Universal Flu mRNA Vaccine

Subjects: Virology

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The seasonal flu vaccine is, essentially, the only known way to prevent influenza epidemics. However, this approach has limited efficacy due to the high diversity of influenza viruses. Several techniques could potentially overcome this obstacle. A recent first-in-human study of a chimeric hemagglutinin-based universal influenza virus vaccine demonstrated promising results. The coronavirus pandemic triggered the development of fundamentally new vaccine platforms that have demonstrated their effectiveness in humans. Currently, there are around a dozen messenger RNA and self-amplifying RNA flu vaccines in clinical or preclinical trials.

Keywords: mRNA vaccine ; universal flu vaccine ; circRNA

1. Development and Use of mRNA Vaccines

Thirty-five years ago, the possibility of protein expression in human cell lines from exogenous mRNA mixed with fat droplets was demonstrated [1]. A year later, the possibility of mRNA delivery into a living organism (frog embryo) was revealed. However, for many years, mRNA was not considered as a viable method to prevent or treat disease due to the perceived technical sophistication. Despite the lack of visible applications of the new technology, several scientific groups continued to consider using mRNA as a delivery vehicle, e.g., for cancer vaccines [2].

An unexpected impetus to the development of mRNA vaccines was provided by the COVID-19 pandemic. There were no ready-made solutions at the time of the emergence of a demand for a vaccine against a novel infection. In such a situation, standard time-tested approaches require comparable, if not more, time in development as compared with new ones. Currently, two major mRNA vaccines in use are manufactured by Moderna and Pfizer [3]. Both vaccines are based on a similar principle. The Moderna and Pfizer vaccines were administered to hundreds of millions of people, efficiently protecting against moderate and symptomatic SARS-CoV-2 infection [4]. It should be noted that over time the effectiveness of vaccination inevitably decreases [5], which necessitates the use of revaccination.

2. mRNA Vaccine Method of Action and Delivery

2.1. Types of mRNA Vaccines

Based on their method of action, mRNA vaccines can be divided into two types: conventional mRNA and self-amplifying mRNA (SAM) vaccines [6]. Conventional mRNA vaccines take advantage of cellular machinery to translate the appropriate protein, whereas SAM vaccines have coding accessory proteins for self-replication (RNA-dependent RNA polymerase, capping enzymes, proteases) beside target RNA [7][8][9][10].

SAM vaccines may be used in the form of formulated in vitro transcribed mRNA or in a form of viral replicon particles produced by packaging SAM with alphavirus structure proteins in cell culture [11]. This was demonstrated through the development of vaccines that prevent CMV infection [12]. Notably, the presence of alphavirus proteins may be toxic for the host cells [13]. At the same time, a recent safety study of the SAM rabies vaccine in rats showed that SAM was well tolerated by the animals [14].

Hekele and colleagues have proven the safety, tolerance, and efficacy of a SAM vaccine coding for influenza hemagglutinin H1N1. This vaccine protected mice from inoculation by the H7N9 strain. Such preparations may be generated within 8 days in a cell-free way after the discovery of a new viral strain [15]. Another advantage of such a platform is the ability to use low concentrations of nucleic acids. Moreover, the amplification of delivered mRNA occurs in situ, generating stronger protective immunity to the antigen as compared with conventional mRNA vaccines.

In addition, the new cationic nanoemulsion (CNE)-formulated SAM vaccine against another RNA-virus, the Zika virus, has recently been shown to protect a non-human primate model in a preclinical trial [16]. Several studies demonstrated the

great potential of SAM vaccine usage for preventing COVID-19. Strong stimulation of either humoral or cellular immune response was revealed [17][18]. Notably, one SAM vaccine is undergoing Stage I clinical trials after confirmation of effectiveness against different SARS-CoV-2 strains [17]. Thus, it can be said that SAM vaccines have shown their immunogenic potency against multiple targets [15][16][17][18][19][20][21]. In addition, based on preclinical data, it can be concluded that SAM-mRNA vaccines could potentially induce a more durable immune response compared to non-replicating mRNA vaccines [22].

