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mRNA vaccines — a new era in vaccinology

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Abstract

mRNA vaccines represent a promising alternative to conventional vaccine approaches because of their high potency, capacity for rapid development and potential for low-cost manufacture and safe administration. However, their application has until recently been restricted by the instability and inefficient *in vivo* delivery of mRNA. Recent technological advances have now largely overcome these issues, and multiple mRNA vaccine platforms against infectious diseases and several types of cancer have demonstrated encouraging results in both animal models and humans. This Review provides a detailed overview of mRNA vaccines and considers future directions and challenges in advancing this promising vaccine platform to widespread therapeutic use.

Vaccines prevent many millions of illnesses and save numerous lives every year[1]. As a result of widespread vaccine use, the smallpox virus has been completely eradicated and the incidence of polio, measles and other childhood diseases has been drastically reduced around the world[2]. Conventional vaccine approaches, such as live attenuated and inactivated pathogens and subunit vaccines, provide durable protection against a variety of dangerous diseases[3]. Despite this success, there remain major hurdles to vaccine development against a variety of infectious pathogens, especially those better able to evade the adaptive immune response[4]. Moreover, for most emerging virus vaccines, the main obstacle is not the effectiveness of conventional approaches but the need for more rapid development and large-scale deployment. Finally, conventional vaccine approaches may not be applicable to non-infectious diseases, such as cancer. The development of more potent and versatile vaccine platforms is therefore urgently needed.

Nucleic acid therapeutics have emerged as promising alternatives to conventional vaccine approaches. The first report of the successful use of *in vitro* transcribed (IVT) mRNA in animals was published in 1990, when reporter gene mRNAs were injected into mice and protein production was detected[5]. A subsequent study in 1992 demonstrated that administration of vasopressin-encoding mRNA in the hypothalamus could elicit a

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Competing interests statement

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physiological response in rats[6]. However, these early promising results did not lead to substantial investment in developing mRNA therapeutics, largely owing to concerns associated with mRNA instability, high innate immunogenicity and inefficient *in vivo* delivery. Instead, the field pursued DNA-based and protein-based therapeutic approaches[7,8].

Over the past decade, major technological innovation and research investment have enabled mRNA to become a promising therapeutic tool in the fields of vaccine development and protein replacement therapy. The use of mRNA has several beneficial features over subunit, killed and live attenuated virus, as well as DNA-based vaccines. First, safety: as mRNA is a non-infectious, non-integrating platform, there is no potential risk of infection or insertional mutagenesis. Additionally, mRNA is degraded by normal cellular processes, and its *in vivo* half-life can be regulated through the use of various modifications and delivery methods[9–12]. The inherent immunogenicity of the mRNA can be down-modulated to further increase the safety profile[9,12,13]. Second, efficacy: various modifications make mRNA more stable and highly translatable[9,12,13]. Efficient *in vivo* delivery can be achieved by formulating mRNA into carrier molecules, allowing rapid uptake and expression in the cytoplasm (reviewed in REFS 10,11). mRNA is the minimal genetic vector; therefore, anti-vector immunity is avoided, and mRNA vaccines can be administered repeatedly. Third, production: mRNA vaccines have the potential for rapid, inexpensive and scalable manufacturing, mainly owing to the high yields of *in vitro* transcription reactions.

The mRNA vaccine field is developing extremely rapidly; a large body of preclinical data has accumulated over the past several years, and multiple human clinical trials have been initiated. In this Review, we discuss current mRNA vaccine approaches, summarize the latest findings, highlight challenges and recent successes, and offer perspectives on the future of mRNA vaccines. The data suggest that mRNA vaccines have the potential to solve many of the challenges in vaccine development for both infectious diseases and cancer.

Basic mRNA vaccine pharmacology

mRNA is the intermediate step between the translation of protein-encoding DNA and the production of proteins by ribosomes in the cytoplasm. Two major types of RNA are currently studied as vaccines: non-replicating mRNA and virally derived, self-amplifying RNA. Conventional mRNA-based vaccines encode the antigen of interest and contain 5′ and 3′ untranslated regions (UTRs), whereas self-amplifying RNAs encode not only the antigen but also the viral replication machinery that enables intracellular RNA amplification and abundant protein expression.

The construction of optimally translated IVT mRNA suitable for therapeutic use has been reviewed previously[14,15]. Briefly, IVT mRNA is produced from a linear DNA template using a T7, a T3 or an Sp6 phage RNA polymerase[16]. The resulting product should optimally contain an open reading frame that encodes the protein of interest, flanking UTRs, a 5′ cap and a poly(A) tail. The mRNA is thus engineered to resemble fully processed mature mRNA molecules as they occur naturally in the cytoplasm of eukaryotic cells.

Complexing of mRNA for *in vivo* delivery has also been recently detailed[10,11]. Naked mRNA is quickly degraded by extracellular RNases[17] and is not internalized efficiently. Thus, a great variety of *in vitro* and *in vivo* transfection reagents have been developed that facilitate cellular uptake of mRNA and protect it from degradation. Once the mRNA transits to the cytosol, the cellular translation machinery produces protein that undergoes post-translational modifications, resulting in a properly folded, fully functional protein. This feature of mRNA pharmacology is particularly advantageous for vaccines and protein replacement therapies that require cytosolic or transmembrane proteins to be delivered to the correct cellular compartments for proper presentation or function. IVT mRNA is finally degraded by normal physiological processes, thus reducing the risk of metabolite toxicity.

Recent advances in mRNA vaccine technology

Various mRNA vaccine platforms have been developed in recent years and validated in studies of immunogenicity and efficacy[18–20]. Engineering of the RNA sequence has rendered synthetic mRNA more translatable than ever before. Highly efficient and non-toxic RNA carriers have been developed that in some cases[21,22] allow prolonged antigen expression *in vivo* (TABLE 1). Some vaccine formulations contain novel adjuvants, while others elicit potent responses in the absence of known adjuvants. The following section summarizes the key advances in these areas of mRNA engineering and their impact on vaccine efficacy.

Optimization of mRNA translation and stability

This topic has been extensively discussed in previous reviews[14,15]; thus, we briefly summarize the key findings (BOX 1). The 5′ and 3′ UTR elements flanking the coding sequence profoundly influence the stability and translation of mRNA, both of which are critical concerns for vaccines. These regulatory sequences can be derived from viral or eukaryotic genes and greatly increase the half-life and expression of therapeutic mRNAs[23,24]. A 5′ cap structure is required for efficient protein production from mRNA[25]. Various versions of 5′ caps can be added during or after the transcription reaction using a vaccinia virus capping enzyme[26] or by incorporating synthetic cap or anti-reverse cap analogues[27,28]. The poly(A) tail also plays an important regulatory role in mRNA translation and stability[25]; thus, an optimal length of poly(A)[24] must be added to mRNA either directly from the encoding DNA template or by using poly(A) polymerase. The codon usage additionally has an impact on protein translation. Replacing rare codons with frequently used synonymous codons that have abundant cognate tRNA in the cytosol is a common practice to increase protein production from mRNA[29], although the accuracy of this model has been questioned[30]. Enrichment of G:C content constitutes another form of sequence optimization that has been shown to increase steady-state mRNA levels *in vitro*[31] and protein expression *in vivo*[12].

Box 1

Strategies for optimizing mRNA pharmacology

A number of technologies are currently used to improve the pharmacological aspects of mRNA. The various mRNA modifications used and their impact are summarized below.

- Synthetic cap analogues and capping enzymes[26,27] stabilize mRNA and increase protein translation via binding to eukaryotic translation initiation factor 4E (EIF4E)
- Regulatory elements in the 5′-untranslated region (UTR) and the 3′-UTR[23] stabilize mRNA and increase protein translation
- Poly(A) tail[25] stabilizes mRNA and increases protein translation
- Modified nucleosides[9,48] decrease innate immune activation and increase translation
- Separation and/or purification techniques: RNase III treatment (N.P. and D.W., unpublished observations) and fast protein liquid chromatography (FPLC) purification[13] decrease immune activation and increase translation
- Sequence and/or codon optimization[29] increase translation
- Modulation of target cells: co-delivery of translation initiation factors and other methods alters translation and immunogenicity

Although protein expression may be positively modulated by altering the codon composition or by introducing modified nucleosides (discussed below), it is also possible that these forms of sequence engineering could affect mRNA secondary structure[32], the kinetics and accuracy of translation and simultaneous protein folding[33,34], and the expression of cryptic T cell epitopes present in alternative reading frames[30]. All these factors could potentially influence the magnitude or specificity of the immune response.

Modulation of immunogenicity

Exogenous mRNA is inherently immunostimulatory, as it is recognized by a variety of cell surface, endosomal and cytosolic innate immune receptors (FIG. 1) (reviewed in REF. 35). Depending on the therapeutic application, this feature of mRNA could be beneficial or detrimental. It is potentially advantageous for vaccination because in some cases it may provide adjuvant activity to drive dendritic cell (DC) maturation and thus elicit robust T and B cell immune responses. However, innate immune sensing of mRNA has also been associated with the inhibition of antigen expression and may negatively affect the immune response[9,13]. Although the paradoxical effects of innate immune sensing on different formats of mRNA vaccines are incompletely understood, some progress has been made in recent years in elucidating these phenomena.

Studies over the past decade have shown that the immunostimulatory profile of mRNA can be shaped by the purification of IVT mRNA and the introduction of modified nucleosides as

well as by complexing the mRNA with various carrier molecules[9,13,36,37]. Enzymatically synthesized mRNA preparations contain double-stranded RNA (dsRNA) contaminants as aberrant products of the IVT reaction[13]. As a mimic of viral genomes and replication intermediates, dsRNA is a potent pathogen-associated molecular pattern (PAMP) that is sensed by pattern recognition receptors in multiple cellular compartments (FIG. 1). Recognition of IVT mRNA contaminated with dsRNA results in robust type I interferon production[13], which upregulates the expression and activation of protein kinase R (PKR; also known as EIF2AK2) and 2′–5′-oligoadenylate synthetase (OAS), leading to the inhibition of translation[38] and the degradation of cellular mRNA and ribosomal RNA[39], respectively. Karikó and colleagues[13] have demonstrated that contaminating dsRNA can be efficiently removed from IVT mRNA by chromatographic methods such as reverse-phase fast protein liquid chromatography (FPLC) or high-performance liquid chromatography (HPLC). Strikingly, purification by FPLC has been shown to increase protein production from IVT mRNA by up to 1,000-fold in primary human DCs[13]. Thus, appropriate purification of IVT mRNA seems to be critical for maximizing protein (immunogen) production in DCs and for avoiding unwanted innate immune activation.

