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RNA Nanotechnology for Reversal of Melanoma Immunotherapy Resistance

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ABSTRACT

Purpose: Cancer immunotherapy works by targeting immune and cancer cell checkpoints to induce long-term anti-tumor responses, showing effectiveness in inhibiting tumor growth and preventing recurrence. However, clinical trials have also reported side effects, as well as resistance in some patients with melanoma, prompting the integration of nanotechnology with immunotherapy to improve outcomes. RNA nanotechnology has emerged as a transformative approach in cancer immunotherapy, particularly in the context of treating melanoma. The review also highlights the challenges and future directions of RNA nanotechnology-mediated cancer immunotherapy in melanoma, underscoring its potential to transform treatment strategies.

Methods: A narrative review of the recent literature was conducted, focusing on RNA nanotechnology applications in melanoma immunotherapy. Studies investigating RNA-based nanoparticles, RNA delivery systems, immune checkpoint modulation, cytokine regulation, tumor antigen vaccines, and immune-cell-targeted therapeutic strategies were evaluated and summerized.

Results: RNA nanotherapeutics have demonstrated the ability to modulate immune checkpoints, reprogram tumor-associated macrophages, enhance antigen presentation, and restore T-cell activity in melanoma models. Lipid nanoparticles, polymeric nanocarriers, and bio-inspired delivery systems have shown improved RNA stability, tumor targeting, and endosomal escape. Emerging clinical evidence from RNA vaccines indicates encouraging immunogenicity and therapeutic potential in advanced melanoma. However, several barriers remain, including tumor heterogeneity, limited penetration into immune-desert metastases, nanoparticle-associated toxicity, manufacturing complexity, and variability of the enhanced permeability and retention effect in human tumors.

Conclusion: RNA nanotechnology represents a promising and rapidly evolving platform for melanoma immunotherapy. By enabling precise modulation of immune responses and improving targeted therapeutic delivery, RNA-based nanostructures may overcome several limitations of current immunotherapeutic approaches. Further preclinical and clinical investigations are required to optimize safety, delivery systems, and therapeutic efficacy before widespread clinical implementation can be achieved.

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Introduction

Melanoma, an aggressive form of skin cancer, arises from the neoplastic conversion of melanocytes in the epidermis.[1,2] Its rising incidence and dismal prognosis contribute to its low survival rates and high mortality rates.[3] Despite standard treatments, melanoma remains difficult to manage, prompting the emergence of novel immunotherapies such as immune checkpoint inhibitors, adoptive cell therapies, oncolytic viruses like T-VEC, and nanotechnology-based delivery systems that enhance efficacy and reduce toxicity.[4,5] Consequently, researchers are exploring promising avenues like immunotherapy and cancer nanotechnology to address this challenge. These innovative approaches have garnered considerable interest due to their potential in melanoma treatment.[6]

Cancer immunotherapy functions by targeting immune checkpoints on both immune and cancer cells to elicit enduring anti-tumor responses. Research has demonstrated its efficacy in inhibiting tumor growth and preventing cancer recurrence.[5,7] However, clinical observations have documented side effects, including fatigue, fever, skin toxicity, diarrhea, and hypotension.[8,9] Moreover, various melanoma patients developing resistance to cancer immunotherapy have been noted.[10] To address these challenges, researchers are integrating nanotechnology with immunotherapy, aiming to mitigate drug resistance and minimize adverse effects.

The utilization of RNA in nanotechnology has catalyzed a significant breakthrough in treating diverse patient populations, including those with cancer. Employing RNA offers numerous advantages, including specificity, cost-effectiveness, and simplified design compared to other drugs or small-molecule proteins.[5] While RNA therapeutics face inherent challenges like instability, nanoparticle-based delivery systems have emerged as an effective solution to these limitations.[11,12]

Immunomodulation and cancer immunotherapy through RNA involve various mechanisms, including the silencing of immune checkpoint genes, modulation of cytokine expression, activation of the immune system, and development of tumor antigen vaccines.[13,14] Numerous studies have explored the application of RNA-based therapy in melanoma immunotherapy, yielding promising outcomes. This review specifically focuses on recent advances in RNA nanotechnology-based strategies for overcoming resistance in melanoma immunotherapy. Particular emphasis is placed on melanoma-associated immune escape mechanisms, RNA-mediated modulation of tumor microenvironment signaling, nanoparticle engineering considerations, translational challenges, and emerging clinical applications of RNA nanotherapeutics designed to enhance immune checkpoint blockade responsiveness and anti-tumor immunity.

Immune Checkpoint Inhibitors and Current Immunotherapy Strategies in Melanoma

The immune system plays a crucial dual role in cancer treatment by targeting tumor cells while sparing normal cells and maintaining a delicate balance. Checkpoints, which are composed of proteins, are very important in regulating the role of the immune system. Tumor cells, including melanoma cells, overexpress checkpoint proteins to evade immune system attack. In this regard, different drugs have been designed to block checkpoint proteins and restore the immune response against melanoma cells.[15,16] The advent of immunotherapy as a therapeutic strategy has revolutionized many cancers, notably melanoma. Immunotherapy by targeting immune checkpoint molecules such as CTLA-4 and PD-1, which are T-cell inhibitors, dramatically improves the anti-tumor response and improves T-cell activity.[17] For example, the use of Ipilimumab, Nivolumab, and Pembrolizumab antibodies that target CTLA-4, PD-1, and PD-L1, respectively. These successes culminated in their approval by the FDA for

melanoma treatment. Furthermore, in order to overcome the treatment of malignancies with drug resistance, such as locally advanced or metastatic melanoma, combination therapies involving immunotherapy drugs or their integration with conventional treatments have been explored.[18-20] Additionally, herpes simplex virus-1 (HSV-1) has emerged as another agent in immunotherapy against melanoma. This genetically engineered oncolytic virus has the capability to multiply in tumor cells and express granulocyte macrophage colony-stimulating factor (GM-CSF) (known as T-VEC). T-VEC is a genetically modified HSV-1 designed to infect and replicate in tumor cells selectively. The replication of T-VEC results in tumor cell lysis, which in turn releases tumor-specific antigens and the GM-CSF. GM-CSF then recruits and activates antigen-presenting cells, thereby enhancing the anti-tumor immune response.[21,22] To optimize treatment efficacy, strategies integrating T-VEC therapy with other modalities such as immune inhibitors, radiation therapy, and targeted therapy have been employed.[21,22] Moreover, the amalgamation of immunotherapy with nanotechnology has garnered significant attention from researchers, yielding promising advancements in melanoma treatment. On the other hand, the use of nanoparticles in melanoma immunotherapy has led to a reduction in toxicity and non-specific immune response against the tumor caused by the administration of immunotherapy drugs.[9]

Although these immunotherapeutic strategies have significantly improved melanoma treatment outcomes, a substantial proportion of patients either fail to respond or eventually develop resistance. Understanding the molecular and immunological basis of this resistance is therefore essential for the development of next-generation therapeutic approaches.