Most ribonucleases involved in mRNA degradation have 5'- or 3'-end-dependent activity. As a result, the stability of linear mRNA is low, impairing mRNA-based vaccine implementation. Notably, circular forms of RNA (circRNA) were demonstrated to be an abundant and prominent type of RNA generated in the result of backsplicing [23]. Due to the lack of a 5'- and 3'-end, circRNA are more stable in comparison with linear mRNA. Recently, several approaches for circRNA generation have been suggested [24][25][26][27].

RNA circularization leads to enhanced stability of the mRNA and greater efficiency in the production of the target protein [24]. Moreover, a circular mRNA vaccine could be stored at room temperature [28]. As a result, manufacturing and implementation costs would be significantly lower. There are currently no data about circular mRNA vaccines. However, some commercial companies are raising funds to implement the concept of protein translation from circular RNA in humans. The Laronde startup raised hundreds of millions of dollars in 2021 for the development of "endless RNA therapeutics" [29].

2.2. Peculiarities of mRNA Vaccine Synthesis

In essence, mRNA vaccine production is an attempt to simulate the changes that mRNA undergoes in a human cell, using a cascade of biochemical reactions in a cell-free environment. For example, mRNA-1273 (Moderna) is generated by an in vitro T7 RNA polymerase-mediated transcription process from plasmid DNA [30]. The DNA template contains a codon-optimized immunogen coding sequence, 5′ untranslated region (UTR), 3′ UTR sequences, and a polyA tail. It should be noted that the miRNA binding sites in the 5' and 3' UTRs decrease the half-life of mRNA as well as vaccine antigen expression in target cells and tissues. Additionally, any non-canonical start codons or strong secondary structures interfering with translation initiation at the 5' UTRs may lower the translation efficiency of a specific antigen. To enhance expression and avoid innate immune hyperactivation, ORF should also be optimized with codon usage and GC content.

In addition, uridine (U) 5′-triphosphate is 100% substituted by 1-methylpseudouridine (m1Ψ) 5′-triphosphate in the transcription reaction mixture [31]. As a result, all Us in the sequence of mRNA-1273 are changed to m1Ψs. It should be noted that U-to-m1Ψ substitution has no effect on the translated protein sequence. Such modification was demonstrated to significantly enhance the mRNA translation level [32]. However, inclusion of m1Ψ guarantees less activation of the innate immune system, compared to mRNA containing canonical uridine [31]. Indeed, in vitro transcribed RNA induces innate immune response through the interaction of cytosolic or endosomal RNA-sensing pattern-recognition receptors. These proteins interact with single- or double-stranded RNA containing exogenous pathogen-associated molecular patterns. Such interactions drive expression of pro-inflammatory cytokines, chemokines, type I IFN, and interferon-stimulated genes [33][34]. Nevertheless, inhibition of early I type IFN-derived immune response is critical for enhancing immunization efficiency by an mRNA vaccine [35]. At the same time, hyperstimulation of the innate response can lead to the elimination of exogenous mRNA or the blockage of its translation mechanism [36]. Therefore, mRNA vaccines must be designed in such a way as to "moderately" stimulate the immune response. Chemical modifications of nucleosides naturally occurring as post-translational modifications in cells may allow the prevention of Toll-like receptor-mediated activation of the immune response [37]. Thus, the use of a novel modification assists in the avoidance of excessive activation of the innate immune response. An example of the potential importance of including a modification in mRNA is the COVID-19 mRNA vaccine produced by CureVac. This vaccine demonstrated low effectiveness (around 48%) [38]. The low efficacy of the drug was likely due to the lack of m1Ψ modification as compared with the Moderna and Pfizer vaccines [39].

The RNA resulting from in vitro transcription undergoes a biochemical 5' capping procedure, imitating the natural process that occurs in the nucleus of the cell [40]. Artificial capping could be conducted by using commercially available kits, e.g. CleanCap (Trilink Biotechnologies) [41]. While such a modification stabilizes the synthesized RNA by shielding from 5' RNA exonucleases, it is necessary for cap-dependent translation initiation. Circular mRNA vaccines that have yet to come into practice are, by definition, incapable of cap-dependent translation initiation. However, there are alternative cap-independent mechanisms of translation initiation, mediated by internal ribosomal entry sites (IRES) or N6-methyladenosine (m6A) modification incorporated into the 5' UTR region of mRNA [42][43].

