine failures.

REFERENCES

1. Francis T. On the doctrine of original antigenic sin. *Proc. Am. Philos. Soc.* 1960, 572–578.
2. Zompi S, Harris E. Original antigenic sin in dengue revisited. *Proc. Natl. Acad. Sci. USA* 2013; 110, 8761–8762.
3. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA. *CDC* (2019).
4. Armentrout EI, Liu GY, Martins GA. T Cell Immunity and the Quest for Protective Vaccines against Staphylococcus aureus Infection. *Microorganisms* 2020; 8(12), 1936.
5. Miller LS, Fowler VG, Shukla SK, Rose WE, Proctor RA. Development of a vaccine against Staphylococcus aureus invasive infections: Evidence based on human immunity, genetics and bacterial

evasion mechanisms. *FEMS Microbiol. Rev.* 2020; 44, 123–153.

6. Lebon A, Labout JA, Verbrugh HA *et al.* Dynamics and determinants of Staphylococcus aureus carriage in infancy: the Generation R Study. *J. Clin. Microbiol.* 2008; 46, 3517–3521.

7. Fowler VG, Jr., Proctor RA. Where does a Staphylococcus aureus vaccine stand? *Clin. Microbiol. Infect.* 2014; 20 Suppl. 5, 66–75.

8. Tsai CM, Hajam IA, Caldera JR, Liu GY. Integrating complex host-pathogen immune environments into *S. aureus* vaccine studies. *Cell Chem. Biol.* 2022; 29, 730–740.

9. Fowler VG, Allen KB, Moreira ED *et al.* Effect of an investigational vaccine for preventing Staphylococcus aureus infections after cardiothoracic surgery: a randomized trial. *JAMA* 2013; 309, 1368–1378.

10. Tsai CM, Caldera JR, Hajam IA *et al.* Non-protective immune imprint underlies failure of Staphylococcus aureus IsdB vaccine. *Cell Host Microbe* 2022; 30, 1163–1172 e6.

11. Tsai CM, Soper N, Bennett M *et al.* Adoptive Transfer of Serum Samples From Children With Invasive Staphylococcal Infection and Protection Against Staphylococcus aureus Sepsis. *J. Infect. Dis.* 2021; 223, 1222–1231.

12. Dekkers G, Bentlage AEH, Stegmann TC *et al.* Affinity of human IgG subclasses to mouse Fc gamma receptors. MAbs 2017; 9, 767–773.

AFFILIATION

George Y Liu, MD & PhD
Department of Pediatrics,
University of California,
San Diego, La Jolla, CA, USA
and
Division of Infectious Diseases,
Rady Children's Hospital,
San Diego, CA, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Liu GY received a grant from Pfizer to study *S. aureus* vaccine.

Funding declaration: This study received funding from the National Institute of Health grant R01AI127406 (GYL), R01AI144694 (GYL).

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Jan 11 2023; **Publication date: Jan** 17 2023

DOI: 10.18609/vac/2023.001

INTERVIEW

Next-generation tuberculosis vaccine discovery

Charlotte Barker, Editor, *Vaccine Insights*, speaks to
Gillian Beamer, Adjunct Associate Professor and Staff Scientist III, Texas Biomedical Research Institute (Texas Biomed) &
Emily Voigt, Principal Scientist at Access to Advanced Health Institute (AAHI)

GILLIAN BEAMER, VMD, PhD, DACVP, is a veterinary pathologist and research scientist studying tuberculosis. She is an Adjunct Associate Professor and Independent Staff Scientist at Texas Biomed. Dr Beamer has about 20 years experience in veterinary medicine, pathology and scientific research. She is a Board Certified Diplomate in the American College of Veterinary Pathologists, having completed a residency in veterinary anatomic pathology at The Ohio State University. She earned her VMD from University of Pennsylvania in 2000 and her PhD from The Ohio State University in 2009. She joined Texas Biomed in 2022.