Mechanisms of Resistance in Melanoma Immunotherapy

Despite the remarkable success of immune checkpoint inhibitors in the treatment of melanoma, innate and acquired resistance remain major clinical challenges.[23,24] Multiple molecular and immunological mechanisms contribute to the failure of treatment against melanoma, including defects in antigen presentation, formation of an immunosuppressive tumor microenvironment, T-cell exhaustion, and aberrant oncogenic signaling pathways.[25,26]

In this regard, loss or downregulation of major histocompatibility complex class I (MHC-I), β2-microglobulin (B2M), and the antigen processing machinery reduce tumor recognition by cytotoxic T lymphocytes and enhance immune evasion.[27,28] Importantly, intrinsic resistance mechanisms involving irreversible loss of antigen presentation machinery, including HLA class I or B2M deficiency, may limit the efficacy of immune checkpoint blockade because tumor cells become poorly recognizable to cytotoxic T lymphocytes.[27,28] In such settings, RNA nanotherapeutics may not fully restore anti-tumor immunity through checkpoint silencing alone. However, several RNA-based approaches may partially overcome these resistance mechanisms indirectly by enhancing dendritic-cell activation, promoting cross-presentation of tumor antigens, stimulating innate immune responses, and increasing recruitment of NK cells and effector T cells within the tumor microenvironment.[29-31] Moreover, mRNA nanovaccines may improve immune recognition through induction of broader neoantigen-specific T-cell responses, although complete restoration of antigen presentation in tumors with permanent HLA or B2M loss remains a significant therapeutic challenge.[31] Furthermore, studies have shown that persistent activation of MAPK signaling and the Wnt/β-catenin pathways has been associated with reduced dendritic cell recruitment and impaired T-cell infiltration into melanoma lesions.[32,33] These alterations contribute to the formation of tumors with poor response to immune checkpoint blockade.[33] RNA therapeutics may directly modulate these resistance-associated molecular pathways and thereby restore anti-tumor immune responses. siRNA-mediated silencing of

PD-L1 can enhance cytotoxic $CD8^+$ T-cell activity by preventing PD-1/PD-L1-mediated T-cell exhaustion, while inhibition of STAT3, IDO, and TGF-β signaling may reduce immunosuppressive cytokine production and promote dendritic-cell maturation and antigen presentation.[34,35] In addition, RNA interference targeting components of the MAPK and Wnt/β-catenin pathways may improve T-cell infiltration into melanoma lesions by reversing immune exclusion and enhancing chemokine-mediated recruitment of dendritic cells and effector lymphocytes.[26,33] mRNA nanovaccines further contribute to immune activation through intracellular translation of tumor-associated antigens or neoantigens, leading to MHC-I and MHC-II antigen presentation and subsequent activation of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells.[29,30] miRNA-based therapeutics may additionally regulate multiple immune escape pathways simultaneously by modulating checkpoint molecule expression, inflammatory signaling, apoptosis, and tumor-associated macrophage polarization within the tumor microenvironment.[36,37]

RNA-based nanotherapies offer unique opportunities to overcome these resistance mechanisms. siRNA-mediated silencing of PD-L1, STAT3, IDO, and TGF-β can reverse immunosuppressive signaling and restore T cell activity.[34,35] Similarly, mRNA nanovaccines enhance antigen presentation and induce sustained CD8+ and CD4+ T cell responses.[31,38] Furthermore, miRNA-based therapies can impact tumor progression and immune evasion by simultaneously regulating multiple oncogenic and immune-related pathways.[36,39]

Importantly, emerging RNA nanoplatforms are increasingly being designed to actively target melanoma metastases with poor angiogenesis.[40,41] Surface-functionalized nanoparticles, receptor-mediated targeting systems, and stimuli-responsive nanocarriers may improve penetration into immunodeficient tumors and enhance therapeutic delivery in clinically refractory melanoma.[42,43]

Given the complexity of these resistance mechanisms, advanced delivery platforms capable of selectively modulating tumor and immune-cell signaling pathways have attracted increasing attention. In this context, nanotechnology-based delivery systems have emerged as promising tools for improving RNA therapeutic stability, targeting, and immunomodulatory efficacy.

Immunotherapy based on nanotechnology in melanoma

Typically, researchers employ passive and active targeting strategies to deliver nanoparticles to tumor cells.[42] Nanoparticles can infiltrate malignant tissues through the enhanced permeation and retention (EPR) mechanism via passive targeting, facilitated by vascular disruption and abnormal lymphatic drainage in tumor tissues.[42] However, the efficacy of this approach varies across different tumor types. Conversely, to enhance nanoparticle optimization, surface modification and active targeting techniques are utilized to pinpoint receptors with heightened expression on tumor cell surfaces.[43-45] The field of nanotechnology has significantly contributed to cancer treatment through the design of diverse nanoparticles aimed at delivering various therapeutic agents such as antigens, proteins, peptides, nucleic acids, and drugs to tumor cells, immune cells, and lymph nodes.[46,47] This approach has led to improvements in the physical and chemical attributes of nanoparticles, including size, shape, and charges, which are crucial determinants of their efficacy.[12,48] Clinical studies have indicated that nanoparticles ranging in size from 20 to 100 nm exhibit favorable characteristics for cancer immunotherapy investigations.[49] It has been determined that these nanoparticles migrate to the lymphatic vessels and lymph nodes by penetrating the extracellular matrix and lead to the stimulation of immune cells and the initiation of an immune response.[50,51] Moreover, the morphology of nanoparticles plays a pivotal role in immunotherapeutic endeavors. Collectively, existing studies suggest that nanoparticle geometry substantially influences biodistribution and immune-cell

