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VACCINE INSIGHTS

SPOTLIGHT

RNA vaccine R&D

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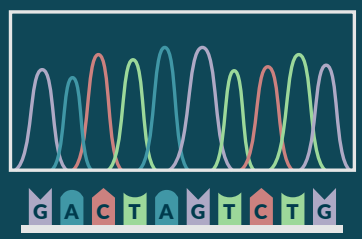
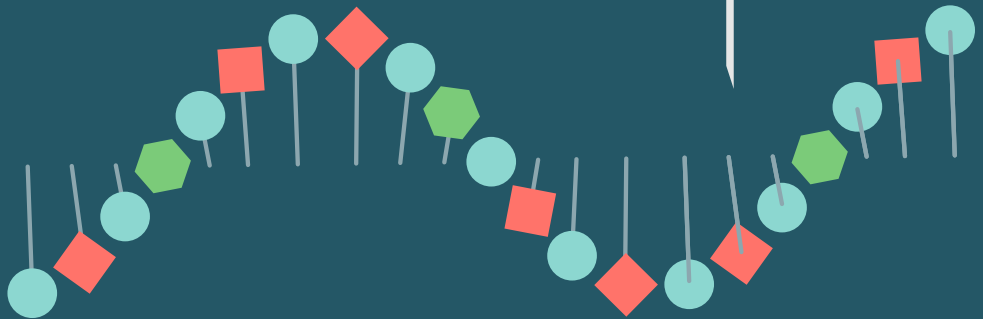
Amit Khandhar, HDT Bio





VACCINE INSIGHTS

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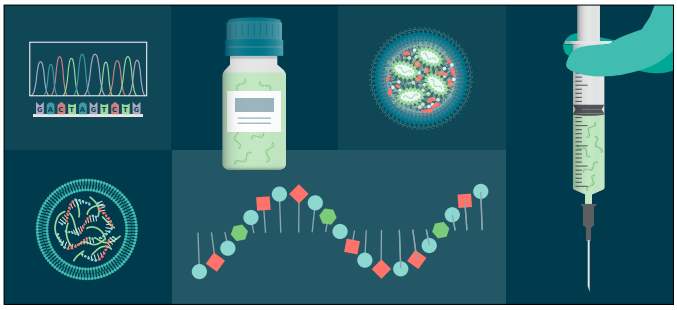

VACCINE INSIGHTS

RNA VACCINE R&D

SPOTLIGHT

Spotlight on RNA–LNP vaccine innovation

Amit Khandhar

FOREWORD

"These articles point to a future where precision, safety, and global equity define the next generation of mRNA vaccines."

Vaccine Insights 2025; 4(2), 75–77 · DOI: 10.18609/vac.2025.011

The global health emergency of the 2019 SARS-CoV2 pandemic spurred significant research and innovation in RNA-based vaccines and therapeutics. With billions receiving mRNA vaccines, there is a vast amount of late-stage clinical and real-world data supporting their safety and efficacy, while also underscoring limitations of first-generation mRNA vaccines.

This month's collection of articles and interviews covers a breadth of innovations in RNA and formulation technologies, ranging from the use of machine learning to discover next-generation precision lipid nanoparticles (LNPs) to use of kinetic models to predict vaccine shelf-life.

HDT Bio's Dr Taishi Kimura presents a viewpoint that underscores the pressing need to enhance RNA vaccine safety. Kimura's focus on localized delivery via a proprietary nanoemulsion that avoids systemic biodistribution and inflammation opens a promising path toward minimizing adverse events such as myocarditis. His insights draw from both clinical data and mechanistic studies, emphasizing the importance of spatially controlled innate immune activation.

Sanofi's mRNA process and its CMC team (Scaccia, *et al*) introduce data-driven advanced kinetic models (AKMs) capable of predicting vaccine shelf-life and stability under a wide range of storage conditions. These initial models are promising, enabling not only shelf-life prediction but also the establishment of internal release limit (IRL)—a key attribute providing assurance that a specific batch will remain stable during its shelf life.

Dr Bowen Li's interview shifts the lens toward the future of LNP development, where artificial intelligence (AI) serves as a catalyst for discovery. At the University of Toronto, Li's AGILE and LUMI-lab platforms exemplify a self-driving, data-rich approach to LNP design, identifying novel, structurally unique lipids optimized for organ-specific delivery.

Complementing this technological frontier is Duccio Medini's strategic vision for the RNA Readiness + Response (R3) program. Medini articulates a global framework that democratizes RNA manufacturing by decoupling product design from manufacturing technologies. Advancements in production capabilities in a product agnostic way promises to enable rapid response to 'disease X' or the next pandemic. The current 'R3 Global' program aims to deploy promising advancements from the first cycle to multiple RNA biofoundries that can be accessed by scientists globally through an RNA service broker.

Finally, the team at Replicate Bioscience (Spasova, *et al*) explore variants of alphavirus based self-replicating RNAs (srRNA) and their ability to affect humoral and cellular immunity. Their data, compared to previously published data, suggest that the optimal srRNA backbone may be antigen-dependent.

Together, these contributions capture a field undergoing rapid change. These articles point to a future where precision, safety, and global equity define the next generation of mRNA vaccines.

BIOGRAPHY

Amit Khandhar earned his PhD in Materials Science and Engineering from the University of Washington, Seattle, WA, USA and, for over a decade now, he has investigated how various nanoparticle technologies interface with biological systems. He is an inventor of HDT Bio's LION™ technology—a clinical-stage formulation used for the delivery of self-amplifying RNA vaccines. His current research interests lie in understanding how routes of vaccine administration affect the quality of immune responses, extending HDT Bio's AMPLIFY technology to diverse infectious disease and oncology targets, and designing formulations that enable nucleic acid modalities beyond saRNA.

Amit Khandhar PhD, Director of Formulations, HDT Bio, Seattle, WA, USA

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VACCINE INSIGHTS

RNA VACCINE R&D

SPOTLIGHT

Enhancing RNA vaccine safety through localized delivery strategies

Taishi Kimura

VIEWPOINT

"...next-generation RNA drug platforms are likely to be optimized for the development and use of RNA vaccines and therapeutics beyond just emergency or pandemic situations."

Vaccine Insights 2025; 4(2), 41–45 · DOI: 10.18609/vac.2025.006

RNA vaccines represent a transformative modality, offering a rapid response to emerging viral threats and exceptional flexibility in development [1,2]. During the unprecedented circumstances of the coronavirus disease-19 (COVID-19) pandemic, RNA vaccines protected humanity, highlighting their potential as a powerful platform for pandemic response against infectious diseases [1]. However, as the pandemic has subsided, it is an opportune time to explore potential improvements in the safety profile of this platform, enabling its broader and more sustainable use as a standard vaccine and therapeutic tool.

Commercial RNA vaccines have been reported to cause relatively frequent adverse events (AEs) [3,4]. Moreover, some of the reported AEs—such as myocarditis/pericarditis and anaphylaxis—are rare but serious reactions [5–7]. After identifying myocarditis and pericarditis risks linked to mRNA vaccination, the US FDA updated Emergency Use Authorization (EUA)

documents, and the CDC revised clinical guidance for healthcare providers [8]. Epidemiological studies have shown that the likelihood of these serious AEs progressing to severe outcomes is extremely low—estimated to be below 0.01%—compared to the risk of severe, critical, or fatal COVID-19 cases [6,7]. Thus, while ongoing monitoring remains important, current data do not indicate that these risks outweigh the benefits of vaccination [9,10]. However, there are also reports that many people who are hesitant about vaccination do so out of fear of side effects [11], and many of the objections raised by anti-vaccine groups focus on AEs. Thus, by recognizing and addressing the risks associated with specific vaccine platforms (particularly RNA vaccines), we may in the long term promote broader social acceptance of vaccination. Based on clinical and non-clinical data accumulated in recent years, methods to achieve this are gradually becoming clearer.

There are two major types of RNA employed in the RNA vaccines currently under clinical investigation and approved uses. Moderna (Spikevax®) and BioNTech/Pfizer (Comirnaty®) use nucleoside-modified conventional RNA, while the RNA used by GENNOVA Biopharmaceuticals (GENNOVA Bio), Arcturus Therapeutics, Imperial College London, VLP Therapeutics, and Replicate Bioscience is known as 'self-amplifying replicon mRNA' (repRNA, also known as saRNA, srRNA, and SAM). The former consists of the minimal components required to express the protein, whereas the latter type additionally encodes a viral replicase to allow the RNA to self-amplify within the cell, thus achieving high potency with a lower dose [12]. Because the reactogenicity of RNA vaccines usually depends on the dose, this dose-sparing effect is theoretically one means of improving safety. Contrary to initial expectations, however, early clinical trials revealed a relatively high frequency of systemic AEs for repRNA vaccines from companies other than GENNOVA Bio. Some of these adverse events were classified as Grade 3 [13,14]. Meanwhile, GENNOVA Bio's vaccine (GEMCOVAC-19, a licensed product under the license agreement between HDT Bio Corp. and GENNOVA Bio) showed extremely high tolerability [15], indicating the need to understand the molecular basis for these differences in systemic reactogenicity.

A delivery formulation is necessary for *in vivo* RNA delivery, and most companies use lipid nanoparticles (LNPs). GENNOVA Bio, however, uses a proprietary cationic nanoemulsion where, instead of being encapsulated, the RNA is complexed through ionic forces to the particles comprising the emulsion. Experiments in mice, conducted by HDT Bio, revealed that repRNA encapsulated in LNP circulates throughout the body and largely accumulates in the liver, whereas repRNA delivered via this proprietary nanoemulsion remains localized at the muscle injection site [16]. Furthermore, the subsequent innate immune responses mirrored this *in vivo* distribution pattern: when repRNA was delivered using the proprietary cationic nanoemulsion, no systemic inflammatory response was induced as determined by serum cytokine levels, and instead, a strong innate response was elicited solely at the injection site and draining lymph nodes. In addition, in the group that received repRNA via LNP, decreases in body weight and increases in serum cardiac troponin levels—a marker of cardiac damage—were observed, while these undesirable responses were significantly less frequent in the group receiving repRNA with the proprietary nanoemulsion. These results are consistent with the extremely high tolerability reported in GENNOVA Bio's clinical trials and demonstrate in a non-clinical model that maintaining the localized biodistribution of RNA is one means of enhancing safety.

The transient elevation of serum cardiac troponin levels we observed in the mouse model suggests unintended cardiac stress when RNA is delivered via LNP. Notably, in a cohort of patients who developed myocarditis after mRNA vaccination, there was an increase in cytokine levels coinciding with the rise in serum cardiac troponin levels, suggesting a possible relationship between a systemic innate inflammatory state and cardiac disease [17]. Another research group has reported the detection of the interleukin 1 receptor antagonist (IL1RA) autoantibodies in some patients who developed myocarditis following RNA vaccination [18]. Given the crucial role of IL1RA in controlling systemic reactogenicity in certain RNA vaccines, as demonstrated in non-clinical studies, further research is needed to elucidate the relationship between systemic inflammatory responses and cardiac pathology. This should include genetic profiling to identify inflammatory signatures and pinpoint factors contributing to cardiac pathology. The clinical and non-clinical data from GENNOVA Bio and HDT Bio (developer and patent holder for a proprietary cationic nanoemulsion called LION™) underscore the high level of safety of localized RNA vaccines delivered by LION from the standpoint of less systemic inflammation. Many developers are currently seeking to improve the safety of the RNA vaccine platform by modifying the lipid composition of LNPs to achieve localized or tissue-targeted delivery [19,20].

Recently, in addition to localizing RNA, new modified nucleosides applicable to repRNA have been discovered [21], and a clinical trial reported their involvement in improved tolerability [22]. Analyses in non-clinical models indicate that controlling systemic cytokine responses is a key mechanism for enhancing safety in these nucleoside-modified repRNA vaccines [21,23]. Thus, controlling systemic inflammatory cytokines is important. Furthermore, beyond using a proprietary cationic nanoemulsion to localize delivery of the RNA, GENNOVA Bio has achieved further enhanced safety and immunogenicity with its COVID-19 vaccines through needle-free injection [15]. Since needle injection mechanically injures cells in the injection sites, which triggers inflammation, needle-free injection is likely less inflammatory. Further studies in the injection site and draining lymph nodes of the vaccinees receiving a needle-free injection of repRNA complexed to a cationic nanoemulsion are warranted to ask this question. Through these ongoing efforts for further safety improvements, next-generation RNA drug platforms are likely to be optimized for the development and use of RNA vaccines and therapeutics beyond just emergency or pandemic situations.

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BIOGRAPHY

Taishi Kimura is a scientist specializing in innate immunity and RNA immunology. His research focuses on the interplay between transfected cells and immune cells, particularly in the context of RNA vaccines. Kimura earned his PhD in Medical Science from Osaka University, Osaka, Japan, where he studied type I interferon-mediated innate immunity to RNA viruses. He then received postdoctoral training at Scripps Research Institute, expanding his research into RNA virus pathogenesis. At HDT Bio, Kimura has played a key role in uncovering the mechanism of action behind HDT's RNA vaccine technology. His recent publication was selected for the Best of Molecular Therapy 2023. Currently serving as a Senior Scientist at HDT Bio, he focuses on elucidating the further molecular details underlying the immunogenicity and reactogenicity of repRNA vaccines using knockout mice, diverse animal models, engineered repRNA, and novel formulations. His research has been supported by an NIH/NIAID R01 grant (1R01AI180195) and the 2025 Career Development Award from the American Society of Gene and Cell Therapy. Through this work, Kimura aims to develop innovative strategies to enhance both the safety and efficacy of repRNA vaccines, contributing to the fight against emerging viral threats and cancer.

