Advanced Pharmaceutical Bulletin

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

Review Article

How to cite this article:
Mashhadi Abolghasem Shirazi M. Next-Generation mRNA Cancer Vaccines: Integrating Innovative Delivery Systems, Personalized Antigen Discovery, and Future Clinical Strategies. Advanced Pharmaceutical Bulletin, doi: 10.34172/apb.46663

Next-Generation mRNA Cancer Vaccines: Integrating Innovative Delivery Systems, Personalized Antigen Discovery, and Future Clinical Strategies

Maryam Mashhadi Abolghasem Shirazi[1*]

[1]Department of Molecular Virology, Pasteur Institute of Iran, Tehran, Iran

ARTICLE INFO

Keywords:
mRNA vaccines,
Cancer immunotherapy,
Tumor antigens,
saRNA,
Adaptive clinical trials

Article History:
Submitted: October 29, 2025
Revised: May 13, 2026
Accepted: July 12, 2026
ePublished: July 14, 2026

ABSTRACT

mRNA has emerged as a transformative platform in vaccine oncology, offering rapid design, non-acquired security and powerful activation of the adaptive immune. Despite encouraging preclinical and clinical results, challenges such as efficient distribution, tumor immune evasion, and patient-specific antigen selection limit their widespread clinical translations. We performed a targeted literature review of relevant preclinical studies and clinical trials to highlight current findings and emerging evidence mRNA vaccines in various cancers, including glioblastoma, melanoma, lungs, breasts, digestive systems, ovarian, prostate, kidney and hematological malignancies. Comparative analysis was carried out between mRNA, DNA and peptide vaccine platforms. Additionally, we critically examined the tumor resistance mechanism and proposed the next generation strategies, including the nanometer-based delivery system, self-amplifying mRNA (saRNA), and bioinformatics-operated antigen discovery, which are using single cell sequencing. Clinical trials continuously display the safety and immunity of mRNA vaccines, with a strong induction of CD8+ T cell reactions and cancer types. However, efficacy outcomes remain variable, with the strongest responses in high-mutation-burden tumors such as melanoma and NSCLC, and limited benefit in low-mutation-burden tumors such as prostate and ovarian cancers. Our conceptual framework introduces the integration of mRNA vaccines with car-T cells and monoclonal antibodies to remove tumor immune resistance. In addition, we present an accurate algorithm, taking advantage of scRNA-seq for patient-specific neoantigen selection, and highlight the benefits of saRNA in increasing antigen expression with low dosage requirements. mRNA vaccine represents a rapidly growing frontier in cancer immunotherapy, yet its success will depend on addressing biological and translation obstacles. This review synthesizes not only current clinical evidence, but also provides innovative conceptual models and future instructions that may redefine the design of the next generation cancer vaccines. By bridging advances in nanotechnology, computational biology and adaptive clinical trial designs, the task offers a roadmap to achieve sustainable, individual and widely accessible cancer immunotherapy.

***Corresponding Author**
Maryam Mashhadi Abolghasem Shirazi, Email: mersedeh_shirazi@yahoo.com, ORCID: 0000-0001-6639-7221

1. Introduction

Globally, cancer ranks as the second most common cause of death. In 2022, the World Health Organization projected that 10 million people would lose their lives to this disease.[1] Furthermore, the International Agency for Research on Cancer estimates that by 2050, the number of individuals affected by cancer may reach 35 million.[1, 2] The high costs associated with current cancer therapies place a significant financial strain on society, highlighting the urgent need for more effective and practical treatment options.[3] Conventional treatments such as surgery, chemotherapy, radiation, and antibody therapy have provided some benefits for patients with various cancers. However, these methods come with limitations and severe side effects. For instance, surgery can be invasive and is often unsuitable for advanced stages of cancer.[4, 5] Both chemotherapy and radiation unintentionally harm healthy cells alongside cancerous ones, leading to distressing side effects for patients. Additionally, antibody therapies can lose their efficacy over time due to genetic mutations or alterations in signaling pathways, resulting in treatment resistance.[6] Consequently, there is a necessity for approaches and therapies that are less invasive but more effective.[7] The COVID-19 pandemic has accelerated the development and widespread implementation of mRNA-based vaccines.[8, 9] In addition, growing clinical evidence indicates that mRNA-based therapeutics hold substantial promise for cancer treatment strategies.[10, 11] mRNA molecules are safe, non-infectious, and do not integrate into the host's DNA, making them better tolerated for cancer interventions with fewer adverse effects than traditional chemotherapy. They can be produced and designed quickly while also fostering both humoral and cell-mediated immune responses aimed at the targeted tumor making mRNA-based vaccines advantageous in cancer care.[12, 13]

The operation of mRNA vaccines involves encoding a tumor-specific antigen in the mRNA, which is then injected into the patient. The patient's cells translate this mRNA into proteins, presenting them to the immune system and stimulating an immune response against the cancer.[12] To date, delivery methods for mRNA to the affected cells have been refined. A promising technique involves encapsulating mRNA in lipid nanoparticles (LNPs) to protect it from degradation and facilitate its entry into the target cells. Numerous studies have been conducted to evaluate the effectiveness and immune response of mRNA vaccines for treating various types of cancer.[14, 15] Reviewing these trials is essential to identify the challenges and advancements necessary for enhancing mRNA vaccine technology in the realm of cancer treatment.

This review highlights the results of clinical trials and the practical difficulties associated with mRNA vaccines. We specifically concentrate on therapies tailored to certain organs, aiming to equip clinicians with detailed information necessary for treating patients effectively. Additionally, we summarize how well mRNA vaccines perform, including their ability to induce cytokines and provoke targeted immune responses against various solid tumors. These tumors include brain cancer, melanoma, lung cancer, breast cancer, cancers of the digestive system, as well as ovarian, prostate, and kidney cancers. Lastly, we thoroughly explore the development of mRNA vaccines, focusing on the most effective delivery methods and combination therapies to better grasp their potential in cancer therapy.

2. The Mechanism, Delivery, and Injection of mRNA Vaccines

Using mRNA vaccines for cancer treatment can assist the immune system in identifying and eliminating cancer cells. The process for creating mRNA vaccines in a lab begins with template DNA, typically a plasmid with a transcription factor like T7, SP6, or T3. This DNA is combined with ribonucleoside triphosphates, RNA

polymerase, and capping enzymes that work together to transcribe the mRNA, starting from the 5′ cap to the 3′ poly A tail, resulting in a synthetic mRNA molecule.[16] To improve stability and reduce immune recognition while protecting against enzymatic degradation, modified nucleosides such as pseudouridine and N1-methylpseudouridine are incorporated throughout the mRNA transcript. Meanwhile, the 5′ cap structure and the 3′ poly(A) tail support efficient translation and enhance mRNA stability.[10, 12]

Lipid nanoparticles (LNPs) are the most widely used delivery system for protecting mRNA and facilitating its efficient uptake by target cells. A typical LNP formulation consists of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol (PEG)-lipids, each contributing to nanoparticle stability, cellular uptake, and intracellular delivery. Ionizable lipids enable efficient mRNA encapsulation and promote endosomal escape, while cholesterol and phospholipids support membrane structure and fusion. PEG-lipids further enhance particle stability and prolong circulation time by reducing rapid immune clearance.[17, 18] LNP formulations can be engineered to improve tissue-specific delivery. For instance, modification of lipid composition has been shown to influence organ targeting following systemic administration, with certain formulations favoring delivery to organs such as the liver, spleen, or lungs.[19, 20] In addition, specific ionizable lipids, such as 113-O12B, have demonstrated enhanced delivery to lymph nodes and improved $CD8^+$ T cell responses.[21] Alternative delivery approaches, including lipoplexes and ex vivo mRNA electroporation of dendritic cells, have also been explored.

Despite these advantages, important safety and pharmacokinetic limitations remain. PEG-lipids have been associated with hypersensitivity reactions, potentially mediated by anti-PEG antibodies, which may also contribute to accelerated blood clearance (ABC phenomenon). Furthermore, LNPs can activate the complement system, leading to complement activation-related pseudoallergy (CARPA), particularly after systemic administration. Another key challenge is their biodistribution profile, as LNPs tend to accumulate predominantly in the liver and spleen following intravenous injection, potentially limiting delivery to target tissues such as tumors.

To overcome these limitations, recent strategies have focused on optimizing PEG density, developing alternative surface coatings, and designing next-generation ionizable lipids with improved targeting efficiency and reduced immunogenicity. These advancements are essential for enhancing the clinical translation of mRNA-based therapeutics[22] (Figure 1).

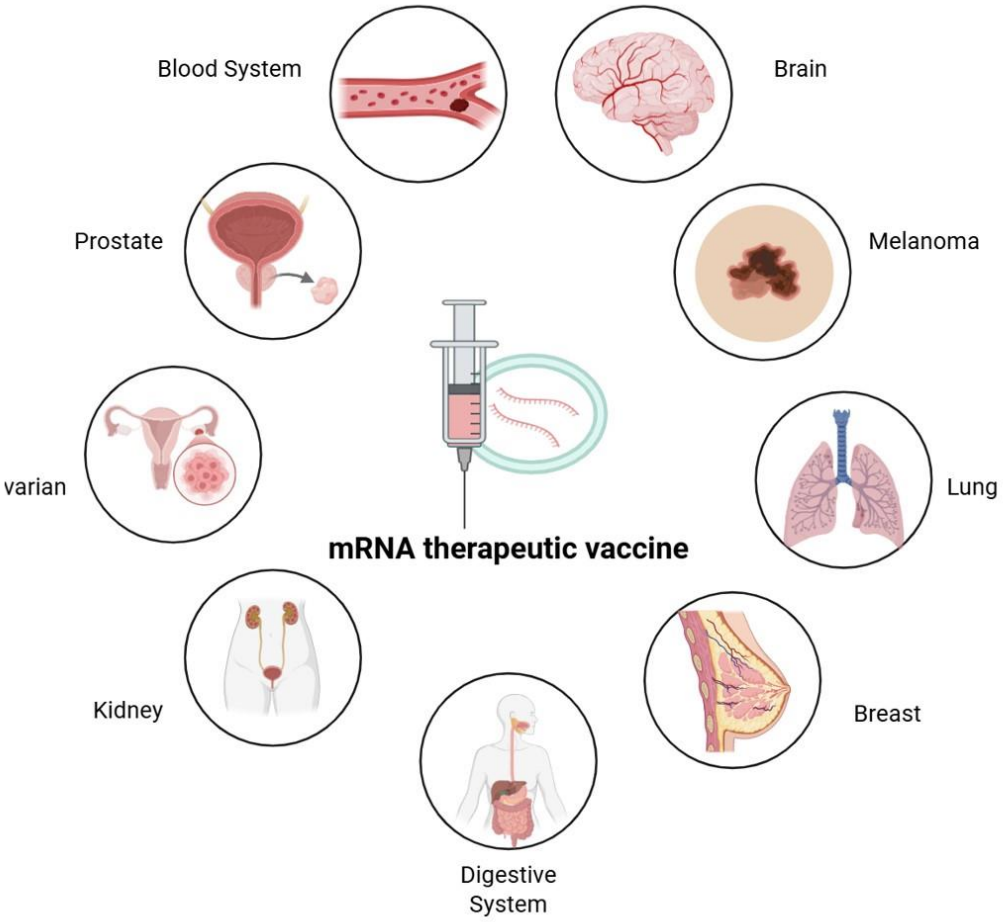

Figure 1. Applications of mRNA therapeutic vaccines across multiple cancer types. This schematic illustrates the broad applicability of mRNA-based therapeutic vaccines in oncology. The central panel depicts an mRNA vaccine construct delivered via injection, highlighting its role in encoding tumor-associated or neoantigen targets. Surrounding panels represent major cancer types in which mRNA vaccine strategies are being investigated, including brain tumors, melanoma, lung cancer, breast cancer, digestive system cancers, kidney cancer, ovarian cancer, prostate cancer, and hematological malignancies. These vaccines are designed to induce antigen-specific immune responses, particularly activating cytotoxic T lymphocytes against tumor cells. The figure emphasizes the versatility of mRNA platforms across both solid tumors and blood cancers.

Once mRNA sequences are designed and enclosed in lipids, the method chosen for administering the vaccine to ensure mRNA reaches the target cells becomes crucial. This decision is influenced by the type of disease and the mRNA therapy involved. Vaccine delivery can occur through several routes, including intravenous (i.v.), intradermal (i.d.), hypodermic (i.h.), intraperitoneal (i.p.), subcutaneous (s.c.), and intranasal (i.n.).[23, 24] When mRNA carrying a tumor antigen reaches the target cell, it is converted into proteins.[25] Major histocompatibility complex (MHC) molecules then present these proteins on the cell surface.[12] Dendritic cells (DCs) play a vital role in the immune response to these antigens. They capture mRNA that contains the antigen and display the resulting proteins on their surface for recognition by T cells.[26] Research is ongoing to explore various injection methods and delivery mechanisms for antigen-laden mRNA. One study evaluated the LPX/RBD-mRNA formulation across five injection routes (i.v., i.m., i.h., i.d., and i.p.) using a mouse model. The findings indicated that i.v., i.m., and i.h. routes produced similar levels of protein expression along with enhanced humoral and cytokine responses, while the i.d. route led to lower protein expression and immune responses compared to the others.[27] Additionally, another study focused on myeloid cells in tumors, utilizing s.c. and i.v. injection methods. It found that the i.v.

route yielded a higher production of tumor-specific CD8+ T cells than the s.c. method.[28] In another investigation, both i.n. and i.m. routes were assessed together, showing that a combination of these methods increased the numbers of humoral, cellular, and resident memory T cells.[29] A separate study examined different lipid particles and mRNA delivery methods using a BALB/c mouse model, comparing solid lipid nanoparticles (SLNs), ionizable lipid nanoparticles (iLNPs), corosolic acid (CA)-modified lipid nanoparticles (cLNPs), and polymeric nanoparticles (PNPs) delivered through i.m., i.d., and i.n. routes. Results indicated that iLNPs produced the highest IgG antibody response, followed by cLNPs and SLNs from i.m. and i.d. injections, respectively.[30] The functioning of the mRNA vaccine concerning different injection methods remains uncertain. However, both the intravenous (i.v.) and intradermal (i.d.) methods have shown antibody reactions similar to those of the intramuscular (i.m.) injection, which is still the most frequently used approach. It is essential to accurately deliver mRNA containing a drug or gene to target cells. To achieve this, numerous strategies have been explored to direct therapeutic mRNA to particular cells. One study indicated that using mannosylated lipoplexes for mRNA delivery improved targeting to splenic dendritic cells compared to standard lipoplexes.[31] Similarly, another investigation focused on lipid nanoparticles (LNPs) with ionizable lipids, which primarily targeted liver sinusoidal endothelial cells (LSECs).[32] Adjusting the PEG lipid content in LNPs allowed for more effective delivery of drug mRNA to liver cells as demonstrated in another study. They also tested mRNA-LNPs with mannose-PEG lipid, confirming specific mRNA delivery to LSECs.[32] A different experiment used lipid 113-O12B in mRNA-containing LNPs that specifically targeted lymph nodes.[21] Research findings indicate that incorporating certain molecules, such as DODAP, 18PA, or DOTAP in the LNP formulation, can target mRNA with specific genes to the liver, spleen, or lungs.[20, 33] To explore colitis treatment, LNPs carrying IL-10 mRNA were injected into a mouse model of inflammatory bowel disease (IBD), leading to an increase in leukocytes in the colon and a decrease in intestinal inflammation.[34] In ovarian cancer research, mRNA-LNPs containing an anti-EGFR antibody were intraperitoneally injected into tumor-bearing mice, resulting in the uptake of the specific mRNA-LNP by ovarian tumor cells and subsequent growth inhibition.[35] Furthermore, the potential of LNPs with mRNA linked to an antibody against CD117 was investigated to modify pro-apoptotic mRNAs in vivo within hematopoietic stem cells. The results highlighted a marked effect on sickle red blood cells, suggesting the possibility of using these pro-apoptotic factors for hematopoietic stem cell transplants in patients.[36]

mRNA-based vaccines and therapies have the potential to play a crucial role in preventing and managing tumors by stimulating both innate and adaptive immune responses. The innate immune response contributes to tumor suppression by activating pattern recognition receptors, including Toll-like receptors (TLRs) such as TLR3 and TLR7/8.[37] They enhance specific immunity through antigen presentation on MHC molecules and cytokine release, thereby activating the adaptive immune response.[26] Cancer vaccines based on mRNA activate both the innate and adaptive immune systems, leading to a robust antitumor response in the body.