Besides dsRNA contaminants, single-stranded mRNA molecules are themselves a PAMP when delivered to cells exogenously. Single-stranded oligoribonucleotides and their degradative products are detected by the endosomal sensors Toll-like receptor 7 (TLR7) and TLR8 (REFS 40,41), resulting in type I interferon production[42]. Crucially, it was discovered that the incorporation of naturally occurring chemically modified nucleosides, including but not limited to pseudouridine[9,43,44] and 1-methylpseudouridine[45], prevents activation of TLR7, TLR8 and other innate immune sensors[46,47], thus reducing type I interferon signalling[48]. Nucleoside modification also partially suppresses the recognition of dsRNA species[46–48]. As a result, Karikó and others have shown that nucleoside-modified mRNA is translated more efficiently than unmodified mRNA *in vitro*[9], particularly in primary DCs, and *in vivo* in mice[45]. Notably, the highest level of protein production in DCs was observed when mRNA was both FPLC-purified and nucleoside-modified[13]. These advances in understanding the sources of innate immune sensing and how to avoid their adverse effects have substantially contributed to the current interest in mRNA-based vaccines and protein replacement therapies.

In contrast to the findings described above, a study by Thess and colleagues found that sequence-optimized, HPLC-purified, unmodified mRNA produced higher levels of protein in HeLa cells and in mice than its nucleoside-modified counterpart[12]. Additionally, Kauffman and co-workers demonstrated that unmodified, non-HPLC-purified mRNA yielded more robust protein production in HeLa cells than nucleoside-modified mRNA, and resulted in similar levels of protein production in mice[49]. Although not fully clear, the discrepancies between the findings of Karikó[9,13] and these authors[12,49] may have arisen from variations in RNA sequence optimization, the stringency of mRNA purification to remove dsRNA contaminants and the level of innate immune sensing in the targeted cell types.

The immunostimulatory properties of mRNA can conversely be increased by the inclusion of an adjuvant to increase the potency of some mRNA vaccine formats. These include

traditional adjuvants as well as novel approaches that take advantage of the intrinsic immunogenicity of mRNA or its ability to encode immune-modulatory proteins. Self-replicating RNA vaccines have displayed increased immunogenicity and effectiveness after formulating the RNA in a cationic nanoemulsion based on the licensed MF59 (Novartis) adjuvant[50]. Another effective adjuvant strategy is TriMix, a combination of mRNAs encoding three immune activator proteins: CD70, CD40 ligand (CD40L) and constitutively active TLR4. TriMix mRNA augmented the immunogenicity of naked, unmodified, unpurified mRNA in multiple cancer vaccine studies and was particularly associated with increased DC maturation and cytotoxic T lymphocyte (CTL) responses (reviewed in REF. 51). The type of mRNA carrier and the size of the mRNA–carrier complex have also been shown to modulate the cytokine profile induced by mRNA delivery. For example, the RNActive (CureVac AG) vaccine platform[52,53] depends on its carrier to provide adjuvant activity. In this case, the antigen is expressed from a naked, unmodified, sequence-optimized mRNA, while the adjuvant activity is provided by co-delivered RNA complexed with protamine (a polycationic peptide), which acts via TLR7 signalling[52,54]. This vaccine format has elicited favourable immune responses in multiple preclinical animal studies for vaccination against cancer and infectious diseases[18,36,55,56]. A recent study provided mechanistic information on the adjuvanticity of RNActive vaccines in mice *in vivo* and human cells *in vitro*[54]. Potent activation of TLR7 (mouse and human) and TLR8 (human) and production of type I interferon, pro-inflammatory cytokines and chemokines after intradermal immunization was shown[54]. A similar adjuvant activity was also demonstrated in the context of non-mRNA-based vaccines using RNAdjuvant (CureVac AG), an unmodified, single-stranded RNA stabilized by a cationic carrier peptide[57].

Progress in mRNA vaccine delivery

Efficient *in vivo* mRNA delivery is critical to achieving therapeutic relevance. Exogenous mRNA must penetrate the barrier of the lipid membrane in order to reach the cytoplasm to be translated to functional protein. mRNA uptake mechanisms seem to be cell type dependent, and the physicochemical properties of the mRNA complexes can profoundly influence cellular delivery and organ distribution. There are two basic approaches for the delivery of mRNA vaccines that have been described to date. First, loading of mRNA into DCs *ex vivo,* followed by re-infusion of the transfected cells[58]; and second, direct parenteral injection of mRNA with or without a carrier. *Ex vivo* DC loading allows precise control of the cellular target, transfection efficiency and other cellular conditions, but as a form of cell therapy, it is an expensive and labour-intensive approach to vaccination. Direct injection of mRNA is comparatively rapid and cost-effective, but it does not yet allow precise and efficient cell-type-specific delivery, although there has been recent progress in this regard[59]. Both of these approaches have been explored in a variety of forms (FIG. 2; TABLE 1).

Ex vivo loading of DCs—DCs are the most potent antigen-presenting cells of the immune system. They initiate the adaptive immune response by internalizing and proteolytically processing antigens and presenting them to $CD8^+$ and $CD4^+$ T cells on major histocompatibility complexes (MHCs), namely, MHC class I and MHC class II, respectively. Additionally, DCs may present intact antigen to B cells to provoke an antibody response[60].

DCs are also highly amenable to mRNA transfection. For these reasons, DCs represent an attractive target for transfection by mRNA vaccines, both *in vivo* and *ex vivo.*

Although DCs have been shown to internalize naked mRNA through a variety of endocytic pathways[61–63], *ex vivo* transfection efficiency is commonly increased using electroporation; in this case, mRNA molecules pass through membrane pores formed by a high-voltage pulse and directly enter the cytoplasm (reviewed in REF. 64). This mRNA delivery approach has been favoured for its ability to generate high transfection efficiency without the need for a carrier molecule. DCs that are loaded with mRNA *ex vivo* are then re-infused into the autologous vaccine recipient to initiate the immune response. Most *ex vivo*-loaded DC vaccines elicit a predominantly cell-mediated immune response; thus, they have been used primarily to treat cancer (reviewed in REF. 58).

Injection of naked mRNA *in vivo*—Naked mRNA has been used successfully for *in vivo* immunizations, particularly in formats that preferentially target antigen-presenting cells, as in intradermal[61,65] and intranodal injections[66–68]. Notably, a recent report showed that repeated intranodal immunizations with naked, unmodified mRNA encoding tumour-associated neoantigens generated robust T cell responses and increased progression-free survival[68] (discussed further in BOX 2).

Box 2

Personalized neoepitope cancer vaccines

Sahin and colleagues have pioneered the use of individualized neoepitope mRNA cancer vaccines[121]. They use high-throughput sequencing to identify every unique somatic mutation of an individual patient's tumour sample, termed the mutanome. This enables the rational design of neoepitope cancer vaccines in a patient-specific manner, and has the advantage of targeting non-self antigen specificities that should not be eliminated by central tolerance mechanisms. Proof of concept has been recently provided: Kreiter and colleagues found that a substantial portion of non-synonymous cancer mutations were immunogenic when delivered by mRNA and were mainly recognized by $CD4^+$ T cells[176]. On the basis of these data, they generated a computational method to predict major histocompatibility complex (MHC) class II-restricted neoepitopes that can be used as vaccine immunogens. mRNA vaccines encoding such neoepitopes have controlled tumour growth in B16-F10 melanoma and CT26 colon cancer mouse models. In a recent clinical trial, Sahin and colleagues developed personalized neoepitope-based mRNA vaccines for 13 patients with metastatic melanoma, a cancer known for its high frequency of somatic mutations and thus neoepitopes. They immunized against ten neoepitopes per individual by injecting naked mRNA intranodally. $CD4^+$ T cell responses were detected against the majority of the neoepitopes, and a low frequency of metastatic disease was observed after several months of follow-up[68]. Interestingly, similar results were also obtained in a study of analogous design that used synthetic peptides as immunogens rather than mRNA[177]. Together, these recent trials suggest the potential utility of the personalized vaccine methodology.

Physical delivery methods in vivo—To increase the efficiency of mRNA uptake *in vivo*, physical methods have occasionally been used to penetrate the cell membrane. An early report showed that mRNA complexed with gold particles could be expressed in tissues using a gene gun, a microprojectile method[69]. The gene gun was shown to be an efficient RNA delivery and vaccination method in mouse models[70–73], but no efficacy data in large animals or humans are available. *In vivo* electroporation has also been used to increase uptake of therapeutic RNA[74–76]; however, in one study, electroporation increased the immunogenicity of only a self-amplifying RNA and not a non-replicating mRNA-based vaccine[74]. Physical methods can be limited by increased cell death and restricted access to target cells or tissues. Recently, the field has instead favoured the use of lipid or polymer-based nanoparticles as potent and versatile delivery vehicles.

Protamine—The cationic peptide protamine has been shown to protect mRNA from degradation by serum RNases[77]; however, protamine-complexed mRNA alone demonstrated limited protein expression and efficacy in a cancer vaccine model, possibly owing to an overly tight association between protamine and mRNA[36,78]. This issue was resolved by developing the RNActive vaccine platform, in which protamine-formulated RNA serves only as an immune activator and not as an expression vector[52].

Cationic lipid and polymer-based delivery—Highly efficient mRNA transfection reagents based on cationic lipids or polymers, such as TransIT-mRNA (Mirus Bio LLC) or Lipofectamine (Invitrogen), are commercially available and work well in many primary cells and cancer cell lines[9,13], but they often show limited *in vivo* efficacy or a high level of toxicity (N.P. and D.W., unpublished observations). Great progress has been made in developing similarly designed complexing reagents for safe and effective *in vivo* use, and these are discussed in detail in several recent reviews[10,11,79,80]. Cationic lipids and polymers, including dendrimers, have become widely used tools for mRNA administration in the past few years. The mRNA field has clearly benefited from the substantial investment in *in vivo* small interfering RNA (siRNA) administration, where these delivery vehicles have been used for over a decade. Lipid nanoparticles (LNPs) have become one of the most appealing and commonly used mRNA delivery tools. LNPs often consist of four components: an ionizable cationic lipid, which promotes self-assembly into virus-sized (~100 nm) particles and allows endosomal release of mRNA to the cytoplasm; lipid-linked polyethylene glycol (PEG), which increases the half-life of formulations; cholesterol, a stabilizing agent; and naturally occurring phospholipids, which support lipid bilayer structure. Numerous studies have demonstrated efficient *in vivo* siRNA delivery by LNPs (reviewed in REF. 81), but it has only recently been shown that LNPs are potent tools for *in vivo* delivery of self-amplifying RNA[19] and conventional, non-replicating mRNA[21]. Systemically delivered mRNA–LNP complexes mainly target the liver owing to binding of apolipoprotein E and subsequent receptor-mediated uptake by hepatocytes[82], and intradermal, intramuscular and subcutaneous administration have been shown to produce prolonged protein expression at the site of the injection[21,22]. The mechanisms of mRNA escape into the cytoplasm are incompletely understood, not only for artificial liposomes but also for naturally occurring exosomes[83]. Further research into this area will likely be of great benefit to the field of therapeutic RNA delivery.

