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REVIEW ARTICLE

Natalia Galant, Natalia Krzyżanowska, Paweł Krawczyk

Department of Pneumonology, Oncology and Allergology Medical University of Lublin, Poland

mRNA vaccines in the treatment of cancer

Address for correspondence:
Natalia Galant, MSc
Department of Pneumonology, Oncology and Allergology Medical University of Lublin
ul. Jaczewskiego 8, 20–090 Lublin, Poland
e-mail: nataliagalant@umlub.pl

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ABSTRACT

Even though studies on mRNA vaccines have been performed since the 1990s, the first clinical trials of their usage were conducted in the late 2010s. The COVID-19 pandemic created an urgent need for effective vaccine development and prompted large-scale research on mRNA vaccines. Large studies enabled the improvement of mRNA vaccine structure and optimization of their delivery platforms, which contributed to a rise in both their efficacy and safety. Currently, mRNA vaccines are used not only in infectious diseases but also are being tested in research on cancer patients. Using next-generation sequencing (NGS) in mRNA vaccine manufacture seems to be beneficial because it enables preparation of personalized vaccines encoding tumor-specific antigens (TSAs). Tumor-specific antigens-based vaccines are associated with a stronger immune response and lower toxicity compared to non-personalized vaccines. Currently, clinical trials on mRNA vaccines are performed in patients with various types of cancer: pancreatic, non-small-cell lung, prostate cancers as well as melanomas. Due to the benefits of mRNA cancer vaccine administration in monotherapy, their combination with chemotherapy, radiotherapy, or immune checkpoint inhibitors (ICIs) have been suggested. In this review, we highlight the latest findings on mRNA vaccine development, including the advantages of using NGS during their production. We also summarize the current Food and Drug Administration (FDA) indications, results of completed clinical trials, and future possibilities of using mRNA vaccines in treatment of cancer patients.

Keywords: mRNA vaccines, neoantigens, immunotherapy, cancer

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Introduction

mRNA vaccines use a messenger ribonucleic acid (mRNA) sequence encoding cancer antigen proteins [1]. mRNA vaccines are administered by using viral vectors (RNA viruses, e.g. retroviruses) [2] or, more often, lipid nanoparticles [3]. It is also possible to inject naked mRNA, however, it is usually unstable [4, 5]. Non-personalized vaccines contain mRNA with the coding sequence for the most common tumor-associated antigens (TAAs) [6, 7]. However, personalized vaccines contain mRNA encoding cancer neoantigens (tumor-specific antigens, TSAs) present in a specific patient. Therefore, in this case, it is necessary to know the nucleotide sequences in DNA and RNA encoding neoantigens in tumor cells [8]. Non-personalized mRNA vaccines usually induce poor T-cell response and immune tolerance due to the wide distribution of TAAs [9]. However, neoantigens, arising from genetic abnormalities, are not present in normal cells and the use of personalized mRNA vaccines usually induces a strong specific immune response [7, 10].

Vaccines containing mRNA are often used with immunotherapy targeting immune checkpoints [11, 12]. Immune checkpoint inhibitors (ICIs), such as antibodies against programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), or cytotoxic T lymphocytes antigen 4 (CTLA-4), restore the activity of lymphocytes that were inhibited by cancer cells. Therefore, lymphocytes which are specific to neoantigens due to mRNA vaccine usage, may perform their action [12]. Moreover, the effect of non-personalized mRNA vaccines can be enhanced by combining them with chemotherapy or radiotherapy [13, 14].

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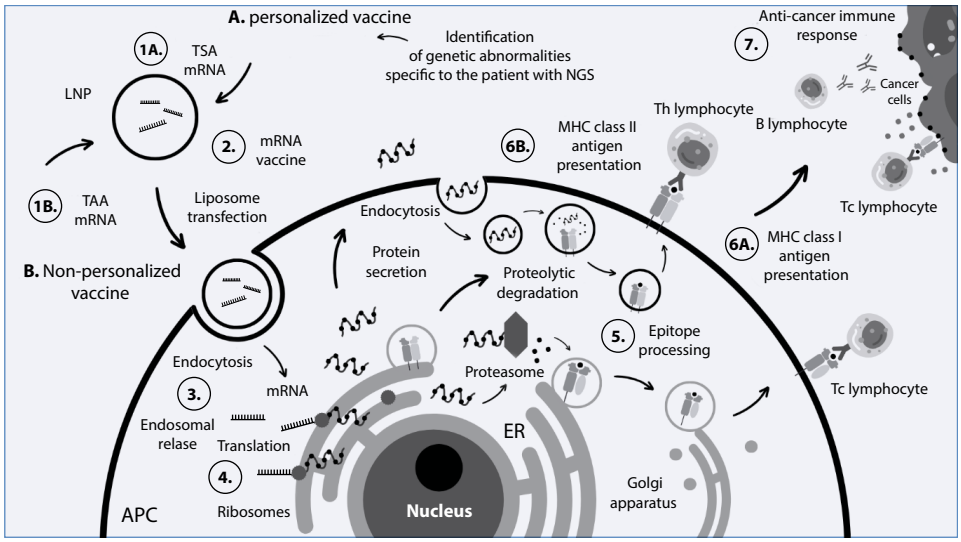

Figure 1. Mechanisms behind mRNA vaccines. Before a personalized vaccine is created, next generation sequencing (NGS) analysis reveals genetic abnormalities specific to the patient [mRNA encoding tumor-specific antigens (TSA)] (**1A**). Non-personalized vaccines contain mRNA encoding tumor-associated antigens (TAA) (**1B**). mRNA is encapsulated in lipid nanoparticles (LNP) (**2**) and transfected into antigen-presenting cells (APC) by endocytosis (**3**). After endosomal escape, ribosomes could translate mRNA to protein (**4**) Protein is further processed (**5**) by a ubiquitin-proteasome system that degrades intracellular antigens into peptides to be presented by major histocompatibility complex (MHC) class I to T cytotoxic cells (Tc) lymphocytes (**6A**). The proteins can also be secreted to extracellular space, degraded after endocytosis, and presented by MHC class II to T helper cells (Th) lymphocytes as an exogenous protein (**6B**). This initiates humoral and cell-mediated anti-cancer immune response (**7**). Figure created by the authors; ER — endoplasmic reticulum

The mRNA vaccines are a relatively safe form of immunotherapy. During their use, genetic material is not incorporated into cell nuclei [4, 15]. Translation takes place in the cytoplasm of antigen-presenting cells (APCs), which protects the cells against genetic modification and cancer transformation [1]. However, the mRNA vaccines are unstable (mRNA requires modification of the 5' end) [1], and their use is prone to embolisms as well as excessive immune reactions and autoimmune disorders (TAAs also occur on normal cells, which may lead to their damage when non-personalized mRNA vaccines are used) [16].