3. mRNA Vaccines for Cancer Treatment

mRNA vaccines offer a hopeful approach to treating different types of cancer. These vaccines, which carry instructions for making proteins linked to tumors or boosting the immune response against tumors, have been developed for many cancer types.[12, 38] In this article, we examined clinical trials focusing on mRNA vaccines and assessed their effectiveness against specific solid tumors, including those of the brain, melanoma, lung, breast, digestive system, kidney, ovarian, prostate, and blood systems through a search in the U.S. National Library of Medicine (ClinicalTrials.gov) (Table 1, Figure 1).

Table 1. Clinical Trials of mRNA therapeutic vaccine for variety cancers

No.	Cancers	NCT-Number	Phase	Status	mRNA therapeutic vaccine/ combination	Injection type
1	Brain	NCT02465268	II	Complete	CMV pp65 LAMP/Saline, Td, GM-CSF	s.c.
2		NCT03688178	II	Recruiting	CMV pp65 LAMP/Varlilumab	i.m.
3		NCT02366728	II	Complete	CMV pp65 LAMP/Basiliximab	i.m.
4		NCT04911621	I	Recruiting	mRNA loaded DC vaccine	i.v.
5		NCT04573140	I	Recruiting	CMV pp65 LAMP, Autologous total tumor mRNA	i.v.
6		NCT05938387	I	Not recruiting	mRNA vaccine	i.m.
7		NCT04534205	II	Recruiting	mRNA vaccine/Pembrolizumab	i.m.
8		NCT00846456	I/II	Complete	mRNA encoded glioblastoma antigens	i.d.
9		NCT00890032	I	Complete	Autologous brain tumor stem cell mRNA encoded antigens	i.d.
10		NCT00626483	I	Complete	CMV pp65 LAMP/Basiliximab, GM-CSF	i.p.
11		NCT02529072	I	Complete	CMV pp65 LAMP/Nivolumab	i.d.
12		NCT03615404	I	Complete	CMV pp65 LAMP, GM-CSF/Tetanus toxoid	i.v.
13		NCT04963413	I	Terminated	CMV pp65 LAMP, GM-CSF	i.v.
14		NCT03548571	II/III	Recruiting	Survivin, hTERT, Autologous tumor stem cell mRNA encoded antigens/Temozolmide	i.d.
15		NCT02649582	I/II	Not recruiting	WT1/Temozolmide	i.d.
16		NCT00639639	I	Complete	CMV pp65 LAMP/Tetanus-Diphtheria Toxoid	i.d.
17		NCT03927222	II	Terminated	CMV pp65 LAMP/Temozolomide, Tetanus-Diphtheria Toxoid, GM-CSF	i.d.
18		NCT04741984	I	Withdrawn	CMV pp65 LAMP	i.v.
19		NCT02808364	I	Complete	Personalized antigens	i.d.
20		NCT02709616	I	Complete	Personalized antigens/Temozolomid, Radiotherapy	i.d.
21		NCT01291420	I/II	Complete	WT1	i.d.

1	Melanoma	NCT01302496	II	Complete	TriMixDC/Ipilimumab	i.d
2		NCT02410733	I	Complete	MAGE-A3, TPTE, NY-ESO-1, Tyrosinase/Pembrolizumab	i.v.
3		NCT04526899	II	Not recruiting	MAGE-A3, TPTE, NY-ESO-1, Tyrosinase/Cemiplimab	i.v.
4		NCT03394937	I	Terminated	TAAs/Pembrolizumab	intranodal
5		NCT03289962	I/II	Not recruiting	Personalized antigens/Atezolizumab	i.v.
6		NCT03815058	II	Complete	mRNA cancer vaccine	i.v.
7		NCT03897881	II	Recruiting	mRNA-4157, Personalized antigens/Pembrolizmab	i.v.
8		NCT00204516	I/II	Complete	MAGE-A1, MAGE-A3, gp100, Melan-A, Tyrosinase, Survivin, Personalized antigens/GM-CSF	i.d.
9		NCT00204607	I/II	Complete	MAGE-A1, MAGE-A3, gp100, Melan-A, Tyrosinase, Survivin/GM-CSF	i.d.
10		NCT01278940	I/II	Complete	mRNA encoded antigens/IL-2	intranodal
11		NCT01530698	I/II	Complete	Tyrosinase, gp100	intranodal
12		NCT01676779	II	Complete	Unknown	i.v./i.d.
13		NCT00243529	I/II	Complete	Tyrosinase, gp100	i.v.
14		NCT01066390	I	Complete	MAGE-A3, MAGE-C2, Tyrosinase, gp100	i.v./i.d.
15		NCT00978913	I	Complete	hTERT, p53, Survivin/Cyclophosphamid	i.d.
16		NCT00940004	I/II	Complete	Tyrosinase, gp100	i.v./i.d.
17		NCT02285413	II	Complete	Tyrosinase, gp100/Cisplatinum	i.v./i.d.
18		NCT00672542	I	Complete	MAGE-3, Melan-A, Tyrosinase, gp100/Proteasome siRNA transfect DC	i.d.
19		NCT01684241	II	Complete	Tyrosinase, NY-ESO-1/Cemiplimab	intranodal
20		NCT03480152	I/II	Terminated	Autologous mRNA encoded antigens/ Pembrolizumab	i.m.
21		NCT03739931	I	Not recruiting	OX40L, IL-23, IL-36γ/Durvalumab	Intratumoral
22		NCT05933577	III	Not recruiting	mRNA-4157/Pembrolizumab	i.m.

23		NCT02410733	I	Complete	MAGE-A3, NY-ESO-1, TPTE, Tyrosinase/Pembrolizumab	i.v.
24		NCT05264974	I	Suspended	RNA-NP	i.v.
25		NCT00126685	I/II	Unknown	Autologous polymerase chain reaction amplified mRNA encoded antigens	i.v.
26		NCT00929019	I/II	Terminated	Tyrosinase, gp100	i.v./i.d.
1	Lung	NCT03948763	I	Complete	KARS, mRNA-5671-V941/Pembrolizumab	i.m.
2		NCT03164772	I/II	Complete	NY-ESO-1, MAGE-C1, MAGE-C2, 5T4, Survivin, Muclin-1/Durvalumab, Tremelimumab	i.d.
3		NCT03908671	Not applocable	Recruiting	Personalized antigens	s.c.
1	Breast	NCT03788083	I	Unknown	TriMix/Placebo	Intratumoral
2		NCT00978913	I	Complete	hTERT, p53, Survivin/Cyclophosphamide	i.d.
3		NCT03739931	I	Not recruiting	OX40L, IL-23, IL-36γ/Durvalumb	Intratumoral
4		NCT01291420	I/II	Complete	WT1	i.d.
5		NCT00003432	I/II	Terminated	CEA	i.v.
1		NCT03948763	I	Complete	KARS, mRNA-5671-V941/Pembrolizumab	i.m.
2		NCT03480152	I/II	Complete	Personalized antigens	i.m.
3		NCT03908671	Not applicable	Recruiting	Personalized antigens	s.c.
4		NCT06026800	I	Recruiting	mRNA encoded antigens/Adjuvant therapy	i.v.
5		NCT06026774	I	Recruiting	Personalized antigens (iNeo-Vac-R01)	
6		NCT06019702	I	Recruiting	mRNA encoded antigens	i.m.
7		NCT05192460	Not applicable	Recruiting	mRNA vaccine	i.m.

8		NCT05227378	I	Not recruiting	mRNA encoded antigens	i.v.
9		NCT05456165	II	Withdrawn	saRNA vaccine/checkpoint blocked	i.v.
10		NCT05141721	II/III	Not recruiting	vaccAde therapeutic vaccine using a saRNA	i.m.
11		NCT04486378	II	Recruiting	Personalized antigens	i.v.
12		NCT03289962	II	Not recruiting	Autologous tumor mRNA encoded antigens/ Atezolizumab	i.v.
13	Digestive system	NCT05761717	Not applicable	Not recruiting	Personalized antigens	s.c.
14		NCT05738447	I	Unknown	mRNA vaccine	i.m.
15		NCT05981066	Not applicable	Recruiting	mRNA encoded antigens	i.m.
16		NCT00228189	I/II	Complete	CEA	i.v.
17		NCT03431311	I/II	Terminated	TGF-β receptor type 2	i.v.
18		NCT03468244	Not applicable	Unknown	Personalized antigens	s.c.
19		NCT06156267	I	Not recruiting	Personalized antigens/Adebrelimab	i.v.
20		NCT06326736	I	Recruiting	mRNA encoded antigens	i.m.
21		NCT04161755	I	Not recruiting	mRNA encoded antigens/Atezolizumab	i.m.
1		NCT00272649	I/II	Complete	Autologous DC vaccine AGS-003/Sunitinib	i.d.
2	Kidney	NCT00678119	I/II	Complete	Autologous DC vaccine AGS-003/Sunitinib	i.d.
3		NCT01582672	III	Terminated	Autologous DC vaccine AGS-003/Sunitinib	i.d.
1		NCT01456065	I	Unknown	hTERT/Survivin	-
2		NCT04163094	I	Terminated	3 OC TAAs/Carboplatin, Paclitaxel, Surgery	i.v.
3	Ovarian	NCT01334047	I/II	Terminated	Stem cell mRNA encoded antigens, hTERT, Survivin	-
4		NCT03323398	I/II	Terminated	OX40L/Durvalumab	Intratumoral

1	Prostate	NCT00010127	I	Terminated	Autologous tumor mRNA encoded antigens	i.d.
2		NCT00906243	II	Terminated	CV9103	i.d.
3		NCT01817738	I/II	Terminated	CV9104 (CV9103,PAP and mucin-1)/ Andergoing androgen suppression, Surgery	i.d.
4		NCT02140138	II	Terminated	STEP, PSA, PSCA, PSMA, PAP, mucin-1	i.d.
5		NCT04382898	I/II	Terminated	BNT112 mRNA, RBLs (038,039,040,041 and 045)/Cemiplimab	i.v.
6		NCT01153113	I/II	Withdrawn	hTERT	i.d.
7		NCT01278914	I/II	Complete	Unknown	s.c.
8		NCT02452307	I/II	Complete	Unknown	s.c.
9		NCT00006430	I	Unknown	Autologous tumor mRNA encoded antigens	i.v./i.d.
10		NCT04382898	I/II	Terminated	BNT112 mRNA	i.v.
11		NCT01446731	II	Complete	hTERT, Survivin, PAP, PSA/Docetaxel	i.d.
12		NCT00004211	I/II	Complete	PSA	i.v.
1	Blood	NCT03739931	I	Not recruiting	OX40L, IL-23, IL-36γ/Durvalumab	Intratumoral
2		NCT00965224	II	Complete	WT1	i.d.
3		NCT01686334	I/II	Not recruiting	WT1	i.d.
4		NCT05169489	I/II	Not recruiting	bbT369; CAR Tcells	i.v.
5		NCT00834002	I	Complete	WT1	i.d.
6		NCT01734304	I/II	Complete	WT1, CMV pp65 LAMP, PRAME	i.d.
7		NCT00514189	I	Terminated	mRNA encoded antigens	intranodal
8		NCT02315118	I/II	Unknown	CD16, 41bb, CD3zeta/Rituximab	i.p.
9		NCT03323398	I/II	Terminated	OX40L/Durvalumab	Intratumoral
10		NCT01995708	I	Complete	WT1, CT7, MAGE-A3/ Autologous stem cell transplantation	i.d.

3.1. mRNA Vaccines for Brain Cancer

Globally, 1–2% of all cancers are brain tumors, with glioma and its subtype, glioblastoma, being the most frequent types.[39, 40] Among early mRNA-based approaches, dendritic cell (DC)-pulsed tumor mRNA vaccines have been extensively investigated in malignant glioma.[41] Early clinical studies have demonstrated encouraging but variable outcomes. For instance, treatment with autologous tumor-loaded DC mRNA was associated with complete tumor resection in a limited number of patients; however, the small sample size and lack of control groups limit the generalizability of these findings.[42] Similarly, enhanced $CD8^+$ cytotoxic T cell responses have been consistently observed following DC-based mRNA vaccination, although the magnitude of cytotoxic activity varied among patients, suggesting heterogeneity in immune responsiveness.

A phase I/II clinical trial evaluating DCs pulsed with cancer stem cell-derived mRNA further supported the immunogenic potential of this strategy, yet robust clinical efficacy data, such as progression-free survival (PFS) and overall survival (OS), remain limited.[43] Ongoing phase II trials investigating vaccines such as CMV pp65-LAMP (NCT02465268, NCT03688178) and DC engraftment strategies (NCT02366728) are expected to provide more definitive insights into clinical benefit. In addition, early-phase trials of intravenously and intramuscularly administered mRNA vaccines have reported acceptable safety profiles and evidence of immune activation, with some studies noting tumor reduction; however, these findings are preliminary and require validation in larger cohorts (NCT04911621, NCT04573140, NCT05938387).

