

Review Article

# Past, Present, and Future of In-Vitro Transcribed RNA Based Modular Vaccine

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**Abstract:** Even though messenger RNA was discovered in 1961, it took another three decades for it to be employed as a vaccine. The search for vaccinations against COVID-19 recently provided significant impetus for the development of mRNA vaccine technology. Self-amplifying mRNA and trans-amplifying mRNA were created to enhance the characteristics of RNA vaccines, particularly their circulation time. Circular RNA vaccines, which were created when the capability of translation on their protein matrix was discovered, are a different area of mRNA technology. Similar to trans-amplifying mRNA, circular RNA is seen to be a fairly promising platform and offers a number of advantages over mRNA vaccines. An overview of the mRNA platform is provided in this paper, along with a critical analysis of the more recent self-amplifying mRNA, trans-amplifying mRNA, and circular RNA platforms built on it. The key characteristics, benefits, and drawbacks of each of the mRNA platforms offered are then examined. This discussion will aid in the choice-making process for the best platform for developing RNA vaccines against viral or cancerous diseases.

**Keywords:** RNA, transcribed vaccine, past, present, future

## 1. INTRODUCTION

In general, there are two types of vaccinations currently in use: classic vaccines like live, attenuated, and inactivated vaccines, and more contemporary vaccines like peptide, DNA, and mRNA vaccines. Each group has a unique set of benefits and drawbacks. Traditional vaccinations offer long-term protection against infection, but their production must be scaled up and their development takes longer. Modern vaccine techniques, however, do not experience these problems, and among them, mRNA vaccines show a number of benefits. The safety of mRNA vaccines is one of its main advantages. mRNA vaccinations do not integrate into the recipient's genome like DNA vaccines do. In contrast to attenuated vaccines, mRNA does not provide a risk of infection, which is another significant benefit. Additionally, mRNA vaccines can be created more quickly and at a lower cost than attenuated or inactivated vaccinations [1, 2]. Additionally, the expression of the protein encoded by mRNA vaccines is very short-lived as a result of physiological mechanisms that naturally degrade proteins, making it simple to manage. Additionally,

mRNA vaccines translate in the cytoplasm rather than having to cross the nuclear barrier, as is the case with DNA vaccines [2, 3]. Additionally, mRNA vaccines do not have the low immunogenicity issue that peptide vaccines do [3-5]. More so than attenuated and inactivated vaccinations, mRNA vaccines may be made more quickly and inexpensively [1, 2]. Last but not least, the intrinsic adaptability of the essential components of mRNA vaccine design makes it simple to alter the encoded protein sequence with little effort and investment of time [6]. This enables the creation of vaccinations with novel features. The primary difficulties with mRNA vaccines stem from the RNA molecule's immunogenicity, which can restrict the amount of protein translated in the body. The vaccine loses its effectiveness if the immune response starts before the mRNA is translated. Reduced dose administration and the use of modified nucleotides have been used to overcome this. Modified caps, nucleotides, and poly (A) tails have been added to mRNA vaccines to increase their intracellular stability. They also have the best regulatory components. Ionizable lipids have additionally improved the administration of mRNA vaccines [3, 7-9]. Consequently, the development of preventative and therapeutic vaccinations is moving in a promising and creative path with the use of mRNA vaccines. Several unique modifications have been developed in addition to conventional mRNA vaccines, including self-amplifying (SAM) mRNA, trans-amplifying mRNA (taRNA), and circular RNA (circRNA). Each of these mRNA-based vaccination variations has unique benefits and drawbacks, which makes it more difficult to choose the best technology for a given application. Numerous mRNA-based vaccines, including one for SARS-CoV-2, are now available to protect against a number of diseases [3]. In order to make it easier to choose the best platform for developing an RNA vaccine against cancers or viral diseases, this review attempts to examine the key distinguishing characteristics of each vaccine type (i.e., mRNA, SAM, taRNA, and circRNA). Before it was utilized as a vaccine, nevertheless, it took decades of scientific research and experimentation [10]. In contrast to viral disorders, it is noteworthy that mRNA vaccines showed more potential for the treatment of oncological conditions. Eli Gilboa and colleagues started investigating mRNA cancer vaccines in humans toward the end of the 20th century. The late-stage cancer vaccine candidate, however, did not produce the anticipated results and was not authorized, which hindered the development of mRNA vaccine technology. However, CureVac and BioNTech began focusing on the creation of mRNA vaccines in the early 2000s thanks to E. Gilboa's research. Direct delivery of mRNA vaccines causes a strong immunological reaction to the mRNA, according to studies, which presents a significant barrier to their use. It wasn't until 2005 that Kariko and Weissman were able to overcome this obstacle by switching out the immunogenic uridine in the vaccine mRNA for pseudouridine [11–14]. The first trans-amplifying mRNA (taRNA), known as SAM, was developed in 2011, marking a significant leap in mRNA technology [16]. Another difficulty in the creation of RNA vaccines was their distribution. In the beginning, positively charged cationic lipids with a head group were used; the head group interacts with the negatively charged RNA [17]. These lipids were harmful to the body due to their continuous positive charge, which prevented their use in vaccinations [18]. Pieter Cullis and his team began experimenting with different liposome lipid compositions for RNA delivery in 2012. In the end, Cullis and his team were able to create an ideal lipid formulation that contained ionizable lipids that could change their charge from positive to neutral under physiological conditions, significantly lowering the toxicity of liposomes while increasing the storage time for mRNA vaccines in this lipid-based delivery system [18]. A tailored mRNA vaccine against melanoma was created by Ugur Sahin in 2017 as the result of these significant breakthroughs. Sahin's research produced encouraging therapeutic outcomes and sparked a great deal of interest in the development of mRNA-based cancer vaccines [19]. While prophylactic vaccinations against viral infections employing mRNA technology lagged behind, mRNA-based therapeutic vaccines for cancer showed