The mRNA vaccines induce an adaptive immune response, humoral and cytotoxic, against cancer cells [2]. Lipid nanoparticles containing mRNA enter the cytoplasm of APCs as a result of endocytosis. In the cytoplasm, translation occurs and proteins containing neoantigens are produced [2, 3]. Polyubiquitinated proteins are degraded in the proteasome, where small peptides, including neoantigens, are formed [5]. Neoantigens in the endoplasmic reticulum attach to major histocompatibility complex (MHC) class I and II molecules. Major histocompatibility complex molecules with antigens are transported through the secretory vesicle to the cell surface, where they present the antigens to cytotoxic T lymphocytes (MHC class I) and helper T lymphocytes (MHC class II) [2, 5]. Lymphocytes T recognize antigens through T-cell receptors. An "immune synapse" is formed by connecting numerous immune checkpoints on the surface of lymphocytes and APCs. Activation of co-stimulatory immune checkpoints leads to the activation of antigen-specific T-cells, which can kill cancer cells. Moreover, B lymphocytes are stimulated to produce specific, anti-cancer antibodies (Fig. 1) [1, 5].

mRNA vaccines development

Studies on mRNA vaccines have been performed since the 1990s [4, 17]. However, due to better stability and longer half-life, wider research has been focused on DNA-based and antigen-based vaccines [18]. Therefore, the first clinical trials with mRNA vaccines occurred only in the late 2010s [17]. Currently, mRNA vaccines are known as safe (since mRNA cannot be integrated into the host genome) and convenient to produce. Moreover, they make it possible to encode multiple specific antigens simultaneously [4, 18]. Problems with mRNA stability are being solved with the addition of a poly(A) tail at the 3' end [4] and a cap at the 5' end [19]. The use of novel delivery technologies, such as lipid nanoparticles, is also beneficial [18, 20].

mRNA synthesis

The mRNA used to prepare the vaccine is transcribed *in vitro* on a linear DNA template, usually plasmid DNA (pDNA) [6, 21, 22] in the presence of RNA polymerase (T3, T7, or SP6) and using nucleoside triphosphates [5]. This step of mRNA preparation is preceded by template DNA synthesis. To achieve satisfactory stability of *in vitro* transcribed (IVT) mRNA, it has to be capped. Capping may be performed simultaneously with transcription (i.e. as co-transcriptional capping) or enzymatically after transcription. Co-transcriptional capping requires adding a cap analog into a transcriptional mixture. Subsequently, capping is performed using the *Vaccinia* capping enzyme (VCE). The poly(A) tail may be manufactured on the basis of a DNA template or added after transcription [6, 21]. Afterward, purification may be necessary. Size exclusion columns and methods based on chromatography are used for this purpose. Additionally, DNase may be used to remove residuals of mRNA templates [21, 23, 24].

Optimization of mRNA structure

Five structures are crucial for mRNA: 5' cap, 5' untranslated region (5'UTR), coding sequence, 3' untranslated region (3'UTR), and poly(A) tail [17]. Vaccine optimization with structural and chemical modifications of those components may improve their properties [25, 26]. For instance, modifications of the 5'cap and poly(A) tail at the 3'end are crucial to achieving high stability of the mRNA structure [27].

Currently, the majority of studies are focused on 5' cap optimization as it is essential to provide resistance to enzymatic degradation of mRNA. The most common approach in mRNA vaccines is to use cap-0 [27]. Cap-0 consists of 7-methylguanosine linked by a 5′,5′-triphosphate chain with the first nucleotide of an RNA chain [19, 28]. Appropriate 5' cap construction prevents mRNA degradation with scavenger decapping enzymes (DcpS): Dcp1 and Dcp2 [19]. Despite cap-0 usage, different caps are being proposed, including cap-1 (possessing a 2'O-methyl group in the first cap-proximal nucleotide) and cap-2 (possessing two 2'O-methyl groups in two first cap-proximal nucleotides) [27, 29]. Wojtaczak et al. proposed the inclusion of 5′-phosphorothiolate (5′-PSL) moiety into the cap structure. The incorporation of 5'-PSL is associated with reduced susceptibility for decapping and improved translational properties of mRNA [28].

The poly(A) tail is involved in transporting mRNA to the cytoplasm. Its association with poly(A)-binding protein (PABP) begins mRNA translation. However, the significance of poly(A) tail modifications during mRNA vaccine manufacture is not as widely researched as 5' cap refinement [19]. Nevertheless, it was suggested that elongation of the poly(A) tail may be advantageous for either mRNA stability or the efficacy of its translation [25, 30].

Despite the abovementioned possibilities regarding the 5' cap and the poly(A) tail, different modifications of mRNA structure are being proposed, including reduction of UU (uracil, uracil) and UA (uracil, adenosine) dinucleotides in the coding region as protection from decapping [26] and to avoid highly stable secondary structures as it may disrupt mRNA transport to ribosomes and elongation [25]. Moreover, purification with HPLC (high-performance liquid chromatography) of manufactured mRNA may be beneficial as it increases the production of encoded protein [25, 31].

mRNA delivery platforms

Various strategies for mRNA delivery have been developed. Viruses and, especially, lipid nanoparticles (LNPs) are most commonly used in mRNA vaccines as vectors. However, delivery may be based on different platforms, such as polymers, peptides, dendritic cells, or cationic nanoemulsions. Apart from those possibilities, naked mRNA may be injected directly. However, it should be borne in mind that naked mRNA administration is associated with its susceptibility to RNase degradation [23].

Viral-based vectors

The use of viral vectors, among others retroviral and adenoviral vectors, for mRNA administration improves both its *in vivo* delivery and transfection efficacy compared to naked mRNA injections. However, the application of non-viral vectors instead of viruses seems to be safer due to reduced pathogenicity and lower capacity for mutagenesis [32]. Moreover, the use of viral vectors may be associated with excessive inflammatory response [33] as they are highly lymphotropic [34]. Another issue limiting the possibility of using those vectors is a problematic large-scale production of viral replicon particles [34]. One of the innovative approaches is the construction of vaccines containing self-amplifying mRNA (SAM). SAM vaccines are based on the alphavirus genome, which encodes 4 genes. One of them, nsP4, encodes RNA replication machinery. In the SAM vaccine, nsP4 is left intact while structural genes are replaced with sequences encoding antigens of interest. DNA prepared in this way is used as a template for transcription into mRNA [35, 36]. Afterward, mRNA may be encapsulated into viral-based vectors (e.g. alphavirus replicon particles) [36] or other selected vectors, such as LNPs [35, 37]. Unlike various other mRNA vaccines, SAM vaccines are intended to be administered in one dose only [38].

Lipid nanoparticles

Lipid nanoparticles are delivery platforms consisting of ionizable amino lipids, polyethylene glycol (PEG), phospholipids, and cholesterol. Ionizable amino lipids enable mRNA release from the endosome to the cytoplasm, PEG prolongs the time of LNP circulation, while phospholipids and cholesterol are essential to stabilize the LNP structure [23, 39, 40]. Encapsulation of nucleic acid in LNP protects it from enzymatic degradation and decreases its clearance during renal filtration. Moreover, it is possible to modify the structure of LNP, for example, by adding coating antibodies to direct mRNA delivery into desirable cells or tissues [41]. One of the crucial issues associated with mRNA-LNP vaccines is their appropriate dosage. LNP application is associated with toxicity that increases with concentration; therefore, the application should be optimized to achieve both good efficacy and low toxicity [41, 42]. Moreover, it was suggested that mRNA-LNP application may cause allergic reactions due to PEG content [42]. Choosing the optimal route of mRNA-LNP vaccine administration seems to be essential [41]. The most commonly used routes are intramuscular and intradermal injections; however, vaccines may be also administrated intravenously [40, 43].

Direct mRNA injection

Naked mRNA can be injected directly into an organism, without a carrier, and formulated in a proper buffer. The uptake of mRNA may be improved by its administration in buffer containing calcium [23]. Direct mRNA injection is more cost-effective compared to using a delivery platform. Moreover, since mRNA translation starts immediately when it is present in the cytoplasm, the vaccine rapidly induces an immune response [23, 40]. However, the most significant disadvantage of this approach is mRNA sensitivity to degradation with RNases present *in vivo* [40, 44], which generates a problem with the proper effective vaccine dosage [45]. Delivery of intradermal, intranodal, or intramuscular mRNA directly to an optimal place is beneficial for its stability [40].

Approaches to encoding antigen selection

Cancer vaccines aim to stimulate patients' immune system to enhance its activity against cancer cells. Cancer vaccine manufacturing may be based on three approaches i.e. encoding tumor-associated antigens, tumor-specific antigens, or immunostimulators [6, 7].