T7 RNA polymerase, in addition to its primary function, is capable of synthesizing RNA based on an RNA template (i.e. RNA template-directed RNA synthesis) in significant quantities [44]. As a result, an RNA strand complementary to the template is synthesized, generating dsRNA. The entry of double-stranded RNA into a cell inevitably leads to a strong immune response, initiated by the binding of a foreign molecule to receptors, such as RIG-I-like receptors [45]. As a result, the efficacy of translation is seriously reduced [46]. The additional step of removing the dsRNA fraction circumvents this problem. Thus, U-to-m1Ψ substitution and dsRNA removal are two necessary and sufficient steps to regulate the interaction between vaccine mRNA and immunity [46]. At the same time, it should be noted that the difficulty of scaling up mRNA vaccine production is of particular concern [47]. This problem remains the subject of active research [48][49].

2.3. Delivery Vehicles

Various mRNA delivery platforms may be used, depending on the task. Viral vectors, gene guns, electroporation, penetrating peptides, polymers, and liposomes are among the most widespread approaches [50][51]. Naked unformulated mRNA was shown to be expressed in cells [52], however cellular uptake is less than 0.01% [53]. Notably, most of these methods are only suitable for in vitro purposes.

Liposomes are the first delivery approach used in nanopharmaceuticals as a kind of universal carrier for both hydrophobic and hydrophilic cargoes: proteins, nucleic acids, and small molecules [54]. A liposome is a 20–1000 nm formation with one or more lipid bilayer containing aqueous and hydrophobic compartments. In 1994, Harashima et al., demonstrated a positive correlation between the size of such particles and the opsonization and macrophage phagocytosis uptake in vivo [55]. This means that liposome size should be carefully measured for delivering purposes [56].

To date, the most common way of delivering mRNA for therapeutic purposes was lipid nanoparticle (LNP) injection. LNP consists of positively charged cationic and ionizable lipids that mediates complexing with negatively charged mRNA. Moreover, such formulation is necessary for cellular uptake and endosomal escape [57][58]. Cationic lipids in LNPs are neutralized with anionic cell lipids and promote nucleic acid entering the cytoplasm through the disruption of LNP complexes. Although LNPs are non-immunogenic and non-toxic in comparison to viral vectors, they still have some cytotoxicity [59] that is dependent on the structure of hydrophilic heads or PEG (polyethylene glycol) modifications of lipids, which are widely used to increase in vivo stability [60]. This may cause membrane damage or vacuolization of the cytoplasm and may affect important cellular pathways and cell cycle stages. There are many new lipids under development for lowering cytotoxic effects without decreasing delivering efficiency.

Producing LNPs is difficult and hardly scalable. LNPs themselves are not sufficiently stable, sterilizable, or bioavailable [54]. Solid lipid nanoparticles (SLNs) containing solid lipids instead of liquid crystalline and nanostructured lipid carriers (NLCs) containing a mix of solid and liquid crystalline lipids were developed for overcoming those limitations.

Another non-viral delivery platform, cationic nanoemulsion (CNE), consists of cationic lipid DOTAP (1,2-dioleoyl-sn-glycero-3-phosphocholine), emulsified with components of Novartis's proprietary adjuvant MF59 and is well tolerated in every age group [19]. CNE's advantages in delivering and enhancing vaccine potency and safety have been proven in over 100 clinical trials. Additionally, it can be stored at +4 °C for up to 3 years [16].

It should be noted that there is a possibility for targeted LNP delivery with the use of incorporated ligands for cell receptors (natural ligands, antibodies, aptamers) and stimuli-responsive (pH, temperature, magnetic fields, laser irradiation) LNPs, as it has been tested for anti-cancer therapies [54].