Notably, combination strategies have also been explored. For example, the mRNA vaccine BNT113 in combination with pembrolizumab in HPV16-positive head and neck squamous cell carcinoma demonstrated favorable tolerability and strong immune activation, although conclusive efficacy data are not yet available (NCT04534205).

Overall, while mRNA-based immunotherapies for glioblastoma show promising immunogenicity, their clinical efficacy remains modest and inconsistent. Key challenges include limited durability of responses, tumor heterogeneity, and the immunosuppressive tumor microenvironment, highlighting the need for improved trial design, combination strategies, and predictive biomarkers.

3.2. mRNA Vaccine for Melanoma

Melanocytes, the pigment-producing cells distributed in the skin and other tissues, can give rise to melanoma upon malignant transformation. Melanoma accounts for a significant proportion of skin cancer-related mortality, representing approximately 0.7% of global cancer deaths.[44] Compared to conventional therapies, mRNA-based vaccines have emerged as a promising immunotherapeutic approach due to their ability to induce tumor-specific and individualized immune responses.[45] Early clinical studies using dendritic cell (DC)-based mRNA vaccines encoding melanoma-associated antigens such as MAGE-A3, MAGE-A2, gp100, and tyrosinase demonstrated that patient-derived tumor RNA-loaded DCs can successfully induce antigen-specific T cell responses.[46, 47] However, these studies were generally limited by small sample sizes and heterogeneity in clinical endpoints. Similarly, combination strategies such as TriMixDC with ipilimumab (NCT01302496) showed enhanced $CD4^+$ and $CD8^+$ T cell activation and increased IFN-γ-producing T cells, suggesting improved immunogenicity, although clear survival benefits remain insufficiently defined.

Key players in the mRNA vaccine development sector include Moderna, BioNTech, and GenenTech, which have reported on mRNA vaccines based on in vitro transcription targeting melanoma. In a phase I/II trial, a protamine-mRNA vaccine designed to encode tumor-associated antigens (TAAs) such as gp100, Melan-A, Tyrosinase, MAGE-A1, MAGE-A3, and survivin was tested on 21 patients with metastatic melanoma.[48] More recent approaches have focused on synthetic mRNA and personalized vaccine platforms. For example, BNT111 (Lipo-MERIT, NCT02410733), targeting multiple melanoma-associated antigens, induced measurable $CD4^+$ and $CD8^+$ T cell responses and demonstrated partial and complete metabolic responses in a subset of patients. Nevertheless, overall response rates remained modest, and not all patients exhibited durable clinical benefit. Similarly, protamine-mRNA vaccines and other early-phase formulations have shown immunogenic potential, but clinical efficacy has been inconsistent, with only a minority of patients achieving complete or partial responses.[49] Patient immune evaluations demonstrated that the BNT111 vaccine successfully elevated the levels of CD4+ and CD8+ T cells targeting antigens and led to durable specific cytotoxic T responses in some individuals (NCT02410733). The BNT111 vaccine was tested on 89 patients, including 42 with stage III/IV melanoma. PET/CT results indicated that three patients treated solely with BNT111 achieved a partial response, seven maintained stable disease, and one reached complete metabolic remission of metastatic sites. This vaccine was also assessed in a Phase II trial involving melanoma patients with stage III/IV who were treated with a PD-1 inhibitor. Clinical outcomes have demonstrated promising efficacy of personalized mRNA melanoma vaccines in phase II studies; however, these approaches have not yet received broad regulatory approval and remain under clinical investigation (NCT04526899).

A study examined the effectiveness of a therapeutic mRNA vaccine that included IFN-I alongside OVA and involved the maturation of DCs transformed with unmodified LNPs. The findings indicated an increase in intratumoral CD40+ DCs and a notable decrease in tumor growth. In 2017, ECI006, a therapeutic vaccine combining TriMix with mRNA that encodes TAA, began a Phase I trial to test its safety and effectiveness (NCT03394937). The therapeutic mRNA vaccines being developed for melanoma, which focus on TAA and central tolerance, offer a promising strategy for treating this disease.

Incorporating personalized lipid-encapsulated mRNA therapeutic vaccines along with either atezolizumab or pembrolizumab from BioNTech and GenenTech has moved into Phase I and II trials. The outcomes of this personalized therapeutic vaccine may open new possibilities for melanoma treatment (NCT03289962 and NCT03815058). Importantly, the most promising outcomes have been observed in personalized mRNA vaccine strategies combined with immune checkpoint inhibitors. In the KEYNOTE-942 trial, mRNA-4157 (V940) combined with pembrolizumab significantly reduced recurrence risk compared to pembrolizumab alone, representing one of the strongest signals of clinical benefit to date (NCT03897881). Despite these encouraging findings, most mRNA vaccine platforms for melanoma remain in early- or mid-phase clinical development and have not yet achieved broad regulatory approval.

Overall, mRNA therapeutic vaccines for melanoma consistently demonstrate strong immunogenicity and acceptable safety profiles; however, clinical outcomes are heterogeneous, with variable response rates and limited long-term survival data. Key challenges include patient-to-patient variability, tumor immune evasion, and optimization of antigen selection. Future progress is likely to depend on improved personalization strategies and rational combination with immune checkpoint inhibitors.

3.3. mRNA Vaccine for Lung Cancer

Lung cancer is the second leading cause of cancer-related deaths globally, accounting for approximately 18–20% of all cancer-related mortality, underscoring the urgent need for more effective therapeutic strategies.[1] In this context, mRNA therapeutic vaccines have emerged as a promising immunotherapeutic approach, particularly for non-small cell lung cancer (NSCLC), although clinical outcomes remain largely preliminary.

Early clinical evaluation of KRAS-targeting mRNA vaccines, such as mRNA-5671/V94, demonstrated that approximately half of treated patients developed measurable immune responses against tumor-associated neoantigens; however, these immune responses did not consistently translate into robust clinical efficacy (NCT03948763). Similarly, multi-epitope vaccines such as BI 1361849, encoding antigens including MUC1, survivin, NY-ESO-1, and MAGE family proteins, were generally well tolerated and capable of inducing antigen-specific immune activation in Phase I/II trials, but clinical endpoints such as progression-free survival (PFS) and overall survival (OS) remain insufficiently characterized (NCT03164772).

Other early-phase studies evaluating mRNA vaccines in NSCLC and related malignancies have reported acceptable safety profiles and limited disease stabilization, yet the overall anti-tumor efficacy has been modest and inconclusive (NCT03908671).[50] Likewise, platforms such as CV9201 and CV9202 have shown immunogenic potential, and combination strategies with immune checkpoint inhibitors (e.g., durvalumab and tremelimumab) have been explored to enhance therapeutic response (NCT03164772). However, clinical benefit remains variable, and definitive evidence of survival improvement is still lacking.

Overall, while mRNA vaccine strategies for lung cancer consistently demonstrate safety and immunogenicity, their clinical efficacy remains limited and heterogeneous. Key challenges include insufficient tumor regression, variability in patient immune responsiveness, and the immunosuppressive tumor microenvironment. These limitations suggest that future progress will likely depend on improved antigen selection, optimized delivery platforms, and rational combination with immunotherapy.

3.4. mRNA vaccine for breast cancer

Breast cancer is the most commonly diagnosed cancer among women globally, accounting for approximately 24.5% of all new cancer cases in women.[1, 2] Early detection significantly improves treatment outcomes and survival rates.[12] Clinically, breast cancer is classified into ER+, HER2+, and triple-negative breast cancer (TNBC), with TNBC representing the most aggressive subtype and associated with the poorest prognosis due to the lack of effective endocrine or targeted therapies.[51, 52]

In this context, mRNA therapeutic vaccines have emerged as a potential immunotherapeutic strategy, particularly for TNBC and high-risk breast cancer. Early development efforts, such as the FixVac platform by BioNTech AG, combined shared tumor-associated antigens (e.g., MUC1, HER2, CEA, WT1) with selected neoantigen targets to enhance immunogenicity. Although early clinical evaluation reported an overall response rate of approximately 33% in TNBC (NCT02316457), these results should be interpreted cautiously given the limited patient numbers and early-phase trial design.[49]

Other strategies, such as the intratumoral TriMix mRNA vaccine encoding CD40L, CD70, and constitutively active TLR4 (caTLR4), demonstrated strong local immune activation and sustained tumor-infiltrating immune responses for up to 30 days (NCT03788083). However, these effects were primarily immunological, and robust

long-term clinical outcome data remain limited. Similarly, dendritic cell-based HER2 mRNA vaccine approaches, including GM-CSF-adjuvanted formulations, have shown enhanced antigen-specific immune responses and reduced recurrence in high-risk patients, although confirmatory large-scale survival data are still lacking (NCT00978913).

Overall, while mRNA-based vaccine strategies in breast cancer consistently demonstrate the ability to induce tumor-specific immune activation, clinical efficacy remains variable and not yet fully established. Particularly in TNBC, durable survival benefits and standardized response endpoints are still insufficiently demonstrated. Current limitations include small cohort sizes, heterogeneity in vaccine platforms, and incomplete understanding of predictive immune biomarkers. Therefore, further well-designed randomized trials are required to validate their clinical utility.

3.5. mRNA vaccines used for gastrointestinal cancers

Gastrointestinal cancers, including tumors of the esophagus, stomach, pancreas, liver, and colon, remain among the leading causes of cancer-related mortality worldwide.[53, 54] In recent years, mRNA-based therapeutic cancer vaccines have emerged as a promising immunotherapeutic strategy for these malignancies. Collectively, early-phase studies suggest that these vaccines are capable of inducing measurable immune activation, such as enhanced IFN-γ secretion or the expansion of antigen-specific T-cell populations, indicating that they can successfully engage both innate and adaptive immunity.[55-57]

The therapeutic mRNA vaccine 4650 was scrutinized for its effectiveness against gastrointestinal and liver cancers.[58] A phase I trial tested the therapeutic mRNA vaccine 4157 containing tumor neoantigens via intramuscular injections in colorectal cancer patients, resulting in both humoral and cellular immune responses being activated.[59] Another phase I trial examined the therapeutic mRNA vaccine that included tumor antigens from the lung and colorectal cancers, successfully prompting immune responses against lung tumors (NCT03948763).

However, a critical limitation becomes apparent when these immunological signals are translated into clinical outcomes. Although phase I and I/II trials frequently demonstrate vaccine-induced immune responses, including humoral and cellular activation, these effects do not consistently correspond to objective tumor regression or meaningful improvements in patient survival (e.g., NCT03480152). This disconnect highlights a central challenge in cancer vaccine development: immune activation alone is not sufficient to overcome the immunosuppressive tumor microenvironment characteristic of gastrointestinal malignancies.

Numerous types of gastrointestinal cancers have been studied concerning the effectiveness of mRNA therapeutic vaccines. For instance, a phase I clinical trial of the personalized neoantigen mRNA vaccine, iNeo-Vac-R01, assessed its safety and effectiveness in patients with gastrointestinal neoplasms (NCT06026800, NCT06026774, and NCT06019702). A clinical study assessed a therapeutic mRNA vaccine featuring tumor-specific antigens, both with and without PD-1/L1, for patients suffering from gastric cancer (NCT05192460). Additionally, another phase I clinical study looked into a therapeutic mRNA1L vaccine (NCT05227378). Furthermore, a phase II study (NCT05456165) and a phase II/III study (NCT05141721) were carried out to examine the effectiveness of the vaccAde therapeutic vaccine utilizing saRNA for individuals with colon and colorectal cancers.[60] Another phase II trial aimed to evaluate the effectiveness of a personalized mRNA vaccine therapy for colorectal cancer patients (NCT04486378). An additional phase II study (NCT03289962) was based on the phase 1a/1b trial of an

autologous mRNA vaccine used alone with atezolizumab. This trial indicated that a T-cell immune response was triggered and confirmed the vaccine's safety.[61, 62]

Moreover, the biological heterogeneity of these cancers, particularly hepatocellular carcinoma (HCC) and colorectal tumors, further complicates vaccine design. Personalized neoantigen approaches and multi-antigen constructs have shown feasibility in eliciting targeted immune responses, and in some preclinical models even tumor growth delay (e.g., NCT05761717). Nevertheless, the clinical evidence remains preliminary, and the durability and therapeutic magnitude of these responses are still uncertain.

A phase I trial evaluating mRNA vaccines for advanced HBV-associated HCC yielded positive results, revealing that long-lasting immune responses were triggered against HBV-related HCC (NCT05738447). Another trial (NCT05981066) focused on an mRNA vaccine featuring a tumor neoantigen in HCC patients, aiming to assess its safety and effectiveness, especially in those who did not respond well to other treatments.[63]

Overall, while mRNA therapeutic vaccines represent a significant conceptual and technological advancement in the treatment of gastrointestinal cancers, current evidence primarily supports their immunogenic potential rather than definitive clinical efficacy. Future progress will likely depend on improving antigen selection, overcoming tumor-mediated immune suppression, and integrating vaccines with complementary immunotherapies to achieve sustained anti-tumor responses.

3.6. mRNA Vaccine for Kidney and Bladder Cancer

Kidney and bladder cancers account for approximately 5% of all cancer cases worldwide,[64] and their management has traditionally relied on a combination of surgery, cystectomy, and immune checkpoint inhibitors (CPIs).[65] Despite these established approaches, long-term control of advanced disease remains challenging, which has driven interest in alternative strategies such as mRNA-based therapeutic cancer vaccines targeting tumor-associated antigens.[66]

From a critical standpoint, early clinical and translational studies in renal cell carcinoma suggest that mRNA or RNA-based vaccination strategies can indeed induce measurable immune activation. For instance, approaches combining synthetic CD40L RNA with amplified tumor RNA and dendritic cell (DC) platforms have demonstrated the capacity to stimulate anti-tumor immunity. Similarly, the AGS-003 vaccine, evaluated in combination with sunitinib in a phase II trial, showed that a subset of patients achieved unusually prolonged survival, with some remaining disease-free for several years.[67] These outcomes indicate that, in selected cases, immune priming via DC-based RNA strategies can translate into meaningful clinical benefit.

However, the overall trajectory of these interventions also reveals important limitations. Despite promising early signals, several related vaccine programs were discontinued in early-phase trials (e.g., NCT00272649, NCT00678119, NCT01582672), primarily due to insufficient clinical efficacy. This lack of sustained success appears to stem not from the concept of RNA vaccination itself, but from deeper design and translational issues, including weak immunogenicity of selected antigens, absence of robust biomarker-driven patient stratification, and limited integration with strategies capable of overcoming tumor-mediated immunosuppression.