Tumor-associated antigens

Tumor-associated antigens show higher expression in tumor tissues compared to normal tissues. Among others, prostate-specific antigen (PSA), human epidermal growth factor receptor 2 (HER2), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) are included in this category [6, 7]. Since TAAs are also expressed on normal cells, the response of T-cells to immunization may be poor [9]. As a result, the administration of stratified drugs (i.e. drugs targeting the most common TAAs in a specific tumor type) may be ineffective in a significant proportion of patients. Therefore, to achieve appropriate vaccine effectiveness, it may be necessary to stimulate patients' immune response by administering adjuvants or co-stimulators [9, 46]. Moreover, it is worth noting that introducing TAAs into therapy may be associated with toxicity. Such problmes occurred during a clinical trial on retrovirus encoding CEA-reactive T-cell receptors in a metastatic colorectal cancer cohort. In that study, part of patients experienced grade 3 diarrhea due to colitis, resulting in their exclusion from this trial [16].

Tumor-specific antigens

Tumor-specific antigens (TSAs) are uniquely expressed in cancer tissue [7]. As neoantigens are not present in normal tissues, immune response to vaccines based on their use is stronger, and the risk of toxicity is lower compared to traditional therapy and TAAs-based vaccines [7, 10]. It is possible to identify and target neoantigens with next-generation sequencing (NGS) and use them to produce personalized vaccines, targeting individual neoantigens present in tumor tissue [8, 10]. This approach seems to be especially beneficial for patients as the majority of mutations harbored by tumors may be patient-specific [8, 47].

Next-generation sequencing for individualized vaccine preparation

The process of preparing individualized vaccines begins with collection of the patient's tumor tissue (material for cancer mutatome assessment) and peripheral blood (source of white blood cells containing healthy, non-cancerous DNA and mRNA) [8, 47, 48]. It was suggested that both fresh and preserved tissues (frozen or formalin-fixed paraffin-embedded) can be used for isolation of nucleic acids. Unfortunately, tissue is usually collected during biopsy in small amounts from one or a limited number of lesions. As a result, it is possible that the samples would not be representative of the whole tumor [8, 49]. It is suggested that in the future, liquid biopsy may be used as an alternative for tumor tissue examination. Liquid biopsy contains circulating tumor DNA (ctDNA) that is shed into circulation by all apoptotic and necrotic tumor cells. Therefore, it is

more representative of the entire tumor compared to single-side biopsy [48, 50].

Afterward, DNA (and possibly RNA to acquire information about the expression of mutated alleles) extracted from samples [47] are sequenced with whole exome sequencing (WES). The tumor and healthy tissues' exomes are compared and analyzed *in silico* to spot cancer neoantigens among germline variants [49, 51]. Neoantigens' selection may be based on analysis of either single nucleotide variants (SNVs), indels (insertions and deletions), fusion genes, or splice variants. Currently, for that purpose, SNV analysis is the most extensively researched [48]. To predict the immunogenicity of neoantigen, which will affect the effectiveness of the vaccine, both its affinity to MHC and level of mutated alleles expression can be analyzed. For that purpose, specific bioinformatic tools and RNA sequencing or RT-PCR may be used, respectively [10, 47, 49].

Lastly, with appropriate neoantigens selected, individualized vaccines can be manufactured under Good Manufacturing Practice conditions and then administrated to patients [10]. Currently, the production of personalized vaccines is expensive (among others, due to high NGS costs) and long-lasting (approximately 2–3 months) —both factors limit the possibilities of performing clinical trials and widespread implementing personalized cancer vaccines [8, 49]. However, further optimization of the process gives hope for wider use of this individualized therapy in the coming years [7, 51].

Immunostimulators

The third approach to mRNA-based vaccine preparation is inclusion of mRNA-encoding immunostimulators, such as cytokines [52–54] or co-stimulatory factors [53, 55]. Immunomodulators can be used to modify tumor microenvironment, promoting infiltration of activated T and B cells or to support effects of therapy with ICIs [11, 54]. It has been pointed out that intratumoral administration of mRNA-encoding immunomodulators is superior to other ways of delivery as it reduces toxicity and provides long-term benefits of treatment [54]. Even though clinical trials on cancer vaccines containing immunostimulators are currently not numerous, it has been demonstrated that their usage potentially improves outcomes for patients treated with ICIs [7].

mRNA vaccines in oncological clinical trials

mRNA vaccines in pancreatic cancer patients

Although patients with pancreatic cancer have a very high risk of death, complete recovery is possible in the early stage of disease using extensive surgery [56]. Chemotherapy, radiotherapy and standard immunotherapy in patients with advanced pancreatic cancer show low effectiveness [57, 58] Whereas molecularly targeted therapies could be used in small groups of patients, e.g. poly (ADP-ribose) polymerase (PARP) inhibitors in patients with *BRCA* genes (breast cancer genes) mutations [58, 59] or neurotrophic tropomyosin receptor kinase (NTRK) inhibitors in patients with *NTRK* gene rearrangements [59]. Therefore, it is reasonable to search for new immunotherapy methods or to strengthen existing immunotherapies, taking into account the strong immunogenicity of pancreatic cancer related to the formation of numerous neoantigens.

Rojas et al. [51] published the results of a phase I single-center study (NCT04161755) on the safety, immunogenicity, and effectiveness of personalized mRNA vaccines (cevumeran, BNT122, RO7198457) in patients with operable pancreatic ductal adenocarcinoma (PDAC). Results of treatment after a median three-year follow-up were presented at the American Association for Cancer Research (AACR) Annual Meeting in 2024 by Sethna et al. [60].

However, earlier, Lopez et al. [61] published the first results from the phase I dose escalation NCT03289962 study on the safety of cevumeran. In this study, cevumeran as a single agent and in combination with atezolizumab (anti-PD-L1 antibody) was used in locally advanced or metastatic solid tumors [patients with non-small-cell lung cancer (NSCLC) and melanoma previously treated with ICIs and immunotherapy-naive patients with melanoma, renal-cell carcinoma, NSCLC, urothelial cancer, and triple-negative breast cancer]. *Ex vivo* T cell responses were detected in 73% of patients. Response to treatment with cevumeran in combination with atezolizumab was observed in 10% of patients with urothelial cancer, 22% of patients with renal-cell carcinoma, 30% of patients with melanoma, 4% of patients with triple-negative breast cancer, and 10% of patients with NSCLC (the majority of patients previously were treated with ICIs). Cevumeran was well tolerated, but its use was associated with more frequent infusion-related reactions, cytokine release syndrome, influenza-like illness, nausea, and pyrexia [61].

In the study conducted by Rojas et al. [51], NGS was performed in the material obtained during pancreatic tumor resection to search for genetic abnormalities responsible for the formation of neoantigens. Bioinformatics analysis allowed the selection of sequences coding for more than 5 neoantigens, which were then used in the mRNA synthesis of the cevumeran vaccine. An individual neoantigen-encoding mRNA-lipoplex vaccine was produced. At that time (6 weeks after surgery), one dose of atezolizumab was administered. Priming doses 1-8 of cevumeran were administered in weeks 9 to 17. Then, from week 21, modified mFOLFIRINOX (irinotecan, fluorouracil,

leucovorin, oxaliplatin) chemotherapy was administered in 12 two-week cycles until week 43 after surgery. Patients received the ninth booster dose of cevumeran at week 46 [51].