The magnitude and duration of *in vivo* protein production from mRNA–LNP vaccines can be controlled in part by varying the route of administration. Intramuscular and intradermal delivery of mRNA–LNPs has been shown to result in more persistent protein expression than systemic delivery routes: in one experiment, the half-life of mRNA-encoded firefly luciferase was roughly threefold longer after intradermal injection than after intravenous delivery[21]. These kinetics of mRNA–LNP expression may be favourable for inducing immune responses. A recent study demonstrated that sustained antigen availability during vaccination was a driver of high antibody titres and germinal centre (GC) B cell and T follicular helper (T_{FH}) cell responses[84]. This process was potentially a contributing factor to the potency of recently described nucleoside-modified mRNA–LNP vaccines delivered by the intramuscular and intradermal routes[20,22,85]. Indeed, T_{FH} cells have been identified as a critical population of immune cells that vaccines must activate in order to generate potent and long-lived neutralizing antibody responses, particularly against viruses that evade humoral immunity[86]. The dynamics of the GC reaction and the differentiation of T_{FH} cells are incompletely understood, and progress in these areas would undoubtedly be fruitful for future vaccine design (BOX 3).

Box 3

The germinal centre and T follicular helper cells

The vast majority of potent antimicrobial vaccines elicit long-lived, protective antibody responses against the target pathogen. High-affinity antibodies are produced in specialized microanatomical sites within the B cell follicles of secondary lymphoid organs called germinal centres (GCs). B cell proliferation, somatic hypermutation and selection for high affinity mutants occur in the GCs, and efficient T cell help is required for these processes[178]. Characterization of the relationship between GC B and T cells has been actively studied in recent years. The follicular homing receptor CXC chemokine receptor 5 (CXCR5) was identified on GC B and T cells in the 1990s[179,180], but the concept of a specific lineage of T follicular helper (T_{FH}) cells was not proposed until 2000 (REFS 181,182). The existence of the T_{FH} lineage was confirmed in 2009 when the transcription factor specific for T_{FH} cells, B cell lymphoma 6 protein (BCL-6), was identified[183–185]. T_{FH} cells represent a specialized subset of $CD4^+$ T cells that produce critical signals for B cell survival, proliferation and differentiation in addition to signals for isotype switching of antibodies and for the introduction of diversifying mutations into the immunoglobulin genes. The major cytokines produced by T_{FH} cells are interleukin-4 (IL-4) and IL-21, which play a key role in driving the GC reaction. Other important markers and functional ligands expressed by T_{FH} cells include CD40 ligand (CD40L), Src homology domain 2 (SH2) domain-containing protein 1A (SH2D1A), programmed cell death protein 1 (PD1) and inducible T cell co stimulator (ICOS)[186]. The characterization of rare, broadly neutralizing antibodies to HIV-1 has revealed that unusually high rates of somatic hypermutation are a hallmark of protective antibody responses against HIV-1 (REF. 187). As T_{FH} cells play a key role in driving this process in GC reactions, the development of new adjuvants or vaccine platforms that can potently activate this cell type is urgently needed.

mRNA vaccines against infectious diseases

Development of prophylactic or therapeutic vaccines against infectious pathogens is the most efficient means to contain and prevent epidemics. However, conventional vaccine approaches have largely failed to produce effective vaccines against challenging viruses that cause chronic or repeated infections, such as HIV-1, herpes simplex virus and respiratory syncytial virus (RSV). Additionally, the slow pace of commercial vaccine development and approval is inadequate to respond to the rapid emergence of acute viral diseases, as illustrated by the 2014–2016 outbreaks of the Ebola and Zika viruses. Therefore, the development of more potent and versatile vaccine platforms is crucial.

Preclinical studies have created hope that mRNA vaccines will fulfil many aspects of an ideal clinical vaccine: they have shown a favourable safety profile in animals, are versatile and rapid to design for emerging infectious diseases, and are amenable to scalable good manufacturing practice (GMP) production (already under way by several companies). Unlike protein immunization, several formats of mRNA vaccines induce strong $CD8^+$ T cell responses, likely owing to the efficient presentation of endogenously produced antigens on MHC class I molecules, in addition to potent $CD4^+$ T cell responses[56,87,88]. Additionally, unlike DNA immunization, mRNA vaccines have shown the ability to generate potent neutralizing antibody responses in animals with only one or two low-dose immunizations[20,22,85]. As a result, mRNA vaccines have elicited protective immunity against a variety of infectious agents in animal models[19,20,22,56,89,90] and have therefore generated substantial optimism. However, recently published results from two clinical trials of mRNA vaccines for infectious diseases were somewhat modest, leading to more cautious expectations about the translation of preclinical success to the clinic[22,91] (discussed further below).

Two major types of RNA vaccine have been utilized against infectious pathogens: self-amplifying or replicon RNA vaccines and non-replicating mRNA vaccines. Non-replicating mRNA vaccines can be further distinguished by their delivery method: *ex vivo* loading of DCs or direct *in vivo* injection into a variety of anatomical sites. As discussed below, a rapidly increasing number of preclinical studies in these areas have been published recently, and several have entered human clinical trials (TABLE 2).

Self-amplifying mRNA vaccines

Most currently used self-amplifying mRNA (SAM) vaccines are based on an alphavirus genome[92], where the genes encoding the RNA replication machinery are intact but the genes encoding the structural proteins are replaced with the antigen of interest. The full-length RNA is ~9 kb long and can be easily produced by IVT from a DNA template. The SAM platform enables a large amount of antigen production from an extremely small dose of vaccine owing to intracellular replication of the antigen-encoding RNA. An early study reported that immunization with 10 μg of naked SAM vaccine encoding RSV fusion (F), influenza virus haemagglutinin (HA) or louping ill virus pre-membrane and envelope (prM-E) proteins resulted in antibody responses and partial protection from lethal viral challenges in mice[93]. The development of RNA complexing agents brought remarkable improvement to the efficacy of SAM vaccines. As little as 100 ng of an RNA replicon vaccine encoding RSV

F, complexed to LNP, resulted in potent T and B cell immune responses in mice, and 1 μg elicited protective immune responses against RSV infection in a cotton rat intranasal challenge system[19]. SAM vaccines encoding influenza virus antigens in LNPs or an oil-in-water cationic nanoemulsion induced potent immune responses in ferrets and conferred protection from homologous and heterologous viral challenge in mice[94–96]. Further studies demonstrated the immunogenicity of this vaccine platform against diverse viruses in multiple species, including human cytomegalovirus (CMV), hepatitis C virus and rabies virus in mice, HIV-1 in rabbits, and HIV-1 and human CMV in rhesus macaques[50,87,97]. Replicon RNA encoding influenza antigens, complexed with chitosan-containing LNPs or polyethylenimine (PEI), has elicited T and B cell immune responses in mice after subcutaneous delivery[98,99]. Chahal and colleagues developed a delivery platform consisting of a chemically modified, ionizable dendrimer complexed into LNPs[89]. Using this platform, they demonstrated that intramuscular delivery of RNA replicons encoding influenza virus, Ebola virus or *Toxoplasma gondii* antigens protected mice against lethal infection[89]. The same group recently demonstrated that vaccination with an RNA replicon encoding Zika virus prM-E formulated in the same manner elicited antigen-specific antibody and $CD8^+$ T cell responses in mice[88]. Another recent study reported immunogenicity and moderate protective efficacy of SAM vaccines against bacterial pathogens, namely *Streptococcus* (groups A and B) spp., further demonstrating the versatility of this platform[100].

One of the advantages of SAM vaccines is that they create their own adjuvants in the form of dsRNA structures, replication intermediates and other motifs that may contribute to their high potency. However, the intrinsic nature of these PAMPs may make it difficult to modulate the inflammatory profile or reactogenicity of SAM vaccines. Additionally, size constraints of the insert are greater for SAM vaccines than for mRNAs that do not encode replicon genes, and the immunogenicity of the replication proteins may theoretically limit repeated use.

Dendritic cell mRNA vaccines

As described above, *ex vivo* DC loading is a heavily pursued method to generate cell-mediated immunity against cancer. Development of infectious disease vaccines using this approach has been mainly limited to a therapeutic vaccine for HIV-1: HIV-1-infected individuals on highly active antiretroviral therapy were treated with autologous DCs electroporated with mRNA encoding various HIV-1 antigens, and cellular immune responses were evaluated[101–106]. This intervention proved to be safe and elicited antigen-specific $CD4^+$ and $CD8^+$ T cell responses, but no clinical benefit was observed. Another study in humans evaluated a CMV pp65 mRNA-loaded DC vaccination in healthy human volunteers and allogeneic stem cell recipients and reported induction or expansion of CMV-specific cellular immune responses[107].

Direct injection of non-replicating mRNA vaccines

Directly injectable, non-replicating mRNA vaccines are an appealing vaccine format owing to their simple and economical administration, particularly in resource-limited settings. Although an early report demonstrated that immunization with liposome-complexed mRNA encoding influenza virus nucleoproteins elicited CTL responses in mice[108], the first

demonstration of protective immune responses by mRNA vaccines against infectious pathogens was published only a few years ago[18]. This seminal work demonstrated that intradermally administered uncomplexed mRNA encoding various influenza virus antigens combined with a protamine-complexed RNA adjuvant was immunogenic in multiple animal models and protected mice from lethal viral challenge.

