HHS Public Access

Author manuscript

ACS Appl Bio Mater. Author manuscript; available in PMC 2024 August 20.

Published in final edited form as:

ACS Appl Bio Mater. 2024 August 19; 7(8): 4894–4913. doi:10.1021/acsabm.3c00721.

Advances in Non-Viral mRNA Delivery Materials and Their Application as Vaccines for Melanoma Therapy

Bevin Neill[†,‡], **Adriana Retamales Romero**[†], **Owen S. Fenton**[†,§,*]

[†]Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[‡]School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

[§]Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

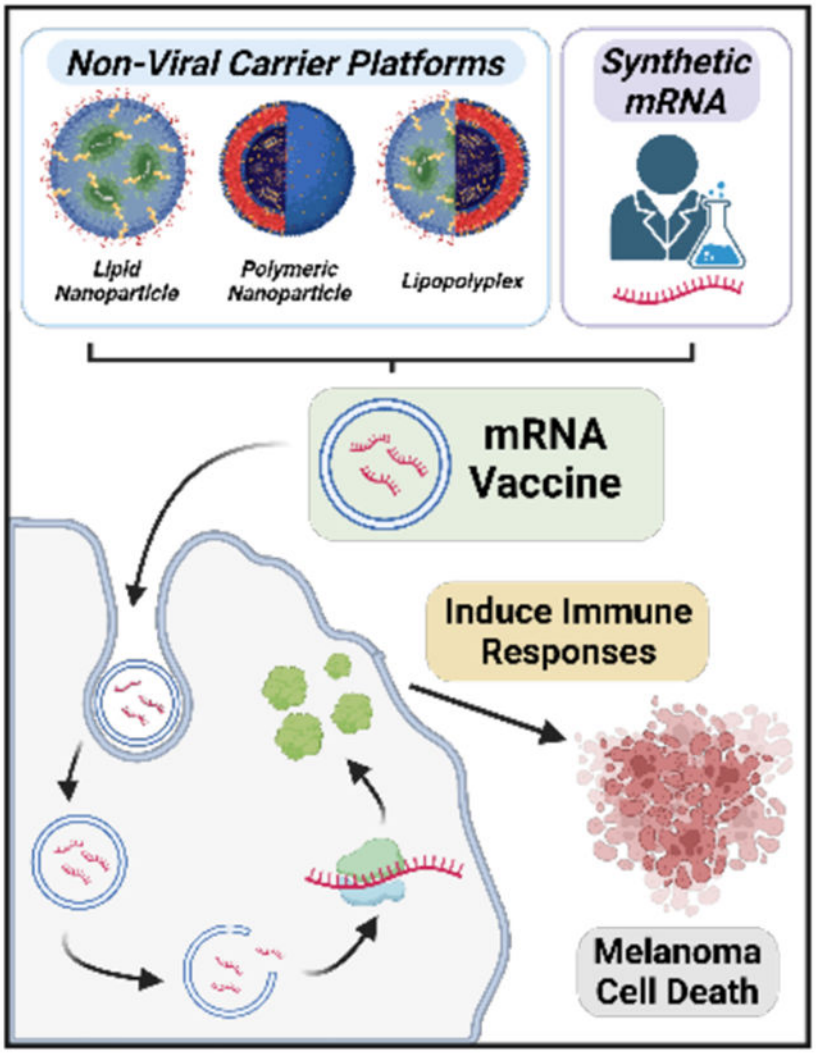
Abstract

Messenger RNA (mRNA) vaccines are promising platforms for cancer immunotherapy because of their potential to encode for a variety of tumor antigens, high tolerability, and capacity to induce strong anti-tumor immune responses. However, the clinical translation of mRNA cancer vaccines can be hindered by the inefficient delivery of mRNA *in vivo.* In this review, we provide an overview of mRNA cancer vaccines by discussing their utility in treating melanoma. Specifically, we begin our review by describing the barriers that can impede mRNA delivery to target cells. We then review native mRNA structure and discuss various modification methods shown to enhance mRNA stability and transfection. Next, we outline the advantages and challenges of three non-viral carrier platforms (lipid nanoparticles, polymeric nanoparticles, and lipopolyplexes) frequently used for mRNA delivery. Last, we summarize preclinical and clinical studies that have investigated non-viral mRNA vaccines for the treatment of melanoma. In writing this review, we aim to highlight innovative non-viral strategies designed to address mRNA delivery challenges while emphasizing the exciting potential of mRNA vaccines as next-generation therapies for the treatment of cancers.

Graphical Abstract

[*]**Corresponding author** osfenton@unc.edu.

The authors declare no other competing financial interests.



Keywords

mRNA vaccines; lipid nanoparticles; polymeric nanoparticles; lipopolyplexes; melanoma

Introduction

Over the past several decades, immunotherapy has gained tremendous attention as a treatment strategy for cancer. Commonly implemented immunotherapies aim to modulate or train the immune system so it can recognize and destroy tumor cells. Various immunotherapy approaches have been developed to dramatically improve outcomes for cancer patients.[1,2] In particular, cancer vaccines have emerged as promising tools to induce strong anti-tumor immune responses. Typically, these vaccines target tumor-associated (TAAs) or tumor-specific antigens (TSAs) to kill malignant cells that overexpress these antigens.[3] Due to immunological memory, the body can recognize and destroy these cells in the future, leading to sustained tumor inhibition.[4] Therefore, cancer vaccines have both

therapeutic and prophylactic potential, making them a versatile and effective strategy for achieving long-lasting anti-tumor immunity.[5]

While there are many types of cancer vaccines, mRNA-based vaccines have recently attracted widespread interest because of their rapid development during the COVID-19 pandemic.[6] Like the COVID-19 vaccines, the main purpose of an mRNA cancer vaccine is to train the body to recognize and destroy cancer via the translation of mRNA into protein antigens. In a cancer vaccine, mRNA encoding a specific tumor antigen is delivered to specialized immune cells called antigen presenting cells (APCs). These cells produce the tumor antigen and present it on its surface by major histocompatibility complexes (MHCs). T cells can then recognize these antigens and activate the adaptive immune system to destroy the cancer cells.[7] Since mRNA vaccines can encode and express a variety of tumor antigens, they are able to induce both humoral and cellular immune responses which is beneficial for efficient tumor eradication (Figure 1).[8] From a safety perspective, mRNA vaccines are advantageous because mRNA does not integrate into the host genome so there is no risk of insertional mutagenesis.[9] Additionally, mRNA is rapidly degraded in the cytoplasm which results in transient antigen presentation and avoids prolonged activation of the immune system.[6] In regards to delivery, mRNA only needs to be delivered into the cytoplasm while DNA vaccines need to be delivered into the nucleus.[10] Furthermore, the worldwide deployment of COVID-19 vaccines led to the development of good manufacturing practice (GMP) protocols to rapidly produce mRNA vaccines.[9] Finally, mRNA vaccines are a simple yet incredibly versatile platform, so their potential to treat a variety of diseases including melanoma is essentially limitless.

Major technological innovations have been made over the past several decades to improve mRNA vaccine stability, delivery, transfection, and large-scale manufacturability. Due to these advancements, more than twenty mRNA-based immunotherapies have entered clinical trials for the treatment of cancer.[11] Recently, mRNA vaccines have emerged as a particularly promising treatment option for melanoma. Melanoma is an aggressive form of skin cancer that originates in melanocytes but can rapidly metastasize to other parts of the body. In the United States, it is estimated that 97,619 people will be newly diagnosed with melanoma and 7,990 people will die from the disease in 2023.[12] The current standard of care for melanoma is typically surgical resection followed by chemotherapy and radiation.[13] However, these methods can be ineffective and are associated with adverse side effects that can greatly reduce the patient's quality of life.[10] Therefore, targeted immunotherapies for melanoma have been developed to overcome these limitations.[14,15] Melanoma is an ideal candidate for immunotherapy because it is highly immunogenic, meaning it provides many different antigens that can be chosen for vaccine formulation.[10] Over the past several years, extensive research has been conducted to investigate the efficacy of mRNA vaccines for melanoma treatment, and several on-going clinical trials indicate the exciting potential of this technology to eradicate the disease. Although these results are promising, there are still many challenges that need to be addressed when optimizing the delivery, uptake, and efficacy of mRNA vaccines to treat metastatic melanoma.

In this review, we provide an overview of non-viral mRNA melanoma vaccines and discuss different approaches used to address these challenges. First, we describe numerous

barriers associated with mRNA delivery. We then review mRNA structure and discuss several modification strategies shown to improve mRNA stability and translation. Next, we detail non-viral carriers used for mRNA vaccines including lipid nanoparticles, polymeric nanoparticles, and lipopolyplexes. After describing the general composition, advantages, and challenges of each carrier, we summarize recent preclinical and clinical data from studies that have evaluated these non-viral mRNA vaccines for the treatment of melanoma. Lastly, we present some remaining challenges and future directions of mRNA cancer vaccines. In writing this review, our goal is to discuss recent developments in non-viral mRNA vaccines for melanoma and highlight the innovative strategies designed to enhance the potential of these vaccines to efficiently treat this deadly disease.

1. Delivery Barriers

Although mRNA is a powerful therapeutic, it must overcome several delivery barriers in order to reach the cytosol of target cells. For cancer applications, the route of administration is an important consideration and typically includes intramuscular or subcutaneous injections for cancer vaccines and intravenous administration for solid tumors. While every route of administration for mRNA therapeutics presents unique challenges and advantages, they are all unified by some central ideas.

First, the therapeutic agent needs to be delivered to the right cells at the proper dosage. For therapeutics administered through the blood, movement across the vascular endothelial barrier is a significant challenge that can greatly hinder delivery to the correct tissue. Generally, a molecule with a diameter larger than 5 nm cannot readily cross the capillary endothelium and will remain in circulation until it is eventually cleared. However, there are certain tissues like the liver, spleen, and some tumors that allow entry of larger molecules with diameters up to 200 nm.[16] Since these organs accommodate the entry of many drug delivery nanocarriers, they may remove the mRNA therapeutic from circulation so there is less available for its intended target. Furthermore, naked mRNA is rapidly degraded by extracellular ribonucleases which further reduces its circulation time.[17] Decreased bioavailability is an issue because it leads to minimal protein production and poor therapeutic efficacy.

The second central idea is that the immune response to exogenous mRNA is incredibly complex. Exogenous mRNA is intrinsically immunogenic which presents another major delivery barrier. Although mRNA's ability to activate the immune system can be beneficial for vaccination purposes, it can paradoxically facilitate mRNA degradation and reduce antigen expression.[5] Exogenous mRNA can be recognized by the innate immune system, leading to activation of Type-1 interferon pathways and secretion of inflammatory cytokines.[18] This adverse immune response can inhibit the translation of mRNA and significantly decrease treatment efficacy. In addition to mRNA immunogenicity, phagocytosis is another immunological barrier both in the bloodstream and in the extracellular matrix of tissues. Phagocytosis is a process in which phagocytic cells such as macrophages and monocytes ingest and eliminate foreign material to protect the body from infection. Exogenous mRNA can be engulfed by phagocytic cells which significantly reduces its circulation time and hampers therapeutic efficacy. Additionally, phagocytes can

efficiently eliminate certain nanoparticle formulations from the body. Proteins absorbed on the nanoparticle surface promote opsonization, leading to aggregation and rapid clearance due to phagocytosis by the mononuclear phagocyte system (MPS).[19] Various strategies can be used when designing drug delivery vehicles to avoid opsonization, and these methods will be discussed later in this review.

Lastly, the mRNA therapeutic needs to be able to efficiently enter the cytoplasm of target cells to undergo translation. However, even if the mRNA drug reaches the correct tissue and evades the immune system, it still faces numerous barriers at the cell surface. First, the mRNA complex must diffuse through the extracellular matrix and then pass through the cell membrane. This is difficult because the negative potential across the cell membrane creates a repulsive force to mRNA due to its negatively charged phosphate backbone.[20] Additionally, mRNA is large and does not readily diffuse through cellular membranes in a passive manner, so its high molecular weight also hinders cellular uptake.[21] Slow entry into the cell provides additional opportunities for mRNA therapeutics to be engulfed by resident macrophages; therefore, efficient uptake is crucial for optimal treatment. Once in the cell, mRNA faces an additional challenge as it needs to exit the endosome to reach the cytoplasm for protein synthesis. If the mRNA complex is unable to escape the endosome, it will be subjected to degradative conditions in the lysosome.[22] Ineffective endosomal escape also imposes higher dosage which can cause toxicity.[23] Finally, mRNA formulated with carriers must be released from their delivery vehicle in order to initiate translation. From initial circulation in the bloodstream to eventual release into the cytoplasm of target cells, mRNA must overcome several hurdles to produce the protein of interest.

Overcoming these delivery barriers is a central goal of the mRNA therapeutic research field. In the following sections, we discuss several clinically relevant strategies that aim to mitigate these delivery challenges and enhance mRNA transfection. Developing new methods to address these limitations will greatly improve the translation of mRNA therapeutics and broaden its clinical utility.

2. Native mRNA Structure and Function

mRNA is a single-stranded polymeric molecule composed of nucleotides attached by phosphodiester bonds. Cells synthesize mRNA during a process called transcription which occurs when an enzyme known as RNA polymerase reads the template (antisense) strand of DNA and creates a complementary strand of RNA. The resulting RNA transcript contains the same genetic information as the non-template (coding) strand of DNA, except RNA contains the base uracil instead of thymine. This transcript is called precursor-mRNA (pre-mRNA) because it is not yet functional and must be modified before it can be used to synthesize protein during translation. Essential pre-mRNA modifications include the addition of cap structures at the 5’ end, polyadenylation at the 3’ end, and removal of noncoding sections (introns) through splicing. Once the pre-mRNA has undergone this additional processing, it becomes mature mRNA which is composed of four distinct regions: 5’ cap, 3’ polyadenylated tail, and a protein-coding sequence flanked by two untranslated regions (UTRs). In the following sections, we provide an overview of these natural pre-mRNA modifications and highlight additional strategies to synthetically modify each region

of *in vitro* transcribed (IVT) mRNA to improve its stability and transfection (Figure 2), thereby enhancing its clinical utility as a cancer vaccine.

2a. 5’ Cap

The addition of the 5’ cap is the initial modification step and occurs during transcription. As soon as the first 20-30 nucleotides of the RNA have been synthesized, an N7-methylated guanosine (m^7G) cap is added to the first transcribed 5’ nucleotide (N) through a reverse 5’ to 5’ triphosphate linkage (ppp).[24] This reaction is mediated by three enzymes: RNA triphosphatase (TPase), RNA guanylyltransferase (GTase), and guanine-N7 methyltransferase (guanine-N7 MTase). RNA TPase first removes the terminal phosphate group from the nucleotide to generate diphosphate. Then, GTase transfers a GMP group from GTP to the diphosphate group to create G cap (GpppNp). Finally, guanine-N7 MTase adds a methyl group to the N7 amine of the G cap to create the “cap 0” structure (m^7GpppNp). An additional methyl group can also be added to the first and second transcribed nucleotides on the 2’-OH group of the ribose molecules to respectively form the cap 1 (m^7GpppNmp) and cap 2 (m^7GpppNmpNmp) structures which exist in higher eukaryotes.[25]

The cap 0 structure has several important biological roles, such as preventing mRNA degradation by exoribonucleases, directing pre-mRNA splicing, facilitating mRNA export from the nucleus to the cytoplasm, and providing molecule stability.[26,27] Notably, the cap 0 structure is necessary for efficient translation because it binds to the eukaryotic translation initiation factor eIF4e which facilitates the recruitment of mRNA to ribosomes for protein synthesis.[28,29] In addition to translation initiation, the binding of eIF4e helps stabilize the mRNA since the “decapping” enzyme DCP2 is no longer able to access the 5’ end.[30] Decapping enzymes remove the 5’ cap which allows exoribonucleases to degrade the mRNA; thus, the 5’ cap also plays a pivotal role in mRNA decay. While the cap 0 structure protects the 5’ end of the mRNA transcript, the poly(A) tail prevents degradation at the 3’ end and is discussed in the next section.

2b. Poly(A) Tail

The poly(A) tail is a long chain of adenine nucleotides that are added to the 3’ end of the mRNA transcript. Similar to the 5’ cap, the poly(A) tail is very important for RNA stability and translational efficiency.[31] Here we provide a brief overview of the poly(A) tail addition but refer to several reviews that offer detailed explanations of 3’ end processing.[32-35] The incorporation of the poly(A) tail consists of two steps: (1) cleavage of the pre-mRNA at the poly(A) site and (2) addition of approximately 100-200 adenine nucleotides. The first step in 3’ end maturation involves a sequence known as the polyadenylation signal (PAS). In higher eukaryotes, the PAS is AAUAAA or a close variant and is located 10-30 nucleotides upstream of the cleavage and polyadenylation site. When the PAS is recognized, a protein complex known as the cleavage and polyadenylation specificity factor (CPSF) binds to the PAS and catalyzes the cleavage of the pre-mRNA. Then, the enzyme poly(A) polymerase (PAP) adds multiple adenosine molecules to the cleaved 3’ end. After 11-14 adenosines have been added, nuclear poly(A) binding protein (PABPN) continues to rapidly grow the poly(A) tail to be approximately 200-250 nucleotides in length.

The poly(A) tail is vital to mRNA function as it improves stability by protecting against degradation, assists with export of mRNA from the nucleus into the cytoplasm, determines mRNA degradation, and provokes transcription termination.[36-40] Additionally, the poly(A) tail has been shown to mediate translation initiation since the poly(A) sequence is recognized by polyadenosyl binding proteins (PABPs) which then interact with the eIF4 complex recruited by the 5' cap.[29] Furthermore, the importance of the poly(A) tail is elucidated by studies that show disruption of poly(A) tail function leads to an increased risk of health conditions such as cancer, immunodeficiency, and hematological diseases.[41,42] Thus, the poly(A) tail is a crucial pre-mRNA modification that plays a pivotal role in maintaining overall health.