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AUTHORSHIP & CONFLICT OF INTEREST

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VACCINE INSIGHTS

RNA VACCINE R&D

SPOTLIGHT

SHORT COMMUNICATION

Novel srRNA vectors for customizable vaccine development

Darina S Spasova*, Shigeki J Miyake-Stoner*, Gaelle Picarda, Annie C Chou, Emily D Brand, Melanie D Olesiuk, Christine C Domingo, Hunter J Little, Thomas T Goodman, Jacqueline L Posy, Jasmin Gonzalez, Terrina L Bayone, Jessica Sparks, Christian J Maine, Nathaniel S Wang, Andrew J Geall, and Parinaz Aliahmad

Recent approvals of self-replicating RNA (srRNA) vaccines have shown the advantages of this platform technology, including generation of durable and comprehensive immune responses at clinical doses approximately 10-fold lower than conventional mRNA. This is in part due to the viral origin of synthetic srRNA vectors which include a replicase that amplifies the co-encoded transgene (i.e. vaccine antigen), allowing for dose-sparing, and adjuvantation of immune responses for vaccines. Interestingly, two approved srRNA vaccines for SARS-CoV-2, as well as many others in late-stage development, share the same base vector derived from the alphavirus Venezuelan Equine Encephalitis virus (VEEV). As with traditional viral vectors, the context in which the host sees an antigen can shape downstream immune responses. Thus, we evaluated if a panel of VEEV and non-VEEV alphaviral vectors result in differential humoral and cell-mediated immunity. Here, we show that although VEEV is advantaged for generation of antibody responses to a viral glycoprotein, a novel, non-VEEV-based srRNA vector can be advantaged for the generation of T cell responses to the same antigen. Thus, similar to traditional viral vectors, we propose that srRNA vectors can also bias downstream immune responses and allow for customizable vaccine design.

Vaccine Insights 2025; 4(2), 55–62 · DOI: 10.18609/vac.2025.008

INTRODUCTION

Manufacturing of traditional vaccines is often costly and cumbersome, requiring large bioreactors or use of cell lines that may pose safety concerns. As seen with the recent SARS-CoV-2 pandemic, synthetic technology, such as RNA, can overcome these challenges with rapid and cost-effective production [1–4]. Within RNA vaccines, two approved platforms exist: messenger RNA (mRNA) and self-replicating RNA (srRNA, also called self-amplifying RNA or replicon). srRNA has the same

advantages as mRNA, namely *in situ* production of vaccine antigens and simplified manufacturing, while having the added advantage of its ability to be dosed orders of magnitude lower. The mechanism relies on copy machinery derived from the parental positive strand RNA virus (typically an alphavirus) that is co-encoded along with the transgene (antigen) of interest [5]. This copy machinery, or viral replicase, can amplify the administered synthetic RNA within host cells, resulting in robust levels of protein expression with low doses.

To date, two srRNA vaccines have obtained regulatory approval for SARS-CoV-2, Kostaive in Japan and EU, and GEMCOVAC in India [6–9]. However historically, srRNA technology has been validated for a multitude of infectious disease and oncology targets both preclinically and clinically [10,11]. Of note, almost all examples of srRNA vaccines, including approved assets, use the same alphaviral vector species derived from Venezuelan equine encephalitis virus (VEEV). Although synthetic srRNA vectors are devoid of genes that encode viral structural proteins, the remaining viral copy machinery, encoded by non-structural proteins (nsPs), is retained in synthetic vectors. In addition to their role in the viral life cycle, nsPs are able to play additional roles, such as interfering with host cell pathways, many of which can affect downstream responses to vaccines, such as protein processing and presentation, innate immune activation, and cellular protein expression [10].

Due to the interplay of viral nsPs and host cellular pathways, we evaluated the ability of different srRNA vaccines, derived from VEEV and non-VEEV alphaviruses, to be functionalized and utilized as vaccines vectors.

RESULTS

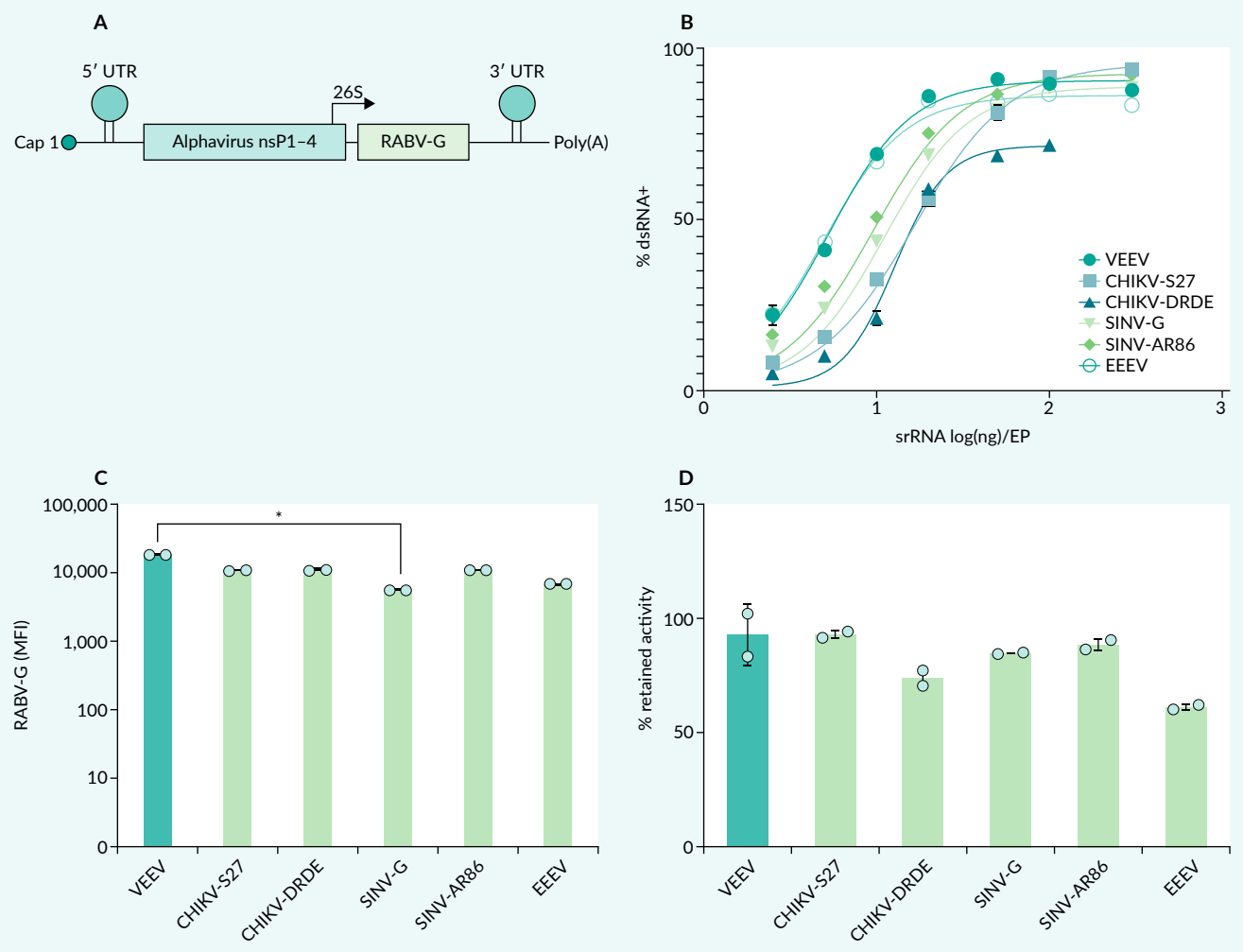
srRNA vectors from non-VEEV alphaviral backbones were selected based on evolutionary diversity and functionally disparate pairs and included CHIKV strain S-27 (CHIKV-S27), CHIKV strain DRDE-06 (CHIKV-DRDE), SINV strain Girdwood (SINV-G), SINV strain AR86 (SINV-AR86) and EEEV strain FL93-939 (EEEV). All vectors were functionalized to replicate in the absence of viral structural proteins and include non-coding region optimizations (Figure 1A) [12, 13]. To assess the utility of novel alphaviral backbones as vaccine vectors, rabies virus glycoprotein (RABV-G) was selected as a model antigen and encoded in each backbone downstream of the subgenomic 26S promoter. Importantly, RABV-G is a validated antigen in the VEEV vector, and has a WHO-defined immune metric that is associated with protective efficacy, specifically a serum titer of 0.5 international units/mL or greater of rabies virus neutralizing antibodies (RVNA) [14].

To assess whether these vectors were able to efficiently replicate in host cells and produce proteins, srRNAs were transfected into BHK-21 cells and the presence of dsRNA, an intermediate of srRNA replication, and expression of RABV-G were measured by flow cytometry. All srRNA vectors were able to successfully replicate in cells, with modest differences to the VEEV-based vector (Figure 1B). Moreover, RABV-G expression was detected in transfected cells, similarly for all alphaviral backbones with the exception of SINV-G, where protein expression was reduced (Figure 1C). The srRNAs were formulated into lipid nanoparticles (LNPs), and retained bioactivity was observed for all the vectors (Figure 1D).

Having shown that novel alphaviral backbones are functional *in vitro*, we evaluated their ability to be efficient vaccine vectors in an *in vivo* immunogenicity study. Mice were administered LNP-formulated srRNA vaccines in a prime-boost strategy and immune responses, including RVNA titers and antigen-specific T cells, were measured. A commercial rabies vaccine, RabAvert®, was included as a

FIGURE 1

Functionalization of novel srRNA vectors.



Alphaviral vectors from indicated species, strains, and subtypes were functionalized and vectorized to encode RABV-G. (A) A general schematic of an alphavirus srRNA vector. *In vitro* activity measured by detection of dsRNA as a replication intermediate at the indicated dose (B), protein expression (C), and retained activity following LNP formulation with 20 ng of srRNA (D) were determined in transfected BHK-21 cells by flow cytometry. Statistical significance was performed using Kruskal-Wallis analysis compared to VEEV, where *p<0.05.

positive comparator. RVNA titers above the WHO-defined indirect immune metric of protection were detected from all srRNA vaccines after a single dose or two doses in all animals (Figure 2A and C). Two srRNA vectors, VEEV and SINV-G elicited statistically superior RVNA responses compared to the inactivated viral vaccine RabAvert after a single administration (Figure 2A). A second administration (boost) of each vaccine led to uniformly equivalent RVNA responses from the majority of srRNA vectors tested as compared to RabAvert, with the exception of CHIKV-DRDE and EEEV vaccines, with RVNA responses from VEEV, CHIKV-S27 and SINV-G and SINV-AR86 demonstrating equivalency to RabAvert (Figure 2C).

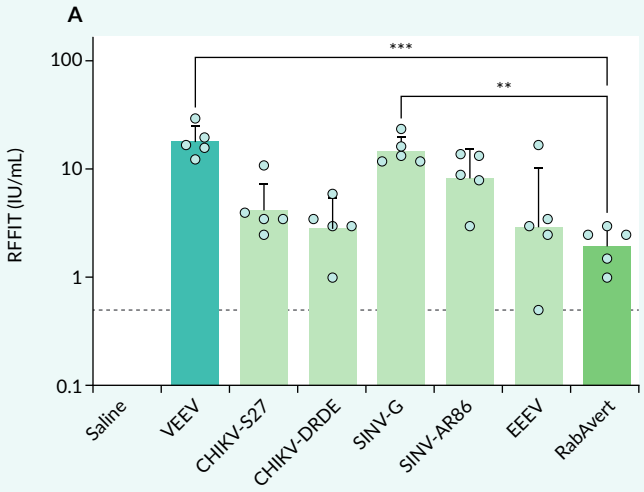
Although humoral immunity is the primary metric to evaluate vaccine efficacy for rabies, we also assessed whether novel srRNA backbones elicit cell-mediated immunity. RABV-G-specific T cell responses were detected after a single or two administrations of both srRNA and RabAvert vaccines (Figure 2B and D). Single

FIGURE 2

Novel srRNA vectors elicit immune responses *in vivo*.