There are different administration routes for mRNA-based therapeutics [58]. Systemic intravenous administration often leads to the accumulation of LNPs in the liver. This is acceptable for replacement therapies and for the production of specific anti-pathogen or anti-cancer antibodies due to inherent liver capability for protein secretion. Otherwise, intravenous delivery may cause a wide distribution of nanoparticles through the lymph nodes, enhancing immune response to the antigen, compared with local administration; this could cause adverse effects, therefore using targeted nanoparticles is preferable. Direct local administration (intramuscular, intradermal, subcutaneous) of mRNA-containing nanoparticles into the target tissue is preferential for achieving a local therapeutic effect and a systemic effect through the recruiting of local antigen presenting cells. Local injection is commonly used for vaccination purposes. Van Lint et al., have shown that direct intranodal administration of tumor-associated antigen mRNA together with mRNA coding for immunomodulatory proteins causes a robust T cell response mediated by dendritic cell mRNA uptake, translation, and antigen presentation [61]. At the same time, intranodal delivery of mRNA-encoded influenza nucleoprotein activates an effective cross-strain T cell response in mice [62].

Some LNP-based therapeutics are already clinically approved and there are also several nucleic acid-based approaches in use. For example, Onpattro by Alnylam Pharmaceuticals, which is a transthyretin-directed siRNA formulated with LNP for the treatment of polyneuropathy caused by hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis), and, of course, the commonly known anti-COVID-19 mRNA vaccines, BNT162b2 by Pfizer/BioNTech and mRNA-1273 by Moderna, although they only received Emergency Use Authorization (EUA) in 2020 [54]. There are also other Stage I and II clinical trials of mRNA-based LNP therapeutics against tumors (melanoma, breast cancer, ovarian cancer, glioblastoma, solid tumors, etc.), viral infections (rabies, Zika virus, CMV, Influenza virus, SARS-CoV-2), tuberculosis, and others [54].

2.4. Flu mRNA Vaccines under Development

There are four influenza mRNA candidate vaccines in clinical trials proposed by Sanofi/TranslateBio, Pfizer, Moderna, and NIAID. The Sanofi/TranslateBio vaccine is a monovalent vaccine that codes for the hemagglutinin protein of A/H3N2. Pfizer's medical has two monovalent vaccines that code for the hemagglutinin of H1N1 and B/Yamagata lineage AIV combined into a singular bivalent vaccine.

Moderna's candidate, mRNA-1010, is a quadrivalent vaccine, encoding the hemagglutinin for two IAVs and two IBVs, selected based on WHO recommendation: A/H1N1, A/H3N2, B/Yamagata-, and B/Victoria-lineages [63]. NIAID's candidate, FluMos-v1, is a universal mRNA vaccine that stimulates antibodies against several different strains of the influenza virus through the display of a hemagglutinin fragment on the surface of a self-assembling nanoparticle scaffold [64].

In addition to the above examples, several other prototypes are being developed [65]; thus, there are at least 10 mRNA vaccines for the influenza virus currently in pre-clinical trials, including a multivalent Moderna vaccine.

In other words, the possibility of using mRNA vaccines is already the subject of active study and several prototypes are being tested in humans. It should be noted that mRNA vaccines for the influenza virus may be a larger challenge to market to the public than for COVID-19 as there are non-mRNA options available in use. However, while current vaccines are safe, their efficacy and coverage leaves space for improvement, which, in theory, can be improved upon by using mRNA vaccines [65].