2c. Untranslated Regions

In addition to the 5' cap and poly(A) tail, the untranslated regions (UTRs) of mRNA largely influence the post-transcriptional regulation of gene expression. The UTRs are located both upstream (5' end) and downstream (3' end) of the coding sequence. Although both regions enhance mRNA stability and transfection, there are several differences between the 5' UTR and 3' UTR. For example, the 5' UTR is approximately 5 times shorter than the 3' UTR (~200 nucleotides vs. ~1000 nucleotides) and has a higher G+C content (60% vs. 45%).[43,44] Structural features of the 5' UTR (ribosome entry sites, upstream open reading frames, upstream start codons, hairpin loops, binding sites for regulatory proteins, and alternative start codons) are integral for translation initiation.[45] Moreover, it has recently been shown that the 5' UTR also impacts the stability, translation efficiency, and turnover of mRNA.[46] On the other hand, evidence suggests the 3' UTR has a diverse set of roles as it mediates subcellular mRNA localization, mRNA export from the nucleus, poly(A) tail status, mRNA stability, and translation efficiency.[47-50] Additionally, the 3' UTR contains target sites for microRNAs (miRNAs), a non-coding RNA that binds to mRNA to regulate its expression.[51] Therefore, the 3' UTR can also affect protein synthesis which in turn can influence many other biological processes. Overall, the UTRs are essential components of mRNA as they have the ability to impact a broad range of cellular functions.

2d. Coding Sequence

The coding sequence is the section of mRNA that is translated into protein. Following transcription, the mRNA transcript contains both coding and noncoding sequences, which are called exons and introns, respectively. To generate mature mRNA transcripts with an uninterrupted coding sequence, a process called splicing must occur to remove the introns and fuse the exons together. Spliceosomes are large RNA-protein complexes that execute this process, and once the mature mRNA has been created, it can be exported to ribosomes for translation. This mature mRNA transcript will contain the continuous coding sequence and the 5' cap, poly(A) tail, and UTRs discussed above. All of these post-transcriptional modifications are vital for proper mRNA function and protein production. Each region of the mRNA transcript can also be further modified to improve mRNA stability and transfection for therapeutic purposes which will be discussed in the following section.

3. Modifications to Improve the Stability and Transfection of Synthetic mRNA

Many mRNA-based therapeutics utilize IVT mRNA due to its advantages over other gene-expressing systems like plasmid DNA and viral vectors. IVT mRNA does not pose the risk of inducing oncogenic mutations, and its synthesis can be robust and scalable.[52] Since IVT mRNA resembles the structure of native mRNA, it can translate proteins in the cytoplasm and can be modified to exhibit various expression patterns. However, even though IVT mRNA is functionally the same as natural mRNA, it can be perceived by the host immune system as a harmful foreign material, thus eliciting a strong inflammatory response.[53] Additionally, IVT mRNA often lacks stability which leads to a short half-life and insufficient protein production. In the following subsections, we will discuss several strategies that have been used to address IVT mRNA instability and immunogenicity.

3a. 5’ Cap Modification

Exogenous mRNA is inherently immunostimulatory because it is recognized by various pattern recognition receptors (PRRs).[18] As a component of the innate immune system, PRRs identify pathogen-associated molecular patterns (PAMPs) that distinguish “self” from “non-self.” One component of IVT mRNA that can prevent recognition by PRRs is the 5’ cap. In viruses, RNA transcripts are uncapped, and the body recognizes the unprotected 5’ end as foreign, triggering an antiviral immune response.[54] Uncapped RNAs are also hydrolyzed by exoribonucleases leading to fast degradation.[17] Thus, adding a 5’ cap to IVT mRNA is useful for manufacturing transcripts with optimal translation efficiency.

There are several enzymatic and chemical methods that have been used for synthetic mRNA 5’ capping. One commonly used in vitro post-translational strategy is the Vaccinia capping system.[55] This is an enzymatic strategy based on the Vaccinia virus Capping Enzyme (VCE) composed of two subunits, D1 and D12. The D1 subunit possesses RNA triphosphatase, guanylyltransferase, and guanine methyltransferase activity, all of which are necessary for the addition of a complete Cap 0 structure. The D12 subunit stimulates the methyltransferase activity to further facilitate the 5’ cap addition. The Vaccinia capping system offers nearly 100% capping efficiency and the cap structures are added in the proper orientation unlike some co-transcriptional methods.[56] However, this system is expensive and can suffer from batch-to-batch variation.

In addition to post-translational enzymatic strategies, chemical capping methods have also been employed to add cap analogs during transcription. However, the conventional Cap 0 analog is susceptible to incorrect attachment to the mRNA sequence as approximately one third of IVT molecules are capped in the reverse direction ($Gpppm^7G$).[57] This inversion results in improper eIF4E binding and low translation efficiency. To prevent reverse cap integration, anti-reverse cap analogs (ARCAs) have been developed. ARCAs are additionally methylated at the 3’ hydroxyl group (closer to m7G) so RNA polymerase can only initiate transcription with the remaining hydroxyl group, forcing cap incorporation in the correct direction. Several studies have shown ARCA-capped mRNAs have higher translational efficiency and prolonged protein production *in vitro*.[58-61] ARCAs can also be

modified to enhance the translational outcome. For example, phosphorothioate substitutions at the β position in the triphosphate bridge confers resistance to decapping enzymes, improves eIF4E binding affinity, and provides additional molecule stabilization.[62,63] Additionally, ARCAs can be modified with boranophosphate (BH3) at the α, β, or γ position of the triphosphate chain. BH3 cap analogs were previously shown to increase protein expression in immune cells which may be favorable for anticancer immunization.[64]

Although ARCAs are beneficial for providing stability and improving translation efficiency, they are still limited in several ways. The capping efficiency is relatively low (~70%), and the cap contains an unnatural methyl group at the C3 position that the immune system can recognize as foreign.[5] The mRNA transcript must also start with guanine which limits its utility. To address the issues associated with ARCAs, a new co-transcriptional cap analog called CleanCap was developed in 2018.[65] CleanCap uses an initiating capped trimer instead of ARCA to yield a naturally occurring Cap 1 structure with an efficiency of 90-99%.[18] Since the Cap 1 structure reduces activation of PRRs, it is beneficial for decreasing the immunogenicity of exogenous mRNA. In addition to the CleanCap technology, a Cap 1 structure can be enzymatically added with guanylyl transferase and 2'-O-methyltransferase.[55] To further reduce the risk of triggering the innate immune system, capped IVT mRNA can be treated with phosphatases to remove any uncapped phosphate, thus preventing PRR recognition and improving mRNA translation.[66]

3b. Poly(A) Tail Modification

The poly(A) tail is a vital component of IVT mRNA as it provides stability and increases translation efficiency. The poly(A) tail is either encoded by the vector from which the mRNA is transcribed or it is enzymatically added through recombinant poly(A) polymerase. Poly(A) sequence length is an important parameter because it has been shown to influence mRNA function. A poly(A) tail of 250 nucleotides is commonly used, but the ideal length may be dependent on cell type. For example, a poly(A) tail longer than 300 nucleotides leads to more efficient translation in human primary T cells while a tail composed of 150-200 nucleotides is optimal for human monocyte-derived dendritic cells.[67,68] Furthermore, it has been shown that poly(A) tail length influences mRNA function through its interactions with poly(A) binding proteins (PABPs). These proteins coat the tail and can interact with the 5' cap to form a closed-loop structure which enhances mRNA stability and translation initiation.[18] A study conducted by Lima and colleagues showed that shorter poly(A) tails promote this closed-loop state for more efficient translation. Their analyses concluded that shorter poly(A) tails are a characteristic of highly expressed genes across eukaryotes.[69] Despite these findings, it is still unclear whether short poly(A) tails are better than long poly(A) tails for improving IVT mRNA translation efficiency. Thus, more studies should be conducted to fully understand the impact of poly(A) size on gene expression.

Similar to the 5' cap, the poly(A) sequence can also be modified to enhance stability and translational yield. Recently, Strzelecka and coworkers modified poly(A) tails with phosphorothioate groups and found these tails were less susceptible to degradation by 3'-deadenylase compared to unmodified tails.[70] Additionally, they studied the translational

properties of mRNAs with boranophosphate-modified poly(A) tails. They showed the presence of the boranophosphate moiety in the poly(A) tail correlated with a decrease in protein expression, indicating boranophosphate modification is not as effective as phosphorothioate functionalization. Bioorthogonal chemistry has also been used to modify poly(A) tails. Anhäuser and colleagues incorporated multiple 2'-azido-modified adenosine nucleotides at the 3' end of mRNA and used click chemistry to label these adenosines with the fluorescent dye sulforhodamine B.[71] Their findings suggest modifying mRNA with fluorophores at the poly(A) tail substantially increases translational efficiency. Although these studies present interesting conclusions, poly(A) tail modifications have yet to be fully explored, and future research may be conducted to continue elucidating the effect of poly(A) tail modifications to further improve mRNA therapeutics.

3c. Optimization of UTRs

The 5' and 3' UTRs play important roles in regulating mRNA degradation rate and translation efficiency by interacting with RNA binding proteins. Each UTR can be optimized to further enhance IVT mRNA stability and translation accuracy. In the 5' UTR, start codons (AUG) and non-canonical start codons (CUG) may be avoided because they disrupt the normal translation of the open reading frame.[45] Highly stable secondary structures may also be avoided in the 5' UTR because they can prevent ribosome recruitment and codon recognition.[45] Furthermore, modifications can be made specifically to the 3' UTR to optimize its functionality. The 3'-UTRs of α- and β-globin mRNAs are often found in IVT mRNA because they contain translation and stability regulatory elements.[72,73] RNA stability can be further improved by placing two human β-globin 3'-UTRs in a head-to-tail orientation.[67] For some applications, destabilizing mRNA can be beneficial for reducing the time needed for protein synthesis. In these situations, AU-rich regions can be inserted into the 3' UTR to ensure quick mRNA degradation and brief protein expression.[74]

In addition to these modifications, UTRs can also be systemically engineered to enhance protein production. Recently, Zeng and colleagues engineered endogenous UTRs by changing the sequence length and nucleotide composition, removing the inhibitory microRNA binding sites, and incorporating additional protein binding motifs that promote translation.[75] Compared to commonly used or control UTRs, their optimized UTRs were 5 to 10 times more efficient. Additionally, bioinformatics and machine learning can be used to design optimal UTRs *in silico* by utilizing genetic algorithms that generate unique synthetic motifs.[76] However, UTR performance is dependent on species, cell type, and cell state, so it is important to thoroughly understand the target cells when designing mRNA UTRs for therapeutic purposes. Overall, UTR modifications are incredibly versatile and can be applied to fine tune the behavior of IVT mRNA.

3d. Base Editing

Another strategy for optimizing IVT mRNA involves replacing natural nucleosides with modified derivatives. Although native mRNA can activate the innate immune system, modified nucleosides do not provoke TLR recognition and have significantly lower immunostimulatory activity.[77,78] Modified nucleosides commonly used in IVT mRNA applications include pseudouridine (Ψ), 1-methylpseudouridine ($m^1Ψ$), and 5-

methylcytidine (m^5C). These nucleotides are often used to replace natural uridine and cytidine, therefore reducing mRNA immunogenicity and prolonging protein production. Additionally, Leppek and colleagues recently found substitution of uridine with either Ψ or $m^1Ψ$ leads to increased IVT mRNA stability in solution.[79] Currently, mRNA vaccines suffer from solution hydrolysis which reduces its efficacy. However, modifying IVT mRNA with Ψ or $m^1Ψ$ could prolong the shelf life of mRNA drugs and improve access to these therapies. In all, these nucleoside modifications are incredibly useful for reducing activation of the innate immune system, increasing the translational capacity of mRNA, and improving overall structural stability.

3e. Purification of IVT mRNA

IVT mRNA purification is necessary to remove all potentially immunogenic contaminants capable of activating the immune system and interfering with adequate protein production. During IVT mRNA synthesis, phage polymerase can produce short RNAs and double stranded RNAs (dsRNAs). These contaminants can activate intracellular PRRs and greatly hinder translation efficiency.[53] Previously, Karikó and colleagues demonstrated that removing these RNA contaminants led to a 10 to 1000 fold increase in protein production in human primary dendritic cells and no induction of Type-1 interferons or inflammatory cytokines.[80] In this study, the contaminants were removed with high-pressure liquid chromatography (HPLC) which is a popular purification method due to its versatility. However, HPLC purification of mRNA can be expensive and relatively low yield (< 50%) so other purification strategies have been explored.[81] Decreasing Mg^{2+} concentration or producing RNA at elevated temperatures have both reduced dsRNA species during IVT mRNA production.[82,83] More recently, Baiersdörfer and coworkers developed a simple, fast, and cost-effective purification method based on the selective binding of dsRNA to cellulose in an ethanol-containing buffer.[81] Their method is capable of removing up to 90% of dsRNA contaminants regardless of the length, coding sequence, or nucleoside composition of the IVT mRNA. Taken together, purification is an important step in IVT mRNA synthesis as it can significantly reduce adverse immune activation and increase protein yields.

4. Non-Viral Delivery Carriers for mRNA

To harness its full therapeutic potential, IVT mRNA needs to be delivered to the cytoplasm of target cells while overcoming several delivery barriers. While modifying synthetic mRNA can greatly enhance its stability and reduce immunogenicity, naked mRNA is still susceptible to enzymatic degradation and elimination by the innate immune system. Furthermore, the large size, hydrophilicity, and negative charge of mRNA hinders its ability to enter cells. Therefore, several platform-based approaches have been developed to protect the mRNA from degradation, allow extravasation, promote uptake in target cells, reduce immunogenicity, prevent renal clearance, and facilitate endosomal escape. These platforms can be grouped into several categories including viral, cell-based, and non-viral. Viral and cell-based carriers have been thoroughly discussed elsewhere, and this review will focus on non-viral vectors for mRNA vaccines.[4,10,20,84,85] In the following section, we highlight several non-viral delivery methods that utilize lipids, polymers, or a combination thereof to elicit strong anti-tumor immune responses.

4a. Lipid Nanoparticles

Currently, lipid nanoparticles (LNPs) are among the most broadly used vectors for RNA delivery. Due to their ability to effectively encapsulate and deliver RNA, they were chosen as the carrier material for the mRNA in the SARS-CoV-2 vaccine by Moderna (mRNA-1273) and Pfizer/BioNTech (BNT162b2).[86] LNPs are composed of an outer lipid shell and a hydrophilic core. Four components form many LNP structures, including an ionizable lipid (IL), helper phospholipid, cholesterol, and lipid-anchored polyethylene glycol (PEG) (Figure 3). Each component has unique qualities and serves a different role in maintaining LNP function.

Ionizable lipids contain an ionizable head group and a long lipid chain.[87] Typically, the ionizable head group is a tertiary amine which modulates the net charge of the IL at different pHs.[88] In acidic environments, the tertiary amine is protonated, and the IL becomes positively charged. During LNP formulation, this positive charge allows the LNP to condense around the negatively charged mRNA and stabilize the molecule. This positive charge also facilitates endosomal escape *in vitro* and *in vivo.* At physiological pH, the IL is at a neutral charge and thus is nontoxic while circulating around the body. When the LNPs are at the cell surface, they are taken up by endocytosis. Since endosomes have an acidic environment, the tertiary amine is once again protonated.[89] The positively charged IL can then interact with phospholipids of the endosome to form an ion pair. As previously described in literature, this ion pair may drive the formation of the nonbilayer inverted hexagonal phase (H_{II}), thereby disrupting the endosomal membrane and facilitating endosomal escape.[90]

Phospholipids are another LNP component that aid in endosomal escape. These zwitterionic lipids are “helper lipids” that also improve overall membrane structure and stability due to their high phase transition temperature.[91,92] Commonly used helper phospholipids include 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), or sterol lipids.[91,93] LNP stability is also improved by cholesterol, a naturally occurring lipid that helps fluidize the lipid membrane.[94] It has also been shown that cholesterol increases the circulation time of lipid-based carriers and enhances transfection efficiency by potentially promoting membrane fusion and endosomal escape.[95-97] Finally, polyethylene glycol-modified (PEGylated) lipids improve LNP circulation time and stability by forming a hydrophilic layer on the LNP surface to prevent particle aggregation and serum protein opsonization.[98,99] It should also be noted that the ratio of each LNP component is very important for creating effective mRNA LNPs, and extensive research has been undertaken to optimize these formulations for ideal mRNA transfection and stability.

Currently, LNPs are widely used as delivery vectors for mRNA therapeutics due to their numerous advantages. To name a few, LNPs are often well tolerated, stabilize mRNA cargo, and can be used without the need for specialized equipment.[100] Additionally, LNPs are quickly and easily prepared by mixing lipids and mRNA in a microfluidic device.[101] Each component of LNPs can also be modified to improve targeted delivery to a tissue of interest.[102,103] Furthermore, COVID-19 clinical trials have shown LNP-based mRNA vaccines have favorable safety profiles and are highly effective in preventing the

development of COVID-19.[104] Despite these advantages and clinical successes, there are some challenges that have limited the utility of LNPs. One major challenge is the low temperature requirement for the storage of LNP-based mRNA vaccines.[105] Rural and suburban areas may not have the necessary infrastructure to store these vaccines at low temperatures which negatively affects vaccine distribution in these areas. Lipid components of LNPs are also capable of activating host immune responses which can lead to adverse side effects. For example, PEG in the Pfizer/BioNTech mRNA COVID-19 vaccine may cause anaphylaxis in patients with IgE-triggered allergies.[106] Another study by Ju and coworkers found LNP mRNA vaccination is associated with an increase in PEG-specific antibodies.[107] It has also been reported that ionizable lipids can stimulate the secretion of proinflammatory cytokines and reactive oxygen species.[108,109] To address these limitations, substantial research has been conducted to modify the physical properties of LNPs to reduce immunogenicity, enhance delivery efficiency, promote endosomal escape, and improve delivery to target cells.[110]

4b. Polymeric Nanoparticles

Aside from lipid nanoparticles, mRNA can also be successfully delivered to target cells using polymers.[111-114] Generally speaking, a polymer is a large molecule composed of small repeating units. An optimal polymeric nanoparticle (PNP) must be able to protect the mRNA from degradation, facilitate cellular uptake, and eventually release the mRNA into the cytoplasm. Polymeric materials commonly employed for mRNA delivery are often highly nitrogenated. Representative examples of these that will be discussed include polyethyleneimines, poly(amidoamines), and poly(β-amino) esters (Figure 4).

Polyethyleneimine (PEI) can be branched or linear and contains many amine groups.[115] Its positive charge allows it to complex with the negatively charged phosphate backbone of mRNA.[116] The amine groups can also form electrostatic interactions with the negatively charged endosome membrane to facilitate endosomal escape and mRNA release into the cytoplasm.[117] While PEI can protect mRNA from degradation and enhance transfection efficiency, molecular weight is an important consideration as it can significantly influence treatment efficacy. Studies have shown PEI with a high molecular weight binds mRNA tightly, leading to decreased protein expression. In contrast, PEI with low molecular weight (~2 kDa) can efficiently deliver mRNA and induce higher levels of protein production.[118] However, PEI often suffers from cytotoxicity, low biodegradability, and poor target specificity, all of which can hinder clinical translation.[119,120] Therefore, various modifications can be made to PEI to improve its safety profile and therapeutic potential. Zhao and coworkers developed a PEI and stearic acid copolymer capable of efficiently delivering mRNA and inducing antigen-specific immune responses.[121] In another study, Li and colleagues discovered linking cyclodextrin to PEI reduced the charge density which decreased the cytotoxicity and increased delivery efficiency.[122] Coupling PEI with polylactide and PEG has also been shown to improve efficacy.[123-125] Furthermore, fluorination of PEI can enhance nucleic acid delivery by lowering systemic toxicity.[126] In summary, PEI can be modified and designed to function as a successful mRNA carrier for therapeutic applications.