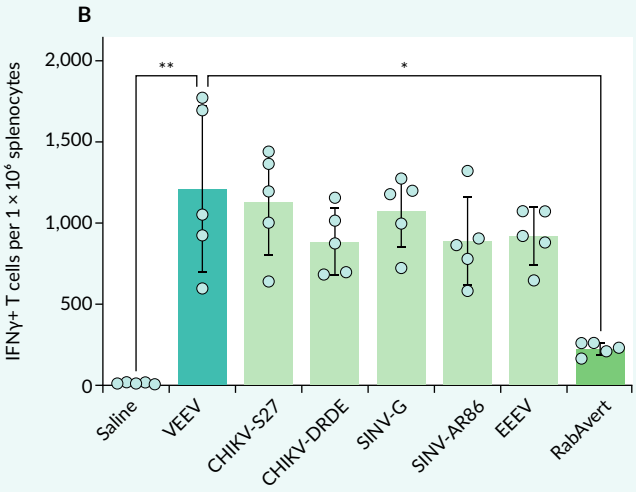
Rabies virus neutralizing antibodies

Post prime



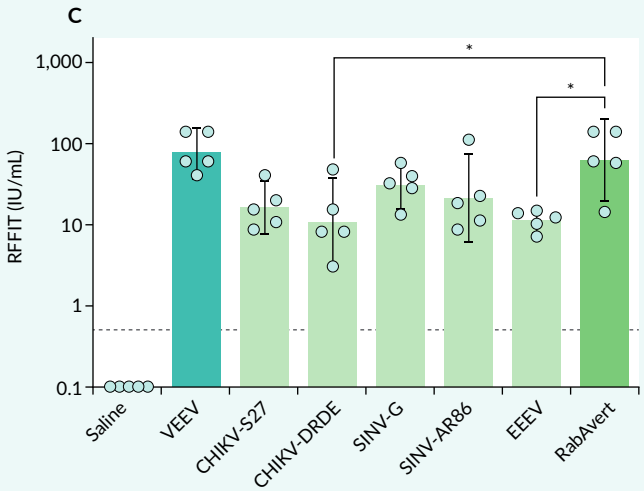
Dunn's multiple comparisons test	Mean rank difference	Summary	Adjusted P value
VEEV vs CHIKV-S27	14.8	*	0.0385
VEEV vs CHIKV-DRDE	17.8	**	0.0067
VEEV vs SINV-G	3.2	ns	>0.9999
VEEV vs SINV-AR86	9.3	ns	0.4699
VEEV vs EEV	16.1	*	0.0187

RABV-G T cells

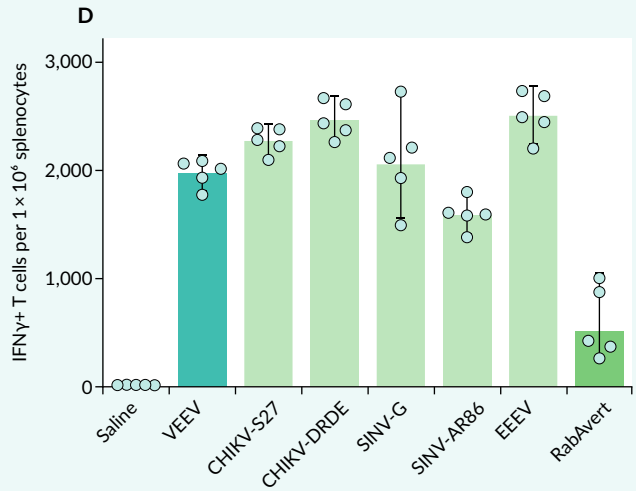


Dunn's multiple comparisons test	Mean rank difference	Summary	Adjusted P value
VEEV vs CHIKV-S27	−0.8	ns	>0.9999
VEEV vs CHIKV-DRDE	6.8	ns	>0.9999
VEEV vs SINV-G	0.0	ns	>0.9999
VEEV vs SINV-AR86	7.4	ns	0.9191
VEEV vs EEV	5.2	ns	>0.9999

Post boost



Dunn's multiple comparisons test	Mean rank difference	Summary	Adjusted P value
VEEV vs CHIKV-S27	13.4	ns	0.0801
VEEV vs CHIKV-DRDE	17.6	**	0.0078
VEEV vs SINV-G	7.7	ns	0.8320
VEEV vs SINV-AR86	11.6	ns	0.1854
VEEV vs EEV	18.1	**	0.0057



Dunn's multiple comparisons test	Mean rank difference	Summary	Adjusted P value
VEEV vs CHIKV-S27	−8.8	ns	0.5699
VEEV vs CHIKV-DRDE	−13.2	ns	0.0888
VEEV vs SINV-G	−4.2	ns	>0.9999
VEEV vs SINV-AR86	5.6	ns	>0.9999
VEEV vs EEV	−14.8	*	0.0393

(See next page for figure legend)

Legend to Figure 2. Serum titers of rabies virus neutralizing antibodies were measured by RFFIT assay after a single (A) or two administrations (C) of srRNA vaccines and RabAvert. Dashed line refers to the established WHO indirect immune measure of protection of 0.5 IU/mL. Splenic RABV-G-specific T cells were measured after a single (B) or two administrations (D) of srRNA vaccines and RabAvert. Statistical significance between VEEV srRNA vaccine compared to non-VEEV srRNA vaccines and RabAvert was performed by a Kruskal-Wallis analysis, where *p<0.05, **p<0.005, ***p<0.0005 on plots and Dunn's multiple test comparisons test performed on VEEV and non-VEEV srRNA vaccines.

administration of srRNA vaccines led to significantly higher RABV-G T cell responses compared to RabAvert with VEEV, while similar among the srRNA vaccine subset (Figure 2B). Interestingly post-boost, T cell responses trended higher with srRNA vaccines compared to RabAvert, and within the srRNA vaccine subsets, EEEV outperformed VEEV for generation of rabies-specific T cell responses (Figure 2D). Remarkably, the subset of srRNA vaccines that were able to induce greater levels of humoral immunity did not fully coincide with vectors that were advantaged for cell-mediated immunity. These data show that that srRNA vector backbones can affect not just the magnitude of host cell immune responses to vaccine antigens, but can also skew these towards humoral or cell-mediated immunity.

TRANSLATION INSIGHT

RNA technology is advantaged for vaccine development, based on its simplified manufacturing, resulting in rapid deployment and lower cost of goods. Within this class, srRNA vaccines have shown superior durability of antibody responses, as well as better induction of T cell-mediated immunity, compared to conventional mRNA vaccines.

To date, the vector backbone in approved srRNA vaccines and late-stage development are derived from the alphavirus VEEV. However, as with traditional viral vectors, the viral backbone is able to affect several host cellular pathways that can in turn result in differential immune responses. We have shown here that within the alphavirus family, vectors can differ in the magnitude of the immune response that they generate, and importantly, how they skew the immune response towards humoral versus cellular immunity. Although the nature of the payload and formulation can also play a role, using an identical antigen, rabies glycoprotein, and LNP formulation, we have identified vectors such as EEEV that can drive more robust T cells when compared to VEEV.

The impact of these findings will result in more potent and fit-for-purpose vaccines, such as utilizing EEEV for applications where T cells are the primary effectors of the immune responses, such as for oncology, chronic viral infections, or targets where antibodies may lead to exacerbated infection via antibody-dependent enhancement of disease such as with flaviviruses.

Although this work is limited to a single antigen, the outcome clearly shows that the context in which the host cell sees an encoded antigen affects downstream immune responses. In parallel to our study, Casmil *et al.* demonstrated the differences in vaccine-elicited antibody and T cell responses to SARS-CoV-2 Spike in mice from a separate set of alphaviral srRNA vectors [15]. Interestingly, in this independent study and for an unrelated antigen, the VEEV-TC83 backbone showed relative superiority for generation of antibody responses but was not the optimal vaccine vector for inducing Spike-specific T cell responses after two doses. Thus, srRNA vectors should not be considered plug-and-play, and a broad library of vectors allows for optimized vaccine design.

METHODS

Preparation of srRNA vaccines

Self-replicating RNA (srRNA) was transcribed *in vitro* using linearized plasmid templates encoding the RABV-G transgene

under the control of alphavirus-based srRNA vectors [13]. The coding sequence for Flury-LEP-C RABV-G (Genbank: ACL98057.1) was codon optimized for human expression (IDT). srRNA drug substance was prepared using *in vitro* transcription (IVT) with the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (NEB, Cat#E2050S) and purified by LiCl precipitation. RNA was capped using Vaccinia Capping System (NEB, Cat# M2080S) and mRNA Cap 2′-O-Methyltransferase (NEB, Cat# M0366S) and followed by LiCl precipitation for purification. For *in vivo* experiments, all srRNA vaccines were formulated in identical LNPs as previously described [12,13].

In vitro activity and transgene expression

srRNA was transformed by electroporation into BHK-21 cells (4D-Nucleofector™, Lonza). At 15–22 h following transformation, the cells were fixed and permeabilized (eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set, Invitrogen) and stained using a PE-conjugated anti-dsRNA mouse monoclonal antibody (J2, Scicons) to quantify the frequency of dsRNA+ cells by fluorescence flow cytometry. Protein expression was determined using an AF647-conjugated anti-RABV-G antibody (1C5, Novus Biologicals). The mean fluorescence intensity of AF647 was used as the readout of relative RABV-G expression. Retained potency was measured as the frequency of dsRNA+ cells post-formulation compared to pre-formulation by extracting srRNA from LNPs (Nucleospin™ RNA, Macherey-Nagel™) and electroporating BHK-21 cells at the same dose.

In vivo immunogenicity

Female BALB/c mice were procured from Charles River Laboratories (CRL). Mice were age-matched (8–12 weeks old) at time study initiation. LNP-formulated srRNA vaccines were administered intramuscularly at a dose of 0.15 µg on days 1 and 21. RabAvert was administered at 1/10th of the human dose intramuscularly (the maximum feasible dose in mice). All procedures were conducted in compliance with all the laws, regulations, and guidelines of the National Institutes of Health (NIH) and with the approval of CRADL Animal Care and Use Committee. CRADL (formerly Explora Biolabs) is an AAALAC accredited facility.

Peptides and proteins

An overlapping peptide library for RABV-G (Flury-LEP-C) protein was custom made, consisting of 15-mer peptides with a 10-amino-acid overlap. Individual peptides were then pooled into a single cocktail for the peptide library stimulations.

ELISpot

Single-cell suspensions of homogenized spleens were stimulated for 16-18 h with indicated peptides. T cell responses were assessed using Mouse IFNg ELISpot PLUS kit (HRP) kit as per the manufacturer's instructions (Mabtech Cat# 33214HST-10). Developed ELISpot plates were sent to Cellular Technology Limited for spot counting and quality control. Data were plotted and statistical analysis was performed using GraphPad Prism.

Neutralization assays

Sera were used to perform rapid fluorescent foci inhibition tests (RFFIT) to quantify RVNA titers using a validated assay [14] by Kansas State Veterinary Diagnostic Laboratory.

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AUTHORSHIP & CONFLICT OF INTEREST

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VACCINE INSIGHTS

RNA VACCINE R&D

SPOTLIGHT

RESEARCH ARTICLE

Predicting stability of conventional and mRNA-based vaccines

Anthony Scaccia, Camille Saade, Pierre Ballesta, Michael Rieger, and Didier Clénet

In recent years, advanced kinetic models applying Arrhenius-based equations successfully predicted stability of biologicals and conventional vaccines during long-term storage under recommended conditions (2–8 °C) but also during unexpected excursions of temperatures during shipments (e.g., cold-chain breaks). Considering the emergence of new products based on mRNA lipid nanoparticle (LNP) technology, we developed kinetic models describing critical quality attributes of mRNA-LNPs, to predict their stability behavior during storage under isothermal and non-isothermal conditions. Then, realistic storage conditions were applied to predict the shelf life of the products when stored in frozen and liquid states. In addition to predicting the long-term stability of vaccines, the evaluation of temperature variations and their impact on shelf life was presented by means of real-time monitoring of temperature fluctuations during their storage and shipment. Finally, the application of kinetic models was proposed for accurate estimation of internal release limits of products.

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INTRODUCTION

Since Arrhenius's seminal work on the temperature dependence of reaction kinetics [1], the Arrhenius law has been widely used to study the stability properties of biopharmaceutical products over several months [2,3] and predict the behavior of an irreversible reaction at a given temperature using experimental data obtained at a higher temperature. To encompass a broader range of reactions, various changes were proposed to capture more complex time—temperature dependencies [4–9]. The S-shape model was summarized by Roduit [9] as a universal Arrhenius-based equation allowing for fitting of both accelerated and decelerated linear rates. The concept behind this model is that one can use critical quality attributes (e.g., antigenicity, infectious titer, purity, high molecular weight species) as an approximation of the reaction's progress. Provided that the change in the chosen attribute is uniform over time, and is measured at various

temperatures, an empirical advanced kinetics model can be obtained that fits experimental data. This model then allows for long-term prediction of the measured component, in various time–temperature scenarios.

In recent years, first-order kinetics with single-step reaction provided a consistent framework to describe the loss of critical attributes for bioproducts [10,11], whereas two-step models were required to accurately predict more complex attributes [9,12,13]. This ongoing advancement in predictive models using advanced kinetics modeling has generalized one-step and two-step models to determine product stability behavior [3,9]. The increasing maturity of advanced kinetic models (AKM) suggests that they could be part of the next version of the ICH and WHO recommendations for stability predictions of biologicals and vaccines [3,14].

In practice, kinetic models are developed from accelerated stability data obtained over brief time periods (i.e., weeks or months) under recommended storage conditions (e.g., 5 °C) and at higher incubation temperatures (e.g., 25 °C, 37 °C, or 40 °C). These kinetic models can predict the long-term stability of pharmaceuticals both during storage and shipment, including unexpected excursions of temperature (cold-chain breaks) [15]. The accuracy of such predictions is frequently estimated using bootstrapping of data [16]. Based on 'good modeling practices' [17], various kinetic models, from simple to more sophisticated, are screened to fit short-term stability data at multiple temperatures. Statistical scores, such as Akaike and Bayesian Information Criteria (AIC/BIC) [18] are used for ranking the models and selecting the most appropriate one. Alternatively, several good models can be considered by applying model averaging approaches [19].