Immunization with the protamine-based RNActive platform encoding rabies virus glycoprotein has also induced protective immunity against a lethal intracerebral virus challenge in mice and potent neutralizing antibody responses in pigs[56]. In a recently published seminal work, Alberer and colleagues evaluated the safety and immunogenicity of this vaccine in 101 healthy human volunteers[91]. Subjects received 80–640 μg of mRNA vaccine three times by needle-syringe or needle-free devices, either intradermally or intramuscularly. Seven days after vaccination, nearly all participants reported mild to moderate injection site reactions, and 78% experienced a systemic reaction (for example, fever, headache and chills). There was one serious adverse event that was possibly related to the vaccine: a transient and moderate case of Bell palsy. Surprisingly, the needle-syringe injections did not generate detectable neutralizing antibodies in 98% of recipients. By contrast, needle-free delivery induced variable levels of neutralizing antibodies, the majority of which peaked above the expected protective threshold but then largely waned after 1 year in subjects who were followed up long term. Elucidating the basis of the disparate immunogenicity between the animals and humans who received this vaccine and between the two routes of delivery will be informative for future vaccine design using this platform.

Other infectious disease vaccines have successfully utilized lipid- or polymer-based delivery systems. Cationic 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) and dioleoylphosphatidylethanolamine (DOPE) lipid-complexed mRNA encoding HIV-1 gag generated antigen-specific $CD4^+$ and $CD8^+$ T cell responses after subcutaneous delivery in mice[109]. Two other studies demonstrated that PEI-complexed mRNAs could be efficiently delivered to mice to induce HIV-1-specific immune responses: subcutaneously delivered mRNA encoding HIV-1 gag elicited $CD4^+$ and $CD8^+$ T cell responses, and intranasally administered mRNA encoding the HIV-1 envelope gp120 subunit crossed the nasal epithelium and generated antigen-specific immune responses in the nasal cavity[110,111]. Kranz and colleagues also performed intravenous immunizations in mice using lipid-complexed mRNA encoding influenza virus HA and showed evidence of T cell activation after a single dose[59].

Nucleoside-modified mRNA vaccines represent a new and highly efficacious category of mRNA vaccines. Owing to the novelty of this immunization platform, our knowledge of efficacy is limited to the results of four recent publications that demonstrated the potency of such vaccines in small and large animals. The first published report demonstrated that a single intradermal injection of LNP-formulated mRNA encoding Zika virus prM-E, modified with 1-methylpseudouridine and FPLC purification, elicited protective immune responses in mice and rhesus macaques with the use of as little as 50 μg (0.02 mg kg^{-1}) of vaccine in macaques[20]. A subsequent study by a different group tested a similarly designed vaccine against Zika virus in mice and found that a single intramuscular immunization elicited moderate immune responses, and a booster vaccination resulted in potent and

protective immune responses[85]. This vaccine also incorporated the modified nucleoside 1-methylpseudouridine, but FPLC purification or other methods of removing dsRNA contaminants were not reported. Notably, this report showed that antibody-dependent enhancement of secondary infection with a heterologous flavivirus, a major concern for dengue and Zika virus vaccines, could be diminished by removing a cross-reactive epitope in the E protein. A recent follow-up study evaluated the same vaccine in a model of maternal vaccination and fetal infection[112]. Two immunizations reduced Zika virus infection in fetal mice by several orders of magnitude and completely rescued a defect in fetal viability.

Another recent report evaluated the immunogenicity of LNP-complexed, nucleoside-modified, non-FPLC-purified mRNA vaccines against influenza HA 10 neuraminidase 8 (H10N8) and H7N9 influenza viruses in mice, ferrets, non-human primates and, for the first time, humans[22]. A single intradermal or intramuscular immunization with low doses (0.4–10 μg) of LNP-complexed mRNA encoding influenza virus HA elicited protective immune responses against homologous influenza virus challenge in mice. Similar results were obtained in ferrets and cynomolgus monkeys after immunization with one or two doses of 50–400 μg of a vaccine containing LNP-complexed mRNA encoding HA, corroborating that the potency of mRNA–LNP vaccines translates to larger animals, including non-human primates.

On the basis of encouraging preclinical data, two phase I clinical trials have recently been initiated to evaluate the immunogenicity and safety of nucleoside-modified mRNA–LNP vaccines in humans for the first time. The mRNA vaccine encoding H10N8 HA is currently undergoing clinical testing (NCT03076385), and interim findings for 23 vaccinated individuals have been reported[22]. Participants received a small amount (100 μg) of vaccine intramuscularly, and immunogenicity was measured 43 days after vaccination. The vaccine proved to be immunogenic in all subjects, as measured by haemagglutination inhibition and microneutralization antibody assays. Promisingly, antibody titres were above the expected protective threshold, but they were moderately lower than in the animal models. Similarly to the study by Alberer *et al.*[91], most vaccinated subjects reported mild to moderate reactogenicity (injection site pain, myalgia, headache, fatigue and chills), and three subjects reported severe injection site reactions or a systemic common cold-like response. This level of reactogenicity appears to be similar to that of more traditional vaccine formats[113,114]. Finally, the Zika virus vaccine described by Richner *et al.*[85,112] is also entering clinical evaluation in a combined phase I/II trial (NCT03014089). Future studies that apply nucleoside-modified mRNA–LNP vaccines against a greater diversity of antigens will reveal the extent to which this strategy is broadly applicable to infectious disease vaccines.

mRNA cancer vaccines

mRNA-based cancer vaccines have been recently and extensively reviewed[115–119]. Below, the most recent advances and directions are highlighted. Cancer vaccines and other immunotherapies represent promising alternative strategies to treat malignancies. Cancer vaccines can be designed to target tumour-associated antigens that are preferentially expressed in cancerous cells, for example, growth-associated factors, or antigens that are unique to malignant cells owing to somatic mutation[120]. These neoantigens, or the

neoepitopes within them, have been deployed as mRNA vaccine targets in humans[121] (BOX 2). Most cancer vaccines are therapeutic, rather than prophylactic, and seek to stimulate cell-mediated responses, such as those from CTLs, that are capable of clearing or reducing tumour burden[122]. The first proof-of-concept studies that not only proposed the idea of RNA cancer vaccines but also provided evidence of the feasibility of this approach were published more than two decades ago[123,124]. Since then, numerous preclinical and clinical studies have demonstrated the viability of mRNA vaccines to combat cancer (TABLE 3).

DC mRNA cancer vaccines

As DCs are central players in initiating antigen-specific immune responses, it seemed logical to utilize them for cancer immunotherapy. The first demonstration that DCs electroporated with mRNA could elicit potent immune responses against tumour antigens was reported by Boczkowski and colleagues in 1996 (REF. 124). In this study, DCs pulsed with ovalbumin (OVA)-encoding mRNA or tumour-derived RNAs elicited a tumour-reducing immune response in OVA-expressing and other melanoma models in mice. A variety of immune regulatory proteins have been identified in the form of mRNA-encoded adjuvants that can increase the potency of DC cancer vaccines. Several studies demonstrated that electroporation of DCs with mRNAs encoding co-stimulatory molecules such as CD83, tumour necrosis factor receptor superfamily member 4 (TNFRSF4; also known as OX40) and 4-1BB ligand (4-1BBL) resulted in a substantial increase in the immune stimulatory activity of DCs[125–128]. DC functions can also be modulated through the use of mRNA-encoded pro-inflammatory cytokines, such as IL-12, or trafficking-associated molecules[129–131]. As introduced above, TriMix is a cocktail of mRNA-encoded adjuvants (CD70, CD40L and constitutively active TLR4) that can be electroporated in combination with antigen-encoding mRNA or mRNAs[132]. This formulation proved efficacious in multiple pre-clinical studies by increasing DC activation and shifting the $CD4^+$ T cell phenotype from T regulatory cells to T helper 1 (T_H1)-like cells[132–136]. Notably, the immunization of patients with stage III or stage IV melanoma using DCs loaded with mRNA encoding melanoma-associated antigens and TriMix adjuvant resulted in tumour regression in 27% of treated individuals[137]. Multiple clinical trials have now been conducted using DC vaccines targeting various cancer types, such as metastatic prostate cancer, metastatic lung cancer, renal cell carcinoma, brain cancers, melanoma, acute myeloid leukaemia, pancreatic cancer and others[138,139] (reviewed in REFS 51,58).

A new line of research combines mRNA electroporation of DCs with traditional chemotherapy agents or immune checkpoint inhibitors. In one trial, patients with stage III or IV melanoma were treated with ipilimumab, a monoclonal antibody against CTL antigen 4 (CTLA4), and DCs loaded with mRNA encoding melanoma-associated antigens plus TriMix. This intervention resulted in durable tumour reduction in a proportion of individuals with recurrent or refractory melanoma[140].

Direct injection of mRNA cancer vaccines

The route of administration and delivery format of mRNA vaccines can greatly influence outcomes. A variety of mRNA cancer vaccine formats have been developed using common

delivery routes (intradermal, intramuscular, subcutaneous or intranasal) and some unconventional routes of vaccination (intranodal, intravenous, intrasplenic or intratumoural).

Intranodal administration of naked mRNA is an unconventional but efficient means of vaccine delivery. Direct mRNA injection into secondary lymphoid tissue offers the advantage of targeted antigen delivery to antigen-presenting cells at the site of T cell activation, obviating the need for DC migration. Several studies have demonstrated that intranodally injected naked mRNA can be selectively taken up by DCs and can elicit potent prophylactic or therapeutic anti-tumour T cell responses[62,66]; an early study also demonstrated similar findings with intrasplenic delivery[141]. Coadministration of the DC-activating protein FMS-related tyrosine kinase 3 ligand (FLT3L) was shown in some cases to further improve immune responses to intranodal mRNA vaccination[142,143]. Incorporation of the TriMix adjuvant into intranodal injections of mice with mRNAs encoding tumour-associated antigens resulted in potent antigen-specific CTL responses and tumour control in multiple tumour models[133]. A more recent study demonstrated that intranodal injection of mRNA encoding the E7 protein of human papillomavirus (HPV) 16 with TriMix increased the number of tumour-infiltrating $CD8^+$ T cells and inhibited the growth of an E7-expressing tumour model in mice[67].

The success of preclinical studies has led to the initiation of clinical trials using intranodally injected naked mRNA encoding tumour-associated antigens into patients with advanced melanoma (NCT01684241) and patients with hepatocellular carcinoma (EudraCT: 2012-005572-34). In one published trial, patients with metastatic melanoma were treated with intranodally administered DCs electroporated with mRNA encoding the melanoma-associated antigens tyrosinase or gp100 and TriMix, which induced limited antitumour responses[144].