References

1. Malone, R.W.; Felgner, P.L.; Verma, I.M. Cationic liposome-mediated RNA transfection. Proc. Natl. Acad. Sci. USA 1989, 86, 6077–6081.
2. Dolgin, E. The tangled history of mRNA vaccines. Nature 2021, 597, 318–324.
3. Fathizadeh, H.; Afshar, S.; Masoudi, M.R.; Gholizadeh, P.; Asgharzadeh, M.; Ganbarov, K.; Köse, Ş.; Yousefi, M.; Kafil, H.S. SARS-CoV-2 (Covid-19) vaccines structure, mechanisms and effectiveness: A review. Int. J. Biol. Macromol. 2021, 188, 740–750.
4. Patel, R.; Kaki, M.; Potluri, V.S.; Kahar, P.; Khanna, D. A comprehensive review of SARS-CoV-2 vaccines: Pfizer, Moderna & Johnson & Johnson. Hum. Vaccin. Immunother. 2022, 18, 2002083.
5. Pegu, A.; O'Connell, S.E.; Schmidt, S.D.; O'Dell, S.; Talana, C.A.; Lai, L.; Albert, J.; Anderson, E.; Bennett, H.; Corbett, K.S.; et al. Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants. Science 2021, 373, 1372–1377.
6. Iavarone, C.; O'hagan, D.T.; Yu, D.; Delahaye, N.F.; Ulmer, J.B. Mechanism of action of mRNA-based vaccines. Expert Rev. Vaccines 2017, 16, 871–881.
7. Magini, D.; Giovani, C.; Mangiavacchi, S.; Maccari, S.; Cecchi, R.; Ulmer, J.B.; De Gregorio, E.; Geall, A.J.; Brazzoli, M.; Bertholet, S. Self-Amplifying mRNA Vaccines Expressing Multiple Conserved Influenza Antigens Confer Protection against Homologous and Heterosubtypic Viral Challenge. PLoS ONE 2016, 11, e0161193.
8. Vogel, A.B.; Lambert, L.; Kinnear, E.; Busse, D.; Erbar, S.; Reuter, K.C.; Wicke, L.; Perkovic, M.; Beissert, T.; Haas, H.; et al. Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. Mol. Ther. 2018, 26, 446–455.
9. Bloom, K.; van den Berg, F.; Arbuthnot, P. Self-amplifying RNA vaccines for infectious diseases. Gene Ther. 2021, 28, 117–129.
10. Blakney, A.K.; Ip, S.; Geall, A.J. An Update on Self-Amplifying mRNA Vaccine Development. Vaccines 2021, 9, 97.

11. Geall, A.J.; Verma, A.; Otten, G.R.; Shaw, C.A.; Hekele, A.; Banerjee, K.; Cu, Y.; Beard, C.W.; Brito, L.A.; Krucker, T.; et al. Nonviral delivery of self-amplifying RNA vaccines. Proc. Natl. Acad. Sci. USA 2012, 109, 14604–14609.

12. Bernstein, D.I.; Reap, E.A.; Katen, K.; Watson, A.; Smith, K.; Norberg, P.; Olmsted, R.A.; Hoeper, A.; Morris, J.; Negri, S.; et al. Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. Vaccine 2009, 28, 484–493.

13. Garmashova, N.; Gorchakov, R.; Frolova, E.; Frolov, I. Sindbis Virus Nonstructural Protein nsP2 Is Cytotoxic and Inhibits Cellular Transcription. J. Virol. 2006, 80, 5686–5696.

14. Stokes, A.; Pion, J.; Binazon, O.; Laffont, B.; Bigras, M.; Dubois, G.; Blouin, K.; Young, J.K.; Ringenberg, M.A.; Ben Abdeljelil, N.; et al. Nonclinical safety assessment of repeated administration and biodistribution of a novel rabies self-amplifying mRNA vaccine in rats: Toxicity and biodistribution of rabies SAM vaccine. Regul. Toxicol. Pharmacol. 2020, 113, 104648.

15. Hekele, A.; Bertholet, S.; Archer, J.; Gibson, D.G.; Palladino, G.; Brito, L.A.; Otten, G.R.; Brazzoli, M.; Buccato, S.; Bonci, A.; et al. Rapidly produced SAM ® vaccine against H7N9 influenza is immunogenic in mice. Emerg. Microbes Infect. 2013, 2, 1–7.

16. Luisi, K.; Morabito, K.M.; Burgomaster, K.E.; Sharma, M.; Kong, W.-P.; Foreman, B.M.; Patel, S.; Fisher, B.; Aleshnick, M.A.; Laliberte, J.; et al. Development of a potent Zika virus vaccine using self-amplifying messenger RNA. Sci. Adv. 2020, 6, eaba5068.

17. Maruggi, G.; Mallett, C.P.; Westerbeck, J.W.; Chen, T.; Lofano, G.; Friedrich, K.; Qu, L.; Sun, J.T.; McAuliffe, J.; Kanitkar, A.; et al. A self-amplifying mRNA SARS-CoV-2 vaccine candidate induces safe and robust protective immunity in preclinical models. Mol. Ther. 2022, in press.

18. Bhattacharya, M.; Sharma, A.R.; Ghosh, P.; Patra, P.; Patra, B.C.; Lee, S.-S.; Chakraborty, C. Bioengineering of Novel Non-Replicating mRNA (NRM) and Self-Amplifying mRNA (SAM) Vaccine Candidates Against SARS-CoV-2 Using Immunoinformatics Approach. Mol. Biotechnol. 2022, 64, 510–525.