More broadly, multi-antigen mRNA vaccine approaches targeting molecules such as MUC1, CEA, Her2/neu, telomerase, survivin, and MAGE-A1 have shown signals of long-term survival benefit in subsets of patients.[68, 69]

Nevertheless, these outcomes remain heterogeneous and difficult to generalize, underscoring that response is highly patient-specific rather than consistently reproducible across populations.

Overall, the current body of evidence suggests that mRNA therapeutic vaccines for kidney and bladder cancers are still at an exploratory stage. While they demonstrate biological plausibility and occasional durable responses, their clinical effectiveness remains inconsistent. This highlights a critical gap between immunogenic potential and therapeutic reliability, emphasizing the need for improved antigen selection, better patient stratification, and rational combination with other immunomodulatory therapies.

3.7. mRNA Vaccine for Ovarian Cancer

Ovarian cancer remains one of the most lethal malignancies affecting women, which has made the search for effective therapeutic vaccines an important but still unresolved clinical goal. Early interest in mRNA-based approaches has largely been driven by their ability to encode tumor-associated antigens such as folate receptor-α (FR-α) and activate dendritic cell mediated immune responses. However, the actual clinical evidence supporting these strategies remains extremely limited and uneven. The most notable early observation comes from a 2004 dendritic cell based mRNA approach targeting FR-α, which was associated with a reported reduction in tumor burden in a single metastatic ovarian cancer patient. Although this result appeared encouraging, it is fundamentally constrained by its case-report nature, meaning it cannot be interpreted as evidence of efficacy. Its value is therefore primarily hypothesis-generating rather than clinically confirmatory, as such isolated responses may reflect individual biological variability rather than a reproducible therapeutic effect.[70]

More recent clinical efforts have attempted to move beyond single-antigen strategies by using multi-target constructs, such as TERT-mRNA and survivin-loaded dendritic cells, as well as liposome-encapsulated mRNA vaccines combined with chemotherapy or adjuvants. However, most of these studies remain in early-phase trials (e.g., NCT01456065, NCT04163094, NCT01334047), and critically, their results are either unpublished or not yet available. This lack of accessible outcome data significantly limits any meaningful assessment of their therapeutic value.

From an interpretive perspective, this pattern highlights a recurring issue in ovarian cancer vaccine development: despite increasing molecular sophistication in vaccine design, clinical translation has not yet occurred. The field is still largely characterized by biological feasibility studies rather than demonstrated clinical efficacy. In particular, challenges such as tumor heterogeneity, immune evasion, and the absence of validated predictive biomarkers likely contribute to the difficulty in achieving consistent therapeutic responses.

Overall, while mRNA therapeutic vaccines represent a promising conceptual strategy for ovarian cancer, current evidence remains preliminary and fragmented. The field is still in an early exploratory phase, and substantial further clinical investigation will be required before any conclusions about efficacy can be drawn.

3.8. mRNA Vaccine for Prostate Cancer

Prostate cancer represents a major global health burden for men, ranking as the second most frequently diagnosed cancer in this population and accounting for an estimated 400,000 deaths annually, with projections suggesting this burden could double by 2040 if more effective therapies are not developed.[71] In this context, mRNA therapeutic vaccines have emerged as an attractive immunotherapeutic strategy; however, their development in prostate cancer reveals a clear gap between strong immunological activity and inconsistent clinical benefit.

Preclinical studies provide initial proof-of-concept that mRNA-based vaccination can induce robust anti-tumor immunity. For example, lipid- or adjuvant-enhanced mRNA constructs targeting prostate cancer models have demonstrated significant tumor growth inhibition in mice, alongside activation of adaptive immune responses.[72] While these findings support biological feasibility, their translational value remains limited, as murine tumor models do not fully capture the immunosuppressive complexity and heterogeneity of human prostate cancer.

Early clinical translation began with vaccines such as CV9103, which incorporated multiple tumor-associated antigens (PSA, PSMA, PSCA, STEAP).[73] Although early-phase studies reported induction of antigen-specific immune responses in a substantial proportion of patients and stabilization of PSA levels in some cases, these outcomes were not consistently associated with objective clinical improvement or survival benefit (NCT00906243). Moreover, dose-escalation studies lacked publicly available results (NCT00831467), limiting the ability to assess reproducibility and optimal dosing strategies.

Subsequent vaccine iterations, such as CV9104, expanded antigen coverage to include PAP and MUC1 in an attempt to enhance immunogenicity. Despite this rational redesign, later clinical evaluation in metastatic castration-resistant prostate cancer (mCRPC) failed to demonstrate meaningful clinical benefit (NCT02140138). This outcome highlights a recurring limitation in prostate cancer vaccine development: increasing antigenic breadth alone does not necessarily overcome immune tolerance or the strongly suppressive tumor microenvironment characteristic of this disease.[73]

More recently, next-generation platforms such as RNA-LPX vaccines (e.g., BNT112) have shown improved immunological performance, induced strong and measurable immune responses while maintained a favorable safety profile in early-phase trials (NCT04382898). Similarly, personalized and dendritic cell-based mRNA vaccine strategies have repeatedly demonstrated the ability to activate tumor-specific T-cell responses across multiple studies. However, these immunological signals have not yet been reliably translated into controlled evidence of clinical superiority over standard treatments, and in some studies no clear advantage over chemotherapy or control conditions was observed.

Even in longer follow-up studies reporting prolonged biochemical remission in subsets of patients, the absence of control arms makes it difficult to attribute outcomes directly to vaccine efficacy. Such designs limit causal interpretation and prevent definitive conclusions regarding therapeutic benefit (e.g., NCT01197625). This underscores a broader methodological issue in the field: immune response biomarkers are often used as primary indicators of success, despite their imperfect correlation with long-term clinical outcomes (NCT01278914, NCT02452307, NCT00006430, and NCT01197625).

A study involving an mRNA vaccine containing the hTERT gene, used alongside DC and docetaxel in patients with mCRPC, indicated that while this combination was safe, it did not yield better safety results compared to chemotherapy alone (NCT01446731).

Overall, the accumulated evidence suggests that mRNA therapeutic vaccines in prostate cancer are highly capable of inducing immune activation but remain insufficiently validated in terms of durable clinical efficacy. The main barriers appear to lie not in vaccine immunogenicity per se, but in tumor immune evasion, antigen selection, and lack of integration with synergistic immunotherapies.[74] Consequently, while the approach remains scientifically promising, its clinical impact is still unproven and requires more rigorously controlled, biomarker-driven trials.

3.9. mRNA vaccine for blood system cancer

Blood cancers, including myeloma, leukemia, and lymphoma, encompass a heterogeneous group of hematopoietic malignancies characterized by the uncontrolled proliferation of abnormal blood or bone marrow derived cells.[1, 75] Despite advances in targeted therapies, these diseases remain clinically challenging due to their biological diversity and capacity for immune evasion. Consequently, immunotherapeutic approaches such as mRNA vaccines, dendritic cell (DC)-based vaccines, and CAR T cell therapies have gained increasing attention as potential treatment modalities.[76]

From a critical perspective, early studies suggest that mRNA-based vaccination strategies can successfully induce measurable anti-tumor immune responses in hematologic malignancies. For instance, DC-pulsed mRNA vaccines targeting AML-associated antigens such as WT1 and PRAME, alongside viral antigen CMV pp65, have demonstrated the ability to elicit antigen-specific cellular immunity in a subset of patients. Notably, some individuals experienced prolonged relapse-free survival, indicating that immune activation may translate into clinically meaningful outcomes in selected cases.[77] However, the small sample size and patient heterogeneity limit the generalizability of these findings, making it difficult to define the true magnitude of therapeutic benefit.

Similarly, preclinical studies in lymphoma models have shown that mRNA vaccines can induce robust CD4+ and CD8+ T-cell responses and suppress tumor growth in mice.[78] While these results provide strong mechanistic support, they remain constrained by the inherent limitations of animal models, which do not fully replicate the immune complexity and disease evolution seen in human hematologic cancers.

More advanced clinical approaches, such as mRNA-2752 delivered via nanoparticles and combined with checkpoint blockade, have further demonstrated that systemic immune activation including expansion of tumor-specific CD8+ T cells and cytokine release (e.g., IFN-γ) is achievable even in heavily pretreated patients (NCT03739931). Nevertheless, these responses primarily reflect immunological activity rather than confirmed long-term clinical benefit.

In contrast, WT1 mRNA/DC-based vaccines have shown more encouraging clinical signals in leukemia, with reports of molecular remission and extended relapse-free survival in a subset of patients over several years (NCT00965224). However, even in these studies, the durability of response appears restricted to a limited proportion of patients, underscoring substantial inter-individual variability. Moreover, the lack of consistently replicated results across trials and the absence of standardized treatment protocols complicate interpretation of efficacy.

Building on earlier research, another trial aimed to assess the safety and effectiveness of the WT1 mRNA vaccine administered intradermally with DCs, focusing on how the method of delivery influenced WT1-adaptive immune responses and relapse prevention (NCT01686334).

Emerging combination strategies, including mRNA vaccines evaluated alongside CAR T cell therapies in refractory B-cell lymphomas, further highlight the field's shift toward multimodal immunotherapy approaches (NCT05169489). While these strategies are conceptually promising, most remain in early-phase evaluation, and robust comparative clinical evidence is still lacking.

Overall, the accumulated data suggest that mRNA therapeutic vaccines in blood cancers are biologically active and capable of inducing strong immune responses, particularly when delivered via dendritic cells or nanoparticle

systems. However, their clinical effectiveness remains variable and insufficiently validated across large, controlled studies. The central challenge moving forward is not merely immune activation, but achieving consistent translation of these responses into durable remission across diverse hematologic malignancies.

4. Novel Conceptual Frameworks and Future Perspectives

This section outlines emerging strategies in mRNA-based cancer immunotherapy, with a clear distinction between clinically supported approaches and hypothesis-driven conceptual frameworks. While several individual components such as mRNA vaccines, immune checkpoint inhibitors (ICIs), and adoptive cell therapies are supported by preclinical and/or clinical evidence, their integration into unified therapeutic platforms remains largely investigational.

4.1. Integration of mRNA Vaccines with Combination Immunotherapy

The integration of mRNA vaccines with other immunotherapeutic modalities represents a promising but still evolving strategy in oncology. Immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1, anti-CTLA-4) and CAR-T cell therapies have demonstrated clinical efficacy in specific settings, while mRNA vaccines are currently being evaluated in early-phase trials. However, the systematic combination of these modalities has not yet been fully validated in clinical practice. Here, we outline a conceptual combinatorial framework (Figure 2) intended to illustrate a potential therapeutic strategy:

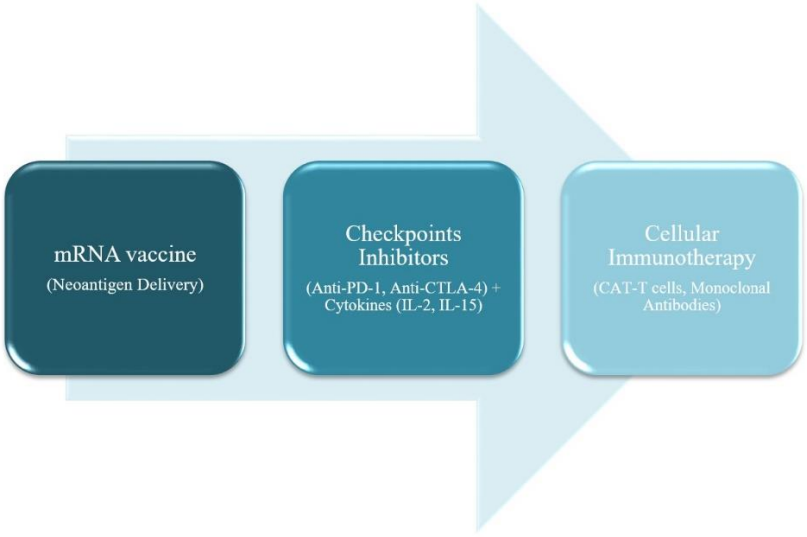

Figure 2. Integrated multi-layered immunotherapeutic framework combining mRNA vaccines with immune-modulating and effector strategies. This schematic illustrates a hierarchical, three-layered immunotherapeutic strategy for cancer treatment. **Layer 1** depicts the administration of mRNA-based vaccines encoding tumor-specific neoantigens, which are processed by antigen-presenting cells to initiate tumor-specific T-cell priming. **Layer 2** integrates immune checkpoint blockade (e.g., PD-1/PD-L1 or CTLA-4 inhibitors) and cytokine-based therapies, which enhance T-cell activation, proliferation, and functional persistence by reversing tumor-induced immunosuppression and modulating the tumor microenvironment. **Layer 3** represents adoptive and antibody-based effector strategies, including CAR-T cell therapy and monoclonal antibodies, which directly mediate tumor cell recognition and cytotoxic elimination. Collectively, this multi-layered framework highlights the synergistic potential of combining antigen priming, immune activation, and effector cell targeting to achieve a robust and sustained anti-tumor immune response.

1. **mRNA vaccine priming (evidence emerging):** induction of tumor-specific CD8+ and CD4+ T cell responses .[79]
2. **Checkpoint blockade (clinically established):** enhancement and maintenance of T cell activity through reversal of exhaustion .[80]
3. **Adoptive cell therapy (partially established):** CAR-T cells targeting tumor-associated antigens, primarily validated in hematologic malignancies.[81] Recent advances further highlight the potential for integrating CAR-T cell therapies with mRNA-based technologies, including transient CAR expression via mRNA electroporation and in vivo immune cell reprogramming strategies. Such approaches may improve safety, controllability, and expand CAR-T applicability beyond hematologic malignancies.[82, 83]

The term *"three-layered integration"* is used here to describe the coordinated or sequential application of complementary immunotherapies, rather than a validated clinical protocol. This approach aims to improve the breadth and durability of anti-tumor immune responses and reduce immune escape.

However, several challenges remain, including safety concerns (e.g., immune-related adverse events), optimal sequencing and dosing, and patient selection. Therefore, this framework should be considered hypothesis-generating, requiring validation in controlled clinical trials.

The concept of *"multi-axis immune reprogramming"* is used in this context to denote the simultaneous modulation of multiple immune pathways such as antigen presentation, T cell activation, and tumor targeting. While biologically plausible, this remains a theoretical construct rather than an established therapeutic paradigm.