considerable advancement. Notably, Moderna, one of the top businesses producing mRNA vaccines, had created nine mRNA vaccines by 2020 for different infectious diseases affecting people, but none had been particularly effective. Similar experiences were reported by other businesses working on mRNA-based vaccinations. However, the SARS-CoV-2 virus's appearance in 2019 fundamentally changed the situation, inspiring big businesses to draw on their wealth of experience and quickly develop mRNA-based vaccines in response to the global epidemic [14,20]. Promising mRNA prototypes of the SARS-CoV-2 virus vaccine were created as early as one year after the start of the pandemic [3]. Because of this, mRNA vaccines are now being produced quickly and are catching up to DNA vaccines in terms of technology.

## 2. LITERATURE REVIEW

The linearization of the DNA template is necessary before mRNA production *in vitro*. The target DNA fragment is often obtained in a linear form suitable for transcription using restriction enzymes or PCR. RNA polymerases from the bacteriophages T3, T7, or SP6 are used to create *in vitro*-transcribed mRNA (IVT mRNA) [21]. At the 5' end of the DNA template is a specific sequence that is recognized by the matching polymerase. For instance, the sequence 5'-TAATACGACTCACTATA-3' is frequently used when bacteriophage T7 is to be used [22]. In order to serve as a template for protein synthesis, each of these components is essential [21, 23]. Although no clear advantages have been found in comparison to Cap 2, studies have shown that mRNA with Cap 1 generally delivers higher protein levels compared to Cap 0. Therefore, the cellular milieu or specific cell line in which the mRNA is to be given determines the appropriate cap alteration [24–25]. The protection of mRNA from 5'-end RNases such the XRN1 family is another crucial role of the cap [26]. In the past, RNA 5'-triphosphatase (RTPase), guanylyltransferase (GTase), and N7-methyltransferase (N7MTase) sequentially catalyzed enzymatic processes to synthesize mRNA *in vitro*. This group of enzymes produces IVT (in vitro-translated) mRNA, which contains Cap 0 [27]. The rates of ribosome interaction with mRNA, which vary by orders of magnitude depending on the sequence, draw attention to the importance of the 5'-UTR [28]. The 5'-UTR's typical length in eukaryotes varies from 53 to 218 nucleotides, with 210 nucleotides serving as the human median [29]. The sequence of the 5'-UTR can help or hurt translation and acts as a platform for transcriptosome formation. The construction of the 43S pre-initiation complex (PIC), which contains eukaryotic initiation factor 2 (eIF2) in conjunction with methionyl initiation tRNA (Met-tRNA<sub>i</sub>), is the first step in the start of eukaryotic translation. The PIC complex is bound by eIFs 1, 1A, 3, and 5, and then mRNA contact is made possible by the eIF4F complex recognizing the cap structure. The RNA helicases eIF4A, eIF4G, eIF4E, and poly(A)-binding protein (PABP) make up the eIF4F complex. Until it comes across an AUG start codon that is complementary to Met-tRNA<sub>i</sub>, the PIC scans the mRNA in a 5'-3' orientation. The 60S ribosomal subunit binds as a result of the interaction between Met-tRNA<sub>i</sub> and AUG, completing the 80S ribosome and making it ready for protein synthesis. The interaction between Met-tRNA<sub>i</sub> and the start codon is significantly influenced by the context in which the AUG codon is located. Met-tRNA<sub>i</sub> may "slip past" the first codon if it is located in a "unfavorable" nucleotide environment. The ideal sequence for the AUG codon in mammals is known as the Kozak consensus sequence, and it is composed of the letters 5'-GCCRCCATGG-3' [30]. The control of transcription is significantly influenced by the secondary structures of mRNA. A hairpin structure that follows the AUG codon can prevent the PIC complex from moving, which lowers the possibility of "slippage" even in unfavorable nucleotide situations. On the other hand, if a hairpin structure is found in the mRNA sequence's coding region, it often causes a reduction in translation efficiency. In particular, the abundance of secondary structures in mammalian 5'-UTRs makes them reliant on the action of the eIF4A RNA helicase to unwind these structures [31]. The PIC interacts