Poly(amidoamine) (PAMAM) dendrimers are another cationic polymer frequently utilized for mRNA delivery.[127] Dendrimers are nanomolecules composed of a core and many branched monomers. They typically contain a high number of functional groups that can be modified to influence physical and chemical properties.[128] The ability to fine tune properties like solubility, pharmacokinetics, and tissue targetability makes dendritic polymers a very valuable tool for drug delivery. As an example, PAMAM dendrimers have been modified to improve therapeutic delivery across the blood brain barrier for the treatment of glioblastoma.[129] Despite their versatility, some drawbacks of PAMAM dendrimers include rapid systemic clearance, insufficient accumulation in target tissues, and cytotoxicity.[130-132] Furthermore, ensuring PAMAM dendrimer sterility can be complicated, so it is necessary to optimize sterilization methods for clinical translation.[133]

Poly(β-amino) esters (PBAEs) are biodegradable polymers that have also successfully been used as nucleic acid carriers. These polymers can be easily synthesized with properties tailored for different therapeutic delivery applications.[134] Moreover, they often reduce toxicity because they are typically degraded into oligomers and monomers that are metabolized and eliminated from the body through normal pathways.[135] PBAEs can also be chemically modified with ligands, peptides, and PEG to further reduce toxicity and improve selective delivery to specific organs.[136-139] To achieve controlled gene delivery, Lee and coworkers designed PBAEs that can be used for on-demand release of nucleic acids in response to UV light and pH triggers.[140] However, PBAEs show some weaknesses as they can be unstable in physiological fluids and in long-term storage.[136,141] PBAEs also have relatively low binding efficiency to nucleic acids and can release nucleic acid cargo off-target.[134,142] New strategies continue to be developed to overcome these challenges and enhance PBAE-based therapeutic delivery.[143]

To summarize, polymers are frequently used as mRNA delivery vehicles because they are structurally diverse and relatively easy to synthesize. Additionally, they can undergo various modifications, so their chemical and physical properties can be optimized for specific therapeutic applications. While every polymer is unique, the challenges associated with polymer-based delivery generally include potential toxicity, colloidal instability, low purity, and poor transfection efficiency.[5,144] As innovative strategies to improve polymer-based delivery systems continue to be evaluated, efforts have also been made to developing hybrid delivery vehicles that combine the benefits of polymers and lipids. In the following section, we discuss several preclinical and clinical studies that have used lipid-based, polymer-based, or hybrid carriers to deliver mRNA vaccines for the treatment of melanoma.

5. Non-Viral mRNA Therapeutic Melanoma Vaccines

Melanoma is a malignant tumor that develops in melanocytes and is the leading cause of skin cancer death. In the United States, the incidence of melanoma has grown 320% since 1975, and around 100,000 people are estimated to be diagnosed with melanoma in 2023.[12,145] Although melanoma treatments have significantly improved over the past several decades, melanoma can still be challenging to manage because it has a high number of mutations and can metastasize rapidly.[146-148] Surgical resection is typically the primary treatment, and while it can be effective for patients with localized disease, it is not curative

for patients with advanced or metastatic melanoma.[149,150] Chemotherapy and radiation therapy are also standard melanoma treatment options, but these methods do not specifically target tumor cells and can lead to adverse side effects.[10] In recent years, immunotherapy has emerged as a promising strategy to improve the efficacy, tolerability, and targetability of melanoma treatment by harnessing the patient's own immune system to kill tumor cells. In particular, mRNA vaccines have become a viable melanoma treatment option because of their numerous advantages and successful clinical translation during the COVID-19 pandemic. Below, we summarize preclinical and clinical studies that have evaluated lipid-, polymer-, or lipid/polymer hybrid-based mRNA vaccines for the treatment of melanoma.

Within the literature, LNPs have been employed for the delivery of mRNA melanoma vaccines. Oberli and colleagues demonstrated their optimized LNP formulation transfected dendritic cells, macrophages, neutrophils, and B cells, all of which are important for activating anti-tumor immune responses.[151] This optimized LNP was used to encapsulate mRNA encoding for the tumor-associated antigens gp100 and TRP2, and its ability to treat aggressive B16F10 melanoma tumors was evaluated. Results showed this mRNA LNP significantly reduced tumor size and prolonged the overall survival of treated mice. Additionally, incorporating the adjuvant lipopolysaccharide (LPS) into this mRNA LNP further enhanced the immune response, leading to slower tumor growth and longer mouse survival compared to mRNA LNPs without LPS.

Through a similar approach, Miao et al. developed a library of ionizable lipid-like materials and tested the top candidate formulations as mRNA delivery vehicles for melanoma vaccines (Figure 5a).[152] These LNP formulations delivered ovalbumin (OVA) mRNA to the OVA-expressing B16F10 mouse melanoma model. OVA is an egg white protein that is commonly used as a model antigen as it has been shown to enhance neoantigen recognition by cytotoxic lymphocytes.[153] The LNP-based mRNA vaccines induced a strong antigen-specific cytotoxic T cell response and significant tumor suppression with only two doses. Furthermore, these LNP formulations stimulated adaptive immune cells through the stimulator of interferon genes (STING) pathway instead of TLRs, leading to potent antigen expression and local pro-inflammatory cytokine release.

In another study, Li and coworkers identified a new CpG-B class oligodeoxynucleotide (CpG2018B) capable of promoting cytokine production and turning "cold tumors" into "hot tumors" by stimulating CD4 and CD8+ T cells.[154] CpG2018B and neoantigen-encoding mRNA were encapsulated in LNPs and intratumorally injected into a melanoma mouse model. While vaccination with CpG2018B or the mRNA vaccine alone inhibited tumor growth, combining the two significantly enhanced the antitumor effect (Figure 5b).

In addition to developing strategies to activate anti-tumor immune responses, efforts have been made to improve the targeting of LNP-based mRNA vaccines. Many LNP formulations show high mRNA expression in the liver which can cause side effects such as reversible hepatic damage and T cell-dominant immune-mediated hepatitis.[155,156] To address this limitation, Chen and colleagues developed a lymph-node (LN) targeting LNP to provide targeted delivery of TRP2 mRNA in a melanoma mouse model.[157] When combined with anti-programmed death-1 (PD-1) antibody, the LN-targeted LNP mRNA vaccine led to

complete response in 40% of treated mice. Furthermore, all surviving mice resisted the rechallenging of a lung metastatic model, indicating the mRNA vaccine generates long-term anti-tumor immunity (Figure 5c).

Aside from delivering mRNAs that produce tumor antigens, LNPs have also been used to encapsulate mRNAs encoding stimulatory cytokines. Liu et al. recently developed novel LNPs to deliver mRNAs encoding interleukin 12 (IL-12), interleukin 27 (IL-27), and granulocyte-macrophage colony-stimulating factor (GM-CSF) to tumors *in vitro* and *in vivo*.[158] Intratumoral administration of this mRNA vaccine resulted in sustained inhibition of B16F10 melanoma growth while avoiding systematic toxicity. This effect was observed due to the infiltration of immune effector cells including natural killer and CD8+ T cells into tumor tissues. While IL-12 mRNA was most potent in reducing tumor growth alone, co-encapsulating IL-12 and IL-27 mRNA significantly reduced tumor size and increased mouse survival compared to controls (Figure 5d). As seen by its broad use within the literature, LNP-based mRNA vaccines show great promise as an effective treatment for melanoma.

Clinical trials have also proven LNP-based mRNA vaccines can improve outcomes for melanoma patients. Currently, an actively recruiting Phase 2 trial is evaluating the efficacy of an LNP-based mRNA vaccine in 157 patients with stage III/IV melanoma (NCT03897881). This vaccine encodes patient-specific neoantigens and is designed to stimulate T cell responses based on the unique mutational signature of a patient's tumor. In addition to studying the efficacy of the vaccine, this trial is investigating whether an anti-programmed death receptor-1 (PD-1) therapy called KEYTRUDA can enhance the antitumor response. After surgical resection, patients were randomized to receive either KEYTRUDA alone or a combination of the mRNA vaccine and KEYTRUDA. It was recently reported that the combination of both the vaccine and KEYTRUDA reduced the risk of reoccurrence or death by 44% compared to KEYTRUDA alone.[159] These promising results are a testament to the development and progress of LNP-based cancer vaccines, and it will be exciting for the field to see how this technology advances in the future.

Besides LNPs, preclinical studies have shown polymer-based carriers can be used to deliver mRNA melanoma vaccines. Recently, Li and colleagues synthesized fluoroalkane modified PEI (F-PEI) to facilitate the intracellular delivery of tumor antigen-encoding mRNA.[160] Their vaccine was capable of inducing antigen-specific CD8 T cell immune responses without the use of additional adjuvants. To evaluate antitumor efficacy, mice were inoculated with OVA-expressing B16 melanoma cells and were treated twice with OVA mRNA encapsulated in F-PEI. Compared to controls, some F-PEI mRNA vaccine formulations were able to prolong mouse survival and inhibit tumor growth (Figure 6a).

PAMAM dendrimers have been utilized in a unique way to enhance delivery of mRNA melanoma vaccines. Interestingly, Zhang and coworkers synthesized cationic lipid-like materials from PAMAM dendrimers and used these lipid-like materials to form LNPs that efficiently delivered antigen-encoding mRNA into dendritic cells.[161] In their animal study, melanoma mouse models were subcutaneously injected with these mRNA LNPs which significantly reduced tumor growth (Figure 6b). It was also noted that the LNP itself induced

the expression of inflammatory cytokines by activating TLR4 signaling. This was beneficial as the LNP served as a self-adjuvant to enhance T cell activation and promote antigen presentation.

Biodegradable polymers like PBAEs have also been employed as carriers for mRNA melanoma vaccines. Zhang and colleagues developed a polymeric nanoparticle composed of PBAE, poly-glutamic acid (PGA), and Di-mannose moieties to deliver mRNAs encoding transcriptional regulatory factor 5 (IRF5) and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ).[162] Initially, it was shown that co-expression of IRF5 and IKKβ can reprogram tumor-associated macrophages to have anti-tumoral properties and a pro-inflammatory phenotype. To evaluate efficacy in an animal model, mice injected with B16F10 melanoma cells were treated with these IRF5/IKKβ mRNA PNPs over three weeks. Compared to controls, this treatment dramatically reduced tumor burden and improved overall survival by 1.3-fold on average.

While lipids and polymers alone are capable of delivering mRNA, combining these materials into hybrid nanoparticles can further promote cellular uptake, facilitate endosomal escape of therapeutic cargo, and improve antigen-specific T cell responses.[163,164] Lipopolyplexes are a commonly used non-viral hybrid carrier for nucleic acid delivery. Since they are composed of a polymeric core and a lipid shell, they are designed to synergize the strengths of both lipids and polymers to generate nanoparticles with better stability, decreased cytotoxicity, and enhanced transfection efficiency.[165] In the literature, there are several reports of lipopolyplex-based mRNA based vaccines for the treatment of melanoma. Mockey and coworkers used PEGylated histidine-rich polylysine and histidylated cationic lipids to formulate histidylated lipopolyplexes.[166] These vectors were used to deliver mRNA encoding the melanoma antigen recognized by T-cells 1 (MART1). On days 0 and 7, mice were intravenously injected with these MART1 mRNA histidylated lipopolyplexes, and at day 14 they were subcutaneously challenged with B16F10 melanoma cells. Their results showed the MART1 mRNA histidylated lipopolyplexes induced specific protection against tumor growth compared to controls. Additionally, they investigated whether MART1 mRNA carrying the lysosomal sorting signal from the LAMP1 protein could enhance melanoma antigen presentation. They found the anti-tumor response was significantly higher when lipopolyplexes containing both MART1 mRNA and MART1-LAMP1 mRNA were used compared to lipopolyplexes containing only MART1 mRNA. Perche and colleagues also formulated mannosylated and histidylated lipopolyplexes to deliver MART1-LAMP1 mRNA.[167] Mice were immunized with these formulations on days 0 and 7 and were challenged with B16F10 cells at day 14. On day 21, the tumor volumes for mice injected with the MART1-LAMP1 mRNA vaccine (~200 mm^3) were lower than those for mice injected with saline solution (~1600 mm^3). Interestingly, this mannosylated formulation prolonged mouse survival and delayed tumor growth more than a nonmannosylated version.

In a similar study, Le Moignic et al. developed a lipopolyplex functionalized with glycolipid containing a tri-antenna of α-D-mannopyranoside to deliver MART1 mRNA.[168] Mice were first subcutaneously inoculated with B16F10 tumors, and 7 days later they received an intradermal injection of the MART1 mRNA lipopolyplex. Compared to controls, this mRNA vaccine significantly reduced tumor volumes and induced efficient stimulatory immune

responses. Furthermore, Persano and coworkers designed a lipopolyplex containing a PBAE polymeric core and phospholipid bilayer shell to facilitate delivery of mRNA into dendritic cells.[169] In their animal study, mice were inoculated with OVA-expressing B16 melanoma cells by tail vein injection to establish lung metastatic tumors. Three days after inoculation, mice were subcutaneously injected three times with the lipopolyplex carrying OVA mRNA. On day 18, mice were euthanized, the lungs were harvested, and the number of metastatic tumor nodules were counted. Mice in the PBS control group developed extensive pulmonary metastases while the mice that received the vaccine showed a 96% decrease in the number of tumor nodules in the lungs (Figure 6c). This vaccine was also shown to stimulate expression of the cytokines IFN-β and IL-12 which influence anti-tumor immunity by promoting dendritic cell maturation.

Lipopolyplexes have also been used to codeliver multiple types of mRNA to elicit anti-tumor responses. Van der Jeught and coworkers utilized a PEGylated derivative of histidylated polylysine and a tri-mannose-bearing diether lipid to form their lipopolyplexes.[170] This carrier was used to encapsulate OVA-encoding mRNA and TriMix mRNA which is a mixture of mRNAs encoding the immune-stimulatory proteins CD40L, CD70, and caTLR4. Mice were inoculated with OVA-expressing B16 tumor cells and received injections of this vaccine on days 5, 10, and 15. The OVA-TriMix mRNA vaccine appeared to evoke T cell responses as tumor growth was efficiently inhibited (Figure 6d). TriMix mRNA has also been used in dendritic cell-based vaccines and appear to be very promising for melanoma treatment.[171-178] This therapy has been evaluated in several clinical trials and has improved outcomes for a meaningful portion of melanoma patients (NCT01066390, NCT01530698, NCT01302496, NCT01676779, NCT03394937). It will be exciting to see how this technology and other non-viral mRNA-based therapies continue to advance melanoma treatment.

6. Conclusion and Future Directions

Cancer immunotherapy holds tremendous potential to improve clinical outcomes without reducing the patient's quality of life. In particular, mRNA cancer vaccines have emerged as a promising tool to evoke strong anti-tumor immune responses. Historically, melanoma served as a promising target for cancer vaccine development given its immunogenic nature and high incidence rate, amongst other factors. Although these studies paved the way for future successes, many of these pioneering approaches were met with challenges due to a number of factors including a limitation in the number of known melanoma antigens against which patients could be vaccinated, a limited number of patients who could be treated given the human leukocyte antigen restricted mode of recognition, and potential safety considerations, amongst other areas. Further, additional studies revealed some of the benefits that arise in treating melanoma with vaccines in tandem with checkpoint inhibitors or other cytokines, findings that have opened up additional research avenues and therapeutic strategies. Lessons learned from these studies have led to the development of improved melanoma vaccines throughout time, ultimately paving the way toward numerous clinical trials which have also confirmed the potential or mRNA vaccines for melanoma treatment (Table 1). The exciting results published by preclinical and clinical studies have provided a solid foundation for future innovative research.

Although this review primarily includes studies utilizing mRNA as a source of tumor antigen, there are many other ways to employ mRNA for cancer treatment. Looking forward, mRNA could be used for additional purposes including but not limited to: (1) encoding monoclonal antibodies that target tumor antigens, immune cells, or immune pathways; (2) encoding antibody fragments to create bispecific antibodies and chimeric antigen receptors; (3) encoding toxic proteins that kill cancer cells; (4) reprograming tumor-associated dendritic cells to activate tumor antigen-specific T cell responses; (5) modulating immunosuppressive cells like tumor-associated macrophages and cancer-associated fibroblasts in the tumor microenvironment; (6) modulating cytokine milieu to enhance cancer cell death; (7) generating chimeric antigen receptor T cells. These mRNA applications are thoroughly discussed in a review by Hoecke et al.[179]

Future work could also focus on using other types of RNA to treat melanoma. Several studies have already investigated the anti-tumor efficacy of short interfering RNA (siRNA) in melanoma models.[180-185] Many of these siRNA therapies utilize the non-viral carriers discussed in this review. From a manufacturing and development standpoint, this is a major advantage because the same delivery materials can be used to efficiently deliver multiple types of nucleic acid cargo. In fact, non-viral carriers could potentially be used to codeliver mRNA and siRNA to simultaneously upregulate and downregulate protein expression to treat melanoma.[186] One possible formulation could include a lipid nanoparticle encapsulating MART1 mRNA and signal transducer and activator of transcription 3 (STAT3) siRNA. STAT3 has been shown to play an important role in melanoma development and is considered a potential target for melanoma therapy.[187] Hypothetically, the mRNA would produce MART1 to induce an antigen-specific T cell response while the siRNA would downregulate STAT3 to inhibit melanoma growth. As both siRNA and mRNA therapies continue to advance, it will be interesting to see how the fields will converge to develop state-of-the-art cancer treatments.

In order to harness the full potential of these therapies, they will need to be designed to overcome delivery barriers. Key considerations include efforts to optimize stability, cellular uptake, endosomal escape, tissue specificity, amongst other areas. Here, we have discussed various mRNA modification and purification strategies used to address these concerns. Additionally, we have reviewed prominent non-viral carriers including lipid nanoparticles, polymeric nanoparticles, and lipopolyplexes. While these strategies have lowered the barriers to mRNA delivery, ongoing clinical trials will continue to advance the field. Future work may focus on identifying highly immunogenic tumor-specific antigens capable of inducing specific and effective immune responses that will not affect normal cells. Developing methods to balance the immunogenicity of mRNA cancer vaccines will also be important for its safety and efficacy. Ideally, mRNA vaccines will activate the immune system enough to kill malignant cells but will not overstimulate the system as this could lead to adverse side effects. Research may also focus on maximizing long-term stability and storage of mRNA vaccines to improve access to these therapies in areas that do not have infrastructure like ultra-low temperature refrigeration.