At the end of this process, the resulting model accurately describes the temporal progress of the chosen indicating attribute.

With new modalities, such as mRNA-based products, LNPs are developed to protect mRNA against enzymatic degradation. However, interactions with and between LNP excipients introduce additional risks for mRNA degradation. Furthermore, the presence of lipid impurities that can facilitate mRNA adduct formation remains a challenge of stabilizing the encapsulated mRNA. Some studies have used simple kinetic models to describe and predict the evolution of stability-indicating attributes, demonstrating their Arrhenius behavior [20–23]. As these results highlight the temperature dependence of mRNA-based product degradation rates, we propose the application of kinetic models to predict and monitor the shelf life of mRNA-based products, much like the process for conventional vaccines. It is noteworthy that this type of product raises an additional challenge for stability modeling because it experiences storage conditions involving a wide temperature range. Using already published data for intact mRNA and adduct contents obtained in both frozen and liquid state conditions [24,25], our work proposes building kinetic models capable of predicting behavior under any time/temperature scenarios for stability-indicating attributes. These models demonstrated accuracy in forecasting mRNA-LNP stability across a wide range of conditions, offering a tool for quality control and logistics planning. Examples of applications for AKM applying frequentist and Bayesian methods are also shown for mRNA-based vaccines. While additional research is required, initial modeling results appear promising for use with mRNA products.

Beyond estimating the shelf life of products and the impact of temperature excursions, we utilized kinetic models combined with bootstrapping to determine internal release limits (IRLs). An IRL is a key attribute estimate established for particular storage conditions (e.g., 24 months at +5 °C) [26–30]. If met by the drug product batch upon release, an IRL provides assurance that the batch will remain within specifications throughout its shelf

life under the tested conditions. This report introduces the application of kinetic modeling and statistical analysis of data to determine realistic IRLs for vaccines.

METHODOLOGY

Stability prediction of products applying advanced kinetics

By applying AKM to multiple observations obtained over a relatively short period of time and at multiple temperatures, successful predictions of the degradation rate can be made over prolonged periods of time. The following steps must be considered to appropriately develop kinetic models:

- perform accelerated thermal aging studies to obtain at least 20 experimental data points collected at different temperatures, with replicates [3,12,31];
- screen kinetic models, including both one-step and two-step equations;
- select the simplest model(s), based on statistical analyses and ranking parameters such as Akaike and Bayesian information criteria; and
- determine the accuracy of predictions by bootstrapping or Bayesian statistics, leading to prediction intervals or posterior predictive intervals, respectively. During screening of kinetic models, the following three categories of models ought to be considered:

One step kinetics

(Eq. 1)

$$\frac{d\alpha}{dt} = A \cdot \exp\left(-\frac{E_a}{RT}\right) \cdot (1-\alpha)^n \cdot \alpha^m$$

Two step kinetics

(Eq. 2)

$$\frac{d\alpha}{dt} = A \cdot \exp\left(-\frac{E_{a1}}{RT}\right) \cdot (1-\alpha)^{n_1} + A_2 \cdot \exp\left(-\frac{E_{a2}}{RT}\right) \cdot (1-\alpha)^{n_2} \cdot \alpha^{m_2}$$

Two step kinetics involving two starting populations

(Eq. 3)

$$\frac{d\alpha}{dt} = r \cdot A_1 \cdot \exp\left(-\frac{E_{a1}}{RT}\right) \cdot (1-\alpha_1)^{n_1} \cdot \alpha_1^{m_1} + (1-r) \cdot A_2 \cdot \exp\left(-\frac{E_{a2}}{RT}\right) \cdot (1-\alpha_2)^{n_2} \cdot \alpha_2^{m_2}$$

$$\alpha = r \cdot \alpha_1 + (1-r) \cdot \alpha_2$$

with the following notations: A_i=pre-exponential factor in s^{-1}; E_{ai}=activation energy in J/mol; r=ratio of the first part over the total reaction (between 0 and 1); n_i=exponents corresponding to the order of the reaction; m_i=exponents mimicking autocatalytic behavior; R=perfect gas constant; T=temperature in Kelvin.

To go from a to the measured attribute y, two other fitting parameters are introduced, y_{init} and y_{end}, which represent the attribute at time 0 and at the end of the reaction, respectively. y is given by:

(Eq. 4)

$$y = (y_{end} - y_{init}) \cdot a + y_{init}$$

When considering AKM as described above, the discussion around statistical uncertainty is of particular interest. The two main options to estimate this uncertainty are frequentist prediction intervals by non-parametric bootstrapping and Bayesian posterior predictive intervals [32]. The frequentist method suggests that if an experiment is repeated many times, the true parameter estimate has a given probability of being captured. It is key to note that this method treats the true parameter as fixed, with a probability distribution depending on the sampling of data. Among frequentist methods, non-parametric bootstrapping makes no assumptions about the true underlying distribution of data, but rather utilizes resampling to build a range of estimates of the parameters. Bootstrapping is most advantageous when the true underlying distribution of data is unknown or difficult to determine. Bootstrapping with a model averaging approach can also be performed by considering not only a single model but several good models (e. g., best model for each category: Equations 1–3). In that case, the number of loops for bootstrap of each model are proportional to the respective values of the weighted information criteria (i.e., AIC, BIC).

Bayesian credible intervals offer another approach to estimating uncertainty. Posterior predictive intervals are derived by considering that the parameters of interest are random variables with their own probability distributions. First, leveraging prior product knowledge, a prior probability distribution of the model parameters is assumed. This prior distribution is then updated with the available data using Bayes' theorem, yielding the so-called posterior distribution. Given this method, a credible interval at specific level (say 95%) means that, given the data and prior information, there is a 95% probability that a parameter lies within that interval. Each method offers its own unique advantages and focus, whether on variability, probability, or non-parametric approach. For the Bayesian approach, based on literature and mechanistic considerations for chemical reactions, prior ranges were defined as follows: n in [0, 6], E in [0, 750] kJ/mol, ln(A) in [0, 100] ln(s^{-1}). These ranges reflect the bulk of the prior probability densities but note that they do not constitute hard boundaries. Priors ranges for n, E and ln(A) are all defined on real positive numbers.

The AKTS-Thermokinetics software (version 6.04) was used to perform AKM and associated stability predictions. Alternatively, an internally developed R-package (rakm version 1.0.2) was used for AKM calculating Bayesian statistics. This package uses the R-package Rstan (version 2.32.6) and its default sampler (NUT-Sampler) to compute the posterior distribution.

Internal release limits

Under the following conditions, IRLs can be determined using AKM. First, the batch to be attributed an IRL should be comparable to the batches used for modeling, assuming a similar temporal evolution of the stability-indicating attribute of interest. Second, if extreme events such as temperature excursion befall the batch before measurement, they must be recorded for modeling.

Under these conditions, the following reasoning can be made. Given a key attribute y and its AKM model, we search to obtain its IRL, i.e., the higher or lower value measured at production from which a batch can be released. For readability purposes, we assume a decreasing attribute and a lower limit at shelf life y_{lim}, but the same approach can be applied to an increasing attribute with a higher limit at shelf life.

In the following, we define success as the probability that *measurement* at shelf life falls within specifications, which is different from the ICHQ1E guidelines that require the actual value to be within specification. This result is more conservative values than those of the ICHQ1E guideline.

From AKM, we can extract the probability density function for y_{init}, $\rho_{init}(y)$ and the corresponding average value $\overline{y_{init}}$. We use this function as an estimation of the probability density function for the real value being yt=0 knowing y_0=the measurement at t=o.

(Eq. 5)

$$\rho(y/y_0) = \rho(y + y_0 + \Delta y \,|\, y_0) = \rho_{init}(\overline{y_{init}} + \Delta y)$$

Because modeling is done on different batches, batch-to-batch variability of y_{init} is included in this term. AKM also provides the probability that, at shelf life (t=t_{SL}), a measurement of y is above y_{lim} knowing the value at t=o: $P(y(t_{SL}) > y_{lim} |\, y_{init})$. Combining these two functions gives the probability of success knowing an initial measurement y_0:

(Eq. 6)

$$\rho_{success}(y_0) = \int_y P(y \,|\, (t_{SL}) > y_{lim} y)\, \rho(y \,|\, y_0)\, dy$$

We define as the IRL the minimal measurement that ensures a given (generally 95%) certainty of success. This change leads us to use prediction rather than confidence interval. This approach is generalist and can be applied with both Bayesian and frequentist modeling. IRLs methodology was developed in MatLab (version 2024a).

It is important to underline that any new batch needs to be analogous to the batches used for modeling to expect a similar temporal progress of the key attribute. Furthermore, to account for some batch variability, a model should have been made following good modeling practices on at least three different and representative samples. External perturbations can meaningfully shift the *effective age* of a batch and should be accounted for by temperature tracking. Under these rather minimal assumptions, IRLs can be determined using AKM.

RESULTS

Stability prediction and real-time shelf-life monitoring of vaccines

To illustrate simple and more complex stability behaviors of vaccines, two use-cases are shown in Figure 1.

A third order reaction was used to describe emergence of free polysaccharide in a commercial conjugate vaccine (Figure 1A and Equation 7), while a two-step reaction including two starting populations was required for accurate prediction of loss of infectious titer for a live attenuated virus-based vaccine (Figure 1B and Equation 8).

(Eq. 7)

$$\frac{da}{dt} = \exp(55.6) \cdot \exp\left(-\frac{192.2e3}{RT}\right) \cdot (1-\alpha)^3$$

(Eq. 8)

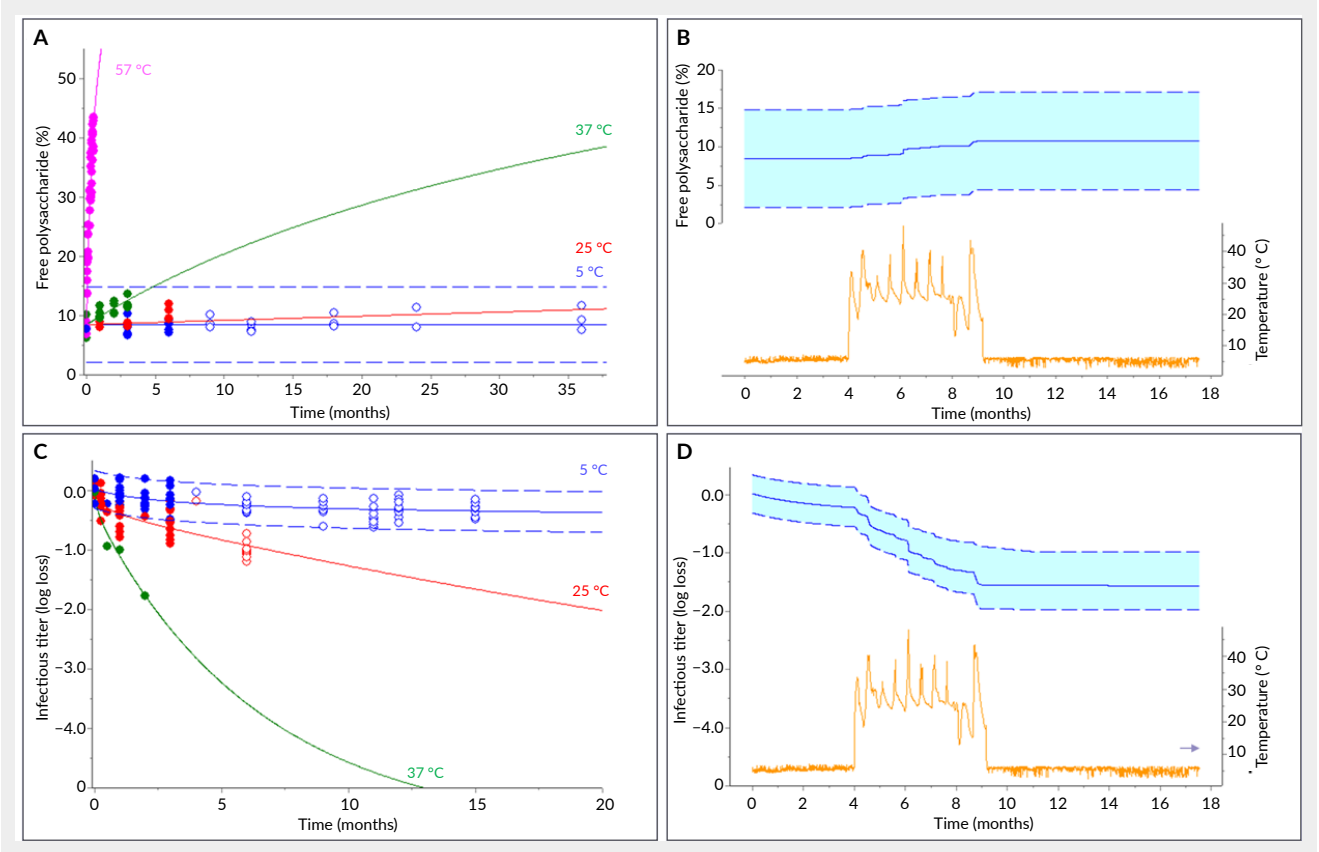
$$\frac{da}{dt} = 0.93 \cdot \exp(36.0) \cdot \exp\left(-\frac{135.8e3}{RT}\right) \cdot (1-\alpha_1) + 0.07 \cdot \exp(20.6) \cdot \exp\left(-\frac{84.6e3}{RT}\right) \cdot (1-\alpha_2)^2$$

Using these kinetic models, 95% prediction bands were constructed for the +5 °C condition. Long-term experimental data (Figure 1, open circles), not used to develop the models, fell within the prediction bands, confirming the accuracy of the predictive models.