uptake, with elongated particles favoring prolonged circulation while spherical systems appear more efficiently internalized by dendritic cells and tumor cells.[52,53] In addition, the surface charge of nanoparticles is effective in the entry of nanoparticles into cancer cells. Cationic nanoparticles may become sequestered in the tumor microenvironment through electrostatic interaction with negatively charged molecules like glycosaminoglycans (e.g., heparan sulfate) and sialic acid-containing glycoproteins at the tumor cell surface and within the extracellular matrix. This nonspecific binding can potentially limit nanoparticle penetration and therapeutic effect.[54-56] However, cationic nanoparticles have been reported to escape endosomal trapping and release their cargoes in the cytoplasm or nucleus using the proton sponge effect.[57] From a pharmaceutical engineering perspective, optimization of surface chemistry, charge density, and endosomal escape mechanisms remains essential for successful RNA nanotherapeutic design. Surface functionalization using PEG, targeting ligands, antibodies, peptides, or biomimetic coatings may improve nanoparticle stability, prolong systemic circulation, reduce opsonization, and enhance selective accumulation within melanoma tissues.[42,43,58] However, excessive PEGylation may simultaneously reduce cellular uptake and endosomal internalization, highlighting the need to balance stealth properties with intracellular delivery efficiency. Similarly, although highly cationic nanoparticles may improve RNA complexation and facilitate endosomal escape through the proton sponge effect, excessive positive surface charge may increase inflammatory toxicity, serum protein adsorption, complement activation, and nonspecific interactions with healthy tissues.[54-57,59] Efficient endosomal escape remains a major translational barrier because intracellular RNA degradation within lysosomes may substantially reduce therapeutic efficacy. Therefore, development of ionizable lipids, pH-responsive polymers, fusogenic lipids, and stimuli-responsive nanocarriers capable of controlled endosomal destabilization has emerged as an important strategy for improving cytosolic RNA delivery and melanoma immunotherapy outcomes.[59-61] Nanoparticle morphology can also influence immune interactions. Elongated or flexible particles may show prolonged circulation, whereas spherical nanoparticles may be more efficiently internalized by dendritic cells and tumor cells.[52,53,62] In melanoma, these properties are directly relevant to therapeutic outcome because successful RNA delivery requires tumor penetration, uptake by immune or tumor cells, endosomal release, and subsequent modulation of antigen presentation or immune checkpoint pathways.[63] Thus, rational engineering of RNA nanocarriers is essential for reversing immune escape and improving response to melanoma immunotherapy.[64] Collectively, current evidence suggests that no single nanoparticle design parameter alone determines therapeutic efficacy in melanoma immunotherapy.[9,61,65] Although smaller nanoparticles may improve tumor penetration, larger or surface-modified systems may prolong circulation and enhance immune-cell interactions.[61] Similarly, highly cationic nanoparticles can improve RNA loading and cellular uptake but may simultaneously increase inflammatory toxicity and off-target immune activation. These findings highlight the need to balance delivery efficiency, immune activation, biocompatibility, and safety during RNA nanocarrier optimization.[9,61] Moreover, differences among experimental models, nanoparticle formulations, and melanoma microenvironment characteristics may partly explain variability across reported studies.[41] Importantly, the enhanced EPR effect observed in murine tumor models is often substantially less pronounced and more heterogeneous in human metastatic melanoma, particularly in poorly vascularized or immune-desert lesions.[41,63] Consequently, passive nanoparticle accumulation alone may be insufficient for effective RNA delivery in clinical settings. To address these limitations, current RNA nanocarrier systems are increasingly engineered with active targeting and microenvironment-responsive properties. Surface-functionalized nanoparticles incorporating antibodies, peptides, mannose ligands, or receptor-targeting moieties may enhance selective uptake by tumor cells, dendritic cells, or tumor-associated macrophages independent of passive EPR-mediated accumulation.[42,66] In addition, stimuli-responsive nanocarriers sensitive to acidic pH, redox conditions, or

enzymatic activity may improve penetration and intracellular RNA release within poorly perfused melanoma metastases.[59,67] Emerging strategies including biomimetic coatings, immune-cell-mediated delivery systems, and nanoparticles designed to recruit or activate dendritic cells and NK cells may further help overcome immune exclusion and limited T-cell infiltration within immune-desert melanoma microenvironments.[63,65] Nevertheless, overcoming the physiological barriers associated with heterogeneous tumor perfusion and stromal resistance remains a major translational challenge for RNA nanomedicine.

Utilizing nanobiotechnology in immunotherapy enhances drug release, facilitates precise localization, optimizes pharmacokinetics, and enables simultaneous delivery of immunomodulatory compounds. This approach can elicit unique responses that may not be achievable through the administration of multiple compounds in combination therapies.[68] Therefore, the use of nanoparticles is effective in improving the effectiveness of immunotherapy drugs, reducing their toxicity and long-term recovery.[69] For example, biomaterial scaffolds can simultaneously deliver tumor-associated antigens and immunostimulatory agents to activate the immune system.[70] To achieve long-term therapeutic effects, it is necessary to induce sufficient immune responses following tumor removal. Zhu et al. utilized a novel nano-system, NPs Al-BSA-Ce6, which comprised of photosensitizer chlorin e6 (Ce6) and aluminum hydroxide (Al) as an immunoadjuvant in bovine serum albumin (BSA) for intravenous injection to target tumor cells. The nanoparticles destroyed tumor cells and prevented recurrence and metastasis by eliciting a systemic antitumor immune response. Notably, the administration of Al-BSA-Ce6 led to the accumulation of T cells in the tumor-site lymph nodes, increased serum antibody levels, cytotoxic T cells and Th1, and cytokine levels.[70] The utilization of copper-cystamine (Cu-Cy) nanoparticles in the administration of photodynamic therapy (PDT) for melanoma has been shown to elicit antitumor immune responses through the maturation of dendritic cells, which subsequently activate CD4+ T cells, CD8+ T cells, and natural killer (NK) cells. Additionally, Cu-Cy nanoparticles induce apoptosis in melanoma cells by increasing the levels of reactive oxygen species (ROS) within the tumor cells.[71] Interleukin-2 (IL-2) has long been a topic of interest for the treatment of melanoma, yet its systemic use is limited due to severe side effects and incomplete responses in only 10% of patients.[72] To overcome these challenges, researchers have turned to nanotechnology to reduce the severity of IL-2 side effects.[73] One study utilized IL-2/Fc nanogel delivery systems that are sensitive to oxidation and reduction, demonstrating that cytokine-containing nanogels could selectively release IL-2 in response to the activation of T cell receptors.[74] In a melanoma mouse model, this delivery system was found to be more efficient than free IL-2, without any apparent toxicity. The use of nanotechnology as a platform for drug delivery systems (DDS) has been successful due to the biocompatibility and non-toxicity of hydrogel systems.[75] Similarly, employing immunoliposomes for IL-2 administration has shown encouraging outcomes.[76]

The field of RNA-based methods in immune modulation and cancer immunotherapy has seen significant progress in recent years, presenting researchers with new opportunities for exploration and discovery.[5] RNA has been successfully employed in immunotherapy-based treatments to silence immune checkpoint genes, activate the innate or adaptive immune system by modulating cytokine expression, and function as tumor antigen vaccines.[13,14] In an effort to enhance the efficacy of these therapeutic approaches, nanotechnology has also been integrated into the process. Various nanocarriers, including liposomes, polymeric nanoparticles (NPs), and inorganic nanoparticles, have been utilized in numerous studies to facilitate the effective delivery of RNAs against a range of tumor cells.[77-80]

Because the therapeutic efficacy of RNA molecules depends heavily on efficient intracellular delivery and protection from degradation, considerable efforts have focused on developing specialized nanocarriers optimized for RNA transport and tumor targeting.