Nonetheless, non-viral mRNA vaccines have incredible potential to become one of the main strategies for cancer treatment. It will be interesting to see how this technology will continue

to evolve and combine with other therapies to provide hope to patients diagnosed with melanoma and other cancers. The exciting results presented in this review are a testament to the progress of mRNA therapeutics, and the future is bright for developing mRNA cancer vaccines capable of dramatically improving patient outcomes.

Acknowledgments

The TOC and Figures 1-4 were created by the authors as original figures on BioRender.com. This work was supported by the NIH This work was supported by an NIH National Institute of Biomedical Imaging and Bioengineering award (1R21EB034942-01). This work was also supported by the NC Translational and Clinical Sciences (NC TraCS) Institute which is supported by the NIH National Center for Advancing Translational Sciences (NCATS) award 1K12TR004416-01.

References:

(1). Riley RS; June CH; Langer R; Mitchell MJ Delivery Technologies for Cancer Immunotherapy. Nat Rev Drug Discov 2019, 18 (3), 175–196. 10.1038/s41573-018-0006-z. [PubMed: 30622344]

(2). Rosenberg SA IL-2: The First Effective Immunotherapy for Human Cancer. J Immunol 2014, 192 (12), 5451–5458. 10.4049/jimmunol.1490019. [PubMed: 24907378]

(3). Buonaguro L; Tagliamonte M Selecting Target Antigens for Cancer Vaccine Development. Vaccines 2020, 8 (4), 615. 10.3390/vaccines8040615. [PubMed: 33080888]

(4). Liu J; Fu M; Wang M; Wan D; Wei Y; Wei X Cancer Vaccines as Promising Immuno-Therapeutics: Platforms and Current Progress. Journal of Hematology & Oncology 2022, 15 (1), 28. 10.1186/s13045-022-01247-x. [PubMed: 35303904]

(5). Miao L; Zhang Y; Huang L mRNA Vaccine for Cancer Immunotherapy. Mol Cancer 2021, 20 (1), 41. 10.1186/s12943-021-01335-5. [PubMed: 33632261]

(6). Vishweshwaraiah YL; Dokholyan NV mRNA Vaccines for Cancer Immunotherapy. Frontiers in Immunology 2022, 13, 1–10. 10.3389/fimmu.2022.1029069.

(7). Iavarone C; O'hagan DT; Yu D; Delahaye NF; Ulmer JB Mechanism of Action of mRNA-Based Vaccines. Expert Review of Vaccines 2017, 16 (9), 871–881. 10.1080/14760584.2017.1355245. [PubMed: 28701102]

(8). Deng Z; Tian Y; Song J; An G; Yang P mRNA Vaccines: The Dawn of a New Era of Cancer Immunotherapy. Frontiers in Immunology 2022, 13, 1–15. 10.3389/fimmu.2022.887125.

(9). Pardi N; Hogan MJ; Porter FW; Weissman D mRNA Vaccines — a New Era in Vaccinology. Nat Rev Drug Discov 2018, 17 (4), 261–279. 10.1038/nrd.2017.243. [PubMed: 29326426]

(10). Bidram M; Zhao Y; Shebardina NG; Baldin AV; Bazhin AV; Ganjalikhany MR; Zamyatnin AA; Ganjalikhani-hakemi M mRNA-Based Cancer Vaccines: A Therapeutic Strategy for the Treatment of Melanoma Patients. Vaccines 2021, 9 (10), 1060. 10.3390/vaccines9101060. [PubMed: 34696168]

(11). Lorentzen CL; Haanen JB; Met Ö; Svane IM Clinical Advances and Ongoing Trials of mRNA Vaccines for Cancer Treatment. The Lancet Oncology 2022, 23 (10), e450–e458. 10.1016/S1470-2045(22)00372-2. [PubMed: 36174631]

(12). Melanoma Skin Cancer Statistics. Accessed online: https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/key-statistics.html (accessed 6 June 2023).

(13). Domingues B; Lopes JM; Soares P; Pópulo H. Melanoma Treatment in Review. ImmunoTargets and Therapy 2018, 7, 35–49. 10.2147/ITT.S134842. [PubMed: 29922629]

(14). Ralli M; Botticelli A; Visconti IC; Angeletti D; Fiore M; Marchetti P; Lambiase A; de Vincentiis M; Greco A Immunotherapy in the Treatment of Metastatic Melanoma: Current Knowledge and Future Directions. Journal of Immunology Research 2020, 2020, 1–12. 10.1155/2020/9235638.

(15). Haanen JBAG Immunotherapy of Melanoma. European Journal of Cancer Supplements 2013, 11 (2), 97–105. 10.1016/j.ejcsup.2013.07.013. [PubMed: 26217118]

(16). Scherphof GL In Vivo Behavior of Liposomes: Interactions with the Mononuclear Phagocyte System and Implications for Drug Targeting. In Targeted Drug Delivery; Juliano RL, Ed.;

Handbook of Experimental Pharmacology; Springer Berlin Heidelberg: Berlin, Heidelberg, 1991; Vol. 100, pp 285–327. 10.1007/978-3-642-75862-1_8.

(17). Houseley J; Tollervey D The Many Pathways of RNA Degradation. Cell 2009, 136 (4), 763–776. 10.1016/j.cell.2009.01.019. [PubMed: 19239894]

(18). Linares-Fernández S; Lacroix C; Exposito J-Y; Verrier B Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol Med 2020, 26 (3), 311–323. 10.1016/j.molmed.2019.10.002. [PubMed: 31699497]

(19). Moghimi SM; Hunter AC; Murray JC Long-Circulating and Target-Specific Nanoparticles: Theory to Practice. Pharmacol Rev 2001, 53 (2), 283–318. [PubMed: 11356986]

(20). Kowalski PS; Rudra A; Miao L; Anderson DG Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. Mol Ther 2019, 27 (4), 710–728. 10.1016/j.ymthe.2019.02.012. [PubMed: 30846391]

(21). Wadhwa A; Aljabbari A; Lokras A; Foged C; Thakur A Opportunities and Challenges in the Delivery of mRNA-Based Vaccines. Pharmaceutics 2020, 12 (2), 102. 10.3390/pharmaceutics12020102. [PubMed: 32013049]

(22). Di Guglielmo GM; Le Roy C; Goodfellow AF; Wrana JL Distinct Endocytic Pathways Regulate TGF-β Receptor Signalling and Turnover. Nat Cell Biol 2003, 5 (5), 410–421. 10.1038/ncb975. [PubMed: 12717440]

(23). Paramasivam P; Franke C; Stöter M; Höijer A; Bartesaghi S; Sabirsh A; Lindfors L; Arteta MY; Dahlén A; Bak A; Andersson S; Kalaidzidis Y; Bickle M; Zerial M Endosomal Escape of Delivered mRNA from Endosomal Recycling Tubules Visualized at the Nanoscale. Journal of Cell Biology 2021, 221 (2), e202110137. 10.1083/jcb.202110137. [PubMed: 34882187]

(24). Shatkin AJ Capping of Eucaryotic mRNAs. Cell 1976, 9 (4 PT 2), 645–653. 10.1016/0092-8674(76)90128-8. [PubMed: 1017010]

(25). Furuichi Y; Shatkin AJ Viral and Cellular mRNA Capping: Past and Prospects. In Advances in Virus Research; Elsevier, 2000; Vol. 55, pp 135–184. 10.1016/S0065-3527(00)55003-9. [PubMed: 11050942]

(26). Ramanathan A; Robb GB; Chan S-H mRNA Capping: Biological Functions and Applications. Nucleic Acids Res 2016, 44 (16), 7511–7526. 10.1093/nar/gkw551. [PubMed: 27317694]

(27). Galloway A; Cowling VH mRNA Cap Regulation in Mammalian Cell Function and Fate. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2019, 1862 (3), 270–279. 10.1016/j.bbagrm.2018.09.011. [PubMed: 30312682]

(28). Amorim IS; Lach G; Gkogkas CG The Role of the Eukaryotic Translation Initiation Factor 4E (EIF4E) in Neuropsychiatric Disorders. Frontiers in Genetics 2018, 9, 1–9. 10.3389/fgene.2018.00561 [PubMed: 29387083]

(29). Mishra RK; Datey A; Hussain T mRNA Recruiting EIF4 Factors Involved in Protein Synthesis and Its Regulation. Biochemistry 2020, 59 (1), 34–46. 10.1021/acs.biochem.9b00788. [PubMed: 31765127]

(30). Borbolis F; Syntichaki P Biological Implications of Decapping: Beyond Bulk mRNA Decay. The FEBS Journal 2022, 289 (6), 1457–1475. 10.1111/febs.15798. [PubMed: 33660392]

(31). Passmore LA; Coller J Roles of mRNA Poly(A) Tails in Regulation of Eukaryotic Gene Expression. Nat Rev Mol Cell Biol 2022, 23 (2), 93–106. 10.1038/s41580-021-00417-y. [PubMed: 34594027]

(32). Sweet TJ; Licatalosi DD 3′ End Formation and Regulation of Eukaryotic mRNAs. In Polyadenylation: Methods and Protocols; Rorbach J, Bobrowicz AJ, Eds.; Methods in Molecular Biology; Humana Press: Totowa, NJ, 2014; pp 3–12. 10.1007/978-1-62703-971-0_1.

(33). Kumar A; Clerici M; Muckenfuss LM; Passmore LA; Jinek M Mechanistic Insights into mRNA 3′-End Processing. Current Opinion in Structural Biology 2019, 59, 143–150. 10.1016/j.sbi.2019.08.001. [PubMed: 31499460]

(34). Nourse J; Spada S; Danckwardt S Emerging Roles of RNA 3′-End Cleavage and Polyadenylation in Pathogenesis, Diagnosis and Therapy of Human Disorders. Biomolecules 2020, 10 (6), 915. 10.3390/biom10060915. [PubMed: 32560344]

(35). Casañal A; Kumar A; Hill CH; Easter AD; Emsley P; Degliesposti G; Gordiyenko Y; Santhanam B; Wolf J; Wiederhold K; Dornan GL; Skehel M; Robinson CV; Passmore LA Architecture

of Eukaryotic mRNA 3′-End Processing Machinery. Science 2017, 358 (6366), 1056–1059. 10.1126/science.aao6535. [PubMed: 29074584]

(36). Proudfoot NJ; Furger A; Dye MJ Integrating mRNA Processing with Transcription. Cell 2002, 108 (4), 501–512. 10.1016/S0092-8674(02)00617-7. [PubMed: 11909521]

(37). Dreyfus M; Régnier P The Poly(A) Tail of mRNAs: Bodyguard in Eukaryotes, Scavenger in Bacteria. Cell 2002, 111 (5), 611–613. 10.1016/S0092-8674(02)01137-6. [PubMed: 12464173]

(38). Fuke H; Ohno M Role of Poly (A) Tail as an Identity Element for mRNA Nuclear Export. Nucleic Acids Research 2008, 36 (3), 1037–1049. 10.1093/nar/gkm1120. [PubMed: 18096623]

(39). Sachs AB; Sarnow P; Hentze MW Starting at the Beginning, Middle, and End: Translation Initiation in Eukaryotes. Cell 1997, 89 (6), 831–838. 10.1016/S0092-8674(00)80268-8. [PubMed: 9200601]

(40). Kühn U; Wahle E Structure and Function of Poly(A) Binding Proteins. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 2004, 1678 (2), 67–84. 10.1016/j.bbaexp.2004.03.008. [PubMed: 15157733]

(41). Ogorodnikov A; Kargapolova Y; Danckwardt S Processing and Transcriptome Expansion at the mRNA 3′ End in Health and Disease: Finding the Right End. Pflugers Arch - Eur J Physiol 2016, 468 (6), 993–1012. 10.1007/s00424-016-1828-3. [PubMed: 27220521]

(42). Danckwardt S; Hentze MW; Kulozik AE 3′ End mRNA Processing: Molecular Mechanisms and Implications for Health and Disease. EMBO J 2008, 27 (3), 482–498. 10.1038/sj.emboj.7601932. [PubMed: 18256699]

(43). Mignone F; Gissi C; Liuni S; Pesole G Untranslated Regions of mRNAs. Genome Biol 2002, 3 (3), reviews0004.1-reviews0004.10.

(44). Pesole G; Liuni S; Grillo G; Saccone C Structural and Compositional Features of Untranslated Regions of Eukaryotic mRNAs. Gene 1997, 205 (1–2), 95–102. 10.1016/S0378-1119(97)00407-1. [PubMed: 9461382]

(45). Leppek K; Das R; Barna M Functional 5′ UTR mRNA Structures in Eukaryotic Translation Regulation and How to Find Them. Nat Rev Mol Cell Biol 2018, 19 (3), 158–174. 10.1038/nrm.2017.103. [PubMed: 29165424]

(46). Jia L; Mao Y; Ji Q; Dersh D; Yewdell JW; Qian S-B Decoding mRNA Translatability and Stability from the 5′ UTR. Nat Struct Mol Biol 2020, 27 (9), 814–821. 10.1038/s41594-020-0465-x. [PubMed: 32719458]

(47). Koh WS; Porter JR; Batchelor E Tuning of mRNA Stability through Altering 3′-UTR Sequences Generates Distinct Output Expression in a Synthetic Circuit Driven by P53 Oscillations. Sci Rep 2019, 9 (1), 5976. 10.1038/s41598-019-42509-y. [PubMed: 30979970]

(48). Conne B; Stutz A; Vassalli J-D The 3′ Untranslated Region of Messenger RNA: A Molecular 'Hotspot' for Pathology? Nat Med 2000, 6 (6), 637–641. 10.1038/76211. [PubMed: 10835679]

(49). Yergert KM; Doll CA; O'Rouke R; Hines JH; Appel B Identification of 3′ UTR Motifs Required for mRNA Localization to Myelin Sheaths in Vivo. PLoS Biol 2021, 19 (1), e3001053. 10.1371/journal.pbio.3001053. [PubMed: 33439856]

(50). Fan J; Wang K; Du X; Wang J; Chen S; Wang Y; Shi M; Zhang L; Wu X; Zheng D; Wang C; Wang L; Tian B; Li G; Zhou Y; Cheng H ALYREF Links 3′-end Processing to Nuclear Export of Non-polyadenylated mRNAs. The EMBO Journal 2019, 38 (9), e99910. 10.15252/embj.201899910. [PubMed: 30858280]

(51). Gu S; Jin L; Zhang F; Sarnow P; Kay MA The Biological Basis for MicroRNA Target Restriction to the 3' Untranslated Region in Mammalian mRNAs. Nat Struct Mol Biol 2009, 16 (2), 144–150. 10.1038/nsmb.1552. [PubMed: 19182800]

(52). Kwon H; Kim M; Seo Y; Moon YS; Lee HJ; Lee K; Lee H Emergence of Synthetic mRNA: In Vitro Synthesis of mRNA and Its Applications in Regenerative Medicine. Biomaterials 2018, 156, 172–193. 10.1016/j.biomaterials.2017.11.034. [PubMed: 29197748]

(53). Mu X; Hur S Immunogenicity of In Vitro-Transcribed RNA. Acc Chem Res 2021, 54 (21), 4012–4023. 10.1021/acs.accounts.1c00521. [PubMed: 34677064]

(54). Hornung V; Ellegast J; Kim S; Brzózka K; Jung A; Kato H; Poeck H; Akira S; Conzelmann K-K; Schlee M; Endres S; Hartmann, G. 5'-Triphosphate RNA Is the Ligand for RIG-I. Science 2006, 314 (5801), 994–997. 10.1126/science.1132505. [PubMed: 17038590]

(55). Muttach F; Muthmann N; Rentmeister A Synthetic mRNA Capping. Beilstein J. Org. Chem 2017, 13 (1), 2819–2832. 10.3762/bjoc.13.274. [PubMed: 30018667]

(56). Fuchs A-L; Neu A; Sprangers R A General Method for Rapid and Cost-Efficient Large-Scale Production of 5′ Capped RNA. RNA 2016, 22 (9), 1454–1466. 10.1261/rna.056614.116. [PubMed: 27368341]

(57). Pasquinelli AE; Dahlberg JE; Lund E Reverse 5' Caps in RNAs Made in Vitro by Phage RNA Polymerases. RNA 1995, 1 (9), 957–967. [PubMed: 8548660]

(58). Kocmik I; Piecyk K; Rudzinska M; Niedzwiecka A; Darzynkiewicz E; Grzela R; Jankowska-Anyszka M Modified ARCA Analogs Providing Enhanced Translational Properties of Capped mRNAs. Cell Cycle 2018, 17 (13), 1624–1636. 10.1080/15384101.2018.1486164. [PubMed: 29954234]

(59). Mockey M; Gonçalves C; Dupuy FP; Lemoine FM; Pichon C; Midoux P mRNA Transfection of Dendritic Cells: Synergistic Effect of ARCA mRNA Capping with Poly(A) Chains in Cis and in Trans for a High Protein Expression Level. Biochemical and Biophysical Research Communications 2006, 340 (4), 1062–1068. 10.1016/j.bbrc.2005.12.105. [PubMed: 16403444]

(60). Zohra FT; Chowdhury EH; Tada S; Hoshiba T; Akaike T Effective Delivery with Enhanced Translational Activity Synergistically Accelerates mRNA-Based Transfection. Biochemical and Biophysical Research Communications 2007, 358 (1), 373–378. 10.1016/j.bbrc.2007.04.059. [PubMed: 17475211]

(61). Stepinski J; Waddell C; Stolarski R; Darzynkiewicz E; Rhoads RE Synthesis and Properties of mRNAs Containing the Novel "Anti-Reverse" Cap Analogs 7-Methyl(3′-O-Methyl)GpppG and 7-Methyl(3′-Deoxy)GpppG. RNA 2001, 7 (10), 1486–1495. [PubMed: 11680853]

(62). Kuhn AN; Diken M; Kreiter S; Selmi A; Kowalska J; Jemielity J; Darzynkiewicz E; Huber C; Türeci Ö; Sahin U Phosphorothioate Cap Analogs Increase Stability and Translational Efficiency of RNA Vaccines in Immature Dendritic Cells and Induce Superior Immune Responses in Vivo. Gene Ther 2010, 17 (8), 961–971. 10.1038/gt.2010.52. [PubMed: 20410931]

(63). Grudzien-Nogalska E; Jemielity J; Kowalska J; Darzynkiewicz E; Rhoads RE Phosphorothioate Cap Analogs Stabilize mRNA and Increase Translational Efficiency in Mammalian Cells. RNA 2007, 13 (10), 1745–1755. 10.1261/rna.701307. [PubMed: 17720878]

(64). Kowalska J; Wypijewska del Nogal A; Darzynkiewicz ZM; Buck J; Nicola C; Kuhn AN; Lukaszewicz M; Zuberek J; Strenkowska M; Ziemniak M; Maciejczyk M; Bojarska E; Rhoads RE; Darzynkiewicz E; Sahin U; Jemielity J Synthesis, Properties, and Biological Activity of Boranophosphate Analogs of the mRNA Cap: Versatile Tools for Manipulation of Therapeutically Relevant Cap-Dependent Processes. Nucleic Acids Research 2014, 42 (16), 10245–10264. 10.1093/nar/gku757. [PubMed: 25150148]

(65). Vaidyanathan S; Azizian KT; Haque AKMA; Henderson JM; Hendel A; Shore S; Antony JS; Hogrefe RI; Kormann MSD; Porteus MH; McCaffrey AP Uridine Depletion and Chemical Modification Increase Cas9 mRNA Activity and Reduce Immunogenicity without HPLC Purification. Mol Ther Nucleic Acids 2018, 12, 530–542. 10.1016/j.omtn.2018.06.010. [PubMed: 30195789]

(66). Pardi N; Muramatsu H; Weissman D; Karikó K In Vitro Transcription of Long RNA Containing Modified Nucleosides. In Synthetic Messenger RNA and Cell Metabolism Modulation: Methods and Protocols; Rabinovich PM, Ed.; Methods in Molecular Biology; Humana Press: Totowa, NJ, 2013; pp 29–42. 10.1007/978-1-62703-260-5_2.