Due to the thermosensitivity of vaccines, monitoring their level of degradation in real-time, during long-term storage or shipments, is of high interest, especially to evaluate the

FIGURE 1

Example of stability predictions for key attributes of conventional vaccines and verification.



Long-term free polysaccharide of a conjugate vaccine (A) and infectious titer of live-attenuated virus in freeze-dried form (C) predicted by kinetic model (lines) at 5 °C (blue), 25 °C (red), 37 °C (green), and 57 °C (pink). Data used for kinetic modeling are displayed as filled circles. At 5 °C, free polysaccharide and infectious titer predictions are shown with prediction band representing 95% PI (dashed lines). Additional experimental stability data (open circles), not used to build the models, are displayed to verify the stability predictions. (B) and (D) display evolution of free polysaccharide (B, blue lines) and infectious titer (D, blue lines), respectively, during storage of vaccines in cold chamber (2–8 °C) with a cold-chain break period exposing vaccines to fluctuation of temperature from 15–47 °C (orange line, bottom).

impact of accidental exposures of products to elevated temperatures [15,31,33]. Figure 1 exemplifies the impact of such a cold chain break including fluctuation of temperature up to 47 °C after 4 months of storage at 5 °C for both vaccines. While low impact was predicted for free polysaccharide content for conjugated vaccine (Figure 1C), a strong loss of infectious titer was observed for live attenuated virus-based vaccine (Figure 1D). Interestingly, a return of both vaccines to recommended storage condition between 2–8 °C immediately stopped evolution of these attributes, as illustrated in Figure 1C and D after 10 months.

For mRNA-based vaccines, data from literature was used to illustrate application of kinetic models. In a study from Raffaele et al. in 2021 [24], an accelerated stability study was performed for a mRNA-LNP prototype by incubating samples at multiple temperature ranging from −20 °C to 60 °C. Over 3 months, evolution of intact mRNA content was periodically determined by capillary electrophoresis method. While stable behavior was observed in the frozen state at −20 °C, progressive decreases of intact mRNA content were shown for storage of samples in liquid state, with this phenomenon being accelerated with rising temperature. As mentioned by authors of this study [24], in a large range of temperatures involving frozen (−20 °C) and liquid (4–60 °C) states, mRNA degradation kinetics follow Arrhenius behavior. Here, it can be demonstrated that the use or not of −20 °C stability data led to comparable models. Applying frequentist or Bayesian method, development of kinetic model using experimental data from 4–60 °C allowed good prediction at −20 °C (Figure 2A and B). Experimental data obtained at −20 °C, not used to develop the model, fell within the prediction bands, confirming the accuracy of the predictive models. These results suggest that, for this product, a single kinetic model can be used to predict evolution of intact mRNA content in a wide range of temperatures (from −20 °C to 60 °C), considering both frozen and liquid states. At this point, it is important to note that AKM is not designed to provide knowledge on the degradation mechanisms of a product. The empirical mathematical model is derived from data collected to capture the degradation progress. It is without any mechanistic basis, but readily allows modeling of both Arrhenius and non-Arrhenius processes to accurately predict stability progression.

Taking advantage of such a kinetic model, various storage scenarios were simulated. Considering 60% of integrity as an arbitrary lower acceptance criterion storage at −20 °C for this mRNA-LNP prototype would ensure a stable behavior with a shelf life of at least 12 months (Figure 2A and B). On the other hand, when the product was stored in a liquid state in a cold chamber and real temperature fluctuation was applied or observed, a progressive drop of mRNA content was predicted, leading to a shelf life of around 1.7 months (Figure 2C). To extend the shelf life of this product, a realistic storage scenario with 6 months in a freezer around −20 °C, then 6 months in a refrigerator around 4 °C can be applied, leading to a predicted shelf life of 7.3 months (Figure 2D).

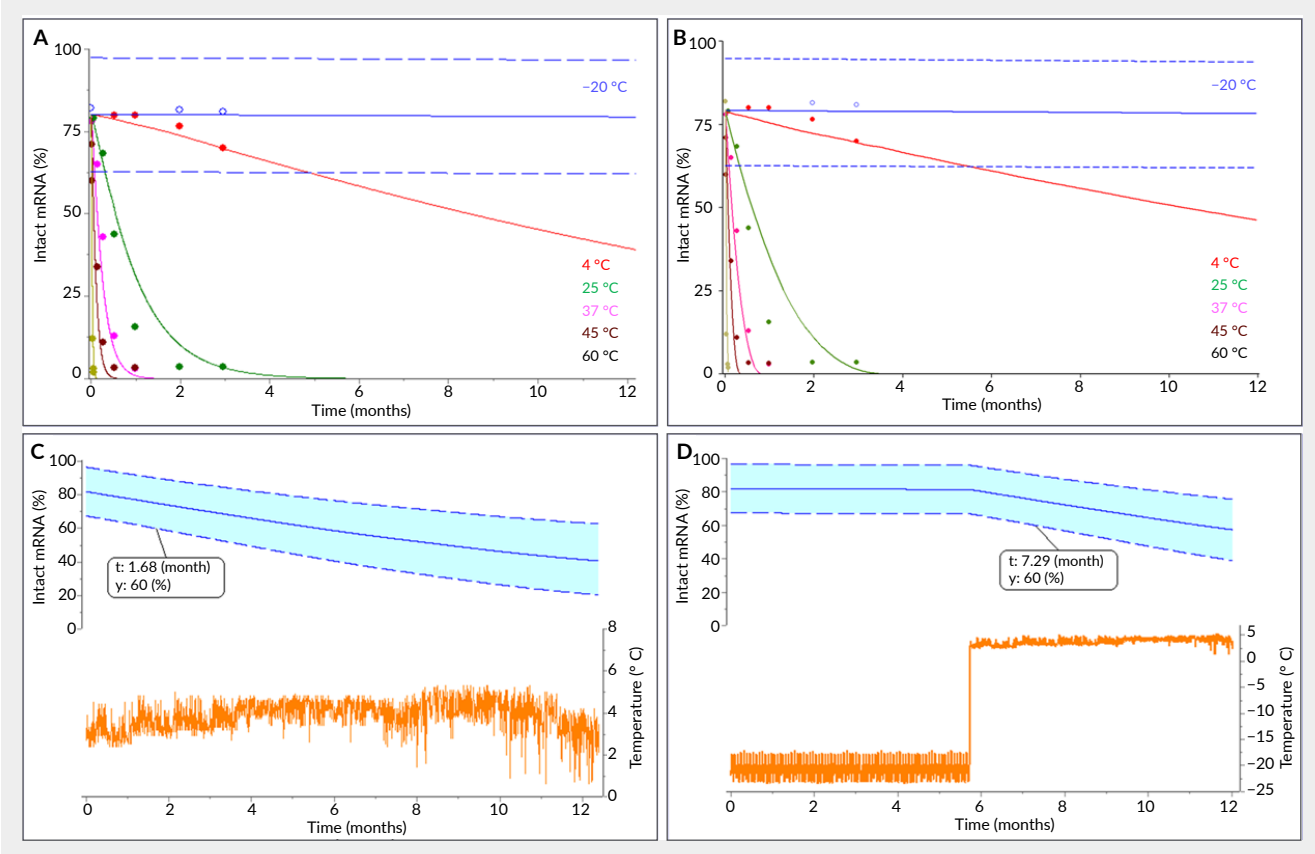
Taking advantage of kinetics models to determine release limits

The IRL methodology uses prediction bands obtained from a model and subsequent bootstrapping to determine what initial conditions could provide assurance that a given product would still meet its specification at the end of its shelf life under specific storage conditions.

To exemplify this application, two different types of vaccines were used. The first one related to a mRNA-LNP prototype characterized by Packer *et al.* [25]. In this study, degradation of mRNA was monitored through adduct formation determined by RP-IP HPLC integrity analysis for samples incubated at different temperatures covering a −20–40 °C range.

FIGURE 2

Example of stability predictions for a key attribute of mRNA-based vaccine prototype.



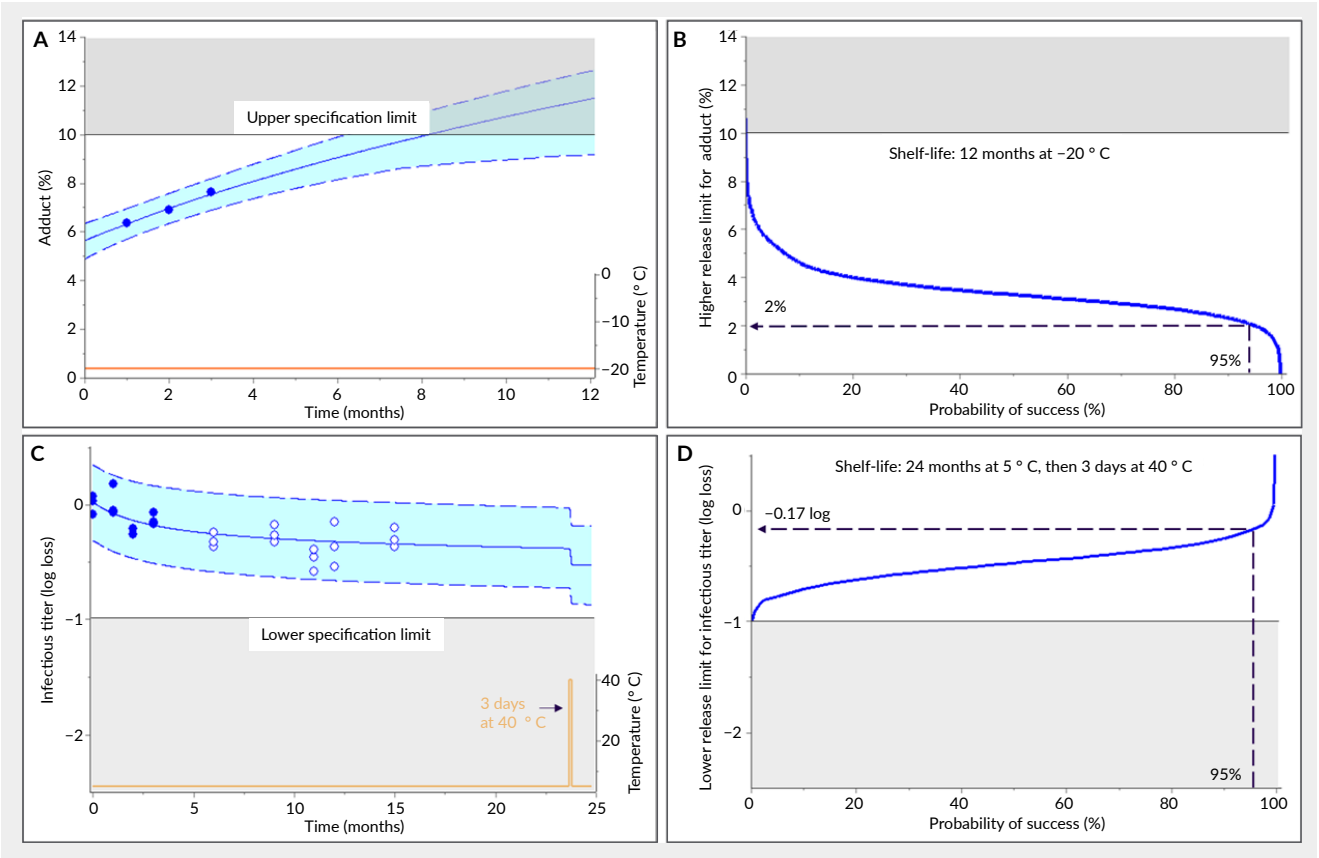
Long-term intact mRNA content predicted by kinetic model (lines) at −20 °C (blue), 4 °C (red), 25 °C (green), 37 °C (pink), 45 °C (maroon), 60 °C (brown) by frequentist (A) and Bayesian (B) approaches. Predictions at −20 °C are displayed with 95% prediction interval (blue dashed lines). Data used for kinetic modeling are displayed as filled symbols. Additional experimental stability data (open symbols at −20 °C), not used to build the models, are displayed to verify the stability predictions. (C) and (D) display evolution of intact mRNA content (blue lines, top), including 95% prediction interval (blue dashed lines), during two different storage conditions. The first one considers real fluctuation of temperature during a storage of product around 4 °C in a cold chamber (C, orange line) while the second one simulates a realistic scenario (D, orange line) including 6 months in a freezer (i.e., around −20°C), then 6 months in a refrigerator (i.e., around 4 °C). Time to reach the 60% of integrity (i.e., lower specification limit) is marked as squared.

Using this dataset, a kinetic model was developed to describe the emergence of adduct as function of time and temperature. Considering 10% of adduct as an arbitrary maximum acceptance criterion (i.e., upper specification limit), a shelf life of 6 months at −20 °C was estimated by the model and its upper prediction band (Figure 3A). To estimate probability of success (PoS) to reach this shelf life of 12 months at −20 °C, depending on release value for adduct content, a graph defining release limit as function of PoS was built (Figure 3B). Less than 10% PoS was predicted for a product released at 5% adduct. To bring this PoS up to 95%, a release limit not higher than 2.0 % must be obtained for this product (Figure 3B), meaning a reduction of around 3% of adduct to be obtained at release.