Overview of nanocarriers for RNA-nanotechnology

Nanotechnology has revolutionized RNA-based therapies by addressing challenges like poor stability and short half-life through the design of targeted carriers.[13,81] These carriers not only protect RNA from degradation and immune responses but also facilitate its specific accumulation at tumor sites.[43] Utilizing the Enhanced Permeability and Retention (EPR) effect, nanocarriers sized between 10 to 200 nm can efficiently penetrate tumor tissues and cells.[81,82] Various types of nanocarriers (Figure 1), including lipid-based, bio-inspired, inorganic, and polymer nanomaterials, have been developed for effective transfer and delivery of RNA molecules, marking significant progress in RNA-based cancer therapies.[5]

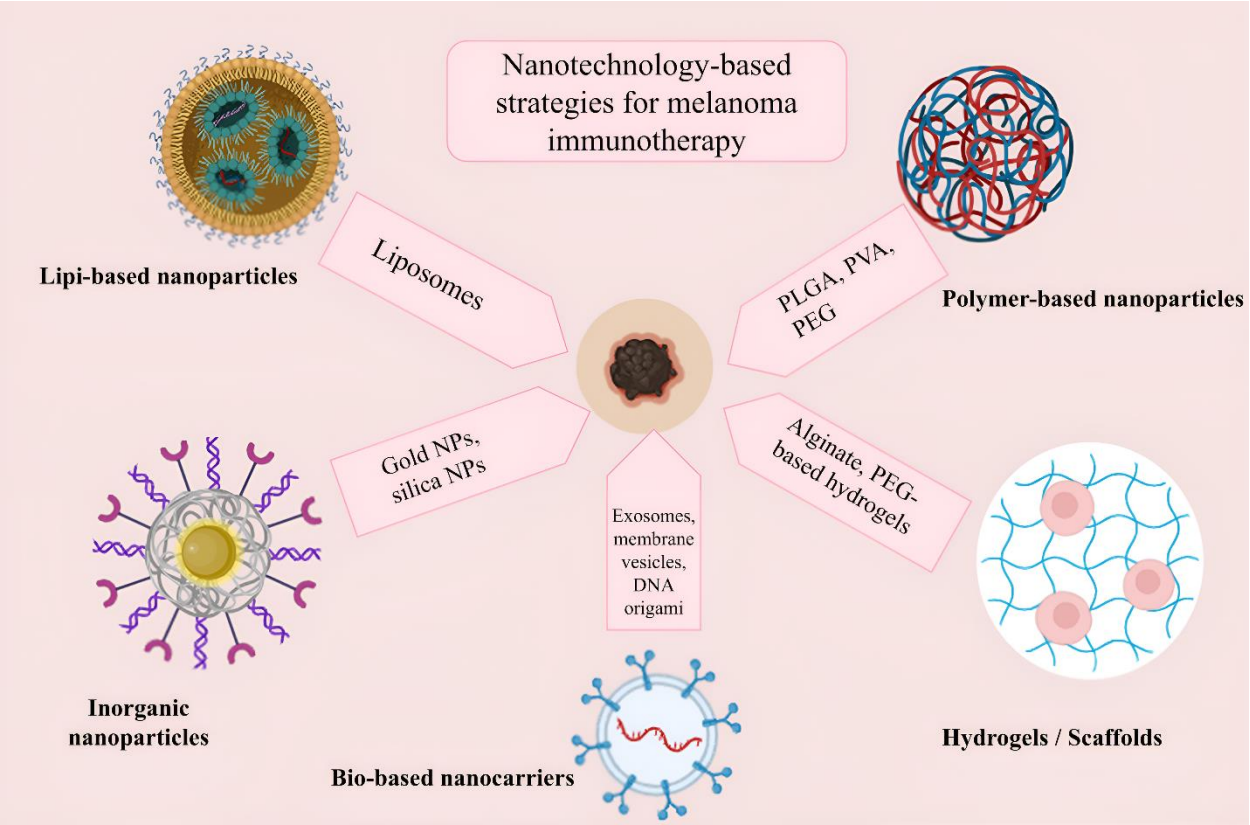


Figure 1: Nanotechnology-based strategies for melanoma immunotherapy. The schematic illustrates five major classes of nanocarriers used in RNA-based melanoma immunotherapy: lipid-based nanoparticles (e.g., liposomes), polymer-based nanoparticles (e.g., PLGA, PVA, PEG), inorganic nanoparticles (e.g., gold and silica NPs), hydrogels or scaffolds (e.g., alginate, PEG-based systems), and bio-based nanocarriers (e.g., exosomes, membrane vesicles, DNA nanostructures). Each platform offers distinct advantages in enhancing delivery, targeting, and immune modulation in melanoma treatment. (Created with BioRender.com)

In recent times, lipid-based nanocarriers have become prevalent for the delivery of drugs and various compounds.[9] These nanocarriers, including liposomes, solid lipid nanoparticles (SLNs), and lipid nanoemulsions, are widely used for RNA delivery purposes.[58] Particularly, nanocarriers based on cationic lipids are recommended for RNA delivery due to their ability to interact with the negatively charged RNA molecules. The homogeneity of lipid and phospholipid-based nanocarriers with cell membranes facilitates the absorption of RNA into cells, enhancing their efficacy.[5] Moreover, these nanocarriers offer advantages such as biocompatibility, biodegradability, and ease of

preparation. However, it's important to note that lipid-based delivery systems also come with certain limitations. These include susceptibility to clearance by organs like the kidneys or liver, potential leakage of cargoes, and a relatively short half-life, which may impact their overall effectiveness in drug delivery.[5] Synthetic polymer-based nanocarriers, exemplified by poly (dl-lactide-co-glycolide) (PLGA), polyvinyl alcohol (PVA), and polyethylene glycol (PEG), have become prevalent in cancer therapy research.[83-87] These nanocarriers are favored for their superior stability, biodegradability, and biocompatibility, making them well-suited for drug delivery applications in oncology.[88] However, their efficacy in RNA delivery is hindered by the absence of cationic units, which diminishes the interaction between the polymers and RNA molecules. To address this challenge, scientists have explored the use of nanocarriers incorporating cationic motifs, aiming to enhance the binding affinity and delivery efficiency of RNA therapeutics.[89]

Researchers employ bio-based nanocarriers, such as exosome-like nanovesicles, DNA-based nanostructures, or red blood cell-derived ghosts, for the transport and administration of RNA molecules.[90-95] The significance of these bio-nanocarriers lies in their minimal toxicity, limited immunogenicity, and adaptability for targeted therapy across diverse medical and therapeutic modalities, including the precise delivery of RNA therapeutics for immunomodulation purposes.[94] Based on existing research findings, nanoparticles crafted from biodegradable polymers and inorganic compounds, such as mesoporous silica materials (MSNs), metallic nanostructures (e.g., iron oxide and gold nanoparticles), quantum dots (QDs), and carbon nanotubes (CNTs), have been utilized as vehicles for RNA transportation.[96-99] These nanoparticles offer facile preparation and design, enabling surface modifications aimed at enhancing cellular uptake and optimization. Nonetheless, the utilization of such nanoparticles necessitates thorough scrutiny concerning potential toxicity and degradability profiles, warranting careful investigation.[87] However, despite their favorable biocompatibility and targeting properties, bio-inspired nanocarriers face several limitations. These include low RNA loading efficiency, reduced storage stability, and rapid clearance by the mononuclear phagocyte system. Moreover, large-scale good manufacturing practice (GMP)-compliant manufacturing remains challenging due to biological variability, and a lack of standardized protocols and defined pharmacokinetics hampers regulatory approval.[100,101] In melanoma immunotherapy, these nanocarrier systems are particularly relevant because they may help overcome major resistance-associated barriers, including poor T-cell infiltration, immunosuppressive tumor microenvironments, inefficient antigen presentation, and limited penetration into metastatic lesions. Rational engineering of melanoma-targeted RNA nanocarriers may therefore improve immune checkpoint blockade responsiveness and facilitate reversal of immune escape mechanisms.