19. Brito, L.A.; Chan, M.; Shaw, C.A.; Hekele, A.; Carsillo, T.; Schaefer, M.; Archer, J.; Seubert, A.; Otten, G.R.; Beard, C.W.; et al. A Cationic Nanoemulsion for the Delivery of Next-generation RNA Vaccines. Mol. Ther. 2014, 22, 2118–2129.

20. Brazzoli, M.; Magini, D.; Bonci, A.; Buccato, S.; Giovani, C.; Kratzer, R.; Zurli, V.; Mangiavacchi, S.; Casini, D.; Brito, L.M.; et al. Induction of Broad-Based Immunity and Protective Efficacy by Self-amplifying mRNA Vaccines Encoding Influenza Virus Hemagglutinin. J. Virol. 2016, 90, 332–344.

21. Bogers, W.M.; Oostermeijer, H.; Mooij, P.; Koopman, G.; Verschoor, E.J.; Davis, D.; Ulmer, J.B.; Brito, L.A.; Cu, Y.; Banerjee, K.; et al. Potent Immune Responses in Rhesus Macaques Induced by Nonviral Delivery of a Self-amplifying RNA Vaccine Expressing HIV Type 1 Envelope With a Cationic Nanoemulsion. J. Infect. Dis. 2015, 211, 947–955.

22. Rappaport, A.R.; Hong, S.-J.; Scallan, C.D.; Gitlin, L.; Akoopie, A.; Boucher, G.R.; Egorova, M.; Espinosa, J.A.; Fidanza, M.; Kachura, M.A.; et al. A self-amplifying mRNA COVID-19 vaccine drives potent and broad immune responses at low doses that protects non-human primates against SARS-CoV-2. bioRxiv 2021, 2021.11.08.467773.

23. Lasda, E.; Parker, R. Circular RNAs: Diversity of form and function. RNA 2014, 20, 1829–1842.

24. Wesselhoeft, R.A.; Kowalski, P.S.; Anderson, D.G. Engineering circular RNA for potent and stable translation in eukaryotic cells. Nat. Commun. 2018, 9, 2629.

25. Meganck, R.M.; Liu, J.; Hale, A.E.; Simon, K.E.; Fanous, M.M.; Vincent, H.A.; Wilusz, J.E.; Moorman, N.J.; Marzluff, W.F.; Asokan, A. Engineering highly efficient backsplicing and translation of synthetic circRNAs. Mol. Ther. - Nucleic Acids 2021, 23, 821–834.

26. Meganck, R.M.; Borchardt, E.K.; Castellanos Rivera, R.M.; Scalabrino, M.L.; Wilusz, J.E.; Marzluff, W.F.; Asokan, A. Tissue-Dependent Expression and Translation of Circular RNAs with Recombinant AAV Vectors In Vivo. Mol. Ther. - Nucleic Acids 2018, 13, 89–98.

27. Litke, J.L.; Jaffrey, S.R. Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts. Nat. Biotechnol. 2019, 37, 667–675.

28. Qu, L.; Yi, Z.; Shen, Y.; Lin, L.; Chen, F.; Xu, Y.; Wu, Z.; Tang, H.; Zhang, X.; Tian, F.; et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell 2022, S0092-8674(22)00394-4.

29. Philippidis, A. IN THE LOOP: “Endless RNA” Takes Its Place in Drug Development. Genet. Eng. Biotechnol. News 2021, 41, 12–13.

30. Corbett, K.S.; Edwards, D.K.; Leist, S.R.; Abiona, O.M.; Boyoglu-Barnum, S.; Gillespie, R.A.; Himansu, S.; Schäfer, A.; Ziwawo, C.T.; DiPiazza, A.T.; et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 2020, 586, 567–571.

31. Nelson, J.; Sorensen, E.W.; Mintri, S.; Rabideau, A.E.; Zheng, W.; Besin, G.; Khatwani, N.; Su, S.V.; Miracco, E.J.; Issa, W.J.; et al. Impact of mRNA chemistry and manufacturing process on innate immune activation. Sci. Adv. 2020, 6, eaaz6893.