(67). Holtkamp S; Kreiter S; Selmi A; Simon P; Koslowski M; Huber C; Türeci Ö; Sahin U Modification of Antigen-Encoding RNA Increases Stability, Translational Efficacy, and T-Cell Stimulatory Capacity of Dendritic Cells. Blood 2006, 108 (13), 4009–4017. 10.1182/blood-2006-04-015024. [PubMed: 16940422]

(68). Grier AE; Burleigh S; Sahni J; Clough CA; Cardot V; Choe DC; Krutein MC; Rawlings DJ; Jensen MC; Scharenberg AM; Jacoby K PEVL: A Linear Plasmid for Generating mRNA IVT Templates With Extended Encoded Poly(A) Sequences. Molecular Therapy - Nucleic Acids 2016, 5, e306. 10.1038/mtna.2016.21. [PubMed: 27093168]

(69). Lima SA; Chipman LB; Nicholson AL; Chen Y-H; Yee BA; Yeo GW; Coller J; Pasquinelli AE Short Poly(A) Tails Are a Conserved Feature of Highly Expressed Genes. Nat Struct Mol Biol 2017, 24 (12), 1057–1063. 10.1038/nsmb.3499. [PubMed: 29106412]

(70). Strzelecka D; Smietanski M; Sikorski PJ; Warminski M; Kowalska J; Jemielity J Phosphodiester Modifications in mRNA Poly(A) Tail Prevent Deadenylation without Compromising Protein Expression. RNA 2020, 26 (12), 1815–1837. 10.1261/rna.077099.120. [PubMed: 32820035]

(71). Anhäuser L; Hüwel S; Zobel T; Rentmeister A Multiple Covalent Fluorescence Labeling of Eukaryotic mRNA at the Poly(A) Tail Enhances Translation and Can Be Performed in Living Cells. Nucleic Acids Res 2019, 47 (7), e42. 10.1093/nar/gkz084. [PubMed: 30726958]

(72). Karikó K; Kuo A; Barnathan ES Overexpression of Urokinase Receptor in Mammalian Cells Following Administration of the in Vitro Transcribed Encoding mRNA. Gene Ther 1999, 6 (6), 1092–1100. 10.1038/sj.gt.3300930. [PubMed: 10455412]

(73). Ross J; Sullivan T Half-Lives of Beta and Gamma Globin Messenger RNAs and of Protein Synthetic Capacity in Cultured Human Reticulocytes. Blood 1985, 66 (5), 1149–1154. 10.1182/blood.V66.5.1149.1149. [PubMed: 4052630]

(74). Chen CY; Shyu AB AU-Rich Elements: Characterization and Importance in mRNA Degradation. Trends Biochem Sci 1995, 20 (11), 465–470. 10.1016/s0968-0004(00)89102-1. [PubMed: 8578590]

(75). Zeng C; Hou X; Yan J; Zhang C; Li W; Zhao W; Du S; Dong Y Leveraging mRNA Sequences and Nanoparticles to Deliver SARS-CoV-2 Antigens In Vivo. Adv. Mater 2020, 32 (40), 2004452. 10.1002/adma.202004452.

(76). Cao J; Novoa EM; Zhang Z; Chen WCW; Liu D; Choi GCG; Wong ASL; Wehrspaun C; Kellis M; Lu TK High-Throughput 5′ UTR Engineering for Enhanced Protein Production in Non-Viral Gene Therapies. Nat Commun 2021, 12 (1), 4138. 10.1038/s41467-021-24436-7. [PubMed: 34230498]

(77). Karikó K; Muramatsu H; Welsh FA; Ludwig J; Kato H; Akira S; Weissman D Incorporation of Pseudouridine into mRNA Yields Superior Nonimmunogenic Vector with Increased Translational Capacity and Biological Stability. Mol Ther 2008, 16 (11), 1833–1840. 10.1038/mt.2008.200. [PubMed: 18797453]

(78). Karikó K; Buckstein M; Ni H; Weissman D Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. Immunity 2005, 23 (2), 165–175. 10.1016/j.immuni.2005.06.008. [PubMed: 16111635]

(79). Leppek K; Byeon GW; Kladwang W; Wayment-Steele HK; Kerr CH; Xu AF; Kim DS; Topkar VV; Choe C; Rothschild D; Tiu GC; Wellington-Oguri R; Fujii K; Sharma E; Watkins AM; Nicol JJ; Romano J; Tunguz B; Diaz F; Cai H; Guo P; Wu J; Meng F; Shi S; Participants E; Dormitzer PR; Solórzano A; Barna M; Das R Combinatorial Optimization of mRNA Structure, Stability, and Translation for RNA-Based Therapeutics. Nat Commun 2022, 13 (1), 1536. 10.1038/s41467-022-28776-w. [PubMed: 35318324]

(80). 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 (21), e142. 10.1093/nar/gkr695. [PubMed: 21890902]

(81). Baiersdörfer M; Boros G; Muramatsu H; Mahiny A; Vlatkovic I; Sahin U; Karikó K A Facile Method for the Removal of DsRNA Contaminant from In Vitro-Transcribed mRNA. Molecular Therapy - Nucleic Acids 2019, 15, 26–35. 10.1016/j.omtn.2019.02.018. [PubMed: 30933724]

(82). Wu MZ; Asahara H; Tzertzinis G; Roy B Synthesis of Low Immunogenicity RNA with High-Temperature in Vitro Transcription. RNA 2020, 26 (3), 345–360. 10.1261/rna.073858.119. [PubMed: 31900329]

(83). Mu X; Greenwald E; Ahmad S; Hur S An Origin of the Immunogenicity of in Vitro Transcribed RNA. Nucleic Acids Research 2018, 46 (10), 5239–5249. 10.1093/nar/gky177. [PubMed: 29534222]

(84). Bafaloukos D; Gazouli I; Koutserimpas C; Samonis G Evolution and Progress of mRNA Vaccines in the Treatment of Melanoma: Future Prospects. Vaccines 2023, 11 (3), 636. 10.3390/vaccines11030636. [PubMed: 36992220]

(85). Santos PM; Butterfield LH Dendritic Cell–Based Cancer Vaccines. The Journal of Immunology 2018, 200 (2), 443–449. 10.4049/jimmunol.1701024. [PubMed: 29311386]

(86). Wilson B; Geetha KM Lipid Nanoparticles in the Development of mRNA Vaccines for COVID-19. J Drug Deliv Sci Technol 2022, 74, 103553. 10.1016/j.jddst.2022.103553. [PubMed: 35783677]

(87). Sun D; Lu Z-R Structure and Function of Cationic and Ionizable Lipids for Nucleic Acid Delivery. Pharm Res 2023, 40 (1), 27–46. 10.1007/s11095-022-03460-2. [PubMed: 36600047]

(88). Hajj KA; Whitehead KA Tools for Translation: Non-Viral Materials for Therapeutic mRNA Delivery. Nat Rev Mater 2017, 2 (10), 17056. 10.1038/natrevmats.2017.56.

(89). Han X; Zhang H; Butowska K; Swingle KL; Alameh M-G; Weissman D; Mitchell MJ An Ionizable Lipid Toolbox for RNA Delivery. Nat Commun 2021, 12 (1), 7233. 10.1038/s41467-021-27493-0. [PubMed: 34903741]

(90). Hafez IM; Maurer N; Cullis PR On the Mechanism Whereby Cationic Lipids Promote Intracellular Delivery of Polynucleic Acids. Gene Ther 2001, 8 (15), 1188–1196. 10.1038/sj.gt.3301506. [PubMed: 11509950]

(91). Cheng X; Lee RJ The Role of Helper Lipids in Lipid Nanoparticles (LNPs) Designed for Oligonucleotide Delivery. Advanced Drug Delivery Reviews 2016, 99, 129–137. 10.1016/j.addr.2016.01.022. [PubMed: 26900977]

(92). Eygeris Y; Gupta M; Kim J; Sahay G Chemistry of Lipid Nanoparticles for RNA Delivery. Acc. Chem. Res 2022, 55 (1), 2–12. 10.1021/acs.accounts.1c00544. [PubMed: 34850635]

(93). Zhang R; El-Mayta R; Murdoch TJ; Warzecha CC; Billingsley MM; Shepherd SJ; Gong N; Wang L; Wilson JM; Lee D; Mitchell MJ Helper Lipid Structure Influences Protein Adsorption and Delivery of Lipid Nanoparticles to Spleen and Liver. Biomater. Sci 2021, 9 (4), 1449–1463. 10.1039/D0BM01609H. [PubMed: 33404020]

(94). Hald Albertsen C; Kulkarni JA; Witzigmann D; Lind M; Petersson K; Simonsen JB The Role of Lipid Components in Lipid Nanoparticles for Vaccines and Gene Therapy. Adv Drug Deliv Rev 2022, 188, 114416. 10.1016/j.addr.2022.114416. [PubMed: 35787388]

(95). Semple SC; Chonn A; Cullis PR Influence of Cholesterol on the Association of Plasma Proteins with Liposomes. Biochemistry 1996, 35 (8), 2521–2525. 10.1021/bi950414i. [PubMed: 8611555]

(96). Magarkar A; Dhawan V; Kallinteri P; Viitala T; Elmowafy M; Róg T; Bunker A. Cholesterol Level Affects Surface Charge of Lipid Membranes in Saline Solution. Sci Rep 2014, 4 (1), 5005. 10.1038/srep05005. [PubMed: 24845659]

(97). Pozzi D; Marchini C; Cardarelli F; Amenitsch H; Garulli C; Bifone A; Caracciolo G Transfection Efficiency Boost of Cholesterol-Containing Lipoplexes. Biochim Biophys Acta 2012, 1818 (9), 2335–2343. 10.1016/j.bbamem.2012.05.017. [PubMed: 22627109]

(98). Suk JS; Xu Q; Kim N; Hanes J; Ensign LM PEGylation as a Strategy for Improving Nanoparticle-Based Drug and Gene Delivery. Adv Drug Deliv Rev 2016, 99 (Pt A), 28–51. 10.1016/j.addr.2015.09.012. [PubMed: 26456916]

(99). Üzgün S; Nica G; Pfeifer C; Bosinco M; Michaelis K; Lutz J-F; Schneider M; Rosenecker J; Rudolph C PEGylation Improves Nanoparticle Formation and Transfection Efficiency of Messenger RNA. Pharm Res 2011, 28 (9), 2223–2232. 10.1007/s11095-011-0464-z. [PubMed: 21594715]

(100). Tenchov R; Bird R; Curtze AE; Zhou Q Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. ACS Nano 2021, 15 (11), 16982–17015. 10.1021/acsnano.1c04996. [PubMed: 34181394]

(101). Maeki M; Uno S; Niwa A; Okada Y; Tokeshi M Microfluidic Technologies and Devices for Lipid Nanoparticle-Based RNA Delivery. J Control Release 2022, 344, 80–96. 10.1016/j.jconrel.2022.02.017. [PubMed: 35183654]

(102). Kularatne RN; Crist RM; Stern ST The Future of Tissue-Targeted Lipid Nanoparticle-Mediated Nucleic Acid Delivery. Pharmaceuticals (Basel) 2022, 15 (7), 897. 10.3390/ph15070897. [PubMed: 35890195]

(103). Qiu M; Tang Y; Chen J; Muriph R; Ye Z; Huang C; Evans J; Henske EP; Xu Q Lung-Selective mRNA Delivery of Synthetic Lipid Nanoparticles for the Treatment of Pulmonary Lymphangioleiomyomatosis. Proceedings of the National Academy of Sciences 2022, 119 (8), e2116271119. 10.1073/pnas.2116271119.

(104). Thomas SJ; Moreira ED; Kitchin N; Absalon J; Gurtman A; Lockhart S; Perez JL; Pérez Marc G; Polack FP; Zerbini C; Bailey R; Swanson KA; Xu X; Roychoudhury S; Koury K; Bouguermouh S; Kalina WV; Cooper D; Frenck RW; Hammitt LL; Türeci Ö; Nell H; Schaefer A; ünal S; Yang Q; Liberator P; Tresnan DB; Mather S; Dormitzer PR; Şahin U; Gruber WC; Jansen KU Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. New England Journal of Medicine 2021, 385 (19), 1761–1773. 10.1056/NEJMoa2110345. [PubMed: 34525277]

(105). Sun J; Zhang M; Gehl A; Fricke B; Nawaz K; Gluesenkamp K; Shen B; Munk J; Hagerman J; Lapsa M COVID 19 Vaccine Distribution Solution to the Last Mile Challenge: Experimental and Simulation Studies of Ultra-Low Temperature Refrigeration System. International Journal of Refrigeration 2022, 133, 313–325. 10.1016/j.ijrefrig.2021.11.005. [PubMed: 34776559]

(106). Kelso JM Anaphylactic Reactions to Novel mRNA SARS-CoV-2/COVID-19 Vaccines. Vaccine 2021, 39 (6), 865–867. 10.1016/j.vaccine.2020.12.084. [PubMed: 33441235]

(107). Ju Y; Lee WS; Pilkington EH; Kelly HG; Li S; Selva KJ; Wragg KM; Subbarao K; Nguyen THO; Rowntree LC; Allen LF; Bond K; Williamson DA; Truong NP; Plebanski M; Kedzierska K; Mahanty S; Chung AW; Caruso F; Wheatley AK; Juno JA; Kent SJ Anti-PEG Antibodies Boosted in Humans by SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine. ACS Nano 2022, 16 (8), 11769–11780. 10.1021/acsnano.2c04543. [PubMed: 35758934]

(108). Kedmi R; Ben-Arie N; Peer D The Systemic Toxicity of Positively Charged Lipid Nanoparticles and the Role of Toll-like Receptor 4 in Immune Activation. Biomaterials 2010, 31 (26), 6867–6875. 10.1016/j.biomaterials.2010.05.027. [PubMed: 20541799]

(109). Dokka S; Toledo D; Shi X; Castranova V; Rojanasakul Y Oxygen Radical-Mediated Pulmonary Toxicity Induced by Some Cationic Liposomes. Pharm Res 2000, 17 (5), 521–525. 10.1023/a:1007504613351. [PubMed: 10888302]

(110). Zhang C; Ma Y; Zhang J; Kuo JC-T; Zhang Z; Xie H; Zhu J; Liu T Modification of Lipid-Based Nanoparticles: An Efficient Delivery System for Nucleic Acid-Based Immunotherapy. Molecules 2022, 27 (6), 1943. 10.3390/molecules27061943. [PubMed: 35335310]

(111). Piotrowski-Daspit AS; Kauffman AC; Bracaglia LG; Saltzman WM Polymeric Vehicles for Nucleic Acid Delivery. Adv Drug Deliv Rev 2020, 156, 119–132. 10.1016/j.addr.2020.06.014. [PubMed: 32585159]

(112). Pack DW; Hoffman AS; Pun S; Stayton PS Design and Development of Polymers for Gene Delivery. Nat Rev Drug Discov 2005, 4 (7), 581–593. 10.1038/nrd1775. [PubMed: 16052241]

(113). Hwang SJ; Davis ME Cationic Polymers for Gene Delivery: Designs for Overcoming Barriers to Systemic Administration. Curr Opin Mol Ther 2001, 3 (2), 183–191. [PubMed: 11338932]

(114). Kumar R; Santa Chalarca CF; Bockman MR; Bruggen CV; Grimme CJ; Dalal RJ; Hanson MG; Hexum JK; Reineke TM Polymeric Delivery of Therapeutic Nucleic Acids. Chem. Rev 2021, 121 (18), 11527–11652. 10.1021/acs.chemrev.0c00997. [PubMed: 33939409]

(115). Zakeri A; Kouhbanani MAJ; Beheshtkhoo N; Beigi V; Mousavi SM; Hashemi SAR; Karimi Zade A; Amani AM; Savardashtaki A; Mirzaei E; Jahandideh S; Movahedpour A Polyethylenimine-Based Nanocarriers in Co-Delivery of Drug and Gene: A Developing Horizon. Nano Rev Exp 2018, 9 (1), 1488497. 10.1080/20022727.2018.1488497. [PubMed: 30410712]

(116). Boussif O; Lezoualc'h F; Zanta MA; Mergny MD; Scherman D; Demeneix B; Behr JP A Versatile Vector for Gene and Oligonucleotide Transfer into Cells in Culture and in Vivo: Polyethylenimine. Proc Natl Acad Sci U S A 1995, 92 (16), 7297–7301. [PubMed: 7638184]

(117). Miyata K; Nishiyama N; Kataoka K Rational Design of Smart Supramolecular Assemblies for Gene Delivery: Chemical Challenges in the Creation of Artificial Viruses. Chem. Soc. Rev 2012, 41 (7), 2562–2574. 10.1039/C1CS15258K. [PubMed: 22105545]

(118). Bettinger T; Carlisle RC; Read ML; Ogris M; Seymour LW Peptide-Mediated RNA Delivery: A Novel Approach for Enhanced Transfection of Primary and Post-Mitotic Cells. Nucleic Acids Res 2001, 29 (18), 3882–3891. [PubMed: 11557821]

(119). Jere D; Jiang HL; Arote R; Kim YK; Choi YJ; Cho MH; Akaike T; Cho CS Degradable Polyethylenimines as DNA and Small Interfering RNA Carriers. Expert Opin Drug Deliv 2009, 6 (8), 827–834. 10.1517/17425240903029183. [PubMed: 19558333]

(120). Schwarz B; Merkel OM Functionalized PEI and its role in gene therapy. Mater Matters 2017, 12 (2). https://scholar.google.com/scholar_lookup?journal=Mater+Matters&title=Functionalized+PEI+and+its+role+in+gene+therapy&author=B+Schwarz&author=OM+Merkel&volume=12&publication_year=2017&pages=2&.