Immunotherapy based on RNA-nanotechnology

RNA structures have been widely utilized in cancer therapies due to their effectiveness. These structures can have either inhibitory such as small interfering RNA (siRNA) and microRNA (miRNA) or enhancing roles (Figure 2).[5] siRNAs function by incorporating into the RNA-induced silencing complex (RISC), where the Argonaute 2 (AGO2) protein facilitates the cleavage of complementary target messenger RNAs (mRNAs).[102,103] This post-transcriptional gene silencing leads to reduced protein expression in a sequence-specific manner.[104] miRNA affects many mRNAs after they're made by attaching to their 3'UTR.[105] mRNA vaccines contain instructions for tumor antigens that cells turn into proteins, which then kick-start immune responses. These approaches allow for pinpoint targeting of immune checkpoints and tumor antigens.[106] Due to the ability to regulate multiple mRNAs by one microRNA, siRNAs are more specific.[5] Furthermore, the employment of mRNA delivery aims to augment the

expression of specific proteins (Figure 2). mRNA therapeutic approaches offer several advantages, including reliable kinetics, heightened likelihood of protein expression, the avoidance of insertional mutagenesis, and enhanced transfection efficiency when utilizing mRNA.[107] Despite notable progress in cancer immunotherapy, the use of antibodies, proteins and engineered immune cells in immunotherapy are not effective enough and have high costs and numerous side effects.[108,109] RNA therapeutics with nanomaterials presents a promising strategy for enhancing the efficacy of the body's immune system in cancer treatment. Moreover, the integration of this approach with other therapeutic combinations holds potential for yielding more efficient and specialized outcomes, thereby establishing itself as a viable therapeutic candidate.[5]

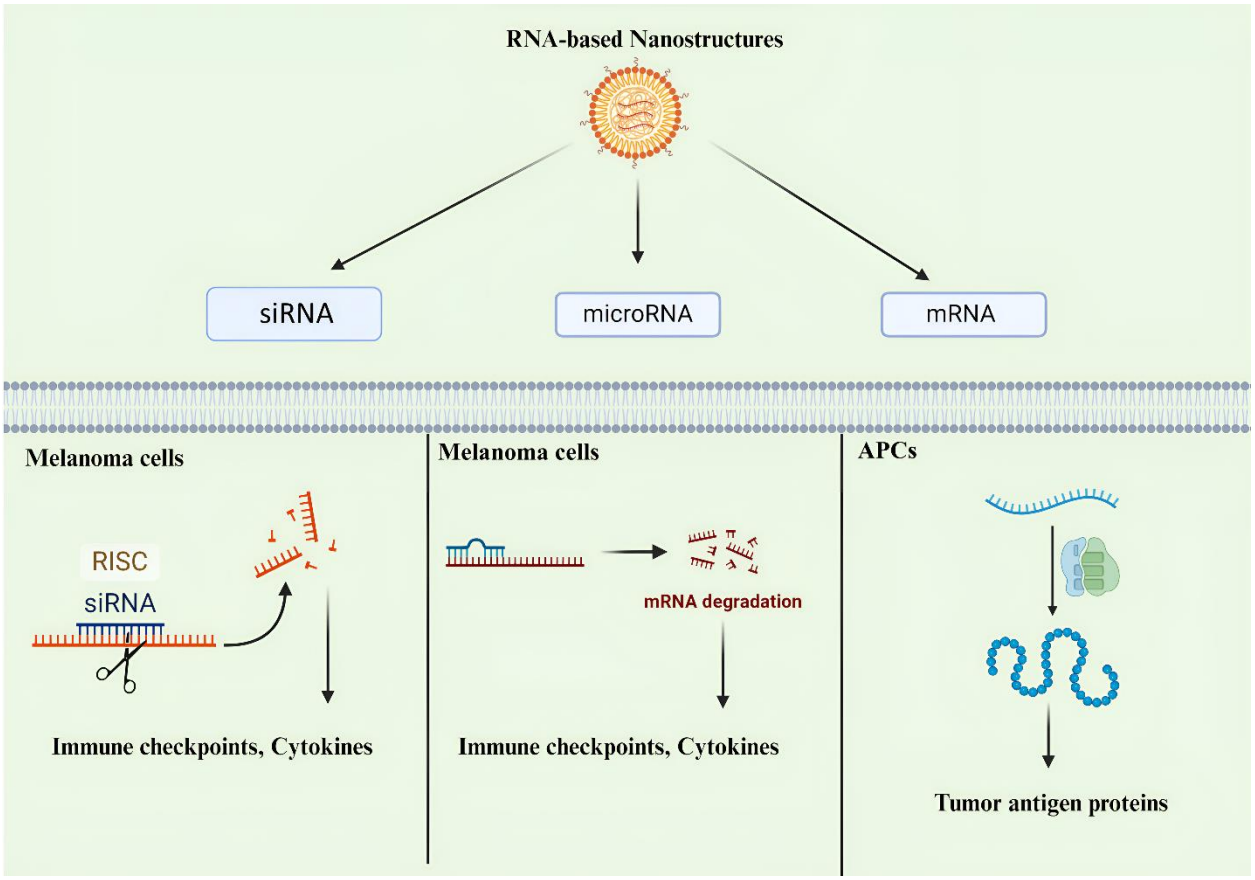


Figure 2: Schematic image of the use of different types of RNA in the treatment of melanoma. Utilizing siRNA and tumor suppressor microRNAs decreases the expression of immune proteins and cytokines, fostering immune system activation for an anti-tumor response. mRNA-based nanomedicines, like nano-vaccines, efficiently deliver tumor antigen proteins to antigen-presenting cells (APCs), enabling subsequent processing of peptide epitopes through the major histocompatibility complex (MHC) class I pathway. This activation enhances CD8+ T cell responses, culminating in an effective anti-tumor immune reaction. (Created with BioRender.com)

siRNA-Based Nanotherapeutics

One of the strategies within RNA nanotechnology-based immunotherapy involves the utilization of siRNA to silence and down-regulate immunosuppressive genes present in cancer cells and cancer-associated immune cells.[110-112] siRNA nanotherapeutics hold the capability to diminish the expression of immune checkpoint proteins and immunosuppressive cytokines, thereby facilitating the activation of the immune system to mount an anti-tumor response (Figure 2).[113] Numerous studies have amassed evidence indicating that the integration of nanoparticles with other therapeutic modalities forms a robust platform for triggering an anti-tumor immune response. Specifically, research has demonstrated that concurrent utilization of siRNA-based nanotechnology in