32. Svitkin, Y.V.; Cheng, Y.M.; Chakraborty, T.; Presnyak, V.; John, M.; Sonenberg, N. N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. Nucleic Acids Res. 2017, 45, 6023–6036.

33. Nair, P.; Sapre, S.U. Significance of RNA Sensors in Activating Immune System in Emerging Viral Diseases. In Dynamics of Immune Activation in Viral Diseases; Springer: Singapore, 2020; pp. 229–242.

34. Uehata, T.; Takeuchi, O. RNA Recognition and Immunity—Innate Immune Sensing and Its Posttranscriptional Regulation Mechanisms. Cells 2020, 9, 1701.

35. Pepini, T.; Pulichino, A.-M.; Carsillo, T.; Carlson, A.L.; Sari-Sarraf, F.; Ramsauer, K.; Debasitis, J.C.; Maruggi, G.; Otten, G.R.; Geall, A.J.; et al. Induction of an IFN-Mediated Antiviral Response by a Self-Amplifying RNA Vaccine: Implications for Vaccine Design. J. Immunol. 2017, 198, 4012–4024.

36. Linares-Fernández, S.; Lacroix, C.; Exposito, J.-Y.; Verrier, B. Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol. Med. 2020, 26, 311–323.

37. Hajj, K.A.; Whitehead, K.A. Tools for translation: Non-viral materials for therapeutic mRNA delivery. Nat. Rev. Mater. 2017, 2, 17056.

38. Kremsner, P.G.; Ahuad Guerrero, R.A.; Arana-Arri, E.; Aroca Martinez, G.J.; Bonten, M.; Chandler, R.; Corral, G.; De Block, E.J.L.; Ecker, L.; Gabor, J.J.; et al. Efficacy and safety of the CVnCoV SARS-CoV-2 mRNA vaccine candidate in ten countries in Europe and Latin America (HERALD): A randomised, observer-blinded, placebo-controlled, phase 2b/3 trial. Lancet Infect. Dis. 2022, 22, 329–340.

39. Morais, P.; Adachi, H.; Yu, Y.-T. The Critical Contribution of Pseudouridine to mRNA COVID-19 Vaccines. Front. Cell Dev. Biol. 2021, 9, 789427.

40. Park, J.W.; Lagniton, P.N.P.; Liu, Y.; Xu, R.-H. mRNA vaccines for COVID-19: What, why and how. Int. J. Biol. Sci. 2021, 17, 1446–1460.

41. Henderson, J.M.; Ujita, A.; Hill, E.; Yousif-Rosales, S.; Smith, C.; Ko, N.; McReynolds, T.; Cabral, C.R.; Escamilla-Powers, J.R.; Houston, M.E. Cap 1 Messenger RNA Synthesis with Co-transcriptional CleanCap ® Analog by In Vitro Transcription. Curr. Protoc. 2021, 1, e39.

42. Sinha, T.; Panigrahi, C.; Das, D.; Panda, A. Circular RNA translation, a path to hidden proteome. WIREs RNA 2022, 13, e1685.

43. Yang, Y.Y.; Fan, X.; Mao, M.; Song, X.; Wu, P.; Zhang, Y.; Jin, Y.; Yang, Y.Y.; Chen, L.-L.; Wang, Y.; et al. Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res. 2017, 27, 626–641.

44. Cazenave, C.; Uhlenbeck, O.C. RNA template-directed RNA synthesis by T7 RNA polymerase. Proc. Natl. Acad. Sci. USA 1994, 91, 6972–6976.

45. Hur, S. Double-Stranded RNA Sensors and Modulators in Innate Immunity. Annu. Rev. Immunol. 2019, 37, 349–375.

46. Karikó, K.; Muramatsu, H.; Ludwig, J.; Weissman, D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. 2011, 39, e142.

47. Rosa, S.S.; Prazeres, D.M.F.; Azevedo, A.M.; Marques, M.P.C. mRNA vaccines manufacturing: Challenges and bottlenecks. Vaccine 2021, 39, 2190–2200.