(121). Zhao M; Li M; Zhang Z; Gong T; Sun X Induction of HIV-1 Gag Specific Immune Responses by Cationic Micelles Mediated Delivery of Gag mRNA. Drug Delivery 2016, 23 (7), 2596–2607. 10.3109/10717544.2015.1038856. [PubMed: 26024387]

(122). Li M; Zhao M; Fu Y; Li Y; Gong T; Zhang Z; Sun X Enhanced Intranasal Delivery of mRNA Vaccine by Overcoming the Nasal Epithelial Barrier via Intra- and Paracellular Pathways. Journal of Controlled Release 2016, 228, 9–19. 10.1016/j.jconrel.2016.02.043. [PubMed: 26941035]

(123). Yang W; Mixich L; Boonstra E; Cabral H Polymer-Based mRNA Delivery Strategies for Advanced Therapies. Advanced Healthcare Materials n/a (n/a), 2202688. 10.1002/adhm.202202688.

(124). Ding G-B; Meng X; Yang P; Li B; Stauber RH; Li Z Integration of Polylactide into Polyethylenimine Facilitates the Safe and Effective Intracellular SiRNA Delivery. Polymers (Basel) 2020, 12 (2), 445. 10.3390/polym12020445. [PubMed: 32074943]

(125). Lutz GJ; Sirsi SR; Williams JH PEG-PEI Copolymers for Oligonucleotide Delivery to Cells and Tissues. Methods Mol Biol 2008, 433, 141–158. 10.1007/978-1-59745-237-3_9. [PubMed: 18679622]

(126). Xue L; Yan Y; Kos P; Chen X; Siegwart DJ PEI Fluorination Reduces Toxicity and Promotes Liver-Targeted SiRNA Delivery. Drug Deliv Transl Res 2021, 11 (1), 255–260. 10.1007/s13346-020-00790-9. [PubMed: 32506259]

(127). Abedi-Gaballu F; Dehghan G; Ghaffari M; Yekta R; Abbaspour-Ravasjani S; Baradaran B; Ezzati Nazhad Dolatabadi J; Hamblin MR PAMAM Dendrimers as Efficient Drug and Gene Delivery Nanosystems for Cancer Therapy. Applied Materials Today 2018, 12, 177–190. 10.1016/j.apmt.2018.05.002. [PubMed: 30511014]

(128). Santos A; Veiga F; Figueiras A Dendrimers as Pharmaceutical Excipients: Synthesis, Properties, Toxicity and Biomedical Applications. Materials (Basel) 2019, 13 (1), 65. 10.3390/ma13010065. [PubMed: 31877717]

(129). Fana M; Gallien J; Srinageshwar B; Dunbar GL; Rossignol J PAMAM Dendrimer Nanomolecules Utilized as Drug Delivery Systems for Potential Treatment of Glioblastoma: A Systematic Review. Int J Nanomedicine 2020, 15, 2789–2808. 10.2147/IJN.S243155. [PubMed: 32368055]

(130). Jain K; Kesharwani P; Gupta U; Jain NK Dendrimer Toxicity: Let's Meet the Challenge. Int J Pharm 2010, 394 (1–2), 122–142. 10.1016/j.ijpharm.2010.04.027. [PubMed: 20433913]

(131). Li X; Naeem A; Xiao S; Hu L; Zhang J; Zheng Q Safety Challenges and Application Strategies for the Use of Dendrimers in Medicine. Pharmaceutics 2022, 14 (6), 1292. 10.3390/pharmaceutics14061292. [PubMed: 35745863]

(132). Bugno J; Hsu H-J; Hong S Tweaking Dendrimers and Dendritic Nanoparticles for Controlled Nano-Bio Interactions: Potential Nanocarriers for Improved Cancer Targeting. J Drug Target 2015, 23 (7–8), 642–650. 10.3109/1061186X.2015.1052077. [PubMed: 26453160]

(133). Tarach P; Janaszewska A Recent Advances in Preclinical Research Using PAMAM Dendrimers for Cancer Gene Therapy. Int J Mol Sci 2021, 22 (6), 2912. 10.3390/ijms22062912. [PubMed: 33805602]

(134). Karlsson J; Rhodes KR; Green JJ; Tzeng SY Poly(Beta-Amino Ester)s as Gene Delivery Vehicles: Challenges and Opportunities. Expert Opin Drug Deliv 2020, 17 (10), 1395–1410. 10.1080/17425247.2020.1796628. [PubMed: 32700581]

(135). Vilar G; Tulla-Puche J; Albericio F Polymers and Drug Delivery Systems. Current Drug Delivery 9 (4), 367–394. [PubMed: 22640038]

(136). Mastorakos P; da Silva AL; Chisholm J; Song E; Choi WK; Boyle MP; Morales MM; Hanes J; Suk JS Highly Compacted Biodegradable DNA Nanoparticles Capable of Overcoming the Mucus Barrier for Inhaled Lung Gene Therapy. Proc Natl Acad Sci U S A 2015, 112 (28), 8720–8725. 10.1073/pnas.1502281112. [PubMed: 26124127]

(137). Fields RJ; Cheng CJ; Quijano E; Weller C; Kristofik N; Duong N; Hoimes C; Egan ME; Saltzman WM Surface Modified Poly(β Amino Ester)-Containing Nanoparticles for Plasmid DNA Delivery. J Control Release 2012, 164 (1), 41–48. 10.1016/j.jconrel.2012.09.020. [PubMed: 23041278]

(138). Segovia N; Dosta P; Cascante A; Ramos V; Borrós S Oligopeptide-Terminated Poly(β-Amino Ester)s for Highly Efficient Gene Delivery and Intracellular Localization. Acta Biomater 2014, 10 (5), 2147–2158. 10.1016/j.actbio.2013.12.054. [PubMed: 24406199]

(139). Dosta P; Ramos V; Borrós S Stable and Efficient Generation of Poly(β-Amino Ester)s for RNAi Delivery. Molecular Systems Design & Engineering 2018, 3 (4), 677–689. 10.1039/C8ME00006A.

(140). Lee JS; Deng X; Han P; Cheng J Dual Stimuli-Responsive Poly(β-Amino Ester) Nanoparticles for On-Demand Burst Release. Macromolecular Bioscience 2015, 15 (9), 1314–1322. 10.1002/mabi.201500111. [PubMed: 26036966]

(141). Negron K; Zhu C; Chen S-W; Shahab S; Rao D; Raabe EH; Eberhart CG; Hanes J; Suk JS Non-Adhesive and Highly-Stable Biodegradable Nanoparticles That Provide Widespread and Safe Transgene Expression in Orthotopic Brain Tumors. Drug Deliv Transl Res 2020, 10 (3), 572–581. 10.1007/s13346-020-00759-8. [PubMed: 32323162]

(142). Deng X; Zheng N; Song Z; Yin L; Cheng J Trigger-Responsive, Fast-Degradable Poly(β-Amino Ester)s for Enhanced DNA Unpackaging and Reduced Toxicity. Biomaterials 2014, 35 (18), 5006–5015. 10.1016/j.biomaterials.2014.03.005. [PubMed: 24674461]

(143). Sadeqi Nezhad M Poly (Beta-Amino Ester) as an in Vivo Nanocarrier for Therapeutic Nucleic Acids. Biotechnology and Bioengineering 2023, 120 (1), 95–113. 10.1002/bit.28269. [PubMed: 36266918]

(144). Niculescu A-G; Bîrcă AC; Grumezescu AM New Applications of Lipid and Polymer-Based Nanoparticles for Nucleic Acids Delivery. Pharmaceutics 2021, 13 (12), 2053. 10.3390/pharmaceutics13122053. [PubMed: 34959335]

(145). Saginala K; Barsouk A; Aluru JS; Rawla P; Barsouk A Epidemiology of Melanoma. Medical Sciences 2021, 9 (4), 63. 10.3390/medsci9040063. [PubMed: 34698235]

(146). Villani A; Potestio L; Fabbrocini G; Troncone G; Malapelle U; Scalvenzi M The Treatment of Advanced Melanoma: Therapeutic Update. IJMS 2022, 23 (12), 6388. 10.3390/ijms23126388. [PubMed: 35742834]

(147). Alexandrov LB; Nik-Zainal S; Wedge DC; Aparicio SAJR; Behjati S; Biankin AV; Bignell GR; Bolli N; Borg A; Børresen-Dale A-L; Boyault S; Burkhardt B; Butler AP; Caldas C; Davies HR; Desmedt C; Eils R; Eyfjörd JE; Foekens JA; Greaves M; Hosoda F; Hutter B; Ilicic T; Imbeaud S; Imielinski M; Jäger N; Jones DTW; Jones D; Knappskog S; Kool M; Lakhani SR; López-Otín C; Martin S; Munshi NC; Nakamura H; Northcott PA; Pajic M; Papaemmanuil E; Paradiso A; Pearson JV; Puente XS; Raine K; Ramakrishna M; Richardson AL; Richter J; Rosenstiel P; Schlesner M; Schumacher TN; Span PN; Teague JW; Totoki Y; Tutt ANJ; Valdés-Mas R; van Buuren MM; van 't Veer L; Vincent-Salomon A; Waddell N; Yates LR; Zucman-Rossi J; Andrew Futreal P; McDermott U; Lichter P; Meyerson M; Grimmond SM; Siebert R; Campo E; Shibata T; Pfister SM; Campbell PJ; Stratton MR Signatures of Mutational Processes in Human Cancer. Nature 2013, 500 (7463), 415–421. 10.1038/nature12477. [PubMed: 23945592]

(148). Huda MN; Deaguro IG; Borrego EA; Kumar R; Islam T; Afrin H; Varela-Ramirez A; Aguilera RJ; Tanner EEL; Nurunnabi M Ionic Liquid-Mediated Delivery of a BCL-2 Inhibitor for Topical Treatment of Skin Melanoma. J Control Release 2022, 349, 783–795. 10.1016/j.jconrel.2022.07.035. [PubMed: 35908622]

(149). Melanoma Treatment - NCI. Accessed online: https://www.cancer.gov/types/skin/patient/melanoma-treatment-pdq (accessed 6 June 2023).

(150). Lee C-S; Thomas CM; Ng KE An Overview of the Changing Landscape of Treatment for Advanced Melanoma. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2017, 37 (3), 319–333. 10.1002/phar.1895.

(151). Oberli MA; Reichmuth AM; Dorkin JR; Mitchell MJ; Fenton OS; Jaklenec A; Anderson DG; Langer R; Blankschtein D Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. Nano Lett 2017, 17 (3), 1326–1335. 10.1021/acs.nanolett.6b03329. [PubMed: 28273716]

(152). Miao L; Li L; Huang Y; Delcassian D; Chahal J; Han J; Shi Y; Sadtler K; Gao W; Lin J; Doloff JC; Langer R; Anderson DG Delivery of mRNA Vaccines with Heterocyclic Lipids Increases Anti-Tumor Efficacy by STING-Mediated Immune Cell Activation. Nat Biotechnol 2019, 37 (10), 1174–1185. 10.1038/s41587-019-0247-3. [PubMed: 31570898]

(153). He M; Huang L; Hou X; Zhong C; Bachir ZA; Lan M; Chen R; Gao F Efficient Ovalbumin Delivery Using a Novel Multifunctional Micellar Platform for Targeted Melanoma Immunotherapy. Int J Pharm 2019, 560, 1–10. 10.1016/j.ijpharm.2019.01.027. [PubMed: 30677484]

(154). Li Q; Ren J; Liu W; Jiang G; Hu R CpG Oligodeoxynucleotide Developed to Activate Primate Immune Responses Promotes Antitumoral Effects in Combination with a Neoantigen-Based mRNA Cancer Vaccine. DDDT 2021, *Volume* 15, 3953–3963. 10.2147/DDDT.S325790. [PubMed: 34566407]

(155). Aldén M; Olofsson Falla F; Yang D; Barghouth M; Luan C; Rasmussen M; De Marinis Y Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. CIMB 2022, 44 (3), 1115–1126. 10.3390/cimb44030073. [PubMed: 35723296]

(156). Boettler T; Csernalabics B; Salié H; Luxenburger H; Wischer L; Salimi Alizei E; Zoldan K; Krimmel L; Bronsert P; Schwabenland M; Prinz M; Mogler C; Neumann-Haefelin C; Thimme R; Hofmann M; Bengsch B SARS-CoV-2 Vaccination Can Elicit a CD8 T-Cell Dominant Hepatitis. J Hepatol 2022, 77 (3), 653–659. 10.1016/j.jhep.2022.03.040. [PubMed: 35461912]

(157). Chen J; Ye Z; Huang C; Qiu M; Song D; Li Y; Xu Q Lipid Nanoparticle-Mediated Lymph Node–Targeting Delivery of mRNA Cancer Vaccine Elicits Robust $CD8^+$ T Cell Response. Proc. Natl. Acad. Sci. U.S.A 2022, 119 (34), e2207841119. 10.1073/pnas.2207841119. [PubMed: 35969778]

(158). Liu J-Q; Zhang C; Zhang X; Yan J; Zeng C; Talebian F; Lynch K; Zhao W; Hou X; Du S; Kang DD; Deng B; McComb DW; Bai X-F; Dong Y Intratumoral Delivery of IL-12 and IL-27 mRNA Using Lipid Nanoparticles for Cancer Immunotherapy. Journal of Controlled Release 2022, 345, 306–313. 10.1016/j.jconrel.2022.03.021. [PubMed: 35301053]

(159). Moderna and Merck Announce mRNA-4157/V940, an Investigational Personalized mRNA Cancer Vaccine, in Combination With KEYTRUDA® (pembrolizumab), Met Primary Efficacy Endpoint in Phase 2b KEYNOTE-942 Trial. Accessed online: https://www.merck.com/news/moderna-and-merck-announce-mrna-4157-v940-an-investigational-personalized-mrna-cancer-vaccine-in-combination-with-keytruda-pembrolizumab-met-primary-efficacy-endpoint-in-phase-2b-keynote-94/ (accessed 5 June 2023).

(160). Li J; Wu Y; Xiang J; Wang H; Zhuang Q; Wei T; Cao Z; Gu Q; Liu Z; Peng R Fluoroalkane Modified Cationic Polymers for Personalized mRNA Cancer Vaccines. Chem Eng J 2023, 456, 140930. 10.1016/j.cej.2022.140930. [PubMed: 36531858]

(161). Zhang H; You X; Wang X; Cui L; Wang Z; Xu F; Li M; Yang Z; Liu J; Huang P; Kang Y; Wu J; Xia X Delivery of mRNA Vaccine with a Lipid-like Material Potentiates Antitumor Efficacy through Toll-like Receptor 4 Signaling. Proc. Natl. Acad. Sci. U.S.A 2021, 118 (6), e2005191118. 10.1073/pnas.2005191118. [PubMed: 33547233]

(162). Zhang F; Parayath NN; Ene CI; Stephan SB; Koehne AL; Coon ME; Holland EC; Stephan MT Genetic Programming of Macrophages to Perform Anti-Tumor Functions Using Targeted mRNA Nanocarriers. Nat Commun 2019, 10 (1), 3974. 10.1038/s41467-019-11911-5. [PubMed: 31481662]

(163). Zhang MH; Scotland BL; Jiao Y; Slaby EM; Truong N; Cottingham AL; Stephanie G; Szeto GL; Pearson RM Lipid–Polymer Hybrid Nanoparticles Utilize B Cells and Dendritic Cells to Elicit Distinct Antigen-Specific CD4+ and CD8+ T Cell Responses. ACS Appl. Bio Mater 2023. 10.1021/acsabm.3c00229.

(164). Chen W; Li H; Liu Z; Yuan W Lipopolyplex for Therapeutic Gene Delivery and Its Application for the Treatment of Parkinson's Disease. Frontiers in Aging Neuroscience 2016, 8, 1–10. 10.3389/fnagi.2016.00068 [PubMed: 26858637]

(165). Karmacharya P; Patil BR; Kim JO Recent Advancements in Lipid–mRNA Nanoparticles as a Treatment Option for Cancer Immunotherapy. J. Pharm. Investig 2022, 52 (4), 415–426. 10.1007/s40005-022-00569-9.