immunotherapy alongside photodynamic or chemical agents elicits synergistic antitumor effects.[114,115] Tumor-associated macrophages (TAMs) are important regulators of the melanoma immune microenvironment and may exhibit either anti-tumor (M1-like) or immunosuppressive (M2-like) phenotypes.[116,117] Consequently, several RNA nanotherapeutic strategies have focused on reprogramming TAMs to restore anti-tumor immunity. For example, dual-targeting nanoparticles delivering anti-CSF-1R siRNA significantly depleted M2-like macrophages, reduced IL-10 and TGF-β expression, increased IL-12 and IFN-γ levels, and enhanced CD8+ T-cell infiltration in melanoma models.[35] Similarly, miR-155-loaded nanoparticles were shown to reactivate macrophage immune function and stimulate anti-tumor immune responses within the tumor microenvironment.[66] Collectively, these findings support macrophage reprogramming as a promising strategy for reversing melanoma-associated immune suppression and improving immunotherapy responsiveness. Tumor antigens are presented to CD8+ or CD4+ T cells by dendritic cells (DCs), a specific type of antigen-presenting cell (APC).[118] Therefore, the role of DCs is pivotal in the anti-tumor immune response. Several inhibitory molecules such as suppressors of cytokine signaling (SOCS) 1, signal transducers and activators of transcription-3 (STAT3), and indoleamine 2,3-dioxygenase (IDO) are expressed on the surface of DCs, thereby inhibiting their function.[60,119-121] A strategy employed in the realm of concerted immunotherapy involves inhibiting the action of these suppressive molecules using siRNA.

miRNA-Based Nanotherapeutics

In addition, tumor suppressor microRNAs prevent the progression of cancer cells by reducing oncogenic mRNA.[122] On the other hand, oncogenic microRNAs have an increased expression in cancer cells, while the expression of tumor suppressor microRNAs decreases.[123,124] Therefore, the regeneration of tumor suppressor microRNAs to increase their expression is a new therapeutic method for cancer treatment to induce apoptosis of cancer cells. Furthermore, the utilization of MicroRNA antagonists has been implemented to suppress the function of oncogenic microRNAs, thereby influencing the immune response against tumor cells.[125,126] Additionally, MicroRNA has been leveraged to downregulate the expression of immune checkpoint proteins and proinflammatory cytokines (Figure 2).[127] For instance, studies have revealed the involvement of miR-34a, miR-200, miR-142-5p, and miR-424 in diminishing the expression of PD-L1 within cancerous cells.[37,128] Numerous studies have explored the use of nanomaterials incorporating microRNA to enhance cancer immunotherapy, yielding encouraging outcomes.[5,129] For instance, lipid-coated calcium phosphonate nanoparticles (CaP/miR@MNPs) loaded with miR155 and mannose-modified were demonstrated to target TAMs, effectively reactivating and reprogramming their functions. This strategy stimulates the immune response within the tumor microenvironment, resulting in reduced tumor growth. Similarly, several analogous investigations have reported promising results utilizing microRNA-based nanomaterials against both tumor cells and the tumor microenvironment, eliciting an immune response.[66]

mRNA Nanovaccines and mRNA Therapeutics

The potential of mRNA as a cancer treatment has gained interest due to its distinctive attributes.[130] However, mRNA instability due to degradation by nucleases has limited the use of mRNAs. Additionally, the hindered entry of larger, negatively charged mRNAs into immune cells, including APCs, has complicated their utilization.[82,131] To address these constraints, nanoparticle-based mRNA carriers have been developed, offering a solution to these limitations. Notably, nanomedicines-based mRNA has found applications in vaccination and cell engineering industries. mRNA-based nano-vaccines are gaining prominence for their potent efficacy, safe administration, and the encouraging outcomes observed in numerous anti-tumor vaccination investigations.[30,132-134] These vaccines

can efficiently deliver an unlimited number of tumor antigen proteins to APCs (Figure 3). Subsequently, the peptide epitopes are processed via the major histocompatibility complex (MHC) class I pathway, triggering immune system activation and fostering CD8+ T cell responses, thereby eliciting an effective anti-tumor immune reaction.[5] Furthermore, mRNA vaccines prompt the activation of the MHC-II pathway, inducing CD4+ T immune responses against cancer cells through protein translation. This suggests that mRNA-based vaccines may effectively trigger both cellular and humoral immune responses against tumors.[135]