4.2. Personalized mRNA Vaccine Algorithm Using scRNA-seq

Personalized antigen selection is a critical determinant of mRNA vaccine efficacy, particularly in the context of tumor heterogeneity. Advances in single-cell RNA sequencing (scRNA-seq) and computational biology have enabled more refined approaches to neoantigen identification.

We propose a bioinformatics-driven workflow, grounded in current technological capabilities:

1. Tumor biopsy acquisition.[84]
2. scRNA-seq profiling to resolve tumor heterogeneity and identify malignant subclones.[85]
3. Computational neoantigen prediction incorporating mutation burden, MHC-binding affinity, and clonality.[86] Recent advances in computational immunology have further improved neoantigen discovery pipelines by integrating deep learning-based MHC binding prediction models, immunopeptidomics datasets, and multi-omics data fusion strategies. These approaches enhance the prioritization of immunogenic and clonally relevant neoantigens, improving the likelihood of clinically effective antigen selection in personalized mRNA vaccine design.[87]
4. Antigen prioritization based on tumor specificity and expression patterns.[88]
5. mRNA construct design encoding multiple selected epitopes.[89]

Each of these steps is individually supported by preclinical and translational studies; however, their integration into fully automated clinical pipelines remains limited.

The term *"digital twin vaccines"* is used here in a conceptual sense, referring to computationally guided vaccine design frameworks that integrate multi-omic data to simulate and optimize patient-specific interventions. At present, such systems are not yet standardized or widely implemented in clinical oncology, and their predictive accuracy and scalability require further validation.

4.3. Self-Amplifying mRNA Vaccines (saRNA) versus Conventional mRNA

Self-amplifying mRNA (saRNA) represents an advanced evolution of conventional mRNA platforms, distinguished by the incorporation of a viral replicase complex that enables intracellular RNA amplification. Following cytoplasmic delivery, the replicase typically derived from alphaviruses drives the synthesis of negative-strand RNA intermediates, which subsequently serve as templates for the production of multiple positive-strand RNA copies, including subgenomic transcripts encoding the antigen of interest.[90] This process results in sustained and amplified antigen expression over time compared with non-replicating mRNA. This prolonged antigen availability has important immunological consequences. Extended expression can enhance dendritic cell activation and maturation, promote efficient antigen processing, and facilitate cross-presentation through MHC class I pathways,[91] thereby strengthening $CD8^+$ T-cell priming.[92] In parallel, persistent antigen exposure may also support $CD4^+$ T-cell help and germinal center formation, contributing to improved B-cell responses and antibody affinity maturation. However, the duration and magnitude of antigen expression must be tightly regulated, as excessive or prolonged exposure may contribute to T-cell exhaustion or tolerance in certain contexts.[93]

At the innate immune level, saRNA replication generates double-stranded RNA (dsRNA) intermediates that are sensed by pattern recognition receptors such as RIG-I, MDA5, and endosomal Toll-like receptors (e.g., TLR3 and TLR7/8). Activation of these pathways induces type I interferon and pro-inflammatory cytokine production, shaping both the magnitude and quality of the adaptive immune response. This intrinsic adjuvant effect can be advantageous, reducing or eliminating the need for external adjuvants. However, excessive interferon signaling can inhibit cap-dependent translation, activate RNA degradation pathways, and ultimately reduce antigen yield. Therefore, fine-tuning innate immune activation through sequence engineering, nucleoside modification, or optimized formulation remains a central challenge in saRNA design.

From a structural and biophysical perspective, the relatively large size of saRNA constructs (~9–12 kb) introduces additional complexities. Larger RNA molecules are generally more susceptible to degradation and may exhibit reduced encapsulation efficiency in lipid nanoparticles (LNPs) or other delivery systems. This necessitates careful optimization of formulation parameters, including lipid composition, particle size, and charge, to ensure efficient cellular uptake, endosomal escape, and cytoplasmic release. Emerging delivery strategies, such as next-generation ionizable lipids, polymer-based carriers, and targeted delivery approaches, aim to improve tissue specificity and reduce off-target effects.

Manufacturing considerations also distinguish saRNA from conventional mRNA. Although saRNA can achieve comparable or superior protein expression at significantly lower doses potentially reducing raw material requirements and cost of goods its longer sequence length and structural complexity may affect in vitro transcription efficiency, purification yield, and product stability. Additionally, controlling the formation of dsRNA byproducts during manufacturing is critical, as these contaminants can exacerbate unwanted innate immune activation.

In the context of oncology, saRNA platforms are being explored for encoding tumor-associated antigens, neoantigens, and immunomodulatory factors such as cytokines or costimulatory molecules. Their ability to sustain antigen expression may be particularly advantageous for overcoming weak immunogenicity in "cold" tumors. Moreover, saRNA vaccines may be combined with immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 therapies, to enhance T-cell infiltration and function within the tumor microenvironment. Nevertheless, tumor-induced immunosuppression, antigen heterogeneity, and variability in patient immune status remain significant barriers to clinical translation.

Despite promising preclinical results particularly in infectious disease models clinical data for saRNA in cancer remain limited. Key translational challenges include balancing immunogenicity with tolerability, optimizing delivery to relevant immune cell populations, and ensuring consistent manufacturing at scale. Future directions may involve hybrid platforms that integrate features of conventional and self-amplifying mRNA, incorporation of regulatory elements to modulate replication kinetics, and the use of personalized antigen designs to improve specificity and efficacy.

Overall, saRNA technologies offer a compelling and mechanistically rich platform for next-generation cancer immunotherapies. However, their successful clinical application will depend on continued advances in RNA engineering, delivery systems, and a deeper understanding of RNA immune system interactions,[94-96] (Table 2).

Table 2: saRNA challenges

No.	Feature	Conventional mRNA	Self-Amplifying mRNA (saRNA)
1	RNA Length	~1–2 kb	~9–12 kb (due to replicase genes)
2	Dose Required	High (50–100 µg)	Low (0.1–1 µg) due to intracellular amplification
3	Antigen Expression	Transient, short-lived	Prolonged and amplified via RNA self-replication
4	Mechanism of Action	Direct cytoplasmic translation	Replicase-driven RNA amplification and subgenomic transcription
5	Manufacturing	Easier, scalable, well-established	More complex (longer transcripts, dsRNA byproducts), less standardized
6	Immune Response	Strong but relatively short-lived	More robust and durable, with intrinsic self-adjuvanting properties
7	Innate Immune Activation	Moderate, can be controlled (e.g., nucleoside modification)	Stronger due to dsRNA intermediates; risk of overactivation
8	Interferon Response	Moderate and tunable	Elevated; can enhance immunity but may suppress translation
9	Risk of Translation Inhibition	Low	Higher (e.g., via interferon signaling and PKR activation)

10	Antigen Presentation	Limited duration	Enhanced cross-presentation (MHC class I) and improved $CD8^+$ T-cell priming
11	Delivery Complexity	Relatively straightforward (standard LNPs)	More challenging; requires advanced delivery systems for larger RNA
12	Stability	Higher molecular stability	Lower stability due to larger size and structural complexity
13	Scalability	Proven at industrial scale	Potentially scalable, but still technically challenging
14	Clinical Maturity	Clinically validated (e.g., approved vaccines)	Mostly preclinical or early clinical stage, especially in oncology
15	Suitability for Cancer	Effective but may require boosting	Promising for "cold" tumors; still considered experimental
16	Design Flexibility	High	High, but constrained by replicase architecture
17	Safety Profile	Well-characterized	Requires further evaluation (e.g., inflammation, reactogenicity)

5. Next-Generation mRNA Vaccines in Oncology

The next era of advances in mRNA-based cancer immunotherapy, bioinformatics-based antigen discovery, and nanotechnology-based drug delivery systems are emerging.[97] These innovations could overcome current limitations such as antigen selection, tumor targeting, and drug delivery efficiency.

5.1. Nanomaterials for Advanced mRNA Delivery

Although lipid nanoparticles (LNPs) have had promising success, they have limitations in immunogenicity, biodistribution, and tumor penetration. New-generation drug delivery platforms are currently being developed to address these challenges:

- **Exosome-based drug delivery systems:** Exosomes are naturally occurring secretory vesicles that can carry RNA, proteins, and lipids. Engineered tumor-targeting exosomes encapsulate mRNA and evade the immune system, and can be biocompatible carriers. Their endogenous homing to specific tissues, such as tumors, makes them superior to synthetic lipids. Exosome-mRNA vaccines can provide simultaneous drug delivery of immune-modulating molecules, providing a dual effect of antigen presentation and immunomodulation.[98]
- **Polymeric nanoparticles (PNPs):** Biodegradable polymers, such as poly(β-amino esters), polylactic-co-glycolic acid (PLGA), and dendrimeric scaffolds, offer tunable delivery properties. PNPs allow for controlled release kinetics, sustained antigen expression, and tumor-specific targeting. PNPs exhibit

increased stability, reduced toxicity, and the ability to incorporate ligands for receptor-mediated uptake compared to conventional LNPs.[99]

- **Hybrid nanoplatforms:** Combining polymers, lipids, and biomimetic coatings (like cancer cell membranes) yields multifunctional nanoparticles. These hybrids can evade the immune system, deliver mRNA with spatiotemporal precision to target tissue, and penetrate the tumor microenvironment.[100]

These hybrids represent a second-generation drug delivery paradigm that aims to achieve precise tumor penetration while minimizing systemic side effects.

5.2. Bioinformatics-Driven Antigen Discovery

Identifying tumor-specific antigens remains a major challenge in mRNA vaccine development. Bioinformatics and AI technologies are central to this process:

- **Neoantigen prediction pipelines:** Integration of genomic and transcriptomic data with machine learning-based MHC-binding models.[101]
- **Single-cell omics integration:** scRNA-seq combined with TCR profiling to capture tumor heterogeneity and immune escape dynamics.[102]
- **Immunopeptidomics validation:** Deep learning models integrating multi-omic and clinical datasets to refine antigen selection.[103]
- **AI-enhanced prioritization:** Deep learning models can integrate genomic, proteomic, and clinical outcome data to rank candidate antigens, ultimately leading to the design of multi-epitope, patient-tailored mRNA vaccines.[102]

To minimize redundancy, algorithmic implementation details are presented in Section 4.2, while clinical applications (e.g., patient stratification) are briefly addressed in Section 6.3.

5.3. Outlook

The convergence of nanotechnology and computational immunology is expected to create a new generation of mRNA-based vaccines that are personalized, efficient, and widely available. Exosome- and polymer-based drug delivery systems can improve biodistribution and minimize toxicity and expand tumor targeting, while AI-based antigen discovery increases precision and adaptability to tumor evolution. The foundation for next-generation oncology vaccines are these innovations that could transform cancer treatment from reactive management to precision-based, proactive immune prevention.

6. Discussion

The mRNA vaccines summarized in Table 1 have shown promising clinical trial results in oncology, but they also have significant limitations that must be addressed before these therapies become standard clinical options. Key themes emerge from the current evidence:

6.1. Critical Analysis of Clinical Trial Outcomes

Most clinical trials of mRNA cancer vaccines, most of which are early-stage (Phase I/II) studies to date, have focused primarily on safety and immunogenicity rather than long-term survival. While many studies have demonstrated robust stimulation of CD8+ T cells and antigen-specific antibody responses, the translation of these immune responses into durable clinical benefits remains inconsistent. For example, trials in glioblastoma (NCT02465268, NCT03688178) and melanoma (NCT02410733, NCT03897881) demonstrated good immunogenicity but had modest improvements in overall survival or progression-free survival. This discrepancy highlights the challenge of overcoming the immunosuppressive tumor microenvironment (TME), which limits the efficacy of mRNA-based immunotherapies.

Another critical observation is the variability of vaccine efficacy across tumor types. Melanoma and lung cancer trials yielded relatively stronger immune responses compared to breast and ovarian cancers, suggesting that tumor mutational burden (TMB) and neoantigen diversity significantly influence vaccine effectiveness. Tumors with low TMB (e.g., prostate, ovarian) may inherently provide fewer immunogenic targets, necessitating alternative antigen discovery approaches or combination therapies.

In addition, variability in administration routes complicates comparisons between trials. The most common drug delivery methods are intramuscular (i.m.) and intradermal (i.d.) injections. However, studies have shown unique benefits, particularly in improving antigen delivery to the tumor microenvironment (TME), in both the intravenous (i.v.) and intratumoral (i.t.) routes. However, standardized protocols are needed to determine the optimal strategy.

6.2. Knowledge Gaps and Unresolved Challenges

Barriers to the clinical application of mRNA vaccines in oncology include:

1. **Durability of response**: Most trials report short-term immunogenicity endpoints, but long-term persistence of vaccine-induced T cells remains unclear.
2. **Patient stratification**: There is a lack of validated biomarkers (beyond PD-L1 expression) to identify which patients will benefit most from mRNA vaccination.
3. **Tumor microenvironment barriers**: Many solid tumors employ immune-evasive mechanisms (like Treg infiltration, myeloid-derived suppressor cells, checkpoint upregulation) that blunt vaccine efficacy.
4. **Safety of repeated dosing**: Although generally well tolerated, the impact of repeated mRNA vaccine administration on systemic inflammation and autoimmunity has not been thoroughly characterized.
5. **Standardization of manufacturing**: Personalized vaccines face logistical hurdles in scaling, quality control, and regulatory harmonization across different healthcare systems.

6.3. Recommendations for Next-Generation Clinical Trials

Future clinical trials should incorporate innovative and integrative strategies:

- **Adaptive trial designs**: Allow dynamic modification of study parameters based on interim analyses.[104, 105]
- **Combination strategies:** Building on the mechanistic framework described in Section 4.1, combining mRNA vaccines with checkpoint inhibitors, cytokines, or CAR-T cells may enhance efficacy.[102]

- **AI-driven patient stratification:** Use of machine learning models to identify responsive patient subgroups and guide neoantigen selection (see Sections 4.2 and 5.2).[106, 107]
- **Real-world evidence (RWE) integration:** Validation of trial outcomes in broader patient populations.
- **Biomarker-guided endpoints**: Incorporation of multi-omic biomarkers (e.g., ctDNA, immune repertoire, TME profiling) to better correlate immune and clinical responses.

Repetitive methodological descriptions have been removed, with emphasis placed on clinical translation.

6.4. Outlook

mRNA vaccines have demonstrated their potential to revolutionize cancer immunotherapy, but their full clinical value has yet to be realized. Moving from promising early-phase results to widespread therapeutic adoption depends on overcoming immunological barriers, refining patient selection, and optimizing trial design. The next generation of mRNA vaccine trials using adaptive, AI-driven, and biomarker-based clinical strategies could fill current knowledge gaps and pave the way for durable, personalized, and universally accessible cancer immunotherapies.