Intranasal vaccine administration is a needle-free, noninvasive manner of delivery that enables rapid antigen uptake by DCs. Intranasally delivered mRNA complexed with Stemfect (Stemgent) LNPs resulted in delayed tumour onset and increased survival in prophylactic and therapeutic mouse tumour models using the OVA-expressing E.G7-OVA T lymphoblastic cell line[145].

Intratumoural mRNA vaccination is a useful approach that offers the advantage of rapid and specific activation of tumour-resident T cells. Often, these vaccines do not introduce mRNAs encoding tumour-associated antigens but simply aim to activate tumour-specific immunity *in situ* using immune stimulatory molecules. An early study demonstrated that naked mRNA or protamine-stabilized mRNA encoding a non-tumour related gene (*GLB1*) impaired tumour growth and provided protection in a glioblastoma mouse model, taking advantage of the intrinsic immunogenic properties of mRNA[146]. A more recent study showed that intratumoural delivery of mRNA encoding an engineered cytokine based on interferon-β (IFNβ) fused to a transforming growth factor-β (TGFβ) antagonist increased the cytolytic capacity of $CD8^+$ T cells and modestly delayed tumour growth in OVA-expressing lymphoma or lung carcinoma mouse models[147]. It has also been shown that intratumoural administration of TriMix mRNA that does not encode tumour-associated antigens results in

activation of $CD8\alpha^+$ DCs and tumour-specific T cells, leading to delayed tumour growth in various mouse models[148].

Systemic administration of mRNA vaccines is not common owing to concerns about aggregation with serum proteins and rapid extracellular mRNA degradation; thus, formulating mRNAs into carrier molecules is essential. As discussed above, numerous delivery formulations have been developed to facilitate mRNA uptake, increase protein translation and protect mRNA from RNases[10,11,79,80]. Another important issue is the biodistribution of mRNA vaccines after systemic delivery. Certain cationic LNP-based complexing agents delivered intravenously traffic mainly to the liver[21], which may not be ideal for DC activation. An effective strategy for DC targeting of mRNA vaccines after systemic delivery has recently been described[59]. An mRNA–lipoplex (mRNA–liposome complex) delivery platform was generated using cationic lipids and neutral helper lipids formulated with mRNA, and it was discovered that the lipid-to-mRNA ratio, and thus the net charge of the particles, has a profound impact on the biodistribution of the vaccine. While a positively charged lipid particle primarily targeted the lung, a negatively charged particle targeted DCs in secondary lymphoid tissues and bone marrow. The negatively charged particle induced potent immune responses against tumour-specific antigens that were associated with impressive tumour reduction in various mouse models[59]. As no toxic effects were observed in mice or non-human primates, clinical trials using this approach to treat patients with advanced melanoma or triple-negative breast cancer have been initiated (NCT02410733 and NCT02316457).

A variety of antigen-presenting cells reside in the skin[149], making it an ideal site for immunogen delivery during vaccination (FIG. 3). Thus, the intradermal route of delivery has been widely used for mRNA cancer vaccines. An early seminal study demonstrated that intradermal administration of total tumour RNA delayed tumour growth in a fibrosarcoma mouse model[65]. Intradermal injection of mRNA encoding tumour antigens in the protamine-based RNActive platform proved efficacious in various mouse models of cancer[36] and in multiple prophylactic and therapeutic clinical settings (TABLE 3). One such study demonstrated that mRNAs encoding survivin and various melanoma tumour antigens resulted in increased numbers of antigen-specific T cells in a subset of patients with melanoma[150]. In humans with castration-resistant prostate cancer, an RNActive vaccine expressing multiple prostate cancer-associated proteins elicited antigen-specific T cell responses in the majority of recipients[151]. Lipid-based carriers have also contributed to the efficacy of intra-dermally delivered mRNA cancer vaccines. The delivery of OVA-encoding mRNA in DOTAP and/or DOPE liposomes resulted in antigen-specific CTL activity and inhibited growth of OVA-expressing tumours in mice[152]. In the same study, coadministration of mRNA encoding granulocyte–macrophage colony-stimulating factor (GM-CSF) improved OVA-specific cytolytic responses. Another report showed that subcutaneous delivery of LNP-formulated mRNA encoding two melanoma-associated antigens delayed tumour growth in mice, and co-delivery of lipopolysaccharide (LPS) in LNPs increased both CTL and antitumour activity[153]. In general, mRNA cancer vaccines have proved immunogenic in humans, but further refinement of vaccination methods, as informed by basic immunological research, will likely be necessary to achieve greater clinical benefits.

The combination of mRNA vaccination with adjunctive therapies, such as traditional chemotherapy, radiotherapy and immune checkpoint inhibitors, has increased the beneficial outcome of vaccination in some preclinical studies[154,155]. For example, cisplatin treatment significantly increased the therapeutic effect of immunizing with mRNA encoding the HPV16 E7 oncoprotein and TriMix, leading to the complete rejection of female genital tract tumours in a mouse model[67]. Notably, it has also been suggested that treatment with antibodies against programmed cell death protein 1 (PD1) increased the efficacy of a neoepitope mRNA-based vaccine against metastatic melanoma in humans, but more data are required to explore this hypothesis[68].

Therapeutic considerations and challenges

Good manufacturing practice production

mRNA is produced by *in vitro* reactions with recombinant enzymes, ribonucleotide triphosphates (NTPs) and a DNA template; thus, it is rapid and relatively simple to produce in comparison with traditional protein subunit and live or inactivated virus vaccine production platforms. Its reaction yield and simplicity make rapid mRNA production possible in a small GMP facility footprint. The manufacturing process is sequence-independent and is primarily dictated by the length of the RNA, the nucleotide and capping chemistry and the purification of the product; however, it is possible that certain sequence properties such as extreme length may present difficulties (D.W., unpublished observations). According to current experience, the process can be standardized to produce nearly any encoded protein immunogen, making it particularly suitable for rapid response to emerging infectious diseases.

All enzymes and reaction components required for the GMP production of mRNA can be obtained from commercial suppliers as synthesized chemicals or bacterially expressed, animal component-free reagents, thereby avoiding safety concerns surrounding the adventitious agents that plague cell-culture-based vaccine manufacture. All the components, such as plasmid DNA, phage polymerases, capping enzymes and NTPs, are readily available as GMP-grade traceable components; however, some of these are currently available at only limited scale or high cost. As mRNA therapeutics move towards commercialization and the scale of production increases, more economical options may become accessible for GMP source materials.

GMP production of mRNA begins with DNA template production followed by enzymatic IVT and follows the same multistep protocol that is used for research scale synthesis, with added controls to ensure the safety and potency of the product[16]. Depending on the specific mRNA construct and chemistry, the protocol may be modified slightly from what is described here to accommodate modified nucleosides, capping strategies or template removal. To initiate the production process, template plasmid DNA produced in *Escherichia coli* is linearized using a restriction enzyme to allow synthesis of runoff transcripts with a poly(A) tract at the 3′ end. Next, the mRNA is synthesized from NTPs by a DNA-dependent RNA polymerase from bacteriophage (such as T7, SP6, or T3). The template DNA is then degraded by incubation with DNase. Finally, the mRNA is enzymatically or chemically capped to enable efficient translation *in vivo*. mRNA synthesis is highly

productive, yielding in excess of 2 g l^{-1} of full-length mRNA in multi-gram scale reactions under optimized conditions.

Once the mRNA is synthesized, it is processed though several purification steps to remove reaction components, including enzymes, free nucleotides, residual DNA and truncated RNA fragments. While LiCl precipitation is routinely used for laboratory-scale preparation, purification at the clinical scale utilizes derivatized microbeads in batch or column formats, which are easier to utilize at large scale[156,157]. For some mRNA platforms, removal of dsRNA and other contaminants is critical for the potency of the final product, as it is a potent inducer of interferon-dependent translation inhibition. This has been accomplished by reverse-phase FPLC at the laboratory scale[158], and scalable aqueous purification approaches are being investigated. After mRNA is purified, it is exchanged into a final storage buffer and sterile-filtered for subsequent filling into vials for clinical use. RNA is susceptible to degradation by both enzymatic and chemical pathways[157]. Formulation buffers are tested to ensure that they are free of contaminating RNases and may contain buffer components, such as antioxidants and chelators, which minimize the effects of reactive oxygen species and divalent metal ions that lead to mRNA instability[159].

Pharmaceutical formulation of mRNAs is an active area of development. Although most products for early phase studies are stored frozen (−70 °C), efforts to develop formulations that are stable at higher temperatures more suitable for vaccine distribution are continuing. Published reports suggest that stable refrigerated or room temperature formulations can be made. The RNActive platform was reported to be active after lyophilization and storage at 5–25 °C for 3 years and at 40 °C for 6 months[91]. Another report demonstrated that freeze-dried naked mRNA is stable for at least 10 months under refrigerated conditions[160]. The stability of mRNA products might also be improved by packaging within nanoparticles or by co-formulation with RNase inhibitors[161]. For lipid-encapsulated mRNA, at least 6 months of stability has been observed (Arbutus Biopharma, personal communication), but longer-term storage of such mRNA–lipid complexes in an unfrozen form has not yet been reported.

Regulatory aspects

There is no specific guidance from the FDA or European Medicines Agency (EMA) for mRNA vaccine products. However, the increasing number of clinical trials conducted under EMA and FDA oversight indicate that regulators have accepted the approaches proposed by various organizations to demonstrate that products are safe and acceptable for testing in humans. Because mRNA falls into the broad vaccine category of genetic immunogens, many of the guiding principles that have been defined for DNA vaccines[162] and gene therapy vectors[163,164] can likely be applied to mRNA with some adaptations to reflect the unique features of mRNA. A detailed review of EMA regulations for RNA vaccines by Hinz and colleagues highlights the different regulatory paths stipulated for prophylactic infectious disease versus therapeutic applications[165]. Regardless of the specific classification within existing guidelines, some themes can be observed in what is stated in these guidance documents and in what has been reported for recently published clinical studies. In particular, the recent report of an mRNA vaccine against influenza virus highlights preclinical and clinical data demonstrating biodistribution and persistence in mice, disease

protection in a relevant animal model (ferrets), and immunogenicity, local reactogenicity and toxicity in humans[22]. As mRNA products become more prominent in the vaccine field, it is likely that specific guidance will be developed that will delineate requirements to produce and evaluate new mRNA vaccines.