(166). Mockey M; Bourseau E; Chandrashekhar V; Chaudhuri A; Lafosse S; Le Cam E; Quesniaux VFJ; Ryffel B; Pichon C; Midoux P mRNA-Based Cancer Vaccine: Prevention of B16 Melanoma Progression and Metastasis by Systemic Injection of MART1 mRNA Histidylated Lipopolyplexes. Cancer Gene Ther 2007, 14 (9), 802–814. 10.1038/sj.cgt.7701072. [PubMed: 17589432]

(167). Perche F; Benvegnu T; Berchel M; Lebegue L; Pichon C; Jaffrès P-A; Midoux P Enhancement of Dendritic Cells Transfection in Vivo and of Vaccination against B16F10 Melanoma with Mannosylated Histidylated Lipopolyplexes Loaded with Tumor Antigen Messenger RNA. Nanomedicine: Nanotechnology, Biology and Medicine 2011, 7 (4), 445–453. 10.1016/j.nano.2010.12.010. [PubMed: 21220051]

(168). Le Moignic A; Malard V; Benvegnu T; Lemiègre L; Berchel M; Jaffrès P-A; Baillou C; Delost M; Macedo R; Rochefort J; Lescaille G; Pichon C; Lemoine FM; Midoux P; Mateo V Preclinical Evaluation of mRNA Trimannosylated Lipopolyplexes as Therapeutic Cancer Vaccines Targeting Dendritic Cells. Journal of Controlled Release 2018, 278, 110–121. 10.1016/j.jconrel.2018.03.035. [PubMed: 29630987]

(169). Persano S; Guevara ML; Li Z; Mai J; Ferrari M; Pompa PP; Shen H Lipopolyplex Potentiates Anti-Tumor Immunity of mRNA-Based Vaccination. Biomaterials 2017, 125, 81–89. 10.1016/j.biomaterials.2017.02.019. [PubMed: 28231510]

(170). Van Der Jeught K; De Koker S; Bialkowski L; Heirman C; Tjok Joe P; Perche F; Maenhout S; Bevers S; Broos K; Deswarte K; Malard V; Hammad H; Baril P; Benvegnu T; Jaffrès P-A; Kooijmans SAA; Schiffelers R; Lienenklaus S; Midoux P; Pichon C; Breckpot K; Thielemans K Dendritic Cell Targeting mRNA Lipopolyplexes Combine Strong Antitumor T-Cell Immunity with Improved Inflammatory Safety. ACS Nano 2018, 12 (10), 9815–9829. 10.1021/acsnano.8b00966. [PubMed: 30256609]

(171). Wilgenhof S; Van Nuffel AMT; Corthals J; Heirman C; Tuyaerts S; Benteyn D; De Coninck A; Van Riet I; Verfaillie G; Vandeloo J; Bonehill A; Thielemans K; Neyns B Therapeutic Vaccination With an Autologous mRNA Electroporated Dendritic Cell Vaccine in Patients With Advanced Melanoma. Journal of Immunotherapy 2011, 34 (5), 448–456. 10.1097/CJI.0b013e31821dcb31. [PubMed: 21577140]

(172). Van Nuffel AM; Benteyn D; Wilgenhof S; Pierret L; Corthals J; Heirman C; van der Bruggen P; Coulie PG; Neyns B; Thielemans K; Bonehill A Dendritic Cells Loaded With mRNA Encoding Full-Length Tumor Antigens Prime CD4+ and CD8+ T Cells in Melanoma Patients. Mol Ther 2012, 20 (5), 1063–1074. 10.1038/mt.2012.11. [PubMed: 22371843]

(173). Wilgenhof S; Nuffel AMTV; Benteyn D; Corthals J; Aerts C; Heirman C; Riet IV; Bonehill A; Thielemans K; Neyns B A Phase IB Study on Intravenous Synthetic mRNA Electroporated Dendritic Cell Immunotherapy in Pretreated Advanced Melanoma Patients. Annals of Oncology 2013, 24 (10), 2686–2693. 10.1093/annonc/mdt245. [PubMed: 23904461]

(174). Wilgenhof S; Corthals J; Van Nuffel AMT; Benteyn D; Heirman C; Bonehill A; Thielemans K; Neyns B Long-Term Clinical Outcome of Melanoma Patients Treated with Messenger RNA-Electroporated Dendritic Cell Therapy Following Complete Resection of Metastases. Cancer Immunol Immunother 2015, 64 (3), 381–388. 10.1007/s00262-014-1642-8. [PubMed: 25548092]

(175). Wilgenhof S; Corthals J; Heirman C; van Baren N; Lucas S; Kvistborg P; Thielemans K; Neyns B Phase II Study of Autologous Monocyte-Derived mRNA Electroporated Dendritic Cells (TriMixDC-MEL) Plus Ipilimumab in Patients With Pretreated Advanced Melanoma. JCO 2016, 34 (12), 1330–1338. 10.1200/JCO.2015.63.4121.

(176). Jansen Y; Kruse V; Corthals J; Schats K; van Dam P-J; Seremet T; Heirman C; Brochez L; Kockx M; Thielemans K; Neyns B A Randomized Controlled Phase II Clinical Trial on mRNA Electroporated Autologous Monocyte-Derived Dendritic Cells (TriMixDC-MEL) as Adjuvant Treatment for Stage III/IV Melanoma Patients Who Are Disease-Free Following the Resection of Macrometastases. Cancer Immunol Immunother 2020, 69 (12), 2589–2598. 10.1007/s00262-020-02618-4. [PubMed: 32591862]

(177). Bonehill A; Van Nuffel AMT; Corthals J; Tuyaerts S; Heirman C; François V; Colau D; van der Bruggen P; Neyns B; Thielemans K Single-Step Antigen Loading and Activation of Dendritic Cells by mRNA Electroporation for the Purpose of Therapeutic Vaccination in Melanoma

Patients. Clinical Cancer Research 2009, 15 (10), 3366–3375. 10.1158/1078-0432.CCR-08-2982. [PubMed: 19417017]

(178). Van Lint S; Goyvaerts C; Maenhout S; Goethals L; Disy A; Benteyn D; Pen J; Bonehill A; Heirman C; Breckpot K; Thielemans K Preclinical Evaluation of TriMix and Antigen mRNA-Based Antitumor Therapy. Cancer Research 2012, 72 (7), 1661–1671. 10.1158/0008-5472.CAN-11-2957. [PubMed: 22337996]

(179). Van Hoecke L; Verbeke R; Dewitte H; Lentacker I; Vermaelen K; Breckpot K; Van Lint S mRNA in Cancer Immunotherapy: Beyond a Source of Antigen. Mol Cancer 2021, 20 (1), 48. 10.1186/s12943-021-01329-3. [PubMed: 33658037]

(180). Pan J; Ruan W; Qin M; Long Y; Wan T; Yu K; Zhai Y; Wu C; Xu Y Intradermal Delivery of STAT3 SiRNA to Treat Melanoma via Dissolving Microneedles. Sci Rep 2018, 8, 1117. 10.1038/s41598-018-19463-2. [PubMed: 29348670]

(181). Zhang X; Cai A; Gao Y; Zhang Y; Duan X; Men K Treatment of Melanoma by Nano-Conjugate-Delivered Wee1 SiRNA. Molecular Pharmaceutics 2021. 10.1021/acs.molpharmaceut.1c00316.

(182). Chen Y; Bathula SR; Yang Q; Huang L Targeted Nanoparticles Deliver SiRNA to Melanoma. Journal of Investigative Dermatology 2010, 130 (12), 2790–2798. 10.1038/jid.2010.222. [PubMed: 20686495]

(183). Ruan R; Chen M; Sun S; Wei P; Zou L; Liu J; Gao D; Wen L; Ding W Topical and Targeted Delivery of SiRNAs to Melanoma Cells Using a Fusion Peptide Carrier. Sci Rep 2016, 6 (1), 29159. 10.1038/srep29159. [PubMed: 27374619]

(184). Poeck H; Besch R; Maihoefer C; Renn M; Tormo D; Morskaya SS; Kirschnek S Gaffal E; Landsberg J; Hellmuth J; Schmidt A; Anz D; Bscheider M; Schwerd T Berking C; Bourquin C; Kalinke U; Kremmer E; Kato H; Akira S; Meyers R; Häcker G; Neuenhahn M; Busch D; Ruland J; Rothenfusser S; Prinz M; Hornung V; Endres S; Tüting T; Hartmann G 5′-Triphosphate-SiRNA: Turning Gene Silencing and Rig-I Activation against Melanoma. Nat Med 2008, 14 (11), 1256–1263. 10.1038/nm.1887. [PubMed: 18978796]

(185). Wang Y; Ji X; Ruan M; Liu W; Song R; Dai J; Xue W Worm-Like Biomimetic Nanoerythrocyte Carrying SiRNA for Melanoma Gene Therapy. Small 2018, 14 (47), 1803002. 10.1002/smll.201803002.

(186). Dong Y; Eltoukhy AA; Alabi CA; Khan OF; Veiseh O; Dorkin JR; Sirirungruang S; Yin H; Tang BC; Pelet JM; Chen D; Gu Z; Xue Y; Langer R; Anderson DG Lipid-Like Nanomaterials for Simultaneous Gene Expression and Silencing In Vivo. Advanced Healthcare Materials 2014, 3 (9), 1392–1397. 10.1002/adhm.201400054. [PubMed: 24623658]

(187). Niu G; Bowman T; Huang M; Shivers S; Reintgen D; Daud A; Chang A; Kraker A; Jove R; Yu H Roles of Activated Src and Stat3 Signaling in Melanoma Tumor Cell Growth. Oncogene 2002, 21 (46), 7001–7010. 10.1038/sj.onc.1205859. [PubMed: 12370822]

(188). Kyte JA; Aamdal S; Dueland S; Sæbøe-Larsen S; Inderberg EM; Madsbu UE; Skovlund E; Gaudernack G; Kvalheim G Immune Response and Long-Term Clinical Outcome in Advanced Melanoma Patients Vaccinated with Tumor-mRNA-Transfected Dendritic Cells. Oncoimmunology 2016, 5 (11), e1232237. 10.1080/2162402X.2016.1232237. [PubMed: 27999747]

(189). Weide B; Pascolo S; Scheel B; Derhovanessian E; Pflugfelder A; Eigentler TK; Pawelec G; Hoerr I; Rammensee H-G; Garbe C Direct Injection of Protamine-Protected mRNA: Results of a Phase 1/2 Vaccination Trial in Metastatic Melanoma Patients. Journal of Immunotherapy 2009, 32 (5), 498–507. 10.1097/CJI.0b013e3181a00068. [PubMed: 19609242]

(190). Aarntzen EHJG; Schreibelt G; Bol K; Lesterhuis WJ; Croockewit AJ; de Wilt JHW; van Rossum MM; Blokx WAM; Jacobs JFM; Duiveman-de Boer T; Schuurhuis DH; Mus R; Thielemans K; de Vries IJM; Figdor CG; Punt CJA; Adema GJ Vaccination with mRNA-Electroporated Dendritic Cells Induces Robust Tumor Antigen-Specific CD4+ and CD8+ T Cells Responses in Stage III and IV Melanoma Patients. Clinical Cancer Research 2012, 18 (19), 5460–5470. 10.1158/1078-0432.CCR-11-3368. [PubMed: 22896657]

(191). Barbier AJ; Jiang AY; Zhang P; Wooster R; Anderson DG The Clinical Progress of mRNA Vaccines and Immunotherapies. Nat Biotechnol 2022, 40 (6), 840–854. 10.1038/s41587-022-01294-2. [PubMed: 35534554]

(192). Bol K; van den Bosch T; Schreibelt G; Punt C; Figdor C; Paridaens D; de Vries J Adjuvant Dendritic Cell Vaccination in High-Risk Uveal Melanoma Patients. J Immunother Cancer 2015, 3 (Suppl 2), P127. 10.1186/2051-1426-3-S2-P127.

(193). Borch TH; Engell-Noerregaard L; Zeeberg Iversen T; Ellebaek E; Met Ö; Hansen M; Andersen MH; Thor Straten P; Svane IM mRNA-Transfected Dendritic Cell Vaccine in Combination with Metronomic Cyclophosphamide as Treatment for Patients with Advanced Malignant Melanoma. OncoImmunology 2016, 5 (9), e1207842. 10.1080/2162402X.2016.1207842. [PubMed: 27757300]

(194). Bol KF; Figdor CG; Aarntzen EH; Welzen ME; Van Rossum MM; Blokx WA; Van De Rakt MW; Scharenborg NM; De Boer AJ; Pots JM; Olde Nordkamp MA; Van Oorschot TG; Mus RD; Croockewit SA; Jacobs JF; Schuler G; Neyns B; Austyn JM; Punt CJ; Schreibelt G; De Vries IJM Intranodal Vaccination with mRNA-Optimized Dendritic Cells in Metastatic Melanoma Patients. OncoImmunology 2015, 4 (8), e1019197. 10.1080/2162402X.2015.1019197. [PubMed: 26405571]

(195). Chung DJ; Carvajal RD; Postow MA; Sharma S; Pronschinske KB; Shyer JA; Singh-Kandah S; Dickson MA; D'Angelo SP; Wolchok JD; Young JW Langerhans-Type Dendritic Cells Electroporated with TRP-2 mRNA Stimulate Cellular Immunity against Melanoma: Results of a Phase I Vaccine Trial. Oncoimmunology 2017, 7 (1), e1372081. 10.1080/2162402X.2017.1372081. [PubMed: 29296525]

(196). Miller M; Sahin U; Derhovanessian E; Kloke B-P; Simon P; Bukur V; Albrecht C; Paruzynski A; Löwer M; Kuhn A; Schreeb K; Attig S; Brueck AK; Bolte S; Grabbe S; Höller C; Utikal J; Huber C; Loquai C; Türeci Ö IVAC MUTANOME: A First-in-Human Phase I Clinical Trial Targeting Individual Mutant Neoantigens for the Treatment of Melanoma. Annals of Oncology 2017, 28, xi1–xi2. 10.1093/annonc/mdx712.003.

(197). Blass E; Ott PA Advances in the Development of Personalized Neoantigen-Based Therapeutic Cancer Vaccines. Nat Rev Clin Oncol 2021, 18 (4), 215–229. 10.1038/s41571-020-00460-2. [PubMed: 33473220]

(198). Patel M; Jimeno A; Wang D; Stemmer S; Bauer T; Sweis R; Geva R; Kummar S; Reagan P; Perets R; LoRusso P; Gupta S; Zacharek S; Laino A; Milberg O; Frederick J; Chen S; Pascarella S; Randolph W; Aanur P; Johansen L; Do K; Meehan R; Sullivan R 539 Phase 1 Study of mRNA-2752, a Lipid Nanoparticle Encapsulating mRNAs Encoding Human OX40L/IL-23/IL-36γ, for Intratumoral (ITu) Injection +/− Durvalumab in Advanced Solid Tumors and Lymphoma. J Immunother Cancer 2021, 9 (Suppl 2). 10.1136/jitc-2021-SITC2021.539.

(199). Cafri G; Gartner JJ; Zaks T; Hopson K; Levin N; Paria BC; Parkhurst MR; Yossef R; Lowery FJ; Jafferji MS; Prickett TD; Goff SL; McGowan CT; Seitter S; Shindorf ML; Parikh A; Chatani PD; Robbins PF; Rosenberg SA mRNA Vaccine–Induced Neoantigen-Specific T Cell Immunity in Patients with Gastrointestinal Cancer. J Clin Invest 130 (11), 5976–5988. 10.1172/JCI134915.

(200). Bauman J; Burris H; Clarke J; Patel M; Cho D; Gutierrez M; Julian R; Scott A; Cohen P; Frederick J; Robert-Tissot C; Zhou H; Mody K; Keating K; Meehan R; Gainor J 798 Safety, Tolerability, and Immunogenicity of mRNA-4157 in Combination with Pembrolizumab in Subjects with Unresectable Solid Tumors (KEYNOTE-603): An Update. J Immunother Cancer 2020, 8 (Suppl 3). 10.1136/jitc-2020-SITC2020.0798.

(201). Adding a Personalized mRNA Cancer Vaccine to Immunotherapy May Prolong Recurrence-free Survival in Patients With High-risk Melanoma. Accessed online: https://www.aacr.org/about-the-aacr/newsroom/news-releases/adding-a-personalized-mrna-cancer-vaccine-to-immunotherapy-may-prolong-recurrence-free-survival-in-patients-with-high-risk-melanoma/ (accessed 15 June 2023).

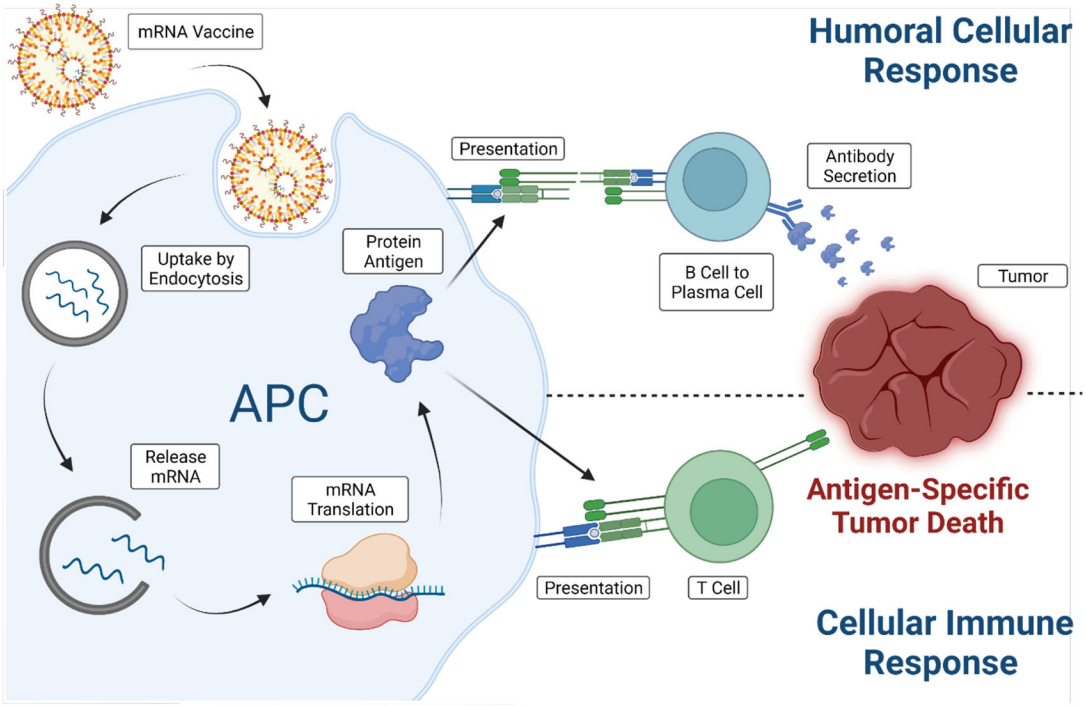


Figure 1.
Cellular and Humoral Immune Response Induced by an mRNA Vaccine

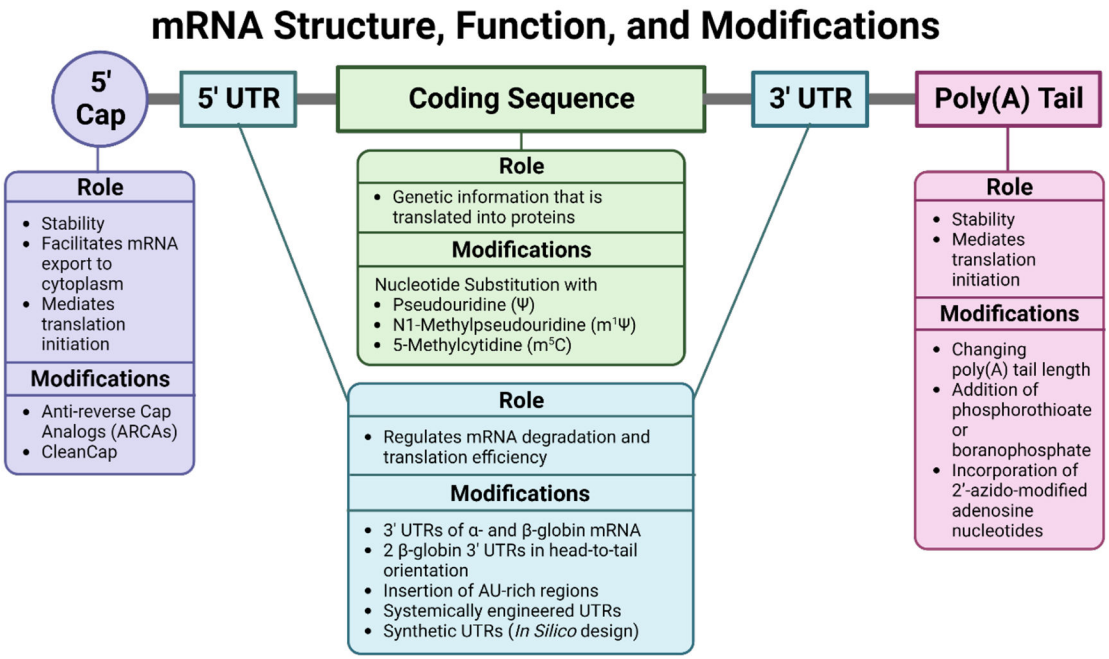


Figure 2.