EMILY VOIGT, is a scientist with 13 years of experience in innate and vaccine immunology. She received her BSc in Chemical Engineering from Kansas State University and her PhD in Chemical and Biological Engineering from University of Wisconsin-Madison. She completed her postdoctoral fellowship at the Mayo Clinic's Vaccine Research Group where she gained experience in conducting, analyzing, and publishing clinical studies of human and mouse immune responses to a variety of vaccines. Dr Voigt joined AAHI in 2018 where her extensive experience in RNA vaccine design and synthesis, viral immunology, and vaccine immunogenicity drives AAHI's RNA platform innovation to enable RNA vaccines and immunotherapies to be equitably accessible to all areas of the world, including resource-limited areas.

Vaccine Insights 2023; 2(1), 21–25

DOI: 10.18609/vac.2023.005

The US National Institute of Allergy and Infectious Diseases recently awarded Texas Biomedical Research Institute (Texas Biomed) and the Access to Advanced Health Institute (AAHI), a joint US $ 3.5 million, 5 year Innovation for Tuberculosis Vaccine Discovery grant. We caught up with two of the scientists involved in the project.

Q Could you introduce yourselves?

GB: I am a veterinary pathologist and scientist by training and hold an adjunct faculty position at Texas Biomedical Research Institute. Our research group is interested in understanding how different individuals respond to infection with *M. tuberculosis*.

EV: I am a Principal Scientist at the Access to Advanced Health Institute (AAHI), where I lead the RNA vaccine team. Our team identifies and aims to solve issues such as thermostability and manufacturability of RNA vaccines, which could cause barriers in distributing vaccines worldwide.

Q Why do we need better vaccines for TB?

GB: Currently, there is one widely used vaccine, Bacille Calmette–Guérin (BCG), which has been used for over 100 years. It is effective for preventing disseminated tuberculosis (TB) in young children and infants, but less effective in preventing pulmonary TB in adults – the most common and contagious form of the disease. Our goal is to create a vaccine to protect adults at risk of pulmonary TB and thus reduce the transmission of bacteria. One case of TB can give rise to more infections – and BCG has helped to reduce transmission but it hasn't eliminated it, so new tools and treatments are needed.

Q Can you explain how AAHI and Texas Biomed will collaborate in the research funded by the Innovation for TB Vaccine Discovery grant?

GB: This is a two-phase project, with vaccine discovery and optimization carried out by research teams at AAHI, and then research teams at Texas Biomed perform pre-clinical efficacy testing. AAHI's goal is to find the best candidates, combinations, and routes of delivery to induce a strong immune response. At Texas Biomed, we will give the candidate vaccines to populations of mice, expose them to *M. tuberculosis*, and test whether the vaccine restricts the growth of bacteria in the lungs, reduces lung-damaging inflammation, and prevents or delays the onset of disease.

"Our goal is to create a vaccine to protect adults at risk of pulmonary TB and thus reduce the transmission of bacteria. One case of TB can give rise to more infections – and BCG has helped to reduce transmission but it hasn't eliminated it, so new tools and treatments are needed."

– **Gillian Beamer**

EV: AAHI has a whole portfolio of next-generation adjuvants that can be paired with TB antigens, and we want to investigate new combinations of adjuvants. We also have a self-amplifying RNA platform that can stimulate robust antibody and T cell responses. These complex technologies are what AAHI brings to the table, and we often look for partner experts in individual diseases to partner with for optimization and efficacy testing of our vaccine candidates.

Q How is AAHI identifying the strongest candidates?

EV: New and improved TB vaccines are in the clinic and showing good results, but we must never stop asking 'how can this be improved?', especially for something as complex as TB. We have a suite of different adjuvant candidates to be tested, as overseen by my colleague Dr Christopher Fox. For example, mucosal-associated invariant T (MAIT) cells have been identified as potentially important targets in protection against COVID. Since TB is also a pulmonary disease, we hypothesize that a new MAIT cell ligand adjuvant may enhance immune response to TB vaccines.