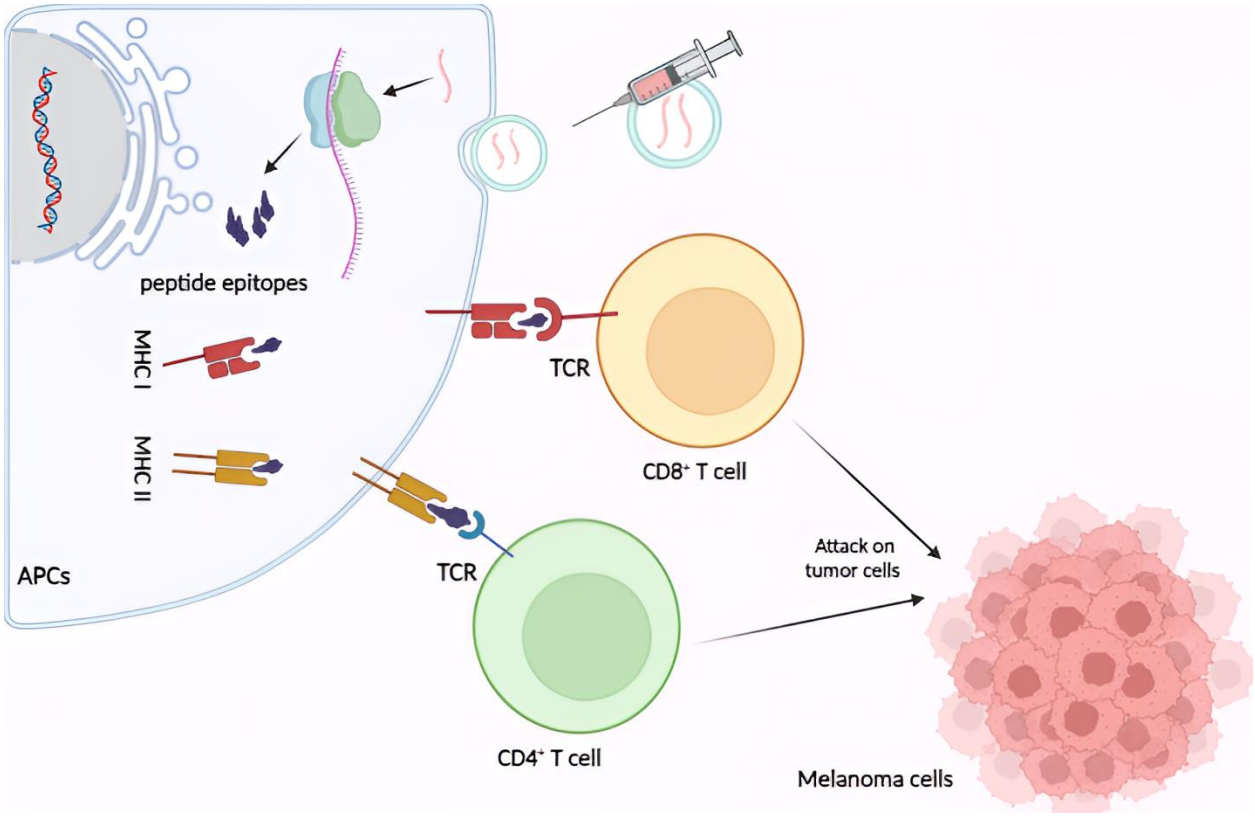


Figure 3: Schematic overview of mRNA-based nanovaccine function in melanoma: nanoparticles deliver mRNA to APCs, leading to tumor antigen expression, MHC class I/II presentation, and activation of $CD8^+$ and $CD4^+$ T-cells that mediate systemic anti-tumor immunity (Created with BioRender.com)

Critical Perspective on RNA Nanotherapeutics

Collectively, preclinical and clinical studies support the therapeutic potential of RNA-based nanotechnology in melanoma immunotherapy. Despite the considerable therapeutic promise of RNA-based nanotechnology in melanoma immunotherapy, important differences exist among RNA modalities regarding specificity, translational maturity, delivery requirements, and clinical applicability.[5,82,86] siRNA nanocarriers provide high target specificity and are particularly suitable for silencing immune checkpoints or immunosuppressive mediators such as PD-L1, STAT3, IDO, and TGF-β.[35,110,112,113,118] However, their efficacy depends strongly on efficient cytosolic delivery and sustained intracellular release.[59,81] In contrast, miRNA-based therapeutics may simultaneously regulate multiple oncogenic and immune-related pathways, which may be advantageous in heterogeneous tumors such as melanoma, although this broader activity may also increase the risk of off-target effects and unpredictable biological responses.[14,122] Among current RNA modalities, mRNA nanovaccines have demonstrated the greatest clinical momentum, particularly due to advances in personalized neoantigen vaccines and liposomal RNA vaccine platforms.[30,132,133,135] Nevertheless, most available evidence remains preclinical, and many studies rely on murine

melanoma models that do not fully recapitulate the vascular heterogeneity, immune suppression, and metastatic complexity of human melanoma.[86] Therefore, future investigations should prioritize clinically relevant models, standardized safety evaluation, and rational combination strategies with immune checkpoint blockade.[23,26] Importantly, the relative advantages of different RNA modalities may depend on tumor immune phenotype, metastatic burden, and treatment context. For example, mRNA vaccines may be particularly effective in immunologically "hot" tumors with preserved antigen presentation, whereas siRNA- or miRNA-based approaches may be more suitable for reversing immunosuppressive signaling within immune-excluded melanoma microenvironments. [23,24,26,31]

RNA-nanotechnology in melanoma immunotherapy

Recently, numerous preclinical and clinical studies have utilized various nanoparticle-based platforms to effectively deliver RNA subtypes for melanoma immunotherapy.

Preclinical Investigations of RNA Nanotechnology in Melanoma

As previously discussed, siRNAs have shown effectiveness in triggering an anti-tumor immune response by targeting key factors involved in tumor progression within cancer cells or by knocking out immunosuppressive genes.[5] Utilizing nanomaterials for siRNA delivery has notably resulted in immune response stimulation in various studies (Table 1). Several studies have investigated nanoparticle-mediated siRNA silencing of PD-L1 as a strategy to enhance melanoma immunotherapy. Tang et al. demonstrated that hybrid polycaprolactone-based nanocarriers delivering PD-L1 siRNA reduced tumor size and enhanced cytotoxic T-cell responses in melanoma-bearing mice.[136] Similarly, Dai et al. and Wang et al. combined PD-L1-targeting siRNA nanoplatforms with photodynamic therapy and reported enhanced immune activation, reduced immune resistance, inhibition of melanoma growth, and lower recurrence rates.[114,115] Collectively, these findings suggest that PD-L1 silencing may synergize with photodynamic therapy by reversing immune exhaustion while simultaneously enhancing immunogenic tumor cell death. Researchers observed that the administration of Au-CGKRK nanoparticles complexed with two siRNAs targeting PD-L1 and STAT3 resulted in enhanced survival and elevated expression of CD8 and CD4 proteins in melanoma-bearing mice. These findings affirm the augmentation of the anti-tumor immune response.[137] Anti-CD47 siRNA in a liposome-protamine-hyaluronic acid (LPH) nanoparticle formulation with suppressor of CD47 function inhibited the growth of melanoma tumors and their metastases.[138] Moreover, the design and administration of nanoparticles incorporating anti-CTLA-4 siRNA for melanoma tumor cell immunotherapy yielded encouraging outcomes, including a rise in the population of anti-tumor CD8 T cells and a decline in the fraction of Tregs within tumor-infiltrating lymphocytes (TILs).[139] Moreover, the incorporation of anti-colony stimulating factor 1 receptor (anti-CSF-1R) siRNA onto dual-targeted M2-like TAM nanoparticles (M2NPs) led to a significant depletion of M2-like TAMs. This resulted in decreased tumor size, prolonged survival, reduced levels of cytokines IL-10 and TGF-β, heightened expression of immune-stimulating cytokines IL-12 and IFN-γ, and enhanced infiltration of CD8+ T cells within the tumor microenvironment of melanoma-bearing mice. On the other hand, investigators have employed mRNA encapsulation within nanocarriers as a nano vaccine strategy targeting melanoma.[35] Oberli et al. encapsulated mRNA encoding tumor-associated antigens such as gp100 and TRP2 into a lipid-based nanovaccine composed of lipid-PEG, a phospholipid, cholesterol, and an adjuvant. Their findings indicated that these nanoparticles were highly efficient in delivering mRNA to dendritic cells, macrophages, and neutrophils, consequently leading to potent activation of CD8+ T cells post-immunization in the B16F10 melanoma model.[140]

Although preclinical investigations have demonstrated encouraging immunomodulatory and anti-tumor effects, translation of RNA nanotherapeutics into clinical practice remains an ongoing challenge. Consequently, several early-phase clinical studies have been initiated to evaluate the safety and therapeutic potential of these approaches in melanoma patients.