Specifically, we now integrate three interrelated determinants of response: tumor mutational burden (TMB), the tumor microenvironment (TME), and the efficiency of antigen presentation.

First, tumors with high TMB (e.g., melanoma and smoking-associated lung cancers) tend to generate a larger repertoire of neoantigens. This increases the probability that immunogenic peptides will be recognized by T cells, thereby enhancing responsiveness to immune checkpoint blockade. However, TMB alone is not sufficient to predict response, as not all mutations produce immunogenic or effectively presented neoantigens.

Second, the composition and functional state of the TME critically shape whether an antitumor immune response can be initiated and sustained. “Inflamed” (hot) tumors, characterized by $CD8^+$ T-cell infiltration, interferon-γ signaling, and the presence of antigen-presenting cells, are more likely to respond to checkpoint inhibitors. In contrast, “cold” or immunosuppressed tumors often exhibit exclusion of T cells or enrichment of suppressive populations such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2-like macrophages, which collectively dampen effective immunity even in the presence of neoantigens.

Third, intact antigen processing and presentation machinery is a critical bottleneck. Defects in components such as MHC class I, β2-microglobulin, or TAP transporters can prevent tumor antigens from being displayed on the cell surface, enabling immune evasion. Thus, even tumors with high neoantigen load may fail to respond if antigen presentation is impaired.

Importantly, these factors are highly interdependent. High TMB does not guarantee response in the context of defective antigen presentation or an immunosuppressive TME. Conversely, therapeutic strategies that modulate the TME (e.g., radiotherapy, anti-angiogenic agents, or combination immunotherapies) may sensitize tumors with intermediate or low TMB. Therefore, response heterogeneity across cancer types is best understood as the result of alignment or misalignment among neoantigen generation, antigen presentation capacity, and immune contexture.

7. Comparative Insights and Resistance Mechanisms

7.1. Comparative Landscape of Cancer Vaccines

mRNA vaccines have emerged as a highly versatile platform in cancer immunotherapy;[108, 109] however, a meaningful evaluation requires comparison with DNA (110) and peptide-based vaccines at mechanistic, immunological, and translational levels.[108, 111]

At the molecular level, mRNA vaccines function in the cytoplasm and are directly translated into antigenic proteins, enabling efficient antigen processing through both MHC class I and class II pathways. This results in robust activation of $CD8^+$ cytotoxic T lymphocytes alongside $CD4^+$ helper responses. In contrast, DNA vaccines must enter the nucleus for transcription prior to antigen expression, making their efficacy dependent on nuclear delivery and cellular proliferation status, which can limit transfection efficiency in vivo. Peptide vaccines, by comparison, bypass intracellular antigen synthesis entirely and rely on exogenous loading onto MHC molecules, restricting their applicability to specific HLA types and often resulting in weaker and less durable immune responses.

From an immunological perspective, mRNA vaccines possess intrinsic adjuvant properties through activation of innate immune sensors such as Toll-like receptors (TLR7/8), leading to type I interferon responses and enhanced dendritic cell maturation. DNA vaccines generally exhibit weaker innate immune activation unless combined with delivery technologies such as electroporation. Peptide vaccines typically require strong external adjuvants to overcome their low immunogenicity.

In terms of safety, mRNA vaccines are non-integrating and transiently expressed, eliminating the risk of genomic insertional mutagenesis associated albeit theoretically with DNA vaccines. Peptide vaccines also have excellent safety profiles but are often limited by poor immunogenicity.

From a manufacturing and translational standpoint, mRNA vaccines offer rapid, cell-free, and highly scalable production, making them particularly suitable for personalized neoantigen approaches. DNA vaccines involve more complex plasmid production and purification processes, while peptide vaccines face logistical challenges in synthesis, especially for multi-epitope personalized formulations.

Regarding clinical performance, mRNA vaccines tend to induce strong effector T-cell responses but may face challenges in durability without boosting strategies. DNA vaccines may provide more sustained antigen expression, potentially contributing to longer-lasting immune stimulation. Peptide vaccines, although historically less effective, have demonstrated selective success in certain tumor types when combined with potent adjuvants or immune checkpoint inhibitors.

Importantly, each platform exhibits distinct vulnerabilities to tumor immune evasion. For instance, peptide vaccines are particularly susceptible to antigen loss variants and HLA downregulation, whereas mRNA and DNA vaccines may better adapt through multi-epitope encoding but remain affected by defects in antigen presentation pathways.

Tumor immune evasion mechanisms such as loss or downregulation of HLA class I molecules, defects in antigen processing and presentation (e.g., β2-microglobulin alterations), upregulation of immune checkpoint ligands such as PD-L1, and recruitment of immunosuppressive cellular components within the tumor microenvironment (including regulatory T cells and myeloid-derived suppressor cells) can collectively attenuate vaccine-induced T cell responses and limit therapeutic durability.[112, 113]

Importantly, this comparison extends beyond descriptive features and highlights how intrinsic platform biology shapes susceptibility to tumor immune evasion mechanisms (Table 3). These comparative features are summarized in Figure 3, which have been expanded to reflect mechanistic, immunological, and translational dimensions.

Table 3. Comparative features of mRNA, DNA, and peptide vaccines in cancer immunotherapy

No.	Feature	mRNA Vaccines	DNA Vaccines	Peptide Vaccines
1	Cellular localization	Cytoplasmic (no nuclear entry required)	Requires nuclear entry for transcription	Extracellular; direct MHC loading
2	Antigen expression mechanism	Direct translation into protein	Transcription → translation	No expression; synthetic peptides
3	Dependence on host cell cycle	No	Yes (enhanced in dividing cells)	No
4	Antigen presentation pathways	MHC I and II (efficient cross-presentation)	MHC I and II (less efficient)	Primarily MHC I ($CD8^+$ biased), limited $CD4^+$
5	$CD4^+$ / $CD8^+$ response balance	Strong $CD8^+$ + $CD4^+$	Moderate $CD8^+$ and $CD4^+$	Often $CD8^+$-restricted (HLA-dependent)
6	Innate immune activation	High (TLR7/8, RIG-I pathways)	Moderate (requires adjuvant/electroporation)	Low (requires strong adjuvants)
7	Intrinsic adjuvant effect	Yes	Limited	No
8	Genomic integration risk	None	Theoretical risk	None
9	Expression duration	Transient	Longer-lasting	Very short
10	Immunogenicity	High	Moderate	Low (unless optimized)
11	HLA restriction	Low (multi-epitope encoding possible)	Low	High
12	Manufacturing speed	Rapid, cell-free	Slower (plasmid production)	Moderate (peptide synthesis)

13	Scalability	High	Moderate	Limited for personalized vaccines
14	Personalization potential	Excellent (neoantigen design)	Good	Challenging (multi-peptide complexity)
15	Stability	Requires cold-chain (LNP-formulated)	Relatively stable	High chemical stability
16	Delivery challenges	LNP delivery systems needed	Efficient delivery (e.g., electroporation) needed	Minimal delivery complexity
17	Susceptibility to antigen loss	Moderate (multi-epitope mitigation possible)	Moderate	High
18	Sensitivity to MHC downregulation	Moderate	Moderate	High
19	TME susceptibility	High (immune suppression limits efficacy)	High	Very high
20	Clinical efficacy (overall trend)	Promising, strong T-cell responses	Variable, often modest	Limited as monotherapy
21	Durability of response	Moderate (boosting needed)	Potentially longer	Limited

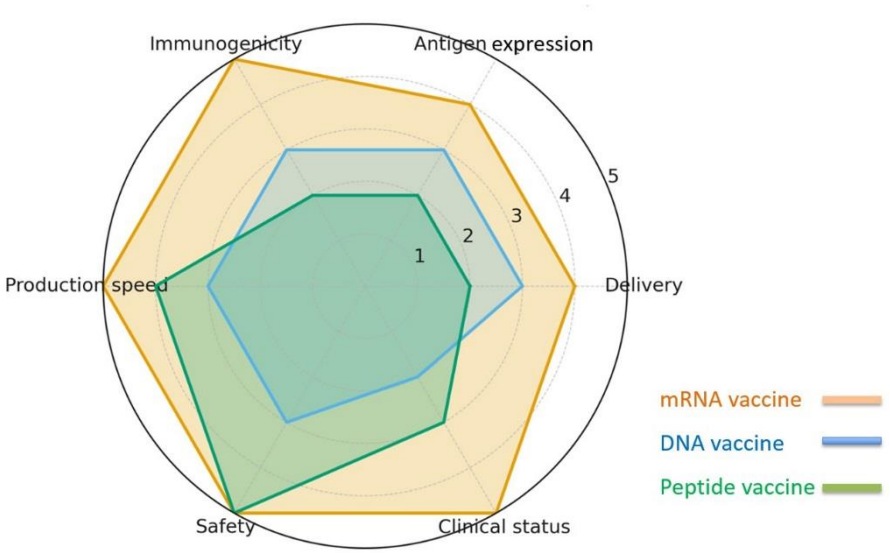

Figure 3. Comparative overview of vaccine platforms including mRNA, DNA, and peptide-based vaccines in cancer immunotherapy. This comparative chart summarizes key functional and translational characteristics of mRNA, DNA, and peptide vaccine platforms. mRNA vaccines demonstrate several advantages, including rapid manufacturing, high translational efficiency, strong immunogenicity, and a favorable safety profile due to their non-integrating, transient expression in the cytoplasm. DNA vaccines offer improved stability and ease of storage but require nuclear entry for transcription, which can limit efficiency. Peptide-based vaccines provide high safety and manufacturing simplicity but generally exhibit lower immunogenicity and limited MHC class I presentation without additional adjuvants. Despite these differences, each platform retains unique strengths and may be optimally applied depending on the clinical context, tumor type, and desired immune response.

7.2. Tumor Resistance Mechanisms Against mRNA Vaccines

Despite their advantages, mRNA vaccines are subject to both intrinsic and extrinsic tumor resistance mechanisms that limit therapeutic efficacy.

Intrinsic resistance arises from tumor cell associated alterations, including antigen loss, downregulation of antigen presentation machinery (e.g., MHC class I), and mutations in interferon gamma (IFNγ) signaling pathways. Additionally, activation of oncogenic pathways such as MAPK and WNT/β-catenin contributes to immune exclusion and reduced T-cell infiltration.[114, 115]

Extrinsic resistance is mediated by the tumor microenvironment (TME), which is often enriched with immunosuppressive cell populations such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2-polarized macrophages. These cells secrete inhibitory cytokines, including IL-10 and TGF-β, which dampen effector T-cell responses.[114, 116]

Another major limitation is **T-cell exhaustion**, driven by chronic antigen exposure and characterized by upregulation of inhibitory receptors such as PD-1, LAG-3, and TIM-3. This dysfunctional state significantly reduces cytotoxic activity and limits sustained anti-tumor immunity.[117]

To overcome these resistance mechanisms (Table 4), several strategies have been proposed:

- **Combination therapies:** Integrating mRNA vaccines with immune checkpoint inhibitors (ICIs), CAR-T cell therapy, or epigenetic modulators can enhance T-cell function and reverse immune suppression.
- **Tumor microenvironment modulation:** Targeting suppressive immune populations or cytokine signaling pathways can improve antigen presentation and immune infiltration.
- **Adaptive vaccine design:** Multi-epitope and neoantigen-based mRNA vaccines may reduce the risk of antigen escape and improve response durability.[116]

Collectively, these findings indicate that mRNA vaccines are unlikely to achieve optimal efficacy as monotherapies in most cancers. Instead, their future clinical success will depend on rational combination strategies and dynamic adaptation to tumor evolution.

Table 4. Mechanisms of tumor resistance against mRNA vaccines and potential solutions

No.	Resistance Mechanism	Description	Potential Solutions
1	Antigen loss/heterogeneity	Tumor cells downregulate or mutate vaccine-targeted antigens, leading to immune escape.	Use of multi-epitope vaccines, incorporation of neoantigen pools, adaptive antigen updating guided by scRNA-seq.
2	Immunosuppressive TME	High infiltration of Tregs, MDSCs, and secretion of TGF-	Combination with checkpoint inhibitors (PD-1/PD-L1, CTLA-

		β/IL-10 suppress effector T cell activity.	4 blockade), cytokine therapies (IL-15, IL-12).
3	Checkpoint upregulation	Tumor upregulates PD-L1 or other inhibitory ligands post-vaccine stimulation.	Concurrent use of immune checkpoint inhibitors; bispecific antibodies targeting PD-L1/TIM-3.
4	Metabolic reprogramming	Tumors exploit hypoxia, lactate accumulation, and nutrient competition to exhaust T cells.	Nanoparticle delivery with metabolic modulators (IDO inhibitors, lactate dehydrogenase blockers).
5	Antigen presentation defects	Downregulation of MHC class I/II molecules reduces tumor visibility to T cells.	Use of mRNA vaccines encoding cytokines (e.g., IFN-γ) or co-stimulatory ligands to enhance MHC expression.
6	Innate immune over-activation	Excessive type I IFN responses to LNPs can cause premature vaccine clearance and inflammation.	Engineering stealth nanoparticles (PEGylation, exosome-mimicking carriers) and optimized nucleoside modifications (pseudouridine).

8. Conclusion

mRNA vaccines have shown significant progress as a novel cancer therapeutic platform. Results from clinical trials have shown that they have the ability to induce strong and antigen-adaptive immune responses in a variety of tumor types, including brain, melanoma, lung, breast, gastrointestinal, kidney, ovarian, prostate, and hematological cancers. mRNA vaccines, encoding tumor- and patient-specific antigens, enable precise targeting and have shown favorable safety profiles compared to other conventional therapies.

However, fundamental challenges remain. The susceptibility to enzymatic degradation, and the complexity of maintaining balanced immune responses continue to limit clinical efficacy. Promising solutions such as lipid nanoparticle (LNP) formulations, chemical modifications of nucleosides, and the use of self-amplifying RNA (saRNA) platforms can help to stabilize mRNA, reduce dosage requirements, and improve therapeutic outcomes. Furthermore, combining mRNA vaccines with immune checkpoint inhibitors, adoptive T cell transfer, or CAR-T therapies may overcome tumor immune resistance and induce durable responses.

Careful optimization of drug delivery platforms, integration of biomarker-based patient stratification, and innovative trial designs that adapt to clinical data in real time will require further research in the future. Importantly, combining mRNA technology with next-generation tools such as single-cell sequencing, AI-guided antigen discovery, and nanomaterial-based carriers will accelerate the development of personalized, scalable, and universally accessible vaccines.