A second product, a virus-based vaccine, was used to exemplify determination of release limit. This conventional vaccine is usually kept at 5 °C, exhibiting a shelf life of at least of 24 months in this recommended storage condition. The key stability-indicating quality attribute, infectious titer of this vaccine, targeted at release at 5.0 Log CCID50/ dose, must not lose more than 1.0 Log CCID50 for 24 months. Considering potential

FIGURE 3

Determination of IRLs for conventional and mRNA-based vaccines.



(A) Emergence of adduct (%) for a mRNA-LNP prototype stored at −20 °C (A, bottom, orange line). Experimental data (filled circles) are overlayed with kinetic model (top, blue line), including 95% prediction interval (dashed lines). Gray zone refers to an unsuitable level of adduct content, higher than upper specification limit marked as horizontal line. (B) Higher release limit for adduct content as function of probability of success to keep this attribute below 10% after 12 months at −20 °C. (C) Loss of infectious titer for a live attenuated virus-based vaccine stored for 24 months at 5 °C, then experiencing a cold-chain break of 3 days at 40 °C (C, bottom, orange line). Experimental data (filled circles) are overlayed with kinetic model (top, blue line), including 95% prediction interval (dashed lines). Additional experimental stability data, not used to build the models, are displayed as open circles. Gray zone refers to an unsuitable adduct contents with lower specification limit marked as horizontal line. (D) Lower release limit for infectious titer loss as function of probability of success to keep this attribute not higher than 1.0 Log after 24 months at 5 °C, then 3 days at 40 °C. For both vaccines, release limits are shown for a 95% PoS (B and D, dashed lines).

exposure to elevated ambient temperature during last mile transportation of such vaccines, a controlled temperature chain label [34] can be implemented to ensure that vaccines can withstand a temperature excursion up to 40 °C for few days. The kinetic-based modeling approach described here can easily, and advantageously, be used to predict degradation profiles of the vaccine during storage at 24 months at 5 °C, then 3 days at 40 °C (Figure 3C). Considering 1.0 Log loss as a maximum acceptance criterion ((i.e., lower specification limit) after 24 months and 3 days of storage, PoS to reach this shelf life was displayed with a graph defining release limit as function of PoS. At 95% of PoS, −0.2 Log was defined for this vaccine (Figure 3D), meaning that the targeted value for infectious titer of this vaccine at release can be obtained with −0.2 Log of margin (e.g., 4.8 Log/dose instead of 5.0 Lg/dose). The result suggests that this vaccine can support 3 days of excursion at 40 °C without the need of an overage at release.

CONCLUSION

Anticipating the stability of biologicals and vaccines is of major concern. By applying AKM to multiple observations obtained over a relatively short storage period at multiple temperatures, the degradation rate over prolonged periods can be successfully predicted. Results presented in this paper show that AKM can also be applied for stability predictions of both conventional and mRNA-based products, thus confirming its suitability for assigning shelf life for a broad range of products.

For the first time, as illustrated in this study using a mRNA-based vaccine, Bayesian statistics were applied to AKM, leading to posterior predictive intervals comparable with prediction intervals usually obtained by the frequentist approach. This proof-of-concept highlights the possibilities arising from the use of recent state-of-the-art statistical methods. Examples of such methods include leveraging information between experiments through informed priors or hierarchical modeling, increasing statistical power and reducing the risk of overfitting [35]. Another promising direction concerns Bayesian Design of Experiments, where available data can mathematically inform the design of future experiments [36].

Beyond long-term stability predictions, kinetic models can also be used for shelf-life monitoring of vaccines, since temperature fluctuations experiencing by products are recorded in real time. Results presented in this paper suggest the emergence of new methods of supply chain management allowing for real-time monitoring of vaccine stocks across the supply chain [15]. Another opportunity is the determination of internal release limits for vaccines. The method presented in this paper can be applied for products maintained under recommended storage conditions (i.e., isotherm) or experiencing any change of temperature during their storage (e.g., 3 days at 40 °C at the end of shelf life). Examples presented in this study illustrate the power of the kinetic models in ensuring the quality of the products throughout their existence, from production to use.

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VACCINE INSIGHTS

RNA VACCINE R&D

SPOTLIGHT

Pandemic preparedness and the future of RNA therapeutics: RNA readiness and response

INTERVIEW

"We believe we can strengthen pandemic preparedness by developing a platform technology and manufacturing infrastructure that extends beyond vaccines, so that there is worldwide R&D and manufacturing capacity to produce RNA products."

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, speaks with **Duccio Medini**, former R3 Program Director, about the RNA Readiness + Response (R3) program, which aims to democratize the manufacturing of RNA therapeutics. They explore the advances made by R3 in scalable, multi-product RNA production, and the program's vision for widespread, accessible RNA vaccines and therapeutics, especially in the face of future pandemics.

Vaccine Insights 2025; 4(2), 47–53 · DOI: 10.18609/vac.2025.007

Q How did you get involved in vaccine development, particularly in the area of RNA-based vaccines?

DM I started out as a theoretical physicist, but after my PhD, I began working in bioinformatics and population genomics. I discovered the pan-genome and became fascinated with bacteria and the diversity of pathogens. This interest gradually led me into vaccine development, where my passion for science could directly impact human lives.

After 20 years in corporate research and development, first with Chiron, then Novartis, and later GlaxoSmithKline, I decided to transition into philanthropy by joining Wellcome Leap, a non-profit that builds and executes bold, unconventional programs, with the aim of increasing the speed and number of breakthroughs in human health. They invited me to develop a transformative program focused on RNA. At the time, we were in the midst of the COVID-19 crisis, and the decision was made to double down on RNA technology to ensure humanity would be better able to respond to a similar crisis in the future.

This became the objective of the R3 program, which I designed and led, and which is now into its second 3-year funding cycle from Wellcome Leap, CEPI, and other co-funders.

Q How have attitudes toward RNA vaccines in the scientific community evolved, especially after the success of the COVID-19 vaccines?

DM In terms of safety and efficacy, these vaccines have been remarkable, serving as a testament to the platform's validity.

RNA changed the timelines to discover and develop a vaccine from over a decade to less than a year. However, the speed and success of COVID-19 vaccine deployment were also due to two key elements. Firstly, there was already a lot of pre-existing research on the specific pathogen and its family, so the antigen was available immediately, and a stabilizing mutation previously developed by the NIH made the vaccine more effective. Without prior research, whether utilizing RNA technology or not, we could not have deployed a vaccine in just 11 months.

Secondly, there was awareness that certain characteristics of the Moderna and BioNTech-Pfizer vaccines were not commercially ideal. Issues such as limited duration of protection and the requirement for cold chain logistics due to a lack of stability at room temperature meant they could not be characterized as mature commercial products.

So, the lesson we learned is that the time between pandemics should be used to fully develop the platform. RNA is increasingly recognized not just as a vaccine delivery platform but as a means to produce virtually any biological product that can be encoded in an RNA sequence, and this is the strategic foundation of the R3 program. We believe we can strengthen pandemic preparedness by developing a platform technology and manufacturing infrastructure that extends beyond vaccines, so that there is worldwide R&D and manufacturing capacity to produce RNA products.

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"...we were in the midst of the COVID-19 crisis, and the decision was made to double down on RNA technology to ensure humanity would be better able to respond to a similar crisis in the future. This became the objective of the R3 program..."

Q What are some of the barriers to global access to RNA vaccines?

DM There are two major barriers. Firstly, when pandemic crises, such as COVID-19, occur, borders close and countries rely on their regional manufacturing and supply chain capacities. This highlighted the urgent need for a globally distributed manufacturing and supply chain network, a major objective of CEPI, which co-funds our program.

The second limitation is intrinsic to biologics. Unlike small-molecule drugs, biologics have traditionally followed the paradigm 'the process is the product.' Any minor change—whether altering several amino acids in a peptide sequence, changing the strain used to derive an outer membrane vesicle, or modifying a monoclonal antibody production method—requires redeveloping the entire manufacturing process. Each new product demands a bespoke manufacturing process, at three or four different scales from research, through clinical up to market, creating a significant barrier due to the immense costs (as much as USD$0.5 billion) and long development timelines.

The first-generation RNA production processes followed the same, outdated biologics model. Consequently, the massive infrastructure that Moderna and BioNTech-Pfizer built for COVID-19 vaccines in emergency, now has utilization challenges, because it's not easy to flex it to other products. However, nucleic acid products, and RNA in particular, have the potential to break this paradigm. Unlike traditional biologics, changing an RNA product does not intrinsically require changing the manufacturing process.

The R3 program aims to eliminate these two obstacles by developing multi-product, multi-scale technologies, allowing developers to manufacture any RNA-based product without the need for tech transfers, re-validation, or major adjustments. This system will be small, standardized, and deployable anywhere in the world.

Q What are the main goals of the R3 program?

DM To develop 'living' RNA- based, distributed, and multi-product biofoundries that provide increased access to diverse biologics in non-emergency times, and economically sustainable, state-of-the-art surge capacity in an emergency. Such a global network would support a 1,000 to 10,000-fold increase in the number of innovators—starting with PhD students worldwide, we called this the "Valentina model"—developing diverse biologics as treatments for cancer, metabolic disorders, cardiovascular conditions and autoimmune diseases, and would provide the capacity to produce up to 20B doses of RNA-based vaccines in a month, equitably, across the world during an outbreak.

Q How does R3 aim to achieve those goals?

DM To achieve these objectives, the R3 program has three major focus areas. The first is the development of novel manufacturing technologies. RNA manufacturing involves three key steps: DNA production, *in vitro* transcription, and RNA encapsulation. We have developed improved methods for each stage. For DNA production, we eliminated one of the biggest sources of variability by shifting from cell-based processes to cell-free DNA printing. For DNA conversion to RNA, we introduced flow-based, continuous production systems. Lastly, for RNA encapsulation, we integrated the process and explored new formulation and encapsulation methods, including novel lipid nanoparticles (LNPs), polymers, and dendrimers. Overall, the systems we have developed can produce 1 mg to 50 g of product per day. This includes the ability to produce up to 1 kg of RNA product per day or 100 different products, all within a cGMP area under 35 m^2, and entirely cell-free.

The second focus area is product development. A technology is only validated when accepted by regulators on actual products. However, the goal was not to develop a vaccine or cancer therapy with the highest revenue or need, but to stretch the product envelope and test whether our manufacturing processes could handle multiple products effectively. We started by developing 20 RNA-based products that we manufactured and developed in the early discovery phase, and then we focused on nine products for the preclinical phase. Eventually, we refined the selection to four products (two vaccines and two therapeutics) that we are now bringing forward to IND/IMPD submission with the US FDA and UK MHRA for clinical validation of the novel manufacturing technologies developed:

- A self-amplifying RNA COVID-19 vaccine, already completing Phase 3 trials in Japan using traditional manufacturing technology.
- A self-amplifying RNA rabies vaccine, developed as a pandemic-response "Product X" platform. Rabies is particularly useful here because of the strong serological correlate of protection, meaning efficacy trials are not needed.
- An mRNA bispecific T cell engager to treat a terrible cancer, multiple myeloma.
- An mRNA monoclonal antibody for respiratory diseases, in this case targeting COVID-19, but with the potential to be adapted to RSV or other viral respiratory pathogens. This RNA-encoded monoclonal antibody is delivered via nebulizer, and encapsulated in polyplexes formed by a novel polymer.

The third focus area is perhaps the most revolutionary from a conceptual point of view—developing standardized design rules and software tools to streamline RNA product development. Some of these tools use artificial intelligence and deep learning, while others rely on model-based simulations, helping scientists determine the optimal nucleotide sequence to encode a given protein while maximizing biological potency and manufacturability. We have developed software models that have achieved up to 20-times increased immunogenicity with the codon-optimized design. Additionally, we have a deep learning model that predicts manufacturability with 80% accuracy in less than one-tenth of a second.

At the end of the initial 3-year program, we decided that the results warranted moving on to a second cycle, which we call R3 Global. In this cycle, we take the products we developed and deploy them into an RNA service broker and multiple RNA biofoundries.

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"The R3 program aims to [develop] multi-product, multi-scale technologies, allowing developers to manufacture any RNA-based product without the need for tech transfers, re-validation, or major adjustments."

Q What are the key features of these RNA biofoundry facilities?

DM A biofoundry is a pure-play foundry—an entity that hosts a full suite of manufacturing technologies, such as cell-free DNA printing and integrated, all-in-one RNA manufacturing machines, without developing their own products. A key characteristic of RNA biofoundries, enabled by the technology R3 developed, is to cover three functions with the same lines: manufacturing for preclinical research (in GMP-like mode, milligram scale), clinical development and market (GMP, grams), pandemic response (kilograms).

These biofoundries are for-profit organizations: they do not develop their own products but rather manufacture for others via standardized processes. Early-phase researchers will access the biofoundries through a service broker who buys capacity at a larger scale and makes it available in single runs to scientists, students, or small biotech companies around the world. A broker also integrates the software, allowing developers to optimize the designs and ensure they are manufacturable with biofoundry technology. The system will operate via a website where customers can access design support for creating the RNA product, and check available manufacturing runs, ensuring they are compatible with the desired design. The run can be purchased directly from the website, and the design can be manufactured by any of the available biofoundries.