Safety

The requirement for safety in modern prophylactic vaccines is extremely stringent because the vaccines are administered to healthy individuals. Because the manufacturing process for mRNA does not require toxic chemicals or cell cultures that could be contaminated with adventitious viruses, mRNA production avoids the common risks associated with other vaccine platforms, including live virus, viral vectors, inactivated virus and subunit protein vaccines. Furthermore, the short manufacturing time for mRNA presents few opportunities to introduce contaminating microorganisms. In vaccinated people, the theoretical risks of infection or integration of the vector into host cell DNA are not a concern for mRNA. For the above reasons, mRNA vaccines have been considered a relatively safe vaccine format.

Several different mRNA vaccines have now been tested from phase I to IIb clinical studies and have been shown to be safe and reasonably well tolerated (TABLES 2,3). However, recent human trials have demonstrated moderate and in rare cases severe injection site or systemic reactions for different mRNA platforms[22,91]. Potential safety concerns that are likely to be evaluated in future preclinical and clinical studies include local and systemic inflammation, the biodistribution and persistence of expressed immunogen, stimulation of auto-reactive antibodies and potential toxic effects of any non-native nucleotides and delivery system components. A possible concern could be that some mRNA-based vaccine platforms[54,166] induce potent type I interferon responses, which have been associated not only with inflammation but also potentially with autoimmunity[167,168]. Thus, identification of individuals at an increased risk of autoimmune reactions before mRNA vaccination may allow reasonable precautions to be taken. Another potential safety issue could derive from the presence of extracellular RNA during mRNA vaccination. Extracellular naked RNA has been shown to increase the permeability of tightly packed endothelial cells and may thus contribute to oedema[169]. Another study showed that extracellular RNA promoted blood coagulation and pathological thrombus formation[170]. Safety will therefore need continued evaluation as different mRNA modalities and delivery systems are utilized for the first time in humans and are tested in larger patient populations.

Conclusions and future directions

Currently, mRNA vaccines are experiencing a burst in basic and clinical research. The past 2 years alone have witnessed the publication of dozens of preclinical and clinical reports showing the efficacy of these platforms. Whereas the majority of early work in mRNA vaccines focused on cancer applications, a number of recent reports have demonstrated the potency and versatility of mRNA to protect against a wide variety of infectious pathogens, including influenza virus, Ebola virus, Zika virus, *Streptococcus* spp. and *T. gondii* (TABLES 1,2).

While preclinical studies have generated great optimism about the prospects and advantages of mRNA-based vaccines, two recent clinical reports have led to more tempered expectations[22,91]. In both trials, immunogenicity was more modest in humans than was expected based on animal models, a phenomenon also observed with DNA-based vaccines[171], and the side effects were not trivial. We caution that these trials represent only two variations of mRNA vaccine platforms, and there may be substantial differences when the expression and immunostimulatory profiles of the vaccine are changed. Further research is needed to determine how different animal species respond to mRNA vaccine components and inflammatory signals and which pathways of immune signalling are most effective in humans.

Recent advances in understanding and reducing the innate immune sensing of mRNA have aided efforts not only in active vaccination but also in several applications of passive immunization or passive immunotherapy for infectious diseases and cancer (BOX 4). Direct comparisons between mRNA expression platforms should clarify which systems are most appropriate for both passive and active immunization. Given the large number of promising mRNA platforms, further head-to-head comparisons would be of utmost value to the vaccine field because this would allow investigators to focus resources on those best suited for each application.

Box 4

mRNA-based passive immunotherapy

Recombinant monoclonal antibodies are rapidly transforming the pharmaceutical market and have become one of the most successful therapeutic classes to treat autoimmune disorders, infectious diseases, osteoporosis, hypercholesterolemia and cancer[188–192]. However, the high cost of protein production and the need for frequent systemic administration pose a major limitation to widespread accessibility. Antibody-gene transfer technologies could potentially overcome these difficulties, as they administer nucleotide sequences encoding monoclonal antibodies to patients, enabling *in vivo* production of properly folded and modified protein therapeutics[193]. Multiple gene therapy vectors have been investigated (for example, viral vectors and plasmid DNA) that bear limitations such as pre-existing host immunity, acquired anti-vector immunity, high innate immunogenicity, difficulties with *in vivo* regulation of antibody production and toxic effects[193,194]. mRNA therapeutics combine safety with exquisite dose control and the potential for multiple administrations with no pre-existing or anti-vector immunity. Two early reports demonstrated that dendritic cells (DCs) electroporated with mRNAs encoding antibodies against immuno-inhibitory proteins secreted functional antibodies and improved immune responses in mice[195,196]. Three recent publications have described the use of injectable mRNA for *in vivo* production of therapeutic antibodies: Pardi and colleagues demonstrated that a single intravenous injection into mice with lipid nanoparticle (LNP)-encapsulated nucleoside-modified mRNAs encoding the heavy and light chains of the anti-HIV-1 neutralizing antibody VRC01 rapidly produced high levels of functional antibody in the serum and protected humanized mice from HIV-1 infection[197]; Stadler and co-workers demonstrated that intravenous administration of low

doses of TransIT (Mirus Bio LLC)-complexed, nucleoside-modified mRNAs encoding various anticancer bispecific antibodies resulted in the elimination of large tumours in mouse models[198]; and Thran and colleagues[199] utilized an unmodified mRNA–LNP delivery system[12] to express three monoclonal antibodies at levels that protected from lethal challenges with rabies virus, botulinum toxin and a B cell lymphoma cell line. No toxic effects were observed in any of these studies. These observations suggest that mRNA offers a safe, simple and efficient alternative to therapeutic monoclonal antibody protein delivery, with potential application to any therapeutic protein.

The fast pace of progress in mRNA vaccines would not have been possible without major recent advances in the areas of innate immune sensing of RNA and *in vivo* delivery methods. Extensive basic research into RNA and lipid and polymer biochemistry has made it possible to translate mRNA vaccines into clinical trials and has led to an astonishing level of investment in mRNA vaccine companies (TABLE 4). Moderna Therapeutics, founded in 2010, has raised almost US$2 billion in capital with a plan to commercialize mRNA-based vaccines and therapies[172,173]. The US Biomedical Advanced Research and Development Authority (BARDA) has committed support for Moderna's clinical evaluation of a promising nucleoside-modified mRNA vaccine for Zika virus (NCT03014089). In Germany, CureVac AG has an expanding portfolio of therapeutic targets[174], including both cancer and infectious diseases, and BioNTech is developing an innovative approach to personalized cancer medicine using mRNA vaccines[121] (BOX 2). The translation of basic research into clinical testing is also made more expedient by the commercialization of custom GMP products by companies such as New England Biolabs and Aldevron[175]. Finally, the recent launch of the Coalition for Epidemic Preparedness Innovations (CEPI) provides great optimism for future responses to emerging viral epidemics. This multinational public and private partnership aims to raise $1 billion to develop platform-based vaccines, such as mRNA, to rapidly contain emerging outbreaks before they spread out of control.

The future of mRNA vaccines is therefore extremely bright, and the clinical data and resources provided by these companies and other institutions are likely to substantially build on and invigorate basic research into mRNA-based therapeutics.

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Glossary

Dendritic cell (DC)

A professional antigen-presenting cell that can potently activate $CD4^+$ and $CD8^+$ T cells by presenting peptide antigens on major histocompatibility complex (MHC) class I and II molecules, respectively, along with co-stimulatory molecules

Pathogen-associated molecular pattern (PAMP)
Conserved molecular structure produced by microorganisms and recognized as an inflammatory danger signal by various innate immune receptors

Type I interferon
A family of proteins, including but not limited to interferon-β (IFNβ) and multiple isoforms of IFNα, released by cells in response to viral infections and pathogen products. Type I IFN sensing results in the upregulation of interferon-stimulated genes and an antiviral cellular state

Fast protein liquid chromatography (FPLC)
A form of liquid chromatography that can be used to purify proteins or nucleic acids. High-performance liquid chromatography (HPLC) is a similar approach, which uses high pressure to purify materials

Nucleoside modification
The incorporation of chemically modified nucleosides, such as pseudouridine, 1-methylpseudouridine, 5-methylcytidine and others, into mRNA transcripts, usually to suppress innate immune sensing and/or to improve translation

Adjuvant
An additive to vaccines that modulates and/or boosts the potency of the immune response, often allowing lower doses of antigen to be used effectively. Adjuvants may be based on pathogen-associated molecular patterns (PAMPs) or on other molecules that activate innate immune sensors

MHC class I
A polymorphic set of proteins expressed on the surface of all nucleated cells that present antigen to $CD8^+$ (including cytotoxic) T cells in the form of proteolytically processed peptides, typically 8–11 amino acids in length

MHC class II
A polymorphic set of proteins expressed on professional antigen-presenting cells and certain other cell types, which present antigen to $CD4^+$ (helper) T cells in the form of proteolytically processed peptides, typically 11–30 amino acids in length

Good manufacturing practice (GMP)
A collection of guidelines and practices designed to guarantee the production of consistently high-quality and safe pharmaceutical products. GMP-grade materials must be used for human clinical trials

Passive immunization or passive immunotherapy
In contrast to traditional (active) vaccines, these therapies do not generate *de novo* immune responses but can provide immune-mediated protection through the delivery of antibodies or

antibody-encoding genes. Passive vaccination offers the advantage of immediate action but at the disadvantage of high cost

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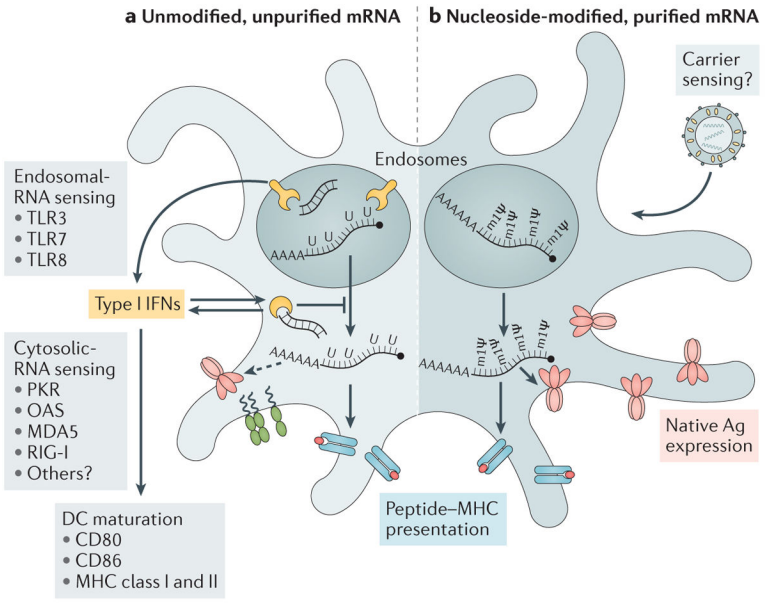

Figure 1. Innate immune sensing of mRNA vaccines

Innate immune sensing of two types of mRNA vaccine by a dendritic cell (DC), with RNA sensors shown in yellow, antigen in red, DC maturation factors in green, and peptide–major histocompatibility complex (MHC) complexes in light blue and red; an example lipid nanoparticle carrier is shown at the top right. A non-exhaustive list of the major known RNA sensors that contribute to the recognition of double-stranded and unmodified single-stranded RNAs is shown. Unmodified, unpurified (part **a**) and nucleoside-modified, fast protein liquid chromatography (FPLC)-purified (part **b**) mRNAs were selected for illustration of two formats of mRNA vaccines where known forms of mRNA sensing are present and absent, respectively. The dashed arrow represents reduced antigen expression. Ag, antigen; PKR, interferon-induced, double-stranded RNA-activated protein kinase; MDA5, interferon-induced helicase C domain-containing protein 1 (also known as IFIH1); IFN, interferon; m1Ψ, 1-methylpseudouridine; OAS, 2′–5′-oligoadenylate synthetase; TLR, Toll-like receptor.

