HHS Public Access

Author manuscript

Vaccine Insights. Author manuscript; available in PMC 2025 May 21.

Published in final edited form as:

Vaccine Insights. 2025 March ; 4(2): 41–45.

Enhancing RNA vaccine safety through localized delivery strategies

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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 out-weigh 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

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

Disclosure and potential conflicts of interest: The author has equity interest in HDT Bio.

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 nucleo-side-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.

Funding declaration:

This work was supported by NIH/NIAID grant R01AI180195-01 and by a Career Development Award from the American Society of Gene & Cell Therapy. The content is solely the responsibility of the author and does not necessarily represent the official views of the funders.

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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