with internal ribosome entry sites (IRES) via a different way from the traditional translation initiation process. Many viral mRNAs contain these IRES sequences, which allow them to recruit the PIC and 40S ribosomal subunit to internal places inside the 5'-UTR without going through the cap structure. It's interesting to note that IRES elements are also found in a number of mRNAs that play a role in mammals' cellular stress responses [32]. To minimize premature translation start, it is critical to avoid secondary structures in the 5'UTR region, especially those rich in GC content, and to omit AUG codons when creating mRNA vaccines [33]. Although increased adenine content in the 5'UTR can initiate translation cap-independently, it can also hasten mRNA breakdown if translation is not occurring [34]. The human or *Xenopus laevis* - and -globin genes, which result in high amounts of protein production, are among the favored alternatives for 5'UTRs [35]. Exploring synthetic sequences is another step in improving 5'UTR design. The analysis of 280,000 randomly generated 5'UTRs using bioinformatics revealed 10 sequences with promising potential [36]. Utilizing minimum 5'UTRs with 12–14 nucleotides is another strategy that has had encouraging results [37]. The search for ideal 5'UTRs for inclusion in mRNA vaccines includes examining highly effective viral IRES elements as potential candidates. The relatively poor capping efficiency while using ARCA for IVT mRNA 5'UTR creation is one of the difficulties. Vaccinia Capping Enzyme (VCE) and m7GmAmG enzyme production is also costly. In order to start cap-independent translation, viral internal ribosome entry sites (IRES) present a potential option [38]. Zhou et al. used the Semliki Forest virus (SFV) in 1994 to apply the theory of self-amplifying mRNA for the first time [15]. SAM is also produced using other well-researched alphaviruses including the Sindbis virus and Venezuelan equine encephalitis virus [39]. A 5' cap, 5'-UTR, lengthy open reading frame (ORF) region, 3'-UTR, and 3' poly(A) tail make up SAM, just like NRM. SAM, however, varies greatly from other vaccine types in that it has the ability to self-replicate using the replicase found downstream of the 5' UTR. IVT mRNA can be capped by nsP1's GTase and N7MTase activities and nsP2's RTPase activity to create Cap 0. Additionally acting as a protease and helicase, nsP2 helps break down the complete nsP complex. Although its function is not entirely understood, nsP3 interacts with a number of host cell proteins and helps to dampen the antiviral response. After the SAM enters the cytoplasm of the target cell, the protein translation process is initiated, primarily involving the nsPs, which are located upstream of the main protein coding region, and which function as an RNA-dependent RNA polymerase (RdRp) responsible for amplifying the number of copies of the original IVT mRNA. The SAM template is utilized by the nsPs to create a complementary RNA strand as part of an early replication complex. A late replication complex is then created once the nsP polypeptide is split up into separate proteins. The original number of IVT RNA copies is increased by this late replication complex by synthesizing a copy of SAM on the RNA template. The inclusion of an alphavirus subgenomic promoter (SGP) region in SAM, which is situated before the gene of interest (GOI), is one distinguishing feature between SAM and NRM. By avoiding the reading of the sequence encoding viral nsP proteins, the SGP speeds up the start of GOI transcription. This process encourages the production of mRNA copies that are translated after only carrying the GOI [39]. In comparison to NRM medications, this replication mechanism enables a 30 to 1000-fold reduction in the needed dose of SAM (0.1-10 versus 30-100 g) [40]. As a result, SAM's lower dose need is less immunogenic, safer, and has a longer duration of action, which helps to create a strong immune response [41]. It also needs less raw materials to be synthesized. SAMs do in fact have some of the problems that come with including viral sequences. The approximately 7 kb length of the nsP segment restricts the length of the gene of interest (GOI) and makes it more difficult to create a vector for bacterial growth for vaccine packaging. Furthermore, viral nsPs may affect the host cell and result in immunological hyperactivation [42]. The production of intracellular double-stranded RNA (dsRNA) during replication,