A key advantage is that as a customer progresses through the development cycle, the scale-up will be seamless, from bench scale to GLP to GMP production. If the developer is satisfied with the product developed for mice, then they can replicate that identical product for hamsters, pigs, and non-human primates. Subsequently, they can continue scaling through Phase 1, Phase 2, and Phase 3 clinical trials, and even to the market, without ever modifying the process.

The flexibility to produce 100 different products at a few miligrams each or produce 1 billion doses of a single vaccine in one day provides huge manufacturing capacity for emergency use, as well as small-scale manufacturing for personalized products. The core of the technology provides flexibility and ubiquity, but to be truly pandemic-ready, you still must make the right investments upstream (supply chain) and downstream (fill & finish).

Q Where do these foundries fit into existing pharmaceutical development pathways?

DM Most pharmaceutical companies are vertically integrated, i.e., they handle everything from design to testing, manufacturing, and commercialization of RNA products. We believe that the technology we have developed has the potential to enable a second business model, parallel to the current one.

It is an ecosystem where, on one side, there are pure-play foundries that only focus on manufacturing, and on the other side, there are developers who only focus on design.

This model is already used in the semiconductor industry. Apple does not manufacture its own microchips. Similarly, Nvidia, the largest producer of graphic processing units in the world, does not have a single manufacturing line of its own. Instead, both companies rely on pure-play foundries. We expect this model to be a huge democratization driver, allowing everyone from small biotechs to PhD students to access high-quality manufacturing services via automated lines, wherever they are in the world.

At the same time, it is a huge driver for geographic distribution of capacity and equitable access to resources. Manufacturing lines that can be deployed in just 35 m^2 and do not require specialized expertise to operate are a game-changer. We expect this model to co-flourish alongside the currently established big pharma model.

We are working with the local governments, CEPI, and other potential funders to demonstrate that this model can be successful in high-, low-, and middle-income countries.

Q What other promising developments in the RNA vaccines field are you excited about?

DM The work being done in the formulation space is extremely exciting. Many people agree that, from a product development perspective, RNA will soon become a formulation industry.

We are starting to understand reasonably well how to encode instructions in an RNA chain. Yet nowadays we only deploy approximately 5% of these instructions to the cells and tissues we want to target. Emerging technologies could allow us to deliver up to 90% of the product to target tissues.

The emergence of alternative encapsulation technologies could also address both the stability issues and the intellectual property challenges surrounding LNPs. For example, the groups of Drew Weissman and Virgil Percec at University of Pennsylvania have developed single-component dendrimers that can match the performance of an LNP, but with a much simpler manufacturing process, consisting of only a handful of synthesis steps [1]. These dendrimers also offer shelf-life stability for months at room temperature. Another example is the single-polymer formulations developed by the team of Phil Santangelo at Emory University—also very simple, stable, and suitable for nebulized delivery directly to the airways [2]. At R3, we are working on producing GMP lots of these materials to assess their performance in humans. If these results are confirmed, I believe formulation will be the area where we see the most exciting surprises.

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BIOGRAPHY

Duccio Medini is a scientist and pharmaceutical executive, currently serving as R3 Global Chief Scientist at Wellcome Leap, a global ARPA for Health, and as Strategic Data Science Director at Toscana Life Sciences Foundation. He led the $60M R3: RNA Readiness + Response program, co-funded by CEPI, to develop standardized, multi-product RNA manufacturing capabilities providing increased access to diverse biologics and sustainable pandemic response. In executive roles of growing responsibility at Chiron, Novartis, and GSK corporations, he led hundreds of data scientists in Vaccines R&D and AI across Europe, US, and Asia, holding Research Board, Innovation Board, Clinical Quality Board, and Data Governance Board responsibilities. Working across biological discovery, clinical development and public health, he discovered the pangenome concept co-founding the pangenomics discipline, contributed to the successful registration of four novel vaccines, including the first universal vaccine against serogroup B meningitis, led the Meningococcal Antigen Typing System (MATS) platform worldwide, developed transformative corporate data strategies, authored over 70 publications, books and patents in data science for health, population genomics and mathematical vaccinology. Medini received his PhD in Physics from the University of Perugia, Italy, with a residency at the Northeastern University in Boston, MA, USA; is habilitated Full Professor of Molecular Biology; has served in international PhD school committees at the Perugia and Turin Universities in Italy; is honorary member of the Cuban Immunology Society, Fellow of the ISI Foundation, board member of the WHO mRNA Hub program, New Zealand National RNA program, and CINI's Italian National Digital Health Lab and is an IMD, ADVAC, and UC Berkley alumnus.

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The future is now: AI-enabled RNA–LNP discovery

INTERVIEW

"I think we will see the emergence of LNPs targeted to the lung and the brain, as well as to immune cells."

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, talks with **Bowen Li**, Assistant Professor at the University of Toronto, about how his groundbreaking work with the AI-driven LUMI-lab platform is delivering a new generation of LNPs with unique capabilities.

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Q How did you first get involved in the area of LNP technologies for mRNA vaccines? And how has your research evolved from there?

BL I started learning about lipid nanoparticle (LNP) technology when I was a graduate student of Bioengineering at the University of Washington. At that time, many researchers were using LNPs specifically to deliver small molecules, but I was also exposed to the use of polymeric nanoparticles to deliver protein therapeutics. My introduction to LNPs for mRNA delivery began during my postdoc training at MIT's Koch Institute with my supervisors, Daniel Anderson, and Robert Langer—both of whom are true pioneers in the field of RNA delivery.

The field was rapidly evolving, particularly with the emergence of the LNP-based mRNA vaccines (the COVID-19 pandemic began just a couple of months after I joined MIT). My

research focused mainly on designing and optimizing novel RNA-LNPs aimed at enhancing the performance of LNPs for delivery of mRNA vaccines and gene therapies. In 2022, I started my own lab at the University of Toronto.

Since then, my work has evolved and migrated into the field of AI-driven biomaterial discovery. Toronto is the place where AI originated—it is the home of the 'Godfather of AI', Nobel Laureate Geoffrey Hinton—and so we are very much encouraged to think about how we can incorporate AI into our work. We leverage deep learning algorithms and high-throughput screening to rationally design next-generation LNPs not only for mRNA delivery, but also emerging modalities such as circular (circ)RNA.

Fundamentally, we seek to integrate traditional approaches, such as combinatorial chemistry and RNA molecule bioengineering, with new approaches (e.g., AI-guided design, self-driving lab) to accelerate the development of safer, more efficient mRNA-based vaccines and therapeutics.

Q Tell us more about your lab's activities at the University of Toronto?

BL Our lab is multidisciplinary. We currently have three main areas of focus for our research.

Firstly, as I have mentioned, we incorporate AI to guide the design of next-generation RNA delivery systems. Right now, LNPs work well for both vaccine delivery via intramuscular injection and systemic hepatic delivery. However, there is high demand for customized LNPs that can access different organs and tissues in the body. We are particularly focused on the discovery of novel LNPs for tissue-specific mRNA delivery.

Secondly, we are engaged in optimizing the RNA molecules themselves. We seek to enhance the stability of mRNA, circRNA, and other nucleic acid modalities such as transfer (t)RNA by optimizing their molecular sequences and introducing new chemical modifications. This, in turn, leads to reduced immunogenicity and improved translation efficiency for these RNA molecules.

Thirdly, we combine these novel nanoparticles and optimized RNA molecules to address specific clinical questions. For example, we are investigating the potential of inhalable RNA therapeutics as a less invasive means of treating cystic fibrosis patients.

Q Can you go deeper on how you apply AI to develop more effective LNP delivery systems for RNA vaccines and therapeutics?

BL Current discovery and development are largely based on existing LNP structures and prior knowledge. When we want to design a new LNP, the first thing we do is refer to the literature and see how others have designed their structures. We then tend to make some incremental changes before we screen a wide range of candidates, identify the ones that can potentially work in a given setting, and advance those for further testing. This is a traditional drug discovery strategy, of course, but we have found this process to be tedious, time-consuming, and expensive.

Instead, in our lab we feed data, including some we have already generated ourselves, to an AI model and ask it to learn from this information, so that it can tell us which kinds of new structures we should try in the future. This helps us get past the previous

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"Rather than us giving the AI model 100, or 1,000, or a million data points, we ask it to decide how many data points it needs."

trial-and-error strategy and gives us a clear pathway forward in terms of the structures we should test, rather than just randomly selecting candidates or trying those from other people's work.

AI can also help us to overcome some limitations regarding human intuition. Human beings tend to stay in their 'comfort zone'. We can be reluctant to try some more unusual structures because we feel like it may be a waste of time. AI can sometimes drive us to try these new structures because it has found a micro-feature that was missed or ignored by humans.

Q What is the LUMI-lab platform and how was it developed?

BL The motivation for us to develop LUMI-lab derives from a persistent question in the field of AI for science, which is: how can we get sufficient data to train the AI model? I have been asked similar questions many times myself. Although others read about our work and are amazed by how we use AI to save time in LNP design, they also express concern about how they can obtain sufficient data to do similar research. This led me to the idea of having the AI model first decide how much data it requires, and then go about generating that data for itself.

Rather than us giving the AI model 100, or 1,000, or a million data points, we ask it to decide how many data points it needs. It then controls robots to perform the experiments required to generate that number of data points. The AI model then digests this information before making a prediction for the next round of studies.

If the model is happy with the result—in this case, that it has identified sufficiently promising LNPs—it will stop. However, if the model realizes that the current number of data points is insufficient to allow it to make an accurate prediction, it will send new demands to the robots to perform another round of experiments, and so on. The model will not stop until it has identified highly promising LNPs, whether that takes one, two, or ten rounds of experiments.

There are two key features of the LUMI-lab platform that are worth highlighting. One is that this is the very first foundation model for LNPs.

The foundation model is currently a popular concept in the AI for science field. Essentially, a foundation model is a very powerful model that has already absorbed a lot of information on a given area. For example, there have been a couple of high-profile papers published recently on foundation models developed for the protein and DNA fields [1].

The benefit of a foundation model is that you don't require a lot of additional wet lab data to train it to do different tasks within its area of expertise. The analogy I like to make is that the model is like a very senior professor in the Department of Chemistry at Harvard University who has no particular knowledge of LNPs. Nonetheless, it would be relatively easy for that professor to understand what is happening in the LNP space and to provide some good suggestions relating to it. Our LNP foundation model has an equivalent knowledge base, having been trained on over 28 million molecular features and structures.

"the AI model can decide on what the next experiment should be, and can then send the relevant commands to the robots in the lab to perform it without any human intervention."

The second feature to highlight is that the LUMI-lab platform is equipped with a self-driving lab, which means a lab that is entirely autonomous and driven by the AI model rather than human beings. As I mentioned previously, the AI model can decide on what the next experiment should be, and can then send the relevant commands to the robots in the lab to perform it without any human intervention. The robots work for the AI model to make it increasingly powerful.

Both of these features—the foundation model and the self-driving lab—are firsts for the LNP field.

Q How successful has LUMI-lab been at generating more effective LNPs? Have the results surprised you?

BL We were hugely surprised. Our logic was that we would train a powerful AI model to help us identify new LNPs. However, the LUMI-lab identified a lipid structure with very good efficiency in human bronchial cells, which has a molecular feature that is completely different from all known lipid structures. It contains a bromine in the lipid tail, which is something that has never been recorded in the literature before. We don't know why the AI model is so keen on this type of structure, but it turns out to be extraordinarily effective. This is the first time the AI model has generated information that was not documented in the literature and had never been observed by humans.

Q What reaction have you had from the field?

BL People are super excited, not least because delivery has been a core challenge for RNA medicine for so long. Without the delivery component, there is no RNA medicine for patients. Many pharma companies are actively looking for new nanoparticles that can satisfy their R&D pipeline needs—for instance, by enabling the extrahepatic delivery of RNA therapeutics. The LUMI-lab is receiving a lot of interest from these companies seeking to collaborate, as well as from venture capital investors.

Q What role does interdisciplinary collaboration play in your research, and how do you integrate different scientific disciplines?

BL I think that our AI-guided platform for LNP design is a perfect example of interdisciplinary research. I had dabbled in machine learning while I was at MIT and saw the promise it held, so once I joined the University of Toronto, I began to actively seek collaborators from the AI field. Fortunately, there is the Vector Institute for Artificial

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Intelligence in Toronto, founded by Geoffrey Hinton. I was able to identify a collaborator there who provided us with a lot of support in terms of the AI algorithms. That is how I started learning about the foundation model.

I really enjoy this kind of interdisciplinary collaboration. In our lab, we incorporate AI, but we also perform a lot of animal studies to validate the potential of the LNPs we discover to address clinical questions. We also work closely with the Hospital for Sick Children in Toronto. They have a cystic fibrosis center, and the physician-scientists there support us by providing us with disease models. This allows us to cover the whole pipeline, from LNP design through RNA drug discovery to *in vivo* testing in animal models.

Q What's next for the LUMI-lab and for your research in general?

BL Regarding the LUMI-lab, we are trying to use it to help us design LNPs for some cells that are notoriously difficult to transfect—for example, T cells. That is what I am working on right now. We want to see if we can develop new nanoparticles that can very efficiently target T cells and transfer mRNA into them, so that we can develop a next-generation CAR-T cell therapy.