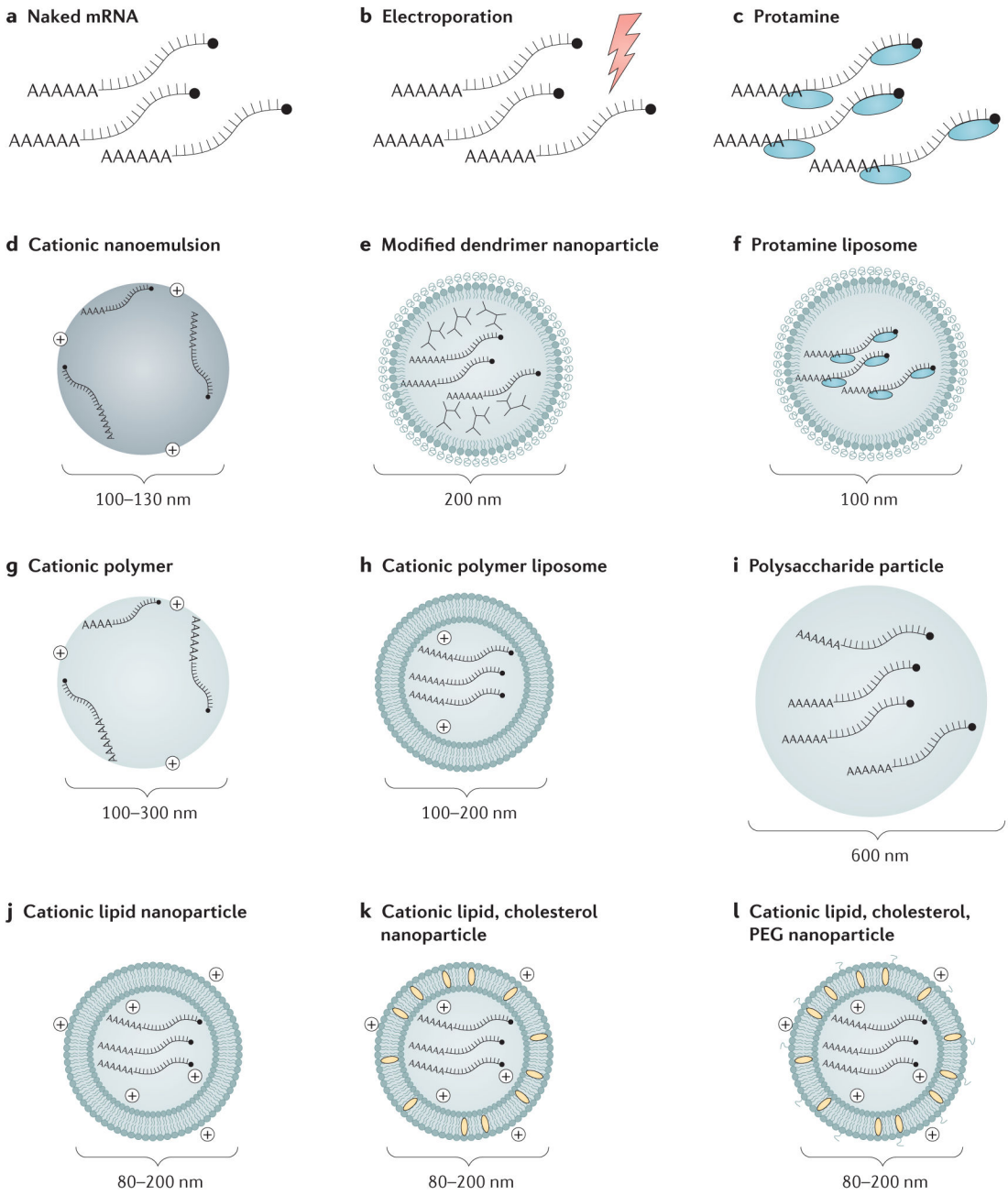

Figure 2. Major delivery methods for mRNA vaccines

Commonly used delivery methods and carrier molecules for mRNA vaccines along with typical diameters for particulate complexes are shown: naked mRNA (part **a**); naked mRNA with *in vivo* electroporation (part **b**); protamine (cationic peptide)-complexed mRNA (part **c**); mRNA associated with a positively charged oil-in-water cationic nanoemulsion (part **d**); mRNA associated with a chemically modified dendrimer and complexed with polyethylene glycol (PEG)-lipid (part **e**); protamine-complexed mRNA in a PEG-lipid nanoparticle (part **f**); mRNA associated with a cationic polymer such as polyethylenimine (PEI) (part **g**); mRNA associated with a cationic polymer such as PEI and a lipid component (part **h**);

mRNA associated with a polysaccharide (for example, chitosan) particle or gel (part **i**); mRNA in a cationic lipid nanoparticle (for example, 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) or dioleoylphosphatidylethanolamine (DOPE) lipids) (part **j**); mRNA complexed with cationic lipids and cholesterol (part **k**); and mRNA complexed with cationic lipids, cholesterol and PEG-lipid (part **l**).

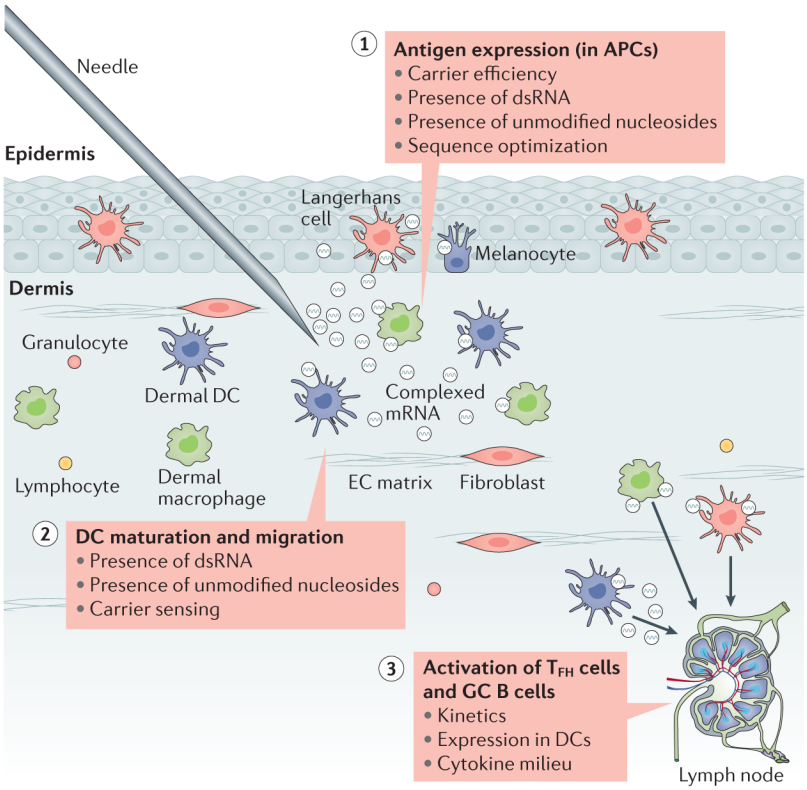

Figure 3. Considerations for effectiveness of a directly injected mRNA vaccine

For an injected mRNA vaccine, major considerations for effectiveness include the following: the level of antigen expression in professional antigen-presenting cells (APCs), which is influenced by the efficiency of the carrier, by the presence of pathogen-associated molecular patterns (PAMPs) in the form of double-stranded RNA (dsRNA) or unmodified nucleosides and by the level of optimization of the RNA sequence (codon usage, G:C content, 5′ and 3′ untranslated regions (UTRs) and so on); dendritic cell (DC) maturation and migration to secondary lymphoid tissue, which is increased by PAMPs; and the ability of the vaccine to activate robust T follicular helper (T_{FH}) cell and germinal centre (GC) B cell responses — an area that remains poorly understood. An intradermal injection is shown as an example. EC, extracellular.

Table 1

mRNA vaccine complexing strategies for *in vivo* use

Delivery system type	Route of delivery	Species	Target
Commercial transfection reagent	i.n.	Mouse	OVA[145]
Protamine	i.d.	Mouse, ferret, pig and human	Influenza virus[18,52], melanoma[150], non-small-cell lung cancer[200], prostate cancer[36,52,151], rabies virus[56], OVA[36,52,155] and Lewis lung cancer[155]
Protamine liposome	i.v.	Mouse	Lung cancer[201]
Polysaccharide particle	s.c.	Mouse and rabbit	Influenza virus[98]
Cationic nanoemulsion	i.m.	Mouse, rabbit, ferret and rhesus macaque	Influenza virus[96], RSV[50], HIV-1 (REFS 50,97), HCMV[50], *Streptococcus* spp.[100], HCV and rabies virus[87]
Cationic polymer	s.c. and i.n.	Mouse	Influenza virus[99], andHIV-1 (REFS 110,111)
Cationic polymer liposome	i.v.	Mouse	Melanoma[202,203], pancreatic cancer[204]
Cationic lipid nanoparticle	i.d., i.v. and s.c.	Mouse	HIV-1 (REF. 109) and OVA[152]
Cationic lipid, cholesterol nanoparticle	i.v., s.c. and i.s.	Mouse	Influenza virus[59,108], melanoma[59,141], Moloney murine leukaemia virus, OVA, HPV andc olon cancer[59]
Cationic lipid, cholesterol, PEG nanoparticle	i.d., i.m. and s.c.	Mouse, cotton rat and rhesus macaque	Zika virus[20,85,112], influenza virus[22,94,95,205], RSV[19], HCMV, rabies virus[87] and melanoma[153]
Dendrimer nanoparticle	i.m.	Mouse	Influenza virus, Ebola virus, *Toxoplasma gondii*[89] and Zika virus[88]

HCMV, human cytomegalovirus; HCV, hepatitis C virus; HPV, human papillomavirus; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.s., intrasplenic; i.v., intravenous; OVA, ovalbumin-expressing cancer models; PEG, polyethylene glycol; RSV, respiratory syncytial virus; s.c., subcutaneous.