which triggers innate immunity and can obstruct protein translation, is another feature of SAM. The features of SAM have been improved by investing in efforts to optimize the viral replicase's codons in order to increase its translation rate [43]. The mRNA that encodes the GOI is known as trans-replicon (TR)-RNA, and the viral replicase can either be nrRNA or saRNA. Conserved sequence elements (5'CSE and 3'CSE) from the alphavirus flank the GOI and are upstream of the GOI to achieve TR-RNA amplification [44]. Pirjo Spuul et al. first put up the idea of the trans-replication system in 2011 [16]. The benefits of SAM are fully incorporated into this design, while some disadvantages are minimized. Notably, the restriction on the GOI length is avoided and the use of changed nucleotides is not restricted by individually encoding the replicase on an RNA platform. This method is also safer since it decreases the possibility of creating recombinant virus particles [45]. It's interesting to note that while taRNA is more immunogenic than saRNA, lower dosages (as low as 50 ng) are required to produce a similar immune response [106]. A change in taRNA has recently been reported. Along with the replicase-encoding RNA, it also contains two extra TR-RNAs [107]. The idea of a modified form of taRNA that includes adenine-rich areas in the 5'UTR has been put out as a result of ongoing advancements in taRNA technology. The alphavirus SGP is absent from this modified taRNA, which results in a shorter RNA and a tenfold lower vaccine dose without lowering in vitro expression levels [46]. The need for at least two different RNAs, one for the replicase and the other for the GOI, is a significant disadvantage of taRNA, though [47]. Though taRNA technology is still in its early phases, its potential for real-world applications are bright. A taRNA vaccine that targets the influenza virus is presently the subject of pre-clinical research [78,106]. Additionally, using this method, a bivalent vaccine [48] against Ross River Virus and Chikungunya was created. The encephalomyocarditis virus (EMCV) IRES is a frequently used IRES for translating synthetic circRNA. It is effective in a variety of cell lines [49], as are IRES derived from coxsackievirus B3 (CVB3) and human rhinovirus B3 (HRV-B3), as well as their modified versions, which have translation levels 3–4 times higher than those of EMCV IRES [131,132,133]. The circularization of linear RNA molecules by chemical or enzymatic processes is an essential step in the creation of IVT circRNA [50]. A significant turning point in the area was the invention of the chemical method for IVT circRNA production in 1988. The 5'-terminal phosphate and 3'-terminal hydroxyl groups of the linear RNA are linked together using cyanogen bromide (BrCN) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The by-products of this process, however, include circRNA variations with 2'-5' phosphodiester linkages at the junction of the linear molecule [51]. Due to its high cost, poor yield, and limited applicability to circularizing RNA longer than 70 nucleotides, the method is no longer widely used [52]. Nucleotide oligomerization domain (NOD)-like receptors (NLRs), oligoadenylate synthetase (OAS) receptors, RNA-dependent protein kinase (PKR), and retinoic acid-inducible gene-I-like (RIG-I-like) receptors (RLRs) are a few of the major pattern recognition receptors (PRRs) in the cytosol. The NLR family member NOD2 is essential for identifying ssRNA. Melanoma differentiation-associated gene 5 (MDA-5) and LGP2 (DEXH-box helicase 58), which identify dsRNA, are included in RLRs together with RIG-I (DEXH/H-box helicase 58), which interacts with both ssRNA and dsRNA. In response to RNA recognition, RLRs, like TLRs and NLRs, trigger the creation of IFN-I and pro-inflammatory cytokines. On the other hand, PRR engages in dsRNA-dependent phosphorylation of eIF2, which suppresses translation. In order to activate RNase L and cause RNA breakdown, oligoadenylate synthetase interacts to dsRNA. Therefore, it is crucial to develop IVT RNA that is immune system-resistant [53]. Numerous modified nucleotides have been proposed and added during IVT mRNA synthesis to lessen its immunogenicity [54] in order to solve this issue. Only a few of the 172 suggested changed bases were able to improve mRNA expression and stability and lessen immunogenicity [55]. The main goal of using these modified nucleotides in combination with natural nucleotides is to increase the