We are also trying to find applications for the LNPs we identify through the LUMI-lab—for example, for gene therapy in the lung. We are seeking to deliver some of the emerging gene editing platforms, such as base or prime editors, in order to rectify the genetic mutations in various different diseases.

On the vaccines side, we also work on engineering vaccine formulations to make them more effective at inducing mucosal immune responses. The current intramuscularly injected mRNA vaccines are quite effective at inducing Immunoglobulin G (IgG) in the blood, but they are not very effective at inducing Immunoglobulin A (IgA) in the mucosal areas. The mucosal immune response plays an important role in protecting from infection, so we are trying to see if we can further optimize the vaccines to make them more potent in this particular regard.

Q Considering the future of RNA delivery systems more generally, how do you expect the field to evolve in the next 5 years?

BL I can foresee that in the next 5 years, more LNPs will be developed for delivering mRNA to extrahepatic tissues. For example, within this timeframe, I think we will see the emergence of LNPs targeted to the lung and the brain, as well as to immune cells. This is already happening right now, and it is set to expand the application of mRNA to more and more clinical scenarios.

I also think we will see greater and greater involvement of AI scientists in the R&D process—for example, in the use of AI to optimize formulations and reduce the need for exhaustive wet-lab screening.

Finally, although most current gene therapies are still based on viral vectors like AAV, I believe the future of this field belongs to non-viral delivery. In the next 5 years, we will see non-viral delivery systems like LNPs finding many more new applications.

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BIOGRAPHY

Bowen Li is an Assistant Professor at the Leslie Dan Faculty of Pharmacy, University of Toronto, and an Affiliate Scientist at the Princess Margaret Cancer Centre. He holds the Tier 2 Canada Research Chair in RNA Vaccines and Therapeutics and the GSK Chair in Pharmaceutics and Drug Delivery. Dr Li earned his PhD in Bioengineering from the University of Washington, Seattle, WA, USA and completed a postdoctoral fellowship under Professors Bob Langer and Daniel Anderson at MIT, Cambridge, MA, USA. His lab employs interdisciplinary approaches including biomolecular engineering, combinatorial chemistry, autonomous high-throughput platforms, and machine learning to develop next-generation delivery systems for nucleic acid medicines. Dr Li's work has resulted in over 60 publications in top journals such as *Nature Biotechnology*, *Nature Materials*, *Nature Biomedical Engineering*, *Nature Medicine*, *Nature Communications*, *Science Advances*, and *PNAS*, as well as ten patents. His research has been recognized with numerous prestigious awards, including the National Sanitarium Association Scholar Award, Oxford-Harrington Rare Disease Scholar Award, AAPS Emerging Leader Award, Biomaterials Science Emerging Investigator Award, CSPS Early Career Award, Marsha Morton Early Career Investigator Award, Gairdner Early Career Investigator Award, ACS Rising Star in Biological, Medicinal, and Pharmaceutical Chemistry, and Connaught New Researcher Award.

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VACCINE CLINICAL UPDATE

Leaving an imprint: maternal immunization to protect newborns

INTERVIEW

"The use of new vaccines in pregnancy, especially those for pathogens with pandemic potential, remains a priority."

Charlotte Barker, Editor, *Vaccine Insights*, speaks with clinician–scientist **Beate Kampmann**, Scientific Director, Charité Center for Global Health, about the evolving maternal immunization landscape, the importance of birth cohort studies, and the challenge of establishing effective pregnancy registries.

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Q How did you become interested in infectious disease and maternal immunization?

BK I trained in pediatrics and specialize in pediatric infectious diseases. Pediatricians generally focus on trying to prevent diseases rather than treating them. The best way to prevent infectious diseases in children is through vaccination. I initially worked on tuberculosis (TB) vaccines, which sparked my interest in vaccine development. I was fortunate to work on vaccines with proven efficacy and see lots of exciting new candidates emerging.

As a clinician, I've seen the detrimental effects of early-onset infectious diseases such as RSV and Group B strep firsthand. I thought, 'If we can't vaccinate the newborns in time,

"We need to determine the best time to immunize and the impact on the evolving immune system of the infant."

why not enhance the natural passive immunity provided by maternal antibodies?' This led me to an interest in maternal immunization, which was already happening successfully in many countries with tetanus. I wondered if we could extend this principle to other diseases.

I felt there was a lot more to learn about maternal immunization from an immunological point of view. We know that if we give vaccines to women during pregnancy, the passive transfer of antibodies can protect the newborn. We've seen this with tetanus, pertussis, and influenza. The question then became, can we extend this principle responsibly? For me, this meant understanding the impact of maternal immunization on the infant's immune system, and this led me to set up the IMmunizing PRegnant women and INfants (IMPRINT) network.

Q Can you tell us more about the IMPRINT network?

BK The IMPRINT Network, funded by UKRI, brings together a global, interdisciplinary community. It covers biological science, midwifery, implementation research, and public engagement. The network has facilitated collaboration and brought together experts from various fields based in over 50 countries.

The network has been particularly beneficial for early career researchers, providing opportunities for funding and collaboration. It has also engaged in grassroots and public engagement, resulting in follow-on funding and successful projects. Unfortunately, funding beyond this year is uncertain, which could leave a significant gap in maternal immunization science.

Q What are the goals of the IMPRINT network?

BK I set up IMPRINT because I felt we needed to look at maternal immunization from three dimensions. First, the biological challenges, which relate to understanding immunology during pregnancy. We need to determine the best time to immunize and the impact on the evolving immune system of the infant. For example, if we vaccinate mothers, there might be less response to the vaccine antigens when we later vaccinate the baby. We need to be careful that there are no unintended consequences for the baby.

Second, there are implementation challenges. Most vaccines are given to small children or babies, and healthcare infrastructure is not set up for maternal vaccines, except for tetanus in low- and middle-income countries (LMICs). In places where vaccines are normally given through general practice, pregnant women might not go there for antenatal care. In Germany, for example, interventions in pregnancy are carried out by obstetricians in specialized clinics, not in general practice. How do we get that group involved in delivering vaccines?

Another question is who records these vaccines? During my work at Imperial College, I spent a lot of time trying to get vaccination records for pregnant women into their antenatal care records, which proved virtually impossible, and it remains a challenge to bring together immunization information from mothers and infants.

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The third dimension is advocacy. We needed advocacy with providers, pregnant women, and healthcare professionals. Midwives, in particular, can be skeptical about interventions during pregnancy unless they are convinced of their necessity.

Understanding vaccine knowledge and acceptance is particularly important in the context of maternal immunizations. It is 'buy one, get one free'—you are protecting both mother and baby, but you also need to consider the safety of both. This conceptual framework required a lot of advocacy. All of these aspects were included in the research we were doing in the IMPRINT Network.

Q What are your key research interests now, and what current projects are you most excited about?

BK The whole agenda of maternal immunization has significantly evolved over the past decade. When we started with this about 10 years ago, maternal immunization was not a prominent topic in vaccine discussions. Now, that's changed completely, and I'm pleased to say that my work has contributed to that change in perception. Pregnant women are no longer automatically excluded from trials, and there is now industry interest in developing vaccines specifically for use in pregnancy. RSV and Group B strep vaccines are prime examples.

My research continues to focus on vaccine trials in pregnancy and understanding the biological impact on infants. We need long-term studies, including birth cohort studies, to fully grasp the clinical relevance of our findings. Funding for such studies is challenging, but essential. The research agenda has moved forward significantly, but many questions remain unanswered. We need to follow up on observational insights from immunological studies to determine their clinical relevance.

The work is not done, but as researchers, nothing ever is fully done. It's like climbing a tree; you always see new branches and have to decide which ones to explore and which to leave to others.

Q Can you describe the current landscape of maternal immunization and its significance in global health?

BK The WHO recommends tetanus, pertussis, and flu vaccines for pregnant women, depending on the geographies. The recently licensed RSV vaccine is a game-changer as it's the first vaccine licensed specifically for use in pregnancy. The challenge now is ensuring implementation platforms are in place to deliver these vaccines effectively. With multiple vaccines now recommended in pregnancy, there may be a role for combination vaccines to reduce the number of injections.

We must also address the knock-on effects of maternal immunization on infant immune responses. For example, pertussis vaccination in mothers can blunt the infant's response, so we need to optimize immunization schedules. Although we haven't seen clinical evidence of any disadvantages, we need to critically examine immunization intervals and schedules for infants.

"Establishing pregnancy registries is essential for tracking the use and impact of vaccines given in pregnancy not only on the pregnant women, but also on her infant."

There are also considerations for vaccines designed for pathogens with pandemic or epidemic potential. None of the COVID trials initially included pregnant women, but attitudes are changing, with Mpox trials now also including pregnant women. We need to design protocols that include pregnant women earlier and consider their increased risk during pregnancy. This is particularly relevant for diseases like Ebola and Lassa fever.

The landscape needs to evolve to ensure safety and effective monitoring of these vaccines. Establishing pregnancy registries is essential for tracking the use and impact of vaccines given in pregnancy not only on the pregnant women, but also on her infant.

Q You recently coauthored a study on creating an electronic pregnancy registry in Gambia using smart paper technology [1]. What were the main findings, and could they be applied to other countries?

BK Smart paper technology links antenatal records and immunization cards through a unique identifier, creating a hybrid system that digitizes existing paper records. This system allows us to monitor pregnancy outcomes and vaccine safety more effectively. The pilot study in Gambia showed promise, and we are evaluating its implementation to potentially scale it up and apply it to other countries.

The technology addresses the issue of linking maternal and infant records, which are often held in separate silos and in LMIC are usually on paper. By scanning existing paper records into a digital system, we can create a more reliable and consistent way to track pregnancy outcomes and vaccine safety. It's not the gold-standard of a fully digitalized electronic medical record system, but it allows us to capture outcomes and give some baseline rates. By establishing baseline data, we can better assess the impact of new interventions, such as the RSV vaccine.

Having completed the pilot study, we are now carrying out further evaluation in partnership with the Gambian Ministry for Health to assess the reliability and implementation of the system, with the goal of scaling it up and potentially extending it to other countries. We would also like to apply the system for pharmacovigilance.

Q What aspects of maternal and infant immune responses to maternal vaccination remain unknown and would benefit from further research?

BK We need more qualitative research among healthcare workers, including obstetricians, gynecologists, and midwives. Understanding their perspectives can help improve vaccine literacy and acceptance among pregnant women and healthcare

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workers. This research is crucial for informed decision-making and addressing skepticism about interventions during pregnancy.

On the systems biology side, we need to determine the clinical relevance of our data and explore optimal immunization schedules. Establishing correlates of protection could help license vaccines more rapidly. We need to bridge effectiveness studies with immunological correlates to understand the impact of maternal immunization on infant health.

Q How can we address vaccine hesitancy and misinformation among pregnant women and healthcare providers?

BK Personal stories and success stories can be powerful tools to counter misinformation. Engaging influencers and sharing positive experiences can help build trust in vaccines. We need to find out what makes people tick and use that to address hesitancy.

Research shows that people who are hesitant about childhood vaccines are likely to be hesitant about maternal immunization as well. Addressing this issue requires engaging with the public in meaningful ways. Expert opinion may not always be persuasive, but personal experiences can make a significant impact.

Q What collaborations or partnerships have been most impactful in your work on maternal immunization?

BK Collaborations with public health institutions and understanding the needs of the countries we work in are crucial. Engaging with these stakeholders early in the research process can help ensure our findings are not just interesting data, but lead to improved care for pregnant women and children. We need to shape our research to align with the health agendas of the countries we work in to get the best 'bang for the buck'.

Q What's next for your work and for maternal vaccination more generally?

BK I've transitioned to a role at the Center for Global Health in Berlin, where I continue to focus on vaccine research and enabling functions.

The use of new vaccines in pregnancy, especially those for pathogens with pandemic potential, remains a priority. Investment in surveillance systems, such as pregnancy registries, is also essential.

I remain passionate about the field and proud of the progress we've made. We've achieved significant buy-in and moved forward on regulatory and equity issues. The goal is to ensure vaccines are not just developed in the Global North for the Global South but that there is ownership and control from the countries that need these vaccines.

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BIOGRAPHY

Beate Kampmann is Professor of Global Health and Scientific Director of the newly established Charite Centre for Global Health and the Institut fur Internationale Gesundheit, Charite Virchow Campus. Beate recently relocated to Berlin from a Chair in Pediatric Infection & Immunity at The London School of Hygiene and Tropical Medicine (LSHTM), where she directed the Vaccine Centre at LSHTM. Over the last 12 years, she divided her working time between London and The Gambia, West Africa, where she led the vaccine research at the MRC Unit—The Gambia in West Africa and where her research projects and PhD student supervision continue. Her translational research portfolio extends from laboratory science to clinical trials of novel vaccines and adjuvants and into health policy guidance, with a scientific focus on innate and acquired immune responses to infection, e.g. tuberculosis and vaccination. She has a special interest in developing strategies for safe and effective vaccines for pregnant women and is the director of IMPRINT, the IMmunising PRegnant women and INfants network, a UKRI-GCRF-funded multi-disciplinary and global network of scientists, clinicians, and public health representatives with a special interest in vaccines for pregnant women and newborns.

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