Twenty-eight patients were resected according to the study protocol, but only 19 patients received atezolizumab. Six patients were excluded from the study because they had an advanced stage of the disease, one patient was not diagnosed with pancreatic ductal carcinoma, one patient withdrew consent, and NGS could not be performed in one patient. Sixteen patients received priming doses of cevumeran. Three patients were excluded from the study due to disease progression, withdrawal of consent, or insufficient number of neoantigens (< 5 neoantigens). Fifteen patients received chemotherapy (one patient had disease progression before chemotherapy). Three of 16 patients did not receive ninth vaccine doses, which was due to progression, death, or mFOLFIRINOX toxicity [51].

In 8 of 16 patients, cevumeran caused an increase in the number of T-cells specific for selected neoantigens. These cells produced IFN-γ *ex vivo* in an enzyme-linked immunospot (ELISpot) assay after incubation with 15-mer neoantigens. Ninety-eight percent of the T-cells targeting individual tumor neoantigens and induced by cevumeran were created *de novo* because they were not detected in the blood or tumor prior to administration of the vaccine. Cevumeran induced 79 clones of cytotoxic T-cells in the blood with an estimated median lifespan of 5.5 years. More than 80% of vaccine-induced neoantigen-specific T-cells could still be detected up to three years after cevumeran administration in patients with an immune response. These patients had longer median recurrence-free survival (RFS) compared to patients who did not respond to treatment [median RFS not reached in responders *vs*. 13.4 months in non-responders; hazard ratio (HR) = 0.14; 95% confidence interval (CI) 0.03–0.6; p = 0.007]. Six of the eight patients who responded to cevumeran remained disease-free during the three-year follow-up period, while seven of the eight patients without an immune response had tumor recurrence. All 16 patients who received cevumeran had grade 1–2 adverse events (AEs). Patients most commonly reported chills, fever, or diarrhea. In individual patients, grade 3 AEs occurred, including fever and hypertension [51, 60].

IMCODE 003 (NCT05968326) global multicenter phase II clinical trial will investigate the efficacy and safety of adjuvant cevumeran in combination with atezolizumab and chemotherapy compared with the current standard of care chemotherapy (mFOLFIRINOX) in PDAC patients [62]. Cevumeran is currently being evaluated in 4 clinical trials: first-line therapy in advanced melanoma patients (NCT03815058), adjuvant therapy in colorectal cancer patients (NCT04486378), subsequent lines of treatment in patients with locally advanced or metastatic solid tumors (NCT03289962), and adjuvant therapy in NSCLC patients who were circulating tumor DNA (ctDNA) positive after surgical resection (NCT04267237, the study was withdrawn) [63].

mRNA vaccines in melanoma patients

Immunotherapy using ICIs has revolutionized the treatment of patients with advanced and operable melanoma. Until recently, patients with advanced melanoma were treated ineffectively with radiotherapy, chemotherapy (dacarbazime), or immunotherapy (gp100 antigen vaccine or cytokine administration) [64, 65]. The discovery of BRAF (B-Raf protooncogen) and MEK (mitogen-activation protein kinase, MAP2K) inhibitors increased chances of longer survival in patients with mutations in codon 600 of the *BRAF* gene. However, immunotherapy has opened up a chance for long-term remissions for a large group of patients with advanced melanoma [64, 66]. Despite high effectiveness, some patients are resistant to ICIs, and mRNA vaccines can overcome this resistance.

The phase I Lipo-MERID trial (NCT02410733) involved 89 melanoma patients in stage IIIB/C or IV previously treated with ICIs. Patients received increasing doses of liposomal mRNA vaccine BNT111 (FixVac) alone or in combination with ICIs. FixVac is composed of mRNA encoding four TAAs: New York oesophageal squamous cell carcinoma 1 (NY-ESO-1), melanoma-associated antigen A3 (MAGE-A3), tyrosinase, and transmembrane phosphatase with tensin homology (TPTE). These antigens have limited expression on normal cells, but are widely expressed on melanoma cells, inducing an anti-tumor immune response. Melanoma cells in enrolled patients had to show the expression of at least one of the TAAs used in the vaccine. FixVac was administered in 8 escalating doses for the first two months and then in 3 booster doses on days 104, 132, and 160 of treatment. Doses and method of administration varied between 7 dose escalation cohorts and 3 expanded cohorts [67].

Interferon-α (IFN-α), interferon- γ (IFN-γ), interleukin 6, IFN-inducible protein 10 (IP-10), and the p70 subunit of IL-12 increased with FixVac doses that were accompanied by a transient fever and chills. Cytokine secretion was pulsatile, transient, and self-limiting. Combining FixVac with anti-PD1 antibodies did not affect cytokines levels. The majority (75%) of patients who were analysed using *ex vivo* IFN-γ ELISpot before and after eight vaccinations showed immune responses against at least one TAAs. The percentage of antigen-specific T-cells continued to increase or remained stable over more than 1 year. Moreover, a significant increase in metabolic activity in lymphocytes, specifically

in the spleen, was seen. In exploratory analysis, 12% and 25% of patients treated with VixVac monotherapy experienced partial response and stabilization, respectively. In a group of patients treated with a combination of VixVac and anti-PD-1 antibodies, the response rate was 35%. Regression of target lesions occurred across all doses, although the partial response rate was highest in patients treated with the medium dose (100 μg) of FixVac in combination with anti-PD1 antibodies [67].

A phase II trial (NCT04526899) with VixVac in combination with cemiplimab (anty-PD-1 antibody) is underway in advanced, unresectable stage III or IV melanoma patients who did not respond to anti-PD-1 therapy [68]. Moreover, FixVac received Food and Drug Administration (FDA) fast-track approval for advanced melanoma patients.

mRNA-4157-P201 (V940) is a lipid-encapsulated personalized vaccine encoding multiple neoantigens selected using NGS examination of tumor tissue. The safety of this mRNA vaccine was examined in the phase I dose escalation study KEYNOTE-603 (NCT03313778) in patients with resected solid tumors and in combination with pembrolizumab in patients with unresectable solid tumors. Of the 13 patients treated with mRNA vaccine monotherapy (3 melanomas, 8 NSCLC, 2 colorectal cancers with high microsatellite instability), 11 patients remained disease-free (12 months follow-up). In the group of the 20 patients receiving combination therapy [1 metastatic cutaneous squamous cell carcinoma, 4 bladder cancers, 2 head and neck carcinomas, 1 melanoma, 7 NSCLC, 2 small-cell lung cancers, 3 microsatellite instability-high (MSI-high) cancers], 5 cases of partial remission and 6 cases of stabilization of the disease were recorded. Thirteen patients from this group had previously received ICI therapy. Neoantigen-specific CD8+ T-cell responses were detected in all patients [69].

Personalized mRNA-4157 vaccine in combination with pembrolizumab (anti-PD-1 antibody) was used in adjuvant therapy in melanoma patients with high risk of recurrence in the phase II KEYNOTE-942 trial. The study involved 224 patients with cutaneous melanoma at stages IIIC, IIID, or IV who underwent complete surgical resection of the tumor; 67 patients were excluded from the study for various reasons, mainly due to problems with performing NGS in DNA and RNA from tumor tissue and the lack of sufficient quality and quantity of tissue for mRNA-4157 vaccine manufacture. Ninety-one percent of patients in the combination arm received a vaccine containing coding sequences for 34 neoantigens. One hundred and seven patients received pembrolizumab in combination with mRNA-4157 vaccine, and 50 patients received pembrolizumab alone. mRNA-4157 vaccine was administered at a dose of 1 mg intramuscularly every 3 weeks for up to 9 cycles, and pembrolizumab at a dose of 200 mg every 3 weeks for up to 18 cycles. The primary endpoint was recurrence-free survival. Fifty-five patients receiving combined treatment and 28 patients treated with pembrolizumab alone completed the full course of therapy [70, 71].