translation of IVT mRNA by inhibiting the activation of PRRs like TLR-3, TLR-7, TLR-8, OAS, RIG-I, and PKR [56–59]. It is crucial to remember that, depending on the kind of cell, the same changed nucleotide can have various impacts on the translation processes. Therefore, it is essential to take into account the particular cell type to which the IVT RNA will be directed when using modified nucleotides and choose the nucleotides accordingly [60]. Modified bases can interfere with replicase activity, therefore SAM is very sensitive to their integration. For this reason, they are typically avoided. Instead of the usage of modified nucleotides, the substantially smaller amounts of SAM used in SAM vaccines compared to NRM are the main cause of their decreased immunogenicity. Notably, reduced immunogenicity and lower vaccination doses were possible when changed nucleotides were combined with improved taRNAs. Although the RNA encoding the replicase can be modified, there are still limitations on the utilization of modified nucleotides in TR-RNA synthesis [61]. Notably, taRNAs provide an additional benefit over SAM because even lower vaccination doses could be utilized, lowering immunogenicity even further [62]. IVT circRNA uses modified nucleotides, similar to mRNA, to lessen immunogenicity while just slightly decreasing translational efficiency. Without affecting translation, the addition of 5% m6A to circRNA dramatically reduces its immunogenicity. CircRNA includes the common RNA modification known as m6A, which is present in about 0.5% of cellular RNA. Notably, m6A within endogenous circRNA in cells interacts to the YTHDF2 protein, which prevents innate immune activation. Additionally, even a single m6A mutation close to the start codon can trigger cap-independent translation of the circRNA by interacting directly with eIF3 [63]. Studies have shown that the presence of numerous copies of the GGACU motif including m6A allows translation to begin without an IRES, allowing the short motifs of about 20 nucleotides to replace the traditional viral sequences. Overall, it has been determined that the RR(m6A)CH motif is very advantageous for starting cap-independent translation of circRNA [65]. Contrarily, the addition of m1 to IVT circRNA does not change its immunogenicity but may have a deleterious impact on translation levels and RNA circularization efficiency [66]. It's significant to note that not enough research has been done on the immunological response to IVT circRNA. RIG-I and PKR are thought to be the main PRRs responsible for identifying circRNA. While PKR inhibits RNA translation, RIG-I activates intracellular signaling pathways, including MAVS, which in turn activates the transcription factor IRF3 and causes the creation of IFN-I. It is speculated that leftover exons from PIE circularization, which can form secondary structures and cause PRR activation, are principally responsible for the immunogenicity of IVT circRNA. IVT circRNAs produced with T4 RNA ligase do not, in fact, trigger the innate immune response. It is important to remember that viral IRESs can also create secondary structures, which may help to activate the PRR. To determine the causes of in vitro-transcribed circRNA's immunogenicity, more research is necessary. The effects of the primary RNA sequence, secondary structure, size, and presence of modified nucleosides should be the main topics of such studies [67]. DNA/RNA heterodimers, dsDNA, and ssDNA are inevitable by-products of the synthesis of synthetic mRNA or circRNA. These by-products are each recognized by particular receptors, which then trigger the innate immune system. For instance, dsDNA and DNA/RNA hybrids are recognized by TLR-9, which is found in the cell endosome, and by cytosolic sensors like cGAS (cyclic GMP-AMP synthase) and AIM2 (missing in melanoma 2), which trigger the production of IFN-I and other cytokines [68]. Therefore, it is essential to remove DNA contaminants and DNA/RNA hybrids from the IVT mRNA. DNA impurities can be effectively removed by DNase-I [69]. IVT mRNA purification from dsRNA and random RNA pieces is still difficult. By using modified nucleotides or lowering the Mg<sup>2+</sup> ion concentration during in vitro transcription, one can reduce the amounts of dsRNA [70]. Additionally, because they are highly immunogenic by-products of the procedure, linear RNA molecules need to be eliminated as well following the creation of IVT circRNA. Exonuclease T or RNase R therapy of IVT circRNA