Table 1: Preclinical Investigations of siRNA Nanotechnology for Immunotherapy in Melanoma

Nanocarriers	Targeted Cells	Targeted Factors	Immunological Function	Ref
polycaprolactone-polyethylene glycol and polycaprolactone-polyethyleneimine	Tumor cells	PD-L1	reduced PD-L1 expression, enhanced cytotoxicity of T cells	[136]
Micellar nanocomposite, modified with folic acid-polyethyleneimine	Tumor cells	PD-L1	triggered an immune response, PD-L1 blockade	[115]
acid-activatable micelle plex	Tumor cells	PD-L1	PD-L1 blockade	[114]
Au-CGKRK nanoparticles	Tumor cells	PD-L1 and STAT3	PD-L1 and STAT3 blockade, elevated CD8 and CD4 proteins	[137]
liposome-protamine-hyaluronic acid (LPH) nanoparticle	Tumor cells	CD47	suppressor of CD47 function, Reducing the escape of tumor cells from the immune system	[138]
PEG-PLA	T cells	CTLA-4	CTLA-4 blockade, rise in the population of anti-tumor CD8 T cells, rise in the population of anti-tumor CD8 T cells	[139]
Peptides NPs	TAMs	CSF-1R	CSF-1R blockade, depletion of M2-like TAMs, reduced levels of cytokines IL-10 and TGF-β, heightened expression of immune-stimulating cytokines IL-12 and IFN-γ, and enhanced infiltration of CD8+ T cells within the tumor microenvironment	[35]

Clinical Trials of RNA-Based Nanomedicines in Melanoma

While numerous RNA nanotherapeutic strategies have demonstrated promising anti-tumor activity in preclinical melanoma models, only a limited number have progressed to human clinical evaluation. Clinical investigations are underway to explore the use of nanocarriers for delivering RNA-mediated immunotherapy to melanoma patients (Table 2). A phase I clinical trial was conducted to assess the safety and efficacy of intravenous RNA-electroporated chimeric antigen receptor (CAR) T cells designed to target the cell surface cMET antigen in patients diagnosed with metastatic melanoma exhibiting a minimum of 30% tumor cMET expression, alongside measurable disease and a history of prior treatment. Findings from this study revealed that the intravenous administration of cMET-targeted CAR T cells elicited immunological adverse events. Among the cohort of seven individuals who underwent treatment, four demonstrated the most favorable response with stable disease, while disease progression was observed in three cases, underscoring the therapeutic efficacy of this modality (NCT03060356).[141] The pivotal role of specific T cell responses to neoantigens in the efficacy of cancer immunotherapy has been underscored by recent research. To harness this potential, the development of an RNA vaccine encoding a neoantigen, termed IVAC® MUTANOME, has been pursued, aiming to stimulate the activation and proliferation of patients' T cells. A Phase I clinical trial was conducted to assess the efficacy and safety of the IVAC® MUTANOME vaccine in patients diagnosed with stage III and IV malignant melanoma.

Encouragingly, treated patients exhibited a robust polyneoepitope immune response directed against the vaccine antigens, with no reports of severe adverse drug reactions (NCT02035956).[142] Furthermore, it has been demonstrated that transfecting dendritic cells with siRNA targeting the immunoproteasome can modulate antigen presentation, enhancing tumor-specific immune responses. In a phase I clinical trial, melanoma patients received dendritic cell vaccines transfected with RNA encoding melanoma antigens (MART-1, MAGE-3, gp100, and tyrosinase). Three groups were evaluated: (1) cells transfected with non-targeting siRNA (control), (2) cells transfected with only tumor antigen RNA, and (3) cells co-transfected with tumor antigen RNA and siRNA targeting immunoproteasome subunits LMP2, LMP7, and MECL1. Antigen-specific T-cell responses were observed in all groups, peaking after 3–4 vaccinations, but only persisted in group 3, which also showed reduced circulating melanoma cells and increased T-cell cytotoxicity (NCT00672542).[143] Besides, a clinical trial is underway to investigate the efficacy and safety of a vaccine containing Autologous tumor RNA on uveal melanoma (NCT01983748). Moreover, a phase 1 clinical trial is presently underway, exploring an intravenous liposomal RNA vaccine (FixVac) which encompasses four non-mutated melanoma tumor-associated antigens (NYESO-1, MAGE-A3, tyrosinase, and TPTE). Initial findings from this investigation have implied that FixVac, either administered alone or coupled with PD1 inhibitor blockade, holds promise in eliciting enduring responses in patients with unresectable melanoma who have undergone prior checkpoint inhibitor therapy. Moreover, the administration of FixVac has been associated with elevated levels of robust CD4+ and CD8+ T cells targeting vaccine antigens, alongside antigen-specific cytotoxic T cell responses (NCT02410733).[31] Recently, personalized neoantigen mRNA vaccines have shown particularly encouraging clinical outcomes in melanoma. In the randomized phase 2b KEYNOTE-942 clinical trial, the individualized neoantigen vaccine mRNA-4157/V940 in combination with pembrolizumab demonstrated improved recurrence-free survival compared with pembrolizumab monotherapy in patients with resected high-risk melanoma (NCT03897881). These findings highlight the growing translational potential of personalized RNA nanovaccine strategies in melanoma immunotherapy.[144] Nevertheless, despite encouraging immunogenicity and therapeutic responses in early-phase clinical studies, many RNA nanotherapeutic platforms that demonstrate efficacy in experimental melanoma models have not yet advanced to large-scale clinical implementation because of delivery inefficiency, tumor heterogeneity, manufacturing complexity, and unresolved long-term safety considerations.[29,61,86] In addition, recent clinical studies suggest that combination approaches integrating RNA nanotherapeutics with immune checkpoint blockade may produce more durable anti-tumor responses than RNA monotherapy alone. However, clinical efficacy remains heterogeneous, emphasizing the importance of patient selection, tumor immune profiling, and optimization of delivery strategies.[23,24,61,144]

Despite these encouraging early clinical findings, several biological, technological, manufacturing, and regulatory barriers continue to limit the widespread clinical implementation of RNA nanotherapeutics.

Table 2: Clinical Investigations of RNA Nanotechnology for Immunotherapy in Melanoma

Cell Targeting	Study type	Number of participants	Intervention	CT no/Ref.
T cells	Early Phase 1	77	T cells modified with RNA anti -cMET CAR	NCT03060356/[141]
Dendritic cells	Phase 1	15	RNA vaccine encoding a neoantigen (IVAC® MUTANOME)	NCT02035956/[142]
Dendritic cells	Phase 1	12	Proteasome siRNA and tumor antigen RNA-transfected dendritic cells	NCT00672542/[143]
Dendritic cells	Phase 3	200	autologous Tumor RNA	NCT01983748
Dendritic cells	Phase 1	119	liposomal RNA vaccine (FixVac) which encompasses four non-mutated melanoma tumor-associated antigens (NYESO-1, MAGE-A3, tyrosinase, and TPTE)	NCT02410733/[31]
Tumor neoantigens/APCs	Phase 2b	157	mRNA-4157/V940 + pembrolizumab	NCT03897881/[144]

Clinical Translation and Regulatory Challenge

Despite promising preclinical and early clinical findings, several barriers continue to limit the widespread clinical translation of RNA nanotherapeutics in melanoma immunotherapy.[9,81,86] One of the major challenges involves the intrinsic instability of RNA molecules, which are highly susceptible to nuclease-mediated degradation and