The majority (83.4%) of patients treated with combination therapy achieved 12-month RFS, and 78.4% of these patients achieved 24-month RFS. In patients treated with pembrolizumab, the percentages were 77.1% and 62.2%, rspectively. The reduction in the risk of disease recurrence was 44% (HR = 0.561; 95% CI 0.309–1.017; p = 0.0266). The greatest benefit from combination therapy compared to monotherapy with pembrolizumab was achieved in patients without PD-L1 expression on tumor cells. The risk of distant metastases occurrence [distant metastases free survival (DMSF)] was also lower in patients receiving mRNA-4157 vaccine and pembrolizumab compared to pembrolizumab alone (HR = 0.347; 95% CI 0.145–0.929; p = 0.0063). mRNA-4157 vaccine in combination with pembrolizumab was well-tolerated without an increase in immune-mediated AEs compared with pembrolizumab monotherapy. Serious AEs and immune-mediated AEs occurred in 14.4% and 35.6% of patients receiving combination therapy, respectively, and in 10.0% and 36% of patients treated with pembrolizumab, respecitively. Grade 1–2 fatigue, diarrhea, pruritis, and nausea occurred slightly more frequently in patients receiving combination therapy, whereas chills and pyrexia occurred almost exclusively in patients receiving the mRNA-4157 vaccine [70, 71].

mRNA-4157 vaccine in combination with pembrolizumab received breakthrough therapy designation in high-risk melanoma patients from the FDA in February 2023 and prime designation from the European Medicine Agency (EMA) in April 2023. Currently, several clinical trials are underway in which mRNA-4157 vaccine is used as monotherapy or in combination with pembrolizumab in patients with solid tumors (NCT03313778), high-risk melanoma (phase III trial NCT05933577), renal-cell carcinoma (NCT06307431), cutaneous squamous cell carcinoma (NCT06295809), NSCLC (NCT06077760), and bladder cancer patients after radical resection (NCT06305767) [72].

mRNA vaccines in non-small-cell lung cancer patients

Non-small-cell lung cancer is the leading cause of cancer death in men and women [73]. Most patients are diagnosed at a stage that does not allow surgical treatment. Fortunately, in patients with NSCLC, progress in treatment methods is the greatest among all cancer patients. Molecularly targeted therapies can be used in

patients with mutations in *EGFR, KRAS, BRAF, MET* genes, and with *ALK, ROS1, RET,* and *NTRK* gene rearrangements. Other patients may benefit from immunotherapy or chemoimmunotherapy. First-line immunotherapy is used in patients with unresectable NSCLC in stage III or IV when PD-L1 is expressed on more than 50% of tumor cells, while chemoimmunotherapy is most often used in patients with lower PD-L1 expression [74].

Unfortunately, immunotherapy is not effective in all patients. More than 25% of patients develop primary resistance to immunotherapy, and further 50% of patients develop secondary resistance to immunotherapy within 1 year of treatment [75]. Therefore, new immunotherapy methods that can be combined with ICIs and used in patients with resistance to immunotherapy are under development.

CV9201 (CureVac) is a non-personalized mRNA vaccine encoding five antigens specific to non-small cell lung cancer: NY-ESO-1, melanoma antigen family C1/C2 (MAGE-C1 and MAGE-C2), surviving, and trophoblast glycoprotein. In a Phase I/IIa dose escalation study (NCT00923312), 46 patients with locally advanced or metastatic NSCLC with stable disease after first-line therapy received five intradermal injections of CV9201 (400–1600 μg mRNA). CV9201 was well tolerated. Most AEs included mild to moderate injection site reactions and flu-like illness. Grade 3 AEs occurred in 7% of patients. The recommended dose of CV9201 in phase IIa was 1,600 μg. In this phase, 63% of patients had an antigen-specific immune response against $\geq$ 1 antigen. The disease was stable in 31% of patients. However, no objective response was observed. Median progression-free survival (PFS) and overall survival (OS) from the first vaccine administration were 5.0 months and 10.8 months, respectively. Two-year OS was achieved in 26.7% of patients, while 3-year OS was found in 20.7% of patients [76].

The phase I/II NCT03164772 study evaluated the safety and preliminary efficacy of mRNA vaccine CV9202 (BI 1361849) encoding 6 tumor-associated antigens: mucin 1 (MUC1), survivin, NY-ESO-1, 5T4 oncofetal antigen, MAGE-C2 and MAGE-C1 in patients with advanced NSCLC. In arm A, patients received the CV9202 vaccine and durvalumab (anti-PD-L1 antibody). In arm B, patients received the CV9202 vaccine, durvalumab, and tremelimumab (anti-CTLA-4 antibody). Patients could be treated with 1 prior line of anti-PD-1 or anti-PD-L1 therapy. The vaccine was intradermally administered in a total of 14 doses during 12 cycles. Twenty-three patients were in arm A and 34 patients in arm B. Patients from both arms had comparable treatment-related AEs (56% *vs*. 57%) and comparable rates of treatment discontinuation (22% *vs*. 24%). Twenty percent of patients treated with CV9202 and 71% of patients treated with durvalumab had partial response and disease control. The median duration of response (DoR) was 10 months, median PFS was 5.7 months, and median OS was not reached. In patients treated with the CV9202 vaccine, durvalumab, and tremelimumab, the overall response rate was 11%, and the disease control rate was 53%. In this group of patients, the median DoR, PFS, and OS were 6 months, 2.5 months, and 10 months, respectively. Based on literature data, the addition of CV9202 to durvalumab yielded comparable or better treatment response, PFS, and OS compared to durvalumab alone or with chemotherapy. However, the addition of tremelimumab did not improve treatment effects [77].

The CV9202 vaccine has been used without much success in clinical trials in NSCLC patients after chemoradiotherapy, after radiotherapy, in the case of progression after EGFR (epidermal growth factor receptor) tyrosine kinase inhibitors, and together with afatinib in patients with mutations in the *EGFR* gene.

Due to the low effectiveness of non-personalized mRNA vaccines in patients with NSCLC, the results of studies using modern personalized mRNA vaccines, including cevumeran and mRNA-4157, will be significant.

mRNA vaccines in prostate cancer

Prostate cancer is the most common cancer in men. Surgery, radiotherapy, and hormone therapy are effective treatments for this cancer. Unfortunately, there are no effective therapeutic options for patients with recurrent, castration-resistant, advanced prostate cancer. Chemotherapy and immunotherapy (e.g., with dendritic cells or ICIs) used so far have had limited effectiveness; however, chemotherapy may be combined with PARP inhibitors in patients with mutations in the *BRCA* genes [78, 79].

CV9104 vaccine contains mRNA encoding the antigens PSA, prostate-specific membrane antigen A (PSMA), prostate stem-cell antigen (PSCA), six transmembrane epithelial antigen of the prostate 1 (STEAP1), prostatic acid phosphatase (PAP), and MUC1. The study NCT01817738 was a double-blind, randomized, placebo-controlled phase I/II trial for men with asymptomatic or minimally symptomatic metastatic castrate-refractory prostate cancer. The phase I part of the trial assessed the safety of CV9104 and determined the dose of CV9104 for the randomized phase II study. The primary objective of the phase II study was to compare OS in patients treated with CV9104 or placebo. One hundred and thirty-four patients received CV9104 vaccine, and 63 patients received placebo. No significant difference in OS was found. Median OS was 35.5 months in patients treated with CV9104 and 33.7 months in patients receiving placebo (HR = 1.1; 95% CI 0.70–1.76; p = 0.33). There were also no significant differences in PFS and time to symptom progression. The incidence

of grade ≥ 3 AEs (51.1% *vs*. 59.7%) and serious AEs (44.5% *vs*. 43.5%) was similar in both arms. Injection site reactions and flu-like symptoms were more frequent in the CV9104-treated patients [80].

Conclusions

Initial failures in the use of mRNA vaccines in cancer treatment resulted from mRNA instability and the lack of personalization of these vaccines. Viral vectors were also not an ideal way to transfer mRNA to APCs. The first vaccines (e.g., CureVac) were based on mRNA-containing TAAs coding sequences, and their immunogenicity was low. The problem of mRNA stability was sorted out during the coronavirus disease 2019 (COVID-19) epidemic, with the development of a stable mRNA vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) containing the virus spike sequence and the development of a technique for creating nanolipid particles. The second problem is being solved thanks to the development of next-generation sequencing techniques, which enable the creation of personalized mRNA vaccines. Understanding the genetic abnormalities responsible for the formation of neoantigens and using these sequences in mRNA vaccines increases the specificity and immunogenicity of these vaccines. Currently, vaccines such as cevumeran, mRNA-4157, or the recently developed NCI-4650 contain sequences coding from several to over 30 neoantigens. They can be used to treat patients with all types of solid tumors that contain mutations responsible for the formation of neoantigens. It has been confirmed that the new NC-4650 vaccine specifically activates the immune system to produce an anti-tumor response in patients with gliomas, gastrointestinal cancers, and hepatocellular carcinoma. The development of personalized mRNA vaccines has made it possible to extend the time to recurrence in surgically resected pancreatic ductal carcinoma and melanoma. An exceptionally effective strategy may be to combine mRNA vaccines with already used immunotherapy with anti-PD-1, anti-PD-L1 and anti-CTLA4 antibodies. mRNA vaccines should be considered breakthrough oncological therapies in solid tumors, similar to therapies using chimeric antigen receptor T (CAR-T) cells in hemato-oncological diseases.

Article Information and Declarations

Author contributions

N.G.: writing — original draft preparation, writing — review and editing; N.K.: writing — original draft preparation, graphic design; P.K.: conceptualization, writing — original draft preparation, writing — review and editing, supervision.

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Competing interests

Authors declare they have no competing interests.

Supplementary material

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References

1. Huang T, Peng L, Han Y, et al. Lipid nanoparticle-based mRNA vaccines in cancers: Current advances and future prospects. Front Immunol. 2022; 13: 922301, doi: 10.3389/fimmu.2022.922301, indexed in Pubmed: 36090974.
2. Gu J, Xu Z, Liu Q, et al. Building a Better Silver Bullet: Current Status and Perspectives of Non-Viral Vectors for mRNA Vaccines. Adv Healthc Mater. 2024; 13(3): e2302409, doi: 10.1002/adhm.202302409, indexed in Pubmed: 37964681.
3. Ramachandran S, Satapathy SR, Dutta T. Delivery Strategies for mRNA Vaccines. Pharmaceut Med. 2022; 36(1): 11–20, doi: 10.1007/s40290-021-00417-5, indexed in Pubmed: 35094366.
4. Rosa SS, Prazeres DMF, Azevedo AM, et al. mRNA vaccines manufacturing: Challenges and bottlenecks. Vaccine. 2021; 39(16): 2190–2200, doi: 10.1016/j.vaccine.2021.03.038, indexed in Pubmed: 33771389.
5. Xu S, Yang K, Li R, et al. mRNA Vaccine Era-Mechanisms, Drug Platform and Clinical Prospection. Int J Mol Sci. 2020; 21(18), doi: 10.3390/ijms21186582, indexed in Pubmed: 32916818.
6. Yao R, Xie C, Xia X. Recent progress in mRNA cancer vaccines. Hum Vaccin Immunother. 2024; 20(1): 2307187, doi: 10.1080/21645515.2024.2307187, indexed in Pubmed: 38282471.
7. Wang B, Pei J, Xu S, et al. Recent advances in mRNA cancer vaccines: meeting challenges and embracing opportunities. Front Immunol. 2023; 14: 1246682, doi: 10.3389/fimmu.2023.1246682, indexed in Pubmed: 37744371.
8. Türeci Ö, Vormehr M, Diken M, et al. Targeting the Heterogeneity of Cancer with Individualized Neoepitope Vaccines. Clin Cancer Res. 2016; 22(8): 1885–1896, doi: 10.1158/1078-0432.CCR-15-1509, indexed in Pubmed: 27084742.
9. Hollingsworth RE, Jansen K. Turning the corner on therapeutic cancer vaccines. NPJ Vaccines. 2019; 4: 7, doi: 10.1038/s41541-019-0103-y, indexed in Pubmed: 30774998.
10. Zhang X, Sharma PK, Peter Goedegebuure S, et al. Personalized cancer vaccines: Targeting the cancer mutanome. Vaccine. 2017; 35(7): 1094–1100, doi: 10.1016/j.vaccine.2016.05.073, indexed in Pubmed: 27449681.
11. Deng Z, Tian Y, Song J, et al. mRNA Vaccines: The Dawn of a New Era of Cancer Immunotherapy. Front Immunol. 2022; 13: 887125, doi: 10.3389/fimmu.2022.887125, indexed in Pubmed: 35720301.
12. Han R, Wang Y, Lu L. Sensitizing the Efficiency of ICIs by Neoantigen mRNA Vaccines for HCC Treatment. Pharmaceutics. 2023; 16(1), doi: 10.3390/pharmaceutics16010059, indexed in Pubmed: 38258070.
13. Fotin-Mleczek M, Zanzinger K, Heidenreich R, et al. Highly potent mRNA based cancer vaccines represent an attractive platform for combination therapies supporting an improved therapeutic effect. J Gene Med. 2012; 14(6): 428–439, doi: 10.1002/jgm.2605, indexed in Pubmed: 22262664.
14. Fotin-Mleczek M, Zanzinger K, Heidenreich R, et al. mRNA-based vaccines synergize with radiation therapy to eradicate established tumors. Radiat Oncol. 2014; 9: 180, doi: 10.1186/1748-717X-9-180, indexed in Pubmed: 25127546.
15. Lorentzen CL, Haanen JB, Met Ö, et al. Clinical advances and ongoing trials on mRNA vaccines for cancer treatment. Lancet Oncol. 2022; 23(10): e450–e458, doi: 10.1016/S1470-2045(22)00372-2, indexed in Pubmed: 36174631.
16. Parkhurst MR, Yang JC, Langan RC, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2011; 19(3): 620–626, doi: 10.1038/mt.2010.272, indexed in Pubmed: 21157437.

17. Subramanian R, Meganathan P, Selvarajan R. mRNA Vaccine - Game Changer against Infectious Disease. Int J Health Sci Res. 2022; 12(7): 250–270, doi: 10.52403/ijhsr.20220737.
18. Liu J, Fu M, Wang M, et al. Cancer vaccines as promising immuno--therapeutics: platforms and current progress. J Hematol Oncol. 2022; 15(1): 28, doi: 10.1186/s13045-022-01247-x, indexed in Pubmed: 35303904.
19. Warminski M, Mamot A, Depaix A, et al. Chemical Modifications of mRNA Ends for Therapeutic Applications. Acc Chem Res. 2023; 56(20): 2814–2826, doi: 10.1021/acs.accounts.3c00442, indexed in Pubmed: 37782471.
20. Chen J, Ye Z, Huang C, et al. 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, doi: 10.1073/pnas.2207841119, indexed in Pubmed: 35969778.
21. Schlake T, Thess A, Fotin-Mleczek M, et al. Developing mRNA--vaccine technologies. RNA Biol. 2012; 9(11): 1319–1330, doi: 10.4161/rna.22269, indexed in Pubmed: 23064118.
22. Beck JD, Reidenbach D, Salomon N, et al. mRNA therapeutics in cancer immunotherapy. Mol Cancer. 2021; 20(1): 69, doi: 10.1186/s12943-021-01348-0, indexed in Pubmed: 33858437.
23. Wang Y, Zhang Z, Luo J, et al. mRNA vaccine: a potential therapeutic strategy. Mol Cancer. 2021; 20(1): 33, doi: 10.1186/s12943-021-01311-z, indexed in Pubmed: 33593376.
24. Kwon S, Kwon M, Im S, et al. mRNA vaccines: the most recent clinical applications of synthetic mRNA. Arch Pharm Res. 2022; 45(4): 245–262, doi: 10.1007/s12272-022-01381-7, indexed in Pubmed: 35426547.
25. Linares-Fernández S, Lacroix C, Exposito JY, et al. Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol Med. 2020; 26(3): 311–323, doi: 10.1016/j.molmed.2019.10.002, indexed in Pubmed: 31699497.
26. Gómez-Aguado I, Rodríguez-Castejón J, Vicente-Pascual M, et al. Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives. Nanomaterials (Basel). 2020; 10(2), doi: 10.3390/nano10020364, indexed in Pubmed: 32093140.
27. McCaffrey A. RNA Epitranscriptome: Role of the 5' Cap. Genetic Engineering & Biotechnology News. 2019; 39(5): 59, 61, doi: 10.1089/gen.39.05.17.
28. Wojtczak BA, Sikorski PJ, Fac-Dabrowska K, et al. 5'-Phosphorothiolate Dinucleotide Cap Analogues: Reagents for Messenger RNA Modification and Potent Small-Molecular Inhibitors of Decapping Enzymes. J Am Chem Soc. 2018; 140(18): 5987–5999, doi: 10.1021/jacs.8b02597, indexed in Pubmed: 29676910.
29. Inagaki M, Abe N, Li Z, et al. Cap analogs with a hydrophobic photocleavable tag enable facile purification of fully capped mRNA with various cap structures. Nat Commun. 2023; 14(1): 2657, doi: 10.1038/s41467-023-38244-8, indexed in Pubmed: 37169757.
30. Litvinova VR, Rudometov AP, Karpenko LI, et al. mRNA Vaccine Platform: mRNA Production and Delivery. Russ J Bioorg Chem. 2023; 49(2): 220–235, doi: 10.1134/S1068162023020152, indexed in Pubmed: 37252004.
31. Karikó K, Muramatsu H, Ludwig J, et al. 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, doi: 10.1093/nar/gkr695, indexed in Pubmed: 21890902.
32. Ho W, Gao M, Li F, et al. Next-Generation Vaccines: Nanoparticle--Mediated DNA and mRNA Delivery. Adv Healthc Mater. 2021; 10(8): e2001812, doi: 10.1002/adhm.202001812, indexed in Pubmed: 33458958.
33. Jenks S. Gene therapy death--"everyone has to share in the guilt". J Natl Cancer Inst. 2000; 92(2): 98–100, doi: 10.1093/jnci/92.2.98, indexed in Pubmed: 10639505.
34. Dailey GP, Crosby EJ, Hartman ZC. Cancer vaccine strategies using self-replicating RNA viral platforms. Cancer Gene Ther. 2023; 30(6): 794–802, doi: 10.1038/s41417-022-00499-6, indexed in Pubmed: 35821284.
35. Pardi N, Hogan MJ, Porter FW, et al. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018; 17(4): 261–279, doi: 10.1038/nrd.2017.243, indexed in Pubmed: 29326426.
36. Savar NS, Vallet T, Arashkia A, et al. Packaging, Purification, and Titration of Replication-Deficient Semliki Forest Virus-Derived Particles as a Self-Amplifying mRNA Vaccine Vector. Iran Biomed J. 2022; 26(4): 269–278, doi: 10.52547/ibj.3535, indexed in Pubmed: 35468712.
37. Morse MA, Crosby EJ, Force J, et al. Clinical trials of self-replicating RNA-based cancer vaccines. Cancer Gene Ther. 2023; 30(6): 803–811, doi: 10.1038/s41417-023-00587-1, indexed in Pubmed: 36765179.
38. Barbier AJ, Jiang AY, Zhang P, et al. The clinical progress of mRNA vaccines and immunotherapies. Nat Biotechnol. 2022; 40(6): 840–854, doi: 10.1038/s41587-022-01294-2, indexed in Pubmed: 35534554.
39. Samaridou E, Heyes J, Lutwyche P. Lipid nanoparticles for nucleic acid delivery: Current perspectives. Adv Drug Deliv Rev. 2020; 154-155: 37–63, doi: 10.1016/j.addr.2020.06.002, indexed in Pubmed: 32526452.
40. Vishweshwaraiah YL, Dokholyan NV. mRNA vaccines for cancer immunotherapy. Front Immunol. 2022; 13: 1029069, doi: 10.3389/fimmu.2022.1029069, indexed in Pubmed: 36591226.
41. Hou X, Zaks T, Langer R, et al. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021; 6(12): 1078–1094, doi: 10.1038/s41578-021-00358-0, indexed in Pubmed: 34394960.
42. Shi Da, Beasock D, Fessler A, et al. To PEGylate or not to PEGylate: Immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. Adv Drug Deliv Rev. 2022; 180: 114079, doi: 10.1016/j.addr.2021.114079, indexed in Pubmed: 34902516.
43. Qin Z, Bouteau A, Herbst C, et al. Pre-exposure to mRNA-LNP inhibits adaptive immune responses and alters innate immune fitness in an inheritable fashion. PLoS Pathog. 2022; 18(9): e1010830, doi: 10.1371/journal.ppat.1010830, indexed in Pubmed: 36054264.
44. Houseley J, Tollervey D. The many pathways of RNA degradation. Cell. 2009; 136(4): 763–776, doi: 10.1016/j.cell.2009.01.019, indexed in Pubmed: 19239894.
45. Diken M, Kreiter S, Selmi A, et al. Antitumor vaccination with synthetic mRNA: strategies for in vitro and in vivo preclinical studies. Methods Mol Biol. 2013; 969: 235–246, doi: 10.1007/978-1-62703-260-5_15, indexed in Pubmed: 23296938.
46. Overwijk WW. Cancer vaccines in the era of checkpoint blockade: the magic is in the adjuvant. Curr Opin Immunol. 2017; 47: 103–109, doi: 10.1016/j.coi.2017.07.015, indexed in Pubmed: 28806603.
47. Vormehr M, Türeci Ö, Sahin U. Harnessing Tumor Mutations for Truly Individualized Cancer Vaccines. Annu Rev Med. 2019; 70: 395–407, doi: 10.1146/annurev-med-042617-101816, indexed in Pubmed: 30691374.
48. Lang F, Schrörs B, Löwer M, et al. Identification of neoantigens for individualized therapeutic cancer vaccines. Nat Rev Drug Discov. 2022; 21(4): 261–282, doi: 10.1038/s41573-021-00387-y, indexed in Pubmed: 35105974.
49. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. Science. 2018; 359(6382): 1355–1360, doi: 10.1126/science.aar7112, indexed in Pubmed: 29567706.
50. Lebofsky R, Decraene C, Bernard V, et al. Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Mol Oncol. 2015; 9(4): 783–790, doi: 10.1016/j.molonc.2014.12.003, indexed in Pubmed: 25579085.
51. Rojas LA, Sethna Z, Soares KC, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. Nature. 2023; 618(7963): 144–150, doi: 10.1038/s41586-023-06063-y, indexed in Pubmed: 37165196.
52. Bechter O, Utikal J, Baurain JF, et al. 391 A first-in-human study of intratumoral SAR441000, an mRNA mixture encoding IL-12sc, interferon alpha2b, GM-CSF and IL-15sushi as monotherapy and in combination with cemiplimab in advanced solid tumors. Regular and young investigator award abstracts. 2020, doi: 10.1136/jitc-2020-sitc2020.0391.
53. Hewitt SL, Bai A, Bailey D, et al. Durable anticancer immunity from intratumoral administration of IL-23, IL-36γ, and OX40L mRNAs. Sci Transl Med. 2019; 11(477), doi: 10.1126/scitranslmed.aat9143, indexed in Pubmed: 30700577.
54. Hotz C, Wagenaar TR, Gieseke F, et al. Local delivery of mRNA-encoded cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models. Sci Transl Med. 2021; 13(610): eabc7804, doi: 10.1126/scitranslmed.abc7804, indexed in Pubmed: 34516826.
55. Jimeno A, Gupta S, Sullivan R, et al. Abstract CT032: A phase 1/2, open-label, multicenter, dose escalation and efficacy study of mRNA-2416, a lipid nanoparticle encapsulated mRNA encoding human OX40L, for intratumoral injection alone or in combination with durvalumab for patients with advanced malignancies. Cancer Research. 2020; 80(16_Supplement): CT032–CT032, doi: 10.1158/1538-7445.am2020-ct032.
56. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol. 2021; 18(7): 493–502, doi: 10.1038/s41575-021-00457-x, indexed in Pubmed: 34002083.
57. Zhao Z, Liu W. Pancreatic Cancer: A Review of Risk Factors, Diagnosis, and Treatment. Technol Cancer Res Treat. 2020; 19:

1533033820962117, doi: 10.1177/1533033820962117, indexed in Pubmed: 33357065.
58. Zhu H, Wei M, Xu J, et al. PARP inhibitors in pancreatic cancer: molecular mechanisms and clinical applications. Mol Cancer. 2020; 19(1): 49, doi: 10.1186/s12943-020-01167-9, indexed in Pubmed: 32122376.
59. Casolino R, Biankin AV. Treatment of pancreatic cancer in 2022. Camb Prism Precis Med. 2023; 1: e14, doi: 10.1017/pcm.2023.2, indexed in Pubmed: 38550932.
60. Sethna Z, Guasp P, Reiche, C, et al. CT025 -Personalized RNA Neoantigen Vaccines Induce Long-Lived CD8+ T Effector Cells in Pancreatic Cancer. Presented at: 2024 AACR Annual Meeting; April 7, 2024; San Diego, CA.
61. Lopez J, Camidge R, Iafolla M, et al. Abstract CT301: A phase Ib study to evaluate RO7198457, an individualized Neoantigen Specific immunoTherapy (iNeST), in combination with atezolizumab in patients with locally advanced or metastatic solid tumors. Cancer Research. 2020; 80(16_Supplement): CT301–CT301, doi: 10.1158/1538-7445.am2020-ct301.
62. A Study of the Efficacy and Safety of Adjuvant Autogene Cevumeran Plus Atezolizumab and mFOLFIRINOX Versus mFOLFIRINOX Alone in Participants With Resected Pancreatic Ductal Adenocarcinoma. https://clinicaltrials.gov/study/NCT05968326 (03.07.2024).
63. https://clinicaltrials.gov/search?cond=RO7198457 (03.07.2024).
64. Jenkins RW, Fisher DE. Treatment of Advanced Melanoma in 2020 and Beyond. J Invest Dermatol. 2021; 141(1): 23–31, doi: 10.1016/j.jid.2020.03.943, indexed in Pubmed: 32268150.
65. Cassarino DS, Miller WJ, Auerbach A, et al. The effects of gp100 and tyrosinase peptide vaccinations on nevi in melanoma patients. J Cutan Pathol. 2006; 33(5): 335–342, doi: 10.1111/j.0303--6987.2006.00449.x, indexed in Pubmed: 16640539.
66. Villani A, Potestio L, Fabbrocini G, et al. The Treatment of Advanced Melanoma: Therapeutic Update. Int J Mol Sci. 2022; 23(12), doi: 10.3390/ijms23126388, indexed in Pubmed: 35742834.
67. Sahin U, Oehm P, Derhovanessian E, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature. 2020; 585(7823): 107–112, doi: 10.1038/s41586-020-2537-9, indexed in Pubmed: 32728218.
68. Trial With BNT111 and Cemiplimab in Combination or as Single Agents in Patients With Anti-PD-1-refractory/Relapsed, Unresectable Stage III or IV Melanoma. https://clinicaltrials.gov/study/NCT04526899 (03.07.2024).
69. Burris H, Patel M, Cho D, et al. A phase I multicenter study to assess the safety, tolerability, and immunogenicity of mRNA-4157 alone in patients with resected solid tumors and in combination with pembrolizumab in patients with unresectable solid tumors. J Clin Oncol. 2019; 37(15_suppl): 2523–2523, doi: 10.1200/jco.2019.37.15_suppl.2523.
70. Khattak A, Carlino M, Meniawy T, et al. Abstract CT001: A personalized cancer vaccine, mRNA-4157, combined with pembrolizumab versus pembrolizumab in patients with resected high-risk melanoma: Efficacy and safety results from the randomized, open-label Phase 2 mRNA-4157-P201/Keynote-942 trial. Cancer Research. 2023; 83(8_Supplement): CT001–CT001, doi: 10.1158/1538-7445.am2023-ct001.
71. Khattak A, Weber J, Meniawy T, et al. Distant metastasis-free survival results from the randomized, phase 2 mRNA-4157-P201/KEYNOTE-942 trial. J Clin Oncol. 2023; 41(17_suppl): LBA9503–LBA9503, doi: 10.1200/jco.2023.41.17_suppl.lba9503.
72. https://clinicaltrials.gov/search?cond=mRNA-4157%20 (03.07.2024).
73. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024; 74(1): 12–49, doi: 10.3322/caac.21820, indexed in Pubmed: 38230766.
74. Planchard D, Popat S, Kerr K, et al. ESMO Guidelines Committee. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018; 29(Suppl 4): iv192–iv237, doi: 10.1093/annonc/mdy275, indexed in Pubmed: 30285222.
75. Zhou S, Yang H. Immunotherapy resistance in non-small-cell lung cancer: From mechanism to clinical strategies. Front Immunol. 2023; 14: 1129465, doi: 10.3389/fimmu.2023.1129465, indexed in Pubmed: 37090727.
76. Sebastian M, Schröder A, Scheel B, et al. A phase I/IIa study of the mRNA-based cancer immunotherapy CV9201 in patients with stage IIIB/IV non-small cell lung cancer. Cancer Immunol Immunother. 2019; 68(5): 799–812, doi: 10.1007/s00262-019-02315-x, indexed in Pubmed: 30770959.
77. Chen DT, Sabari J, Thompson J, et al. Abstract CT052: A phase IB study of mRNA-based active cancer vaccine, BI1361849, combined with durvalumab and tremelimumab immunotherapy in patients with non-small cell lung cancer (NSCLC). Cancer Research. 2024; 84(7_Supplement): CT052–CT052, doi: 10.1158/1538-7445.am2024-ct052.
78. Wang G, Zhao Di, Spring DJ, et al. Genetics and biology of prostate cancer. Genes Dev. 2018; 32(17-18): 1105–1140, doi: 10.1101/gad.315739.118, indexed in Pubmed: 30181359.
79. Sekhoacha M, Riet K, Motloung P, et al. Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. Molecules. 2022; 27(17), doi: 10.3390/molecules27175730, indexed in Pubmed: 36080493.
80. Stenzl A, Feyerabend S, Syndikus I, et al. Results of the randomized, placebo-controlled phase I/IIB trial of CV9104, an mRNA based cancer immunotherapy, in patients with metastatic castration-resistant prostate cancer (mCRPC). Ann Oncol. 2017; 28: v408–v409, doi: 10.1093/annonc/mdx376.014.