Mature mRNA structure and function and synthetic modification strategies used to optimize mRNA performance

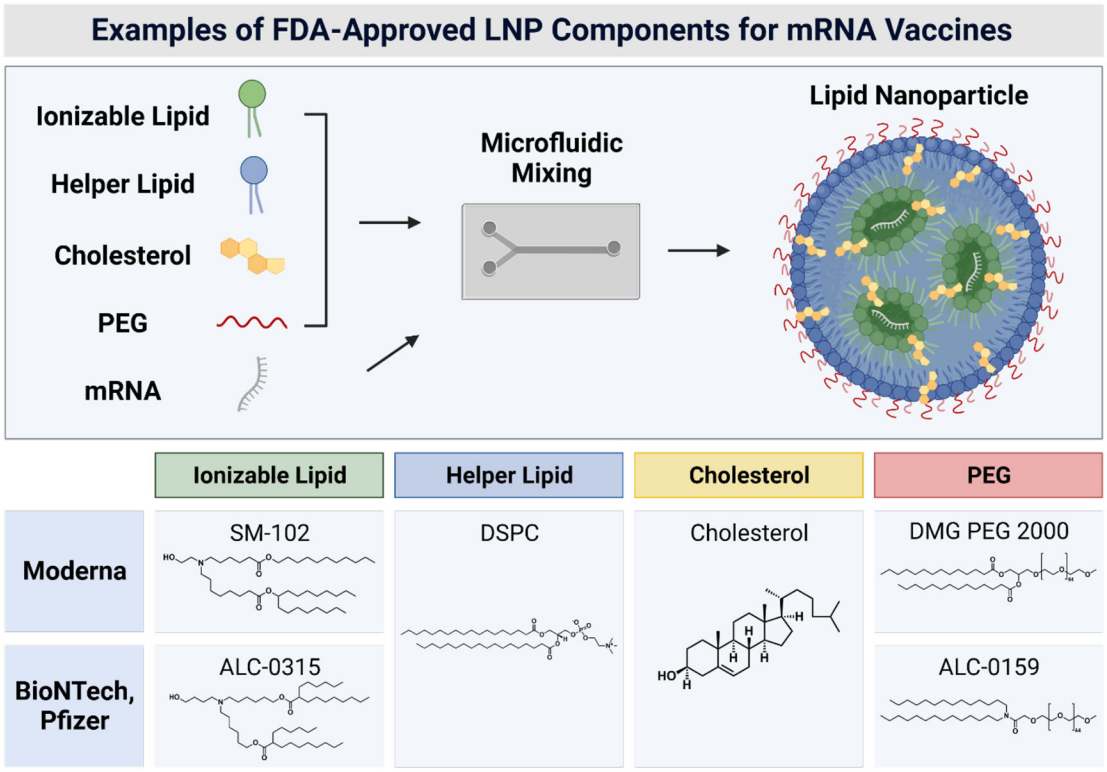


Figure 3.
Examples of FDA-approved lipid nanoparticle components used in mRNA vaccines

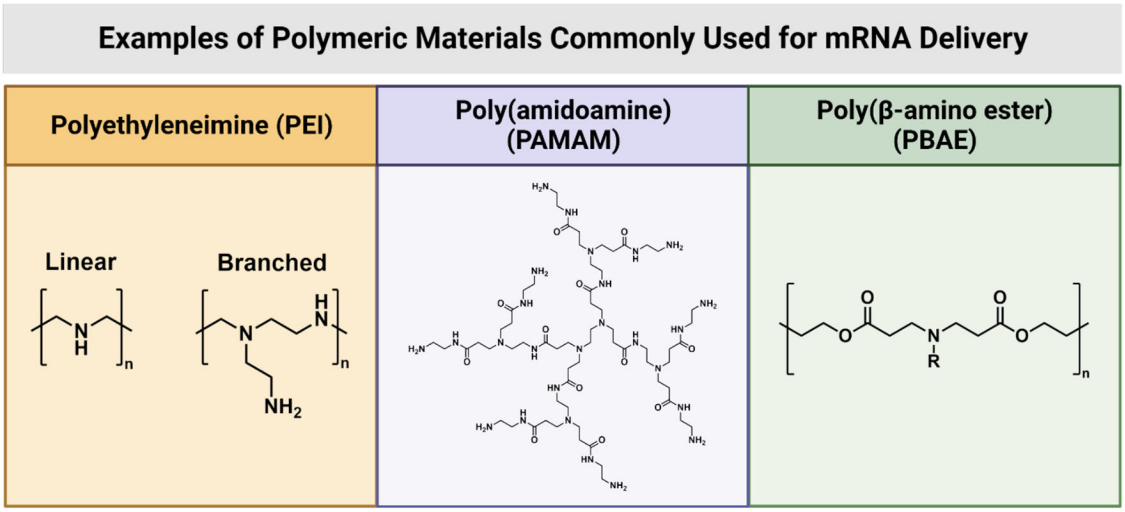


Figure 4.
Representative examples of polymeric materials commonly used for mRNA delivery

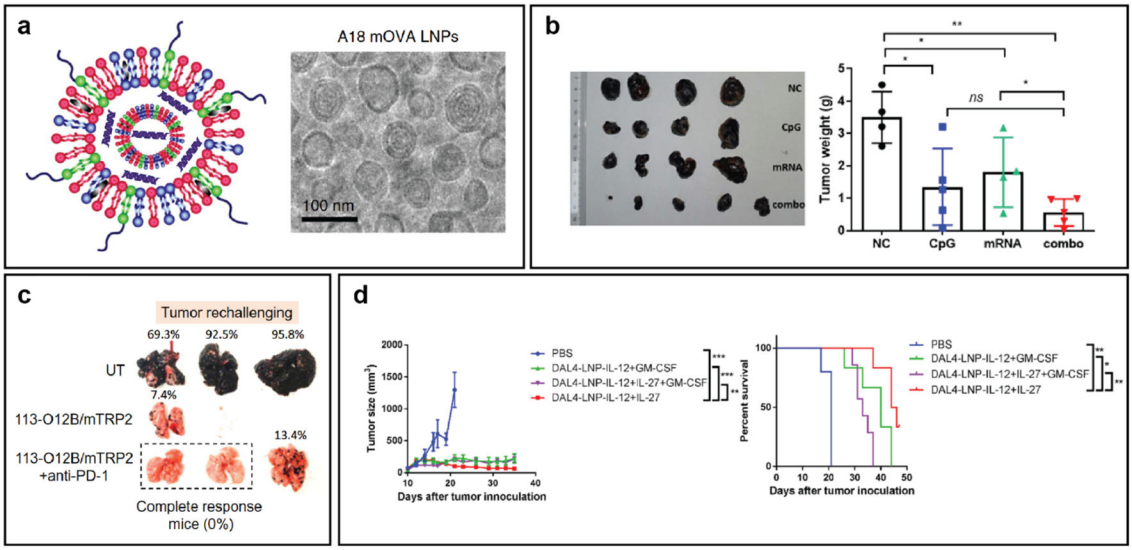


Figure 5.

a) Schematic and cryogenic electron microscopy image of optimized mOVA LNP formulation. Reproduced with permission from reference (152), Copyright 2019 Springer Nature. **b)** Anti-tumor effect of CpG2018B and mRNA LNP combination therapy. Images (left) and weights (right) of tumors resected from mice treated with negative control (NC), CpG, LNP-based mRNA vaccine, or CpG2018B combined with LNP-based mRNA vaccine. Reproduced with permission from reference (154), Copyright 2021 Dove Medical Press Limited. **c)** Tumor rechallenging with IV injection of B16F10-OVA cells in untreated (UT) and surviving mice. The surviving mice were previously treated with the mRNA vaccine alone or in combination with anti-PD-1 therapy. Reproduced with permission from reference (157), Copyright 2022 PNAS. **d)** Tumor size and survival curve of mice treated with LNPs encapsulating multiple cytokine mRNAs. Reproduced with permission from reference (158), Copyright 2022 Elsevier.

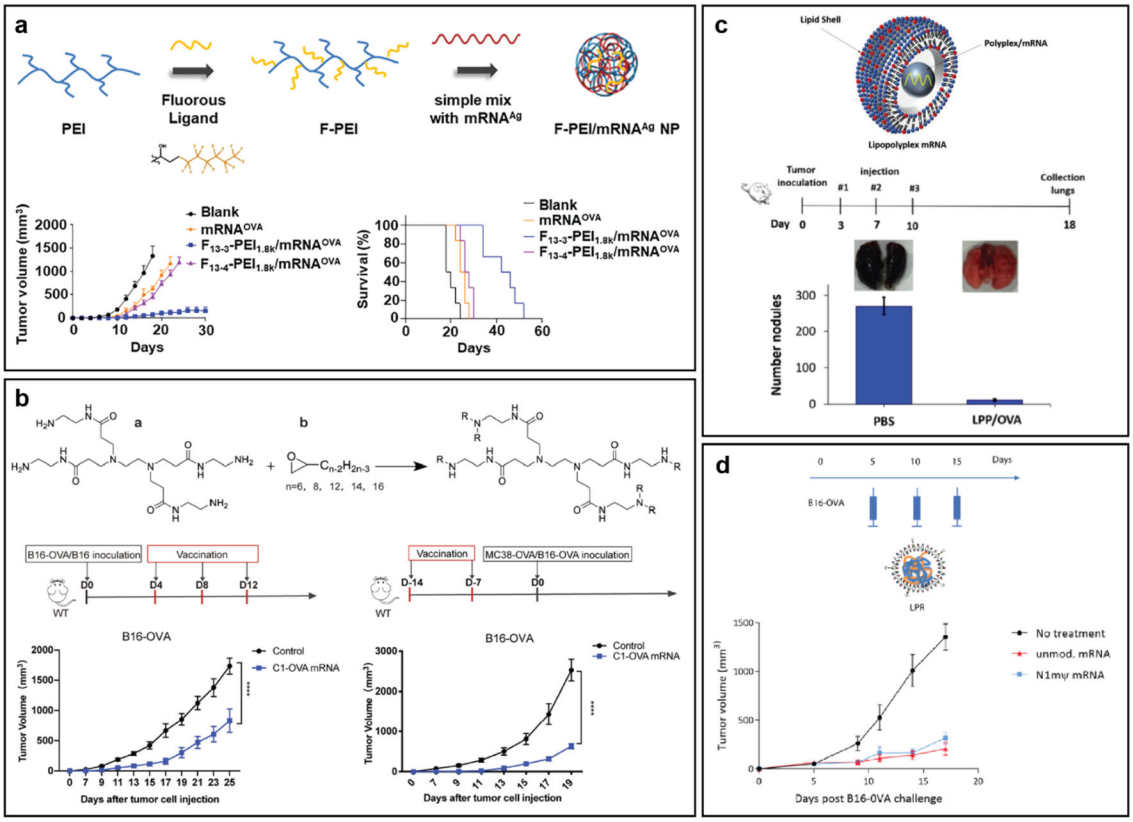


Figure 6.

a) Graphical overview of F-PEI mRNA vaccine preparation (top) and tumor volumes and survival curves of mice bearing B16-OVA tumors in different treatment groups (bottom). Reproduced with permission from reference (160), Copyright 2022 Elsevier. **b)** Synthesis of lipid-like materials from PAMAM dendrimers (top) and B16-OVA tumor volumes from mice treated with OVA mRNA LNPs for therapeutic (bottom left) and prophylactic (bottom right) purposes. Reproduced with permission from reference (161), Copyright 2021 PNAS. **c)** Schematic of lipopolyplex mRNA vaccine (top) and number of tumor nodules in the lungs following treatment with lipopolyplex mRNA vaccine (LPP/OVA) in B16-OVA melanoma lung metastasis model (bottom). Reproduced with permission from reference (169), Copyright 2017 Elsevier. **d)** Vaccination schedule and tumor growth curve of B16-OVA inoculated mice following treatment with a lipopolyplex vaccine carrying either unmodified or N1-methylpseudouridine (N1mΨ) modified mRNA. Reproduced with permission from reference (170), Copyright 2018 American Chemical Society.

Table 1.

Clinical Trials Using Non-Viral mRNA Vaccines for the Treatment of Melanoma

Study Start Date	mRNA-encoded antigen	Delivery Carrier	Co-Treatment	Investigator/ Sponsor	Status	Phase	NCT	Additional References
2002	Autologous tumor-mRNA	Dendritic cells	IL-2	Steinar Aamdal, Oslo University Hospital - Norwegian Radium Hospital	Completed	I/II	NCT01278940	188
2004	Melan-A, Mage-A1, Mage-A3, Survivin, gp100, Tyrosinase	Protamine-stabilized	GM-CSF	Claus Garbe, University of Tuebingen, University Hospital Tuebingen	Completed	I/II	NCT00204607	189
2004	Tyrosinase, gp100	Dendritic cells	N/A	Cornelis JA Punt, G.J. Adema, Radboud University Nijmegen Medical Centre/ Nijmegen Center for Molecular Life Sciences, Dutch Cancer Society	Completed	I/II	NCT00243529	190
2007	Melan-A, Mage-A1, Mage-A3, Survivin, gp100, Tyrosinase, Autologous tumor-mRNA	Naked	GM-CSF	Claus Garbe, University of Tuebingen, University Hospital Tuebingen, German Research Foundation	Completed	I/II	NCT00204516	191
2009	hTERT, Survivin, tumor-derived mRNA	Dendritic cells	Temozolomide	Steinar Aamdal, Oslo University Hospital - Norwegian Radium Hospital	Terminated, logistical problems	I/II	NCT00961844	
2009	Tyrosinase, gp100	Dendritic cells	N/A	Cornelis JA Punt, Radboud University Nijmegen Medical Centre, Rotterdam Eye Hospital	Terminated, slow accrual	I/II	NCT00929019	192
2009	Tyrosinase, gp100	Dendritic cells	N/A	Cornelis JA Punt, Radboud University Nijmegen Medical Centre	Completed	I/II	NCT00940004	
2009	hTERT, Survivin, p53	Dendritic cells	Cyclophosphamide	Inge Marie Svane, Department of Oncology, Herlev University Hospital	Completed	I	NCT00978913	193
2009	TriMix mRNA, MAGE-A3, MAGE-C2,	Dendritic cells	N/A	Bart Neyns, Universitair Ziekenhuis Brussel	Completed	I	NCT01066390	173

Study Start Date	mRNA-encoded antigen	Delivery Carrier	Co-Treatment	Investigator/ Sponsor	Status	Phase	NCT	Additional References
	Tyrosinase, gp100							
2010	TriMix mRNA, gp100, Tyrosinase	Dendritic cells	N/A	Cornelis JA Punt, Radboud University Medical Center	Completed	I/II	NCT01530698	194
2011	TRP2	Dendritic cells	N/A	James Young, Memorial Sloan Kettering Cancer Center, Rockefeller University	Completed	I	NCT01456104	195
2011	Tyrosinase, gp100	Dendritic cells	Cisplatinum	Winette van der Graaf, Radboud University Medical Center	Completed	II	NCT02285413	
2011	TriMix mRNA, MAGE-A3, MAGE-C2, Tyrosinase, gp100	Dendritic cells	Ipilimumab	Bart Neyns Vrije Universiteit Brussel	Completed	II	NCT01302496	
2012	Melanoma antigens	Naked	N/A	Ugur Sahin, Ribological GmbH, BioNTech	Completed	I	NCT01684241	
2012	TriMix mRNA, MAGE-A3, MAGE-C2, Tyrosinase, gp100	Dendritic cells	N/A	Bart Neyns, Universitair Ziekenhuis Brussel, RIZIV	Completed	II	NCT01676779	
2013	Personalized neoantigens	Naked	RBL001/RBL002 (encodes melanoma antigens)	Ugur Sahin, BioNTech RNA Pharmaceuticals GmbH	Completed	I	NCT02035956	196
2015	NY-ESO-1, MAGE-A3, TPTE, Tyrosinase	Lipoplex	Anti-PD-1	BioNTech	Active, not recruiting	I	NCT02410733	
2017	TriMix mRNA, Tyrosinase, gp100, MAGE-A3, MAGE-C2, PRAME	Naked	Anti-PD-1	eTheRNA immunotherapies	Terminated, expiry of study medication	I	NCT03394937	
2017	20 patient-specific tumor neoantigens	Lipoplex	Atezolizumab	Genentech, Inc., BioNTech	Active, not recruiting	I	NCT03289962	197
2018	OX40L, IL-23, IL-36γ	nanoparticle	Durvalumab	ModernaTX, Inc., AstraZeneca	Recruiting	I	NCT03739931	198
2018	20 patient-specific tumor neoantigens	Lipid nanoparticle	N/A	Steven A Rosenberg, National Cancer Institute (NCI)	Terminated, slow accrual	I/II	NCT03480152	199
2019	20 patient-specific	Lipoplex	Pembrolizumab	Genentech, Inc., BioNTech	Active, not recruiting	II	NCT03815058	

Study Start Date	mRNA-encoded antigen	Delivery Carrier	Co-Treatment	Investigator/ Sponsor	Status	Phase	NCT	Additional References
	tumor neoantigens							
2019	34 patient-specific tumor neoantigens	Lipid nanoparticle	Pembrolizumab	ModernaTX, Inc., Merck Sharp & Dohme LLC	Recruiting	II	NCT03897881	200,201
Estimated June 2023	Autologous tumor-mRNA	DOTAP liposome	Anti-PD-1	Bently Doonan, University of Florida	Not yet recruiting	I	NCT05264974	