has been suggested [71–73]. IVT mRNA and circRNA are now isolated from dsRNA using two major methods. Reversed-phase high-performance liquid chromatography (HPLC) is used in the first method while cellulose is used in the second [74–80]. However, there are a number of drawbacks to reversed-phase HPLC, such as low scalability, toxicity of the eluant (acetonitrile), and significant losses of IVT mRNA and circRNA [81-85]. The cellulose-based approach for IVT mRNA purification, on the other hand, is not affected by these disadvantages. Up to 90% of dsRNA contaminants are removed, while small RNA fragments might not be completely removed [86]. Although cellulose has not been used widely to purify IVT circRNA, it has the potential to be used in such applications. Studies showing that purification alone can increase translated protein levels by 10-1000 times relative to unpurified mRNA [78,166,201] make clear the importance of separating IVT mRNA from dsRNA contaminants. Similar studies with IVT CircRNA emphasize how crucial it is to eliminate reaction byproducts in order to decrease immunogenicity [87–100].

### 3. CONCLUSION

In the sixty-two years since its discovery, mRNA has changed from a research tool to a tool for the treatment and prevention of a variety of ailments. mRNA-based vaccines have emerged as a promising technology area that is currently undergoing active development. There are currently self- and trans-amplifying IVT RNA vaccines based on mRNA available. Viral vaccines based on them were created in 2015 after it was discovered that mammalian cells might translate proteins from circRNA. The four platforms (mRNA, self-amplifying mRNA, trans-amplifying mRNA, and circRNA) used for vaccine development each have a number of benefits and drawbacks. The most researched platform for the in vitro production of RNA for use as a vaccine is non-amplifying mRNA. It enables the choice of several regulatory components to boost antigen translation in various cell types and tissues. To lessen the immunogenicity of the mRNA and stop immune activation prior to translation, several modified bases can also be employed. The relatively brief half-life of the matrix and the resulting tiny amount of antigen generated are the main drawbacks of mRNA-based vaccines, though. Because mRNA is amplified inside the cell, self-amplifying mRNA is a logical extension of mRNA and is intended to address its primary issues. The amount of antigen that is translated grows as a result of the mRNA inside the cell being amplified, which raises the number of mRNAs there initially. As a result, self-amplifying mRNA vaccines have a long half-life inside cells, may be administered at low dosages, and induce the production of a sizable amount of antigen. The presence of a sequence encoding four non-structural proteins (nsP1, nsP2, nsP3, and nsP4) derived from alphaviruses is what gives self-amplifying mRNA its distinctive characteristics. However, the fundamental shortcomings of this platform are these viral sequences. The amount of the antigen in such a vaccination is limited since the viral proteins are highly immunogenic and the sequence encoding them contains more than 7000 nucleotide pairs. Additionally, the mRNA amplification's double-stranded by-products are immunogenic. The inability to employ modified nitrogenous bases is yet another drawback of self-amplifying mRNA. Using two different types of mRNA, a trans-amplifying mRNA vaccination platform was developed to address the self-amplifying mRNA platform's numerous issues. The first encodes non-structural alphavirus proteins required for the amplification of the target mRNA, which also contains components crucial for the antigen's recognition by RNA-dependent RNA polymerase. This platform lacks some of the drawbacks of self-amplifying mRNA but has all of its benefits. Modified nitrogenous bases can be used with the trans-amplifying mRNA platform to lessen the immunogenicity of the mRNA. However, this mRNA platform is still poorly known, needs at least two different kinds of mRNA, and still contains viral sequences. The final kind of RNA vaccine is called circRNA, and it has a

special structure that prevents exonucleases from breaking it down and gives it a longer half-life, which results in higher quantities of the translated antigen. The circRNA platform also permits the use of modified nitrogenous bases because it lacks a cap and poly A tail, which provide a number of challenges in the production of vaccines. The level of produced antigen is lower in this platform than in self-replicating mRNA platforms, and both of these platforms (trans-amplifying mRNA and this one) are currently insufficiently investigated. A larger quantity of antigen is produced by the circRNA platform than the mRNA platform, and it is also safer than self-replicating mRNAs, which contain viral sequences. Nevertheless, at this point, it appears that this platform is the most promising. It is possible to use any mRNA-based technology for both prophylactic vaccinations against different viral infections and therapeutic cancer vaccines.

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