Ultimately, despite the aforementioned challenges, the rapid pace of innovation places mRNA vaccines at the forefront of next-generation cancer immunotherapy. With continued refinement and integration into multimodal treatment strategies, these vaccines have the potential to transform cancer care from conventional management to truly precise and durable immunotherapy solutions.

Author contributions

M.M.A.S wrote and reviewed the main manuscript.

Data availability

All data generated or analysed during the study are included in the submitted manuscript information files. There are no restrictions on data availability.

Declaration of interest statement

The author declares that she has no conflict of interest to declare in relation to this article.

Funding

There was no funding.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2024;74(3):229-63. DOI: 10.3322/caac.21834
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA: a cancer journal for clinicians. 2024;74(1). DOI: 10.3322/caac.21820
3. Kreier F. Cancer will cost the world $25 trillion over next 30 years. Nature Publishing Group; 2023. DOI: 10.1038/d41586-023-00883-6
4. Wyld L, Audisio RA, Poston GJ. The evolution of cancer surgery and future perspectives. Nature reviews Clinical oncology. 2015;12(2):115-24. DOI: 10.1038/nrclinonc.2014.191
5. Nuwer R. Advances in highly targeted radiation treatment for cancer have ignited interest in a once obscure field. Nature. 2024;629(8014):S7-S9. DOI: 10.1038/d41586-024-01466-2
6. Labrie M, Brugge JS, Mills GB, Zervantonakis IK. Therapy resistance: opportunities created by adaptive responses to targeted therapies in cancer. Nature Reviews Cancer. 2022;22(6):323-39. DOI: 10.1038/s41568-022-00474-0
7. Zhang P, Xiao Y, Sun X, Lin X, Koo S, Yaremenko AV, et al. Cancer nanomedicine toward clinical translation: Obstacles, opportunities, and future prospects. Med. 2023;4(3):147-67. DOI: 10.1016/j.medj.2023.02.007
8. Dolgin E. Covid vaccine Flop Spotlights Mrna Design Challenges. Nature. 2021;594:483-. DOI: 10.1038/d41586-021-01353-1
9. Hogan MJ, Pardi N. mRNA vaccines in the COVID-19 pandemic and beyond. Annual review of medicine. 2022;73:17-39. DOI: 10.1146/annurev-med-042420-112725
10. Huang X, Kong N, Zhang X, Cao Y, Langer R, Tao W. The landscape of mRNA nanomedicine. Nature Medicine. 2022;28(11):2273-87. DOI: 10.1038/s41591-022-02061-1
11. Parhiz H, Atochina-Vasserman EN, Weissman D. mRNA-based therapeutics: looking beyond COVID-19 vaccines. The Lancet. 2024;403(10432):1192-204. DOI: 10.1016/S0140-6736(24)00182-5

12. Liu C, Shi Q, Huang X, Koo S, Kong N, Tao W. mRNA-based cancer therapeutics. Nature Reviews Cancer. 2023;23(8):526-43. DOI: 10.1038/s41568-023-00583-5

13. Guan S, Rosenecker J. Nanotechnologies in delivery of mRNA therapeutics using nonviral vector-based delivery systems. Gene therapy. 2017;24(3):133-43. DOI: 10.1038/gt.2017.5

14. Rojas LA, Sethna Z, Soares KC, Olcese C, Pang N, Patterson E, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. Nature. 2023;618(7963):144-50. DOI: 10.1038/s41586-023-06063-y

15. Sayour EJ, Boczkowski D, Mitchell DA, Nair SK. Cancer mRNA vaccines: clinical advances and future opportunities. Nature Reviews Clinical Oncology. 2024;21(7):489-500. DOI: 10.1038/s41571-024-00874-5

16. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. Nature reviews Drug discovery. 2014;13(10):759-80. DOI: 10.1038/nrd4278

17. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nature Reviews Materials. 2021;6(12):1078-94. DOI: 10.1038/s41578-021-00358-0

18. Zhou F, Huang L, Li S, Yang W, Chen F, Cai Z, et al., editors. From structural design to delivery: mRNA therapeutics for cancer immunotherapy. Exploration; 2024: Wiley Online Library. DOI: 10.1002/EXP.20230189

19. Wang X, Liu S, Sun Y, Yu X, Lee SM, Cheng Q, et al. Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. Nature protocols. 2023;18(1):265-91. DOI: 10.1038/s41596-022-00790-5

20. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. Nature nanotechnology. 2020;15(4):313-20. DOI: 10.1038/s41565-020-0669-6

21. Chen J, Ye Z, Huang C, Qiu M, Song D, Li Y, et al. Lipid nanoparticle-mediated lymph node–targeting delivery of mRNA cancer vaccine elicits robust CD8+ T cell response. Proceedings of the National Academy of Sciences. 2022;119(34):e2207841119. DOI: 10.1073/pnas.2207841119

22. Xiao Y, Tang Z, Huang X, Chen W, Zhou J, Liu H, et al. Emerging mRNA technologies: delivery strategies and biomedical applications. Chemical Society Reviews. 2022;51(10):3828-45. DOI: 10.1039/D1CS00822A

23. Shi J, Huang M-W, Lu Z-D, Du X-J, Shen S, Xu C-F, et al. Delivery of mRNA for regulating functions of immune cells. Journal of Controlled Release. 2022;345:494-511. DOI: 10.1016/j.jconrel.2022.03.012

24. Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. Frontiers in immunology. 2019;10:594. DOI: 10.3389/fimmu.2019.00594

25. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. Nature Reviews Cancer. 2021;21(5):298-312. DOI: 10.1038/s41568-020-00339-z

26. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nature Reviews Immunology. 2020;20(1):7-24. DOI: 10.1038/s41577-019-0210-z

27. Huang H, Zhang C, Yang S, Xiao W, Zheng Q, Song X. The investigation of mRNA vaccines formulated in liposomes administrated in multiple routes against SARS-CoV-2. Journal of Controlled Release. 2021;335:449-56. DOI: 10.1016/j.jconrel.2021.04.024

28. Baharom F, Ramirez-Valdez RA, Khalilnezhad A, Khalilnezhad S, Dillon M, Hermans D, et al. Systemic vaccination induces CD8+ T cells and remodels the tumor microenvironment. Cell. 2022;185(23):4317-32. e15. DOI: 10.1016/j.cell.2022.10.014

29. Künzli M, O'Flanagan SD, LaRue M, Talukder P, Dileepan T, Stolley JM, et al. Route of self-amplifying mRNA vaccination modulates the establishment of pulmonary resident memory CD8 and CD4 T cells. Science immunology. 2022;7(78):eadd3075. DOI: 10.1016/j.jconrel.2021.12.004

30. Anderluzzi G, Lou G, Woods S, Schmidt ST, Gallorini S, Brazzoli M, et al. The role of nanoparticle format and route of administration on self-amplifying mRNA vaccine potency. Journal of Controlled Release. 2022;342:388-99. DOI: 10.1016/j.jconrel.2021.12.004

31. Perche F, Benvegnu T, Berchel M, Lebegue L, Pichon C, Jaffrès P-A, et al. 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):445-53. DOI: 10.1016/j.nano.2011.01.007

32. Kim M, Jeong M, Hur S, Cho Y, Park J, Jung H, et al. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. Science advances. 2021;7(9):eabf4398. DOI: 10.1126/sciadv.abf4398

33. Dilliard SA, Cheng Q, Siegwart DJ. On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. Proceedings of the National Academy of Sciences. 2021;118(52):e2109256118. DOI: 10.1073/pnas.2109256118

34. Veiga N, Goldsmith M, Granot Y, Rosenblum D, Dammes N, Kedmi R, et al. Cell specific delivery of modified mRNA expressing therapeutic proteins to leukocytes. Nature communications. 2018;9(1):4493. DOI: 10.1038/s41467-018-06947-7

35. Rosenblum D, Gutkin A, Kedmi R, Ramishetti S, Veiga N, Jacobi AM, et al. CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Science advances. 2020;6(47):eabc9450. DOI: 10.1126/sciadv.abc9450

36. Breda L, Papp TE, Triebwasser MP, Yadegari A, Fedorky MT, Tanaka N, et al. In vivo hematopoietic stem cell modification by mRNA delivery. Science. 2023;381(6656):436-43. DOI: 10.1126/science.adh3480

37. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. Nature reviews Drug discovery. 2018;17(4):261-79. DOI: 10.1038/nrd.2017.243

38. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Molecular cancer. 2021;20(1):41. DOI: 10.1186/s12943-021-01335-5

39. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-49. DOI: 10.3322/caac.21660

40. McNeill KA. Epidemiology of brain tumors. Neurologic clinics. 2016;34(4):981-98. DOI: 10.1016/j.ncl.2016.06.014

41. Melnick K, Dastmalchi F, Mitchell D, Rahman M, Sayour EJ. Contemporary RNA therapeutics for glioblastoma. NeuroMolecular Medicine. 2022;24(1):8-12. DOI: 10.1007/s12017-021-08682-6

42. Kobayashi T, Yamanaka R, Homma J, Tsuchiya N, Yajima N, Yoshida S, et al. Tumor mRNA–loaded dendritic cells elicit tumor-specific CD8+ cytotoxic T cells in patients with malignant glioma. Cancer Immunology, Immunotherapy. 2003;52(10):632-7. DOI: 10.1007/s00262-003-0406-7

43. Vik-Mo EO, Nyakas M, Mikkelsen BV, Moe MC, Due-Tønnesen P, Suso EMI, et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunology, Immunotherapy. 2013;62(9):1499-509. DOI: 10.1007/s00262-013-1453-3

44. Schadendorf D, Van Akkooi AC, Berking C, Griewank KG, Gutzmer R, Hauschild A, et al. Melanoma. The Lancet. 2018;392(10151):971-84. DOI: 10.1016/S0140-6736(18)31559-9

45. Robertson BM, Fane ME, Weeraratna AT, Rebecca VW. Determinants of resistance and response to melanoma therapy. Nature Cancer. 2024;5(7):964-82. DOI: 10.1038/s43018-024-00783-6

46. Bidram M, Zhao Y, Shebardina NG, Baldin AV, Bazhin AV, Ganjalikhany MR, et al. mRNA-based cancer vaccines: a therapeutic strategy for the treatment of melanoma patients. Vaccines. 2021;9(10):1060. DOI: 10.3390/vaccines9101060

47. Aarntzen EH, Schreibelt G, Bol K, Lesterhuis WJ, Croockewit AJ, De Wilt JH, et al. 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):5460-70. DOI: 10.1158/1078-0432.CCR-11-3368

48. Weide B, Pascolo S, Scheel B, Derhovanessian E, Pflugfelder A, Eigentler TK, et al. 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. DOI: 10.1097/CJI.0b013e3181a00068

49. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature. 2020;585(7823):107-12. DOI: 10.1038/s41586-020-2537-9

50. Khan P, Siddiqui JA, Lakshmanan I, Ganti AK, Salgia R, Jain M, et al. RNA-based therapies: A cog in the wheel of lung cancer defense. Molecular cancer. 2021;20(1):54. DOI: 10.1186/s12943-021-01338-2

51. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, et al. Recent advances in therapeutic strategies for triple-negative breast cancer. Journal of hematology & oncology. 2022;15(1):121. DOI: 10.1186/s13045-022-01341-0

52. Bonotto M, Gerratana L, Poletto E, Driol P, Giangreco M, Russo S, et al. Measures of outcome in metastatic breast cancer: insights from a real-world scenario. The oncologist. 2014;19(6):608-15. DOI: 10.1634/theoncologist.2014-0002

53. Islam F, Mitra S, Emran TB, Khan Z, Nath N, Das R, et al. Natural small molecules in gastrointestinal tract and associated cancers: molecular insights and targeted therapies. Molecules. 2022;27(17):5686. DOI: 10.3390/molecules27175686

54. Cao W, Qin K, Li F, Chen W. Comparative study of cancer profiles between 2020 and 2022 using global cancer statistics (GLOBOCAN). Journal of the National Cancer Center. 2024;4(2):128-34. DOI: 10.1016/j.jncc.2024.01.001

55. Forghanifard MM, Gholamin M, Moaven O, Farshchian M, Ghahraman M, Aledavood A, et al. Neoantigen in esophageal squamous cell carcinoma for dendritic cell-based cancer vaccine development. Medical oncology. 2014;31(10):191. DOI: 10.1007/s12032-014-0191-7

56. Gholamin M, Moaven O, Farshchian M, Mahmoudi M, Sankian M, Memar B, et al. Induction of cytotoxic T lymphocytes primed with tumor RNA-loaded dendritic cells in esophageal squamous cell carcinoma: preliminary step for DC vaccine design. BMC cancer. 2010;10(1):261. DOI: 10.1186/1471-2407-10-261

57. Cafri G, Gartner JJ, Zaks T, Hopson K, Levin N, Paria BC, et al. mRNA vaccine–induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. The Journal of clinical investigation. 2020;130(11):5976-88. DOI: 10.1172/JCI134248

58. Cafri G, Gartner JJ, Hopson K, Meehan RS, Zaks TZ, Robbins P, et al. Immunogenicity and tolerability of personalized mRNA vaccine mRNA-4650 encoding defined neoantigens expressed by the autologous cancer. American Society of Clinical Oncology; 2019. DOI: 10.1200/JCO.2019.37.15_suppl.2514

59. Burris HA, Patel MR, Cho DC, Clarke JM, Gutierrez M, Zaks TZ, 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. American Society of Clinical Oncology; 2019. DOI: 10.1200/JCO.2019.37.15_suppl.2523

60. Hecht JR, Shergill A, Goldstein MG, Fang B, Cho MT, Lenz H-J, et al. Phase 2/3, randomized, open-label study of an individualized neoantigen vaccine (self-amplifying mRNA and adenoviral vectors) plus immune checkpoint blockade as maintenance for patients with newly diagnosed metastatic colorectal cancer (GRANITE). American Society of Clinical Oncology; 2022. DOI: 10.1200/JCO.2022.40.4_suppl.TPS186

61. Kopetz S, Morris VK, Alonso-Orduna V, Garcia-Alfonso P, Reboredo M, Fernandez Montes A, et al. A phase 2 multicenter, open-label, randomized, controlled trial in patients with stage II/III colorectal cancer who are ctDNA positive following resection to compare efficacy of autogene cevumeran versus watchful waiting. American Society of Clinical Oncology; 2022. DOI: 10.1200/JCO.2022.40.4_suppl.TPS261

62. Lopez JS, Camidge R, Iafolla M, Rottey S, Schuler M, Hellmann 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-CT. DOI: 10.1158/1538-7445.AM2020-CT301