48. Whitley, J.; Zwolinski, C.; Denis, C.; Maughan, M.; Hayles, L.; Clarke, D.; Snare, M.; Liao, H.; Chiou, S.; Marmura, T.; et al. Development of mRNA manufacturing for vaccines and therapeutics: mRNA platform requirements and development of a scalable production process to support early phase clinical trials. Transl. Res. 2022, 242, 38–55.

49. Shepherd, S.J.; Warzecha, C.C.; Yadavali, S.; El-Mayta, R.; Alameh, M.G.; Wang, L.; Weissman, D.; Wilson, J.M.; Issadore, D.; Mitchell, M.J. Scalable mRNA and siRNA Lipid Nanoparticle Production Using a Parallelized Microfluidic Device. Nano Lett. 2021, 21, 5671–5680.

50. Tan, L.; Sun, X. Recent advances in mRNA vaccine delivery. Nano Res. 2018, 11, 5338–5354.

51. Kowalski, P.S.; Rudra, A.; Miao, L.; Anderson, D.G. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. Mol. Ther. 2019, 27, 710–728.

52. Wolff, J.A.; Malone, R.W.; Williams, P.; Chong, W.; Acsadi, G.; Jani, A.; Felgner, P.L. Direct Gene Transfer into Mouse Muscle in Vivo. Science (80-.). 1990, 247, 1465–1468.

53. Sahin, U.; Karikó, K.; Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. Nat. Rev. Drug Discov. 2014, 13, 759–780.
54. Tenchov, R.; Bird, R.; Curtze, A.E.; Zhou, Q. Lipid Nanoparticles─From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. ACS Nano 2021, 15, 16982–17015.
55. Harashima, H.; Sakata, K.; Funato, K.; Kiwada, H. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. Pharm. Res. 1994, 11, 402–406.
56. Nagayasu, A.; Uchiyama, K.; Kiwada, H. The size of liposomes: A factor which affects their targeting efficiency to tumors and therapeutic activity of liposomal antitumor drugs. Adv. Drug Deliv. Rev. 1999, 40, 75–87.
57. Oberli, M.A.; Reichmuth, A.M.; Dorkin, J.R.; Mitchell, M.J.; Fenton, O.S.; Jaklenec, A.; Anderson, D.G.; Langer, R.; Blankschtein, D. Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. Nano Lett. 2017, 17, 1326–1335.
58. Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. Nat. Rev. Mater. 2021, 6, 1078–1094.
59. Lv, H.; Zhang, S.; Wang, B.; Cui, S.; Yan, J. Toxicity of cationic lipids and cationic polymers in gene delivery. J. Control. Release 2006, 114, 100–109.
60. Ryals, R.C.; Patel, S.; Acosta, C.; McKinney, M.; Pennesi, M.E.; Sahay, G. The effects of PEGylation on LNP based mRNA delivery to the eye. PLoS ONE 2020, 15, e0241006.
61. Van Lint, S.; Goyvaerts, C.; Maenhout, S.; Goethals, L.; Disy, A.; Benteyn, D.; Pen, J.; Bonehill, A.; Heirman, C.; Breckpot, K.; et al. Preclinical Evaluation of TriMix and Antigen mRNA-Based Antitumor Therapy. Cancer Res. 2012, 72, 1661–1671.
62. Joe, P.T.; Christopoulou, I.; van Hoecke, L.; Schepens, B.; Ysenbaert, T.; Heirman, C.; Thielemans, K.; Saelens, X.; Aerts, J.L. Intranodal administration of mRNA encoding nucleoprotein provides cross-strain immunity against influenza in mice. J. Transl. Med. 2019, 17, 242.
63. Abbasi, J. Moderna’s mRNA Vaccine for Seasonal Flu Enters Clinical Trials. JAMA 2021, 326, 1365.
64. Boyoglu-Barnum, S.; Ellis, D.; Gillespie, R.A.; Hutchinson, G.B.; Park, Y.-J.; Moin, S.M.; Acton, O.J.; Ravichandran, R.; Murphy, M.; Pettie, D.; et al. Quadrivalent influenza nanoparticle vaccines induce broad protection. Nature 2021, 592, 623–628.
65. Dolgin, E. mRNA flu shots move into trials. Nat. Rev. Drug Discov. 2021, 20, 801–803.