An adjuvant formulation was developed at AAHI with collaboration from the company 3M. TLR 7/8 agonist 3M-052 has shown great promise in COVID-19 and HIV vaccines, and we want to see if it can be harnessed for a TB vaccine. We will similarly be testing a novel nanoalum adjuvant, also developed at AAHI. By changing the structure of alum to a nanoparticle, you not only change its physical characteristics but how it stimulates immune responses. Overall, we will be testing a whole host of such different adjuvant formulation approaches with TB antigens to see which works the best, including adjuvant combinations that trigger immune responses from multiple different directions, which often has the effect of strengthening and diversifying the immune responses.

We are also planning to test combinations of protein and RNA vaccines, as well as combinations of mucosal and intramuscular delivery methods. We'll start with a round of testing to identify the lead adjuvants, followed by a round of RNA vaccine testing and investigation of mucosal immune responses. And finally, we'll move into a mix-and-match

approach; for example, an adjuvanted protein vaccine, with an intramuscular primer and an intranasal RNA boost.

> "Our work so far has focused on developing the next-generation adjuvants, ensuring we formulate them appropriately for the specific protein antigens involved, and producing RNA vaccines that express TB antigens."
>
> **– Emily Voigt**

Q How will the leading candidates be tested at Texas Biomed? Why use the Diversity Outbred mouse model?

GB: Initial vaccine testing at AAHI will use a recombinant inbred strain of mice from the Collaborative Cross collection, which may better model human responses than standard laboratory C57BL/6 inbred strain. At Texas Biomed, we will include Diversity Outbred mice because it is a population model of genetic diversity and the DO population has the same eight 'parent' strains used to make the Collaborative Cross collection.

The benefit of using genetically diverse mice is that it models the genetic diversity of humans, which subsequently captures diverse phenotypes, and we can model aspects of TB that do not occur or are very rare in standard inbred mouse strains. For example, when we infect the Diversity Outbred mouse population with *M. tuberculosis*, some of them develop granuloma necrosis and cavitation, which are also observed in vulnerable people with diverse genetic backgrounds. Our goal with AAHI's vaccine is to show efficacy by protecting the 25–30% of the Diversity Outbred population that we know is especially vulnerable to TB.

Q What are the next steps?

EV: Our work so far has focused on developing the next-generation adjuvants, ensuring we formulate them appropriately for the specific protein antigens involved, and producing RNA vaccines that express TB antigens. We have now initiated the first set of mouse studies that compare and contrast the immune response developed to each of the new vaccine formulations. Over the coming 6–12 months we will be running those studies and gathering the data that allows us to investigate how these new adjuvants and vaccines are working, and select lead vaccine candidates for the next phase, which is the mixing and matching approach followed by efficacy tests, first in collaborative cross inbred mice and then in the Diversity Outbred mouse model.

GB: The next step for Texas Biomed is simply patience while AAHI makes a great vaccine.

DOI: 10.18609/vac.2023.005

AFFILIATIONS

Gillian Beamer, VMD, PhD & DACVP
Adjunct Associate Professor and Staff Scientist III,
Texas Biomedical Research Institute (Texas Biomed)

Emily Voigt, PhD
Principal Scientist,
Access to Advanced Health Institute (AAHI)

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Voigt E is author on pending patent applications regarding intranasally delivered RNA vaccines and thermostable nanostructured lipid carrier-delivered self-amplifying RNA vaccines, technologies being utilized in this work. Beamer G discloses she receives salary from Aiforia Inc.

Funding declaration: The authors received financial support for the research, authorship and/or publication of this article from National Institutes of Health (award R61AI169026).

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an interview with Gillian Beamer and Emily Voigt carried out on Jan 26 2023.

Interview held: Jan 26 2023**; Revised manuscript received:** Feb 21 2023; **Publication date:** Feb 24 2023.