Table 2

Clinical trials with mRNA vaccines against infectious diseases

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Argos Therapeutics	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	• NCT00672191 (II) • NCT01069809 (II) • NCT02042248 (I)	• Completed[105] • Completed; results NA • Completed; results NA
CureVac AG	RNActive viral Ag mRNA (i.m., i.d.)	Rabies virus	NCT02241135 (I)	Active[56,91]
Erasmus Medical Center	DC loaded with viral Ag mRNA with TriMix (i.nod.)	HIV-1	NCT02888756 (II)	Recruiting
Fundació Clínic per la Recerca Biomèdica	Viral Ag mRNA with TriMix (NA)	HIV-1	NCT02413645 (I)	Active
Massachusetts General Hospital	DC loaded with viral Ag mRNA (i.d.)	HIV-1	NCT00833781 (II)	Completed[104]
McGill University Health Centre	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	NCT00381212 (I/II)	Completed[102]
Moderna Therapeutics	Nucleoside-modified viral Ag mRNA (i.m.)	Zika virus	NCT03014089 (I/II)	Recruiting[85]
		Influenza virus	NCT03076385 (I)	Ongoing[22]

The table summarizes the clinical trials registered at ClinicalTrials.gov as of 5 May 2017. Ag, antigen; CD40L, CD40 ligand; DC, dendritic cell; EP, electroporated; i.d., intradermal; i.m., intramuscular; i.nod., intranodal; NA, not available.

Table 3

Clinical trials with mRNA vaccines against cancer

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Antwerp University Hospital	DC EP with TAA mRNA (i.d. or NA)	AML	• NCT00834002 (I) • NCT01686334 (II)	• Completed[206,207] • Recruiting
		AML, CML, multiple myeloma	NCT00965224 (II)	Unknown
		Multiple solid tumours	NCT01291420 (I/II)	Unknown[208]
		Mesothelioma	NCT02649829 (I/II)	Recruiting
		Glioblastoma	NCT02649582 (I/II)	Recruiting
Argos Therapeutics	DC EP with autologous tumour mRNA with or without CD40L mRNA (i.d. or NA)	Renal cell carcinoma	• NCT01482949 (II) • NCT00678119 (II) • NCT00272649 (I/II) • NCT01582672 (III) • NCT00087984 (I/II)	• Ongoing • Completed[209] • Completed; results NA • Ongoing • Completed; results NA
		Pancreatic cancer	NCT00664482 (NA)	Completed; results NA
Asterias Biotherapeutics	DC loaded with TAA mRNA (NA)	AML	NCT00510133 (II)	Completed[210]
BioNTech RNA Pharmaceuticals GmbH	Naked TAA or neo-Ag mRNA (i.nod.)	Melanoma	• NCT01684241 (I) • NCT02035956 (I)	• Completed; results NA • Ongoing
	Liposome-complexed TAA mRNA (i.v.)	Melanoma	NCT02410733 (I)	Recruiting[59]
	Liposome-formulated TAA and neo-Ag mRNA (i.v.)	Breast cancer	NCT02316457 (I)	Recruiting
CureVac AG	RNActive TAA mRNA (i.d.)	Non-small-cell lung cancer	• NCT00923312 (I/II) • NCT01915524 (I)	• Completed[211] • Terminated[200]
		Prostate cancer	• NCT02140138 (II) • NCT00831467 (I/II) • NCT01817738 (I/II)	• Terminated • Completed[151] • Terminated[212]
Duke University	DC loaded with CMV Ag mRNA (i.d. or ing.)	Glioblastoma, malignant glioma	• NCT00626483 (I) • NCT00639639 (I) • NCT02529072 (I) • NCT02366728 (II)	• Ongoing[213] • Ongoing[138,139] • Recruiting • Recruiting
	DC loaded with autologous tumour mRNA (i.d.)	Glioblastoma	NCT00890032 (I)	Completed; results NA
	DC, matured, loaded with TAA mRNA (i.nod.)	Melanoma	NCT01216436 (I)	Terminated
Guangdong 999 Brain Hospital	DC loaded with TAA mRNA (NA)	Glioblastoma	• NCT02808364 (I/II) • NCT02709616 (I/II)	• Recruiting • Recruiting
		Brain metastases	NCT02808416 (I/II)	Recruiting
Herlev Hospital	DC loaded with TAA mRNA (i.d.)	Breast cancer, melanoma	NCT00978913 (I)	Completed[214]

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
		Prostate cancer	NCT01446731 (II)	Completed[215]
Life Research Technologies GmbH	DC, matured, loaded with TAA mRNA (NA)	Ovarian cancer	NCT01456065 (I)	Unknown
Ludwig-Maximilian-University of Munich	DC loaded with TAA and CMV Ag mRNA (i.d.)	AML	NCT01734304 (I/II)	Recruiting
MD Anderson Cancer Center	DC loaded with AML lysate and mRNA (NA)	AML	NCT00514189 (I)	Terminated
Memorial Sloan Kettering Cancer Center	DC (Langerhans) EP with TAA mRNA (i.d.)	Melanoma	NCT01456104 (I)	Ongoing
		Multiple myeloma	NCT01995708 (I)	Recruiting
Oslo University Hospital	DC loaded with autologous tumour or TAA mRNA (i.d. or NA)	Melanoma	• NCT00961844 (I/II) • NCT01278940 (I/II)	• Terminated • Completed[216]
		Prostate cancer	• NCT01197625 (I/II) • NCT01278914 (I/II)	• Recruiting • Completed; results NA
		Glioblastoma	NCT00846456 (I/II)	Completed[217]
		Ovarian cancer	NCT01334047 (I/II)	Terminated
Radboud University	DC EP with TAA mRNA (i.d. and i.v. or i.nod)	Colorectal cancer	NCT00228189 (I/II)	Completed[218]
		Melanoma	• NCT00929019 (I/II) • NCT00243529 (I/II) • NCT00940004 (I/II) • NCT01530698 (I/II) • NCT02285413 (II)	• Terminated • Completed[219,220] • Completed[220,221] • Completed[144,220,221] • Completed; results NA
Universitair Ziekenhuis Brussel	DC EP with TAA and TriMix mRNA (i.d. and i.v.)	Melanoma	• NCT01066390 (I) • NCT01302496 (II) • NCT01676779 (II)	• Completed[137] • Completed[140] • Completed; results NA
University Hospital Erlangen	DC, matured, loaded with autologous tumour RNA (i.v.)	Melanoma	NCT01983748 (III)	Recruiting
University Hospital Tübingen	Autologous tumour mRNA with GM-CSF protein (i.d. and s.c.)	Melanoma	NCT00204516 (I/II)	Completed[222]
	Protamine-complexed TAA mRNA with GM-CSF protein (i.d. and s.c.)	Melanoma	NCT00204607 (I/II)	Completed[150]
University of Campinas, Brazil	DC loaded with TAA mRNA (NA)	AML, myelodysplastic syndromes	NCT03083054 (I/II)	Recruiting
University of Florida	RNActive* TAA mRNA (i.d.)	Prostate cancer	NCT00906243 (I/II)	Terminated

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
	DC loaded with CMV Ag mRNA with GM-CSF protein (i.d.)	Glioblastoma, malignant glioma	NCT02465268 (II)	Recruiting

The table summarizes the clinical trials registered at ClinicalTrials.gov as of 5 May 2017. Ag, antigen; AML, acute myeloid leukaemia; CD40L, CD40 ligand; CML, chronic myeloid leukaemia; CMV, cytomegalovirus; DC, dendritic cell; EP, electroporated; GM-CSF, granulocyte–macrophage colony-stimulating factor; i.d., intradermal; ing., inguinal injection; i.nod., intranodal injection; i.v., intravenous; NA, not available; neo-Ag, personalized neoantigen; s.c., subcutaneous; TAA, tumour-associated antigen.

* Developed by CureVac AG.

Table 4

Leading mRNA vaccine developers: research focus, partners and therapeutic platforms

Institution	mRNA technology	Partners	Indication (disease target)
Argos Biotechnology	mRNA neoantigens (Arcelis platform)	NA	Individualized cancer vaccines, HIV-1
BioNTech RNA Pharmaceuticals GmbH	Nucleoside-modified mRNA (IVAC Mutanome, FixVAC)	Genentech/Roche	Individualized cancer vaccines
		Bayer AG	Veterinary vaccines
CureVac AG	Sequence-optimized, purified mRNA (RNActive, RNArt, RNAdjuvant)	Boehringer Ingelheim GmbH	Cancer vaccines (lung cancer)
		Johnson & Johnson	Viral vaccines
		Sanofi Pasteur	Infectious disease vaccines
		BMGF	Infectious disease vaccines
		IAVI	HIV vaccines
eTheRNA Immunotherapies	Purified mRNA (TriMix)	NA	Cancer (melanoma, breast), viral vaccines (HBV and/or HPV)
GlaxoSmithKline/Novartis	Self-amplifying mRNA (SAM) (alphavirus replicon)	NA	Infectious disease vaccines
Moderna Therapeutics	Nucleoside-modified mRNA	Merck & Co.	Individualized cancer vaccines, viral vaccines
		BMGF, DARPA, BARDA	Viral vaccines (influenza virus, CMV, HMPV, PIV, chikungunya virus, Zika virus)
University of Pennsylvania	Nucleoside-modified, purified mRNA	NA	Infectious disease vaccines

BARDA, Biomedical Advanced Research and Development Authority; BMGF, Bill & Melinda Gates Foundation; CMV, cytomegalovirus; DARPA, Defense Advanced Research Projects Agency; HBV, hepatitis B virus; HMPV, human metapneumovirus; HPV, human papillomavirus; IAVI, International AIDS Vaccine Initiative; NA, not available; PIV, parainfluenza virus.