63. Xing L, Wang Z-K, Li D-M, Li J, Liu M. RNA-based therapies in hepatocellular carcinoma: state of the art and clinical perspectives. Hepatoma Research. 2024;10:N/A-N/A. DOI: 10.20517/2394-5079.2023.115

64. Scélo G, Brennan P. The epidemiology of bladder and kidney cancer. Nature clinical practice urology. 2007;4(4):205-17. DOI: 10.1038/ncpuro0721

65. Tran L, Xiao J-F, Agarwal N, Duex JE, Theodorescu D. Advances in bladder cancer biology and therapy. Nature Reviews Cancer. 2021;21(2):104-21. DOI: 10.1038/s41568-020-00313-9

66. Xu H, Zheng X, Zhang S, Yi X, Zhang T, Wei Q, et al. Tumor antigens and immune subtypes guided mRNA vaccine development for kidney renal clear cell carcinoma. Molecular cancer. 2021;20(1):159. DOI: 10.1186/s12943-021-01461-0

67. Amin A, Dudek AZ, Logan TF, Lance RS, Holzbeierlein JM, Knox JJ, et al. Survival with AGS-003, an autologous dendritic cell–based immunotherapy, in combination with sunitinib in unfavorable risk patients with advanced renal cell carcinoma (RCC): phase 2 study results. Journal for immunotherapy of cancer. 2015;3(1):14. DOI: 10.1186/s40425-015-0055-3

68. Rittig SM, Haentschel M, Weimer KJ, Heine A, Müller MR, Brugger W, et al. Long-term survival correlates with immunological responses in renal cell carcinoma patients treated with mRNA-based immunotherapy. Oncoimmunology. 2016;5(5):e1108511. DOI: 10.1080/2162402X.2015.1108511

69. Rittig SM, Haentschel M, Weimer KJ, Heine A, Muller MR, Brugger W, et al. Intradermal vaccinations with RNA coding for TAA generate CD8+ and CD4+ immune responses and induce clinical benefit in vaccinated patients. Molecular Therapy. 2011;19(5):990-9. DOI: 10.1038/mt.2010.289

70. Hernando JJ, Park T-W, Fischer H-P, Zivanovic O, Braun M, Pölcher M, et al. Vaccination with dendritic cells transfected with mRNA-encoded folate-receptor-α for relapsed metastatic ovarian cancer. The lancet oncology. 2007;8(5):451-4. DOI: 10.1016/S1470-2045(07)70109-8

71. Rastogi I, Muralidhar A, McNeel DG. Vaccines as treatments for prostate cancer. Nature Reviews Urology. 2023;20(9):544-59. DOI: 10.1038/s41585-023-00780-9

72. Islam MA, Rice J, Reesor E, Zope H, Tao W, Lim M, et al. Adjuvant-pulsed mRNA vaccine nanoparticle for immunoprophylactic and therapeutic tumor suppression in mice. Biomaterials. 2021;266:120431. DOI: 10.1016/j.biomaterials.2020.120431

73. Rausch S, Schwentner C, Stenzl A, Bedke J. mRNA vaccine CV9103 and CV9104 for the treatment of prostate cancer. Human vaccines & immunotherapeutics. 2014;10(11):3146-52. DOI: 10.4161/hv.29882

74. Tryggestad AM, Axcrona K, Axcrona U, Bigalke I, Brennhovd B, Inderberg EM, et al. Long-term first-in-man phase I/II study of an adjuvant dendritic cell vaccine in patients with high-risk prostate cancer after radical prostatectomy. The Prostate. 2022;82(2):245-53. DOI: 10.1002/pros.24257

75. Bowman RL, Busque L, Levine RL. Clonal hematopoiesis and evolution to hematopoietic malignancies. Cell stem cell. 2018;22(2):157-70. DOI: 10.1016/j.stem.2018.01.011

76. González-Herrero I, Rodríguez-Hernández G, Luengas-Martínez A, Isidro-Hernández M, Jiménez R, García-Cenador MB, et al. The making of leukemia. International journal of molecular sciences. 2018;19(5):1494. DOI: 10.3390/ijms19051494

77. Lichtenegger FS, Schnorfeil FM, Rothe M, Deiser K, Altmann T, Bücklein VL, et al. Toll-like receptor 7/8-matured RNA-transduced dendritic cells as post-remission therapy in acute myeloid leukaemia: results of a phase I trial. Clinical & Translational Immunology. 2020;9(3):e1117. DOI: 10.1002/cti2.1117

78. Tusup M, Läuchli S, Jarzebska NT, French LE, Chang Y-T, Vonow-Eisenring M, et al. mRNA-based anti-TCR CDR3 tumour vaccine for T-cell lymphoma. Pharmaceutics. 2021;13(7):1040. DOI: 10.3390/pharmaceutics13071040

79. Gao Y, Yang L, Li Z, Peng X, Li H. mRNA vaccines in tumor targeted therapy: Mechanism, clinical application, and development trends. Biomarker research. 2024;12(1):93. DOI: 10.1186/s40364-024-00735-0

80. Li Y-R, Lyu Z, Shen X, Fang Y, Yang L. Boosting CAR-T cell therapy through vaccine synergy. Trends in Pharmacological Sciences. 2025;46(2):180-99. DOI: 10.1016/j.tips.2024.11.003

81. Hushmandi K, Imani Fooladi AA, Reiter RJ, Farahani N, Liang L, Aref AR, et al. Next-generation immunotherapeutic approaches for blood cancers: Exploring the efficacy of CAR-T and cancer vaccines. Experimental Hematology & Oncology. 2025;14(1):75. DOI: 10.1186/s40164-025-00639-0

82. Wang T, Jin F, Xing W, Yu S. Non-Viral and mRNA-Based Strategies for Next-Generation CAR-T Cell Therapy. Pathology-Research and Practice. 2026:156455. DOI: 10.1016/j.prp.2025.156455

83. Kitte R, Rabel M, Geczy R, Park S, Fricke S, Koehl U, et al. Lipid nanoparticles outperform electroporation in mRNA-based CAR T cell engineering. Molecular Therapy Methods & Clinical Development. 2023;31. DOI: 10.1016/j.omtm.2023.09.009

84. Noé A, Cargill TN, Nielsen CM, Russell AJ, Barnes E. The application of single-cell RNA sequencing in vaccinology. Journal of Immunology Research. 2020;2020(1):8624963. DOI: 10.1155/2020/8624963

85. Sahin U, Derhovanessian E, Miller M, Kloke B-P, Simon P, Löwer M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222-6. DOI: 10.1038/nature23003

86. Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. Nature Reviews Immunology. 2018;18(3):168-82. DOI: 10.1038/nri.2017.131

87. Guarra F, Colombo G. Computational methods in immunology and vaccinology: design and development of antibodies and immunogens. Journal of Chemical Theory and Computation. 2023;19(16):5315-33. DOI: 10.1021/acs.jctc.3c00591

88. Vieth B, Parekh S, Ziegenhain C, Enard W, Hellmann I. A systematic evaluation of single cell RNA-seq analysis pipelines. Nature communications. 2019;10(1):4667. DOI: 10.1038/s41467-019-12266-7

89. Alburquerque-González B, López-Abellán MD, Luengo-Gil G, Montoro-García S, Conesa-Zamora P. Design of personalized neoantigen RNA vaccines against cancer based on next-generation sequencing data. Pharmacogenomics in Drug Discovery and Development: Springer; 2022. p. 165-85. DOI: 10.1007/978-1-0716-2208-7_9

90. Zhou Y, Wei Y, Tian X, Wei X. Cancer vaccines: current status and future directions. Journal of Hematology & Oncology. 2025;18(1):18. DOI: 10.1186/s13045-025-01641-0

91. Huang S, Que H, Wang M, Wei X. mRNA vaccines as cancer therapies. Chinese Medical Journal. 2024;137(24):2979-95. DOI: 10.1097/CM9.0000000000003291

92. Wilbrink MF, Herfst S, de Vries RD. Steps towards licensure of self-amplifying RNA vaccines. The Lancet Infectious Diseases. 2025;25(3):247-8. DOI: 10.1016/S1473-3099(24)00779-8

93. Blakney AK, Ip S, Geall AJ. An update on self-amplifying mRNA vaccine development. Vaccines. 2021;9(2):97. DOI: 10.3390/vaccines9020097

94. Wei Z, Zhang S, Wang X, Xue Y, Dang S, Zhai J. Technological breakthroughs and advancements in the application of mRNA vaccines: a comprehensive exploration and future prospects. Frontiers in Immunology. 2025;16:1524317. DOI: 10.3389/fimmu.2025.1524317

95. Casmil IC, Jin J, Won E-J, Huang C, Liao S, Cha-Molstad H, et al. The advent of clinical self-amplifying RNA vaccines. Molecular Therapy. 2025;33(6):2565-82. DOI: 10.1016/j.ymthe.2025.03.021

96. Blakney A. The next generation of RNA vaccines: self-amplifying RNA. The Biochemist. 2021;43(4):14-7. DOI: 10.1042/BIO20210040

97. Ho W, Gao M, Li F, Li Z, Zhang XQ, Xu X. Next-generation vaccines: nanoparticle-mediated DNA and mRNA delivery. Advanced healthcare materials. 2021;10(8):2001812. DOI: 10.1002/adhm.202001812

98. Iqbal Z, Rehman K, Mahmood A, Shabbir M, Liang Y, Duan L, et al. Exosome for mRNA delivery: strategies and therapeutic applications. Journal of Nanobiotechnology. 2024;22(1):395. DOI: 10.1186/s12951-024-02632-6

99. Lin Y, Chen X, Wang K, Liang L, Zhang H. An overview of nanoparticle-based delivery platforms for mRNA vaccines for treating cancer. Vaccines. 2024;12(7):727. DOI: 10.3390/vaccines12070727

100. Jiang Z, Xu Y, Du G, Sun X. Emerging advances in delivery systems for mRNA cancer vaccines. Journal of Controlled Release. 2024;370:287-301. DOI: 10.1016/j.jconrel.2024.04.028

101. Biswas N, Chakrabarti S, Padul V, Jones LD, Ashili S. Designing neoantigen cancer vaccines, trials, and outcomes. Frontiers in immunology. 2023;14:1105420. DOI: 10.3389/fimmu.2023.1105420

102. Ni L. Advances in mRNA-based cancer vaccines. Vaccines. 2023;11(10):1599. DOI: 10.3390/vaccines11101599

103. Floudas CS, Sarkizova S, Ceccarelli M, Zheng W. Leveraging mRNA technology for antigen based immuno-oncology therapies. Journal for Immunotherapy of Cancer. 2025;13(1):e010569. DOI: 10.1136/jitc-2024-010569

104. Jiménez JL, Zheng H. A Bayesian adaptive design for dual-agent phase I–II oncology trials integrating efficacy data across stages. Biometrical Journal. 2023;65(7):2200288. DOI: 10.1002/bimj.202200288

105. Alexander B, Berry S, Viele K, Buxton M, Paoloni M, Lewis R, et al. Author Correction: Adaptive platform trials: definition, design, conduct and reporting considerations (Nature Reviews Drug Discovery,(2019), 18, 10,(797-807), 10.1038/s41573-019-0034-3). Nature Reviews Drug Discovery. 2019;18(10):808-. DOI: 10.1038/s41573-019-0042-3

106. Kong H. Advances in personalized Cancer vaccine development: AI applications from neoantigen discovery to mRNA formulation. BioChem. 2025;5(2):5. DOI: 10.3390/biochem5020005

107. Elfatimi E, Lekbach Y, Prakash S, BenMohamed L. Artificial intelligence and machine learning in the development of vaccines and immunotherapeutics—yesterday, today, and tomorrow. Frontiers in Artificial Intelligence. 2025;8:1620572. DOI: 10.3389/frai.2025.1620572

108. Wang B, Pei J, Xu S, Liu J, Yu J. Recent advances in mRNA cancer vaccines: meeting challenges and embracing opportunities. Frontiers in immunology. 2023;14:1246682. DOI: 10.3389/fimmu.2023.1246682

109. Beck JD, Reidenbach D, Salomon N, Sahin U, Türeci Ö, Vormehr M, et al. mRNA therapeutics in cancer immunotherapy. Molecular cancer. 2021;20(1):69. DOI: 10.1186/s12943-021-01377-9

110. Jahanafrooz Z, Baradaran B, Mosafer J, Hashemzaei M, Rezaei T, Mokhtarzadeh A, et al. Comparison of DNA and mRNA vaccines against cancer. Drug discovery today. 2020;25(3):552-60. DOI: 10.1016/j.drudis.2019.10.003

111. Nguyen CM, Vu TT, Nguyen MN, Tran-Nguyen T-S, Huynh CT, Ha QT, et al. Neoantigen-based mRNA vaccine exhibits superior anti-tumor activity compared to synthetic long peptides in an in vivo lung carcinoma model. Cancer Immunology, Immunotherapy. 2025;74(4):1-17. DOI: 10.1007/s00262-024-03975-4

112. Sari G, Rock KL. Tumor immune evasion through loss of MHC class-I antigen presentation. Current opinion in immunology. 2023;83:102329. DOI: 10.1016/j.coi.2023.102329

113. Kawazu M, Ueno T, Saeki K, Sax N, Togashi Y, Kanaseki T, et al. HLA class I analysis provides insight into the genetic and epigenetic background of immune evasion in colorectal cancer with high microsatellite instability. Gastroenterology. 2022;162(3):799-812. DOI: 10.1053/j.gastro.2021.11.041

114. Katopodi T, Petanidis S, Grigoriadou E, Anestakis D, Charalampidis C, Chatziprodromidou I, et al. Immune specific and tumor-dependent mRNA vaccines for cancer immunotherapy: reprogramming clinical translation into tumor editing therapy. Pharmaceutics. 2024;16(4):455. DOI: 10.3390/pharmaceutics16040455

115. Vu SH, Vetrivel P, Kim J, Lee M-S. Cancer resistance to immunotherapy: molecular mechanisms and tackling strategies. International journal of molecular sciences. 2022;23(18):10906. DOI: 10.3390/ijms231810906

116. Giri S, Lamichhane G, Pandey J, Khadayat R, KC S, Devkota HP, et al. Immune Modulation and Immunotherapy in Solid Tumors: Mechanisms of Resistance and Potential Therapeutic Strategies. International Journal of Molecular Sciences. 2025;26(7):2923. DOI: 10.3390/ijms26072923

117. Anderson KG, Braun DA, Buqué A, Gitto SB, Guerriero JL, Horton B, et al. Leveraging immune resistance archetypes in solid cancer to inform next-generation anticancer therapies. Journal for immunotherapy of cancer. 2023;11(6):e006533. DOI: 10.1136/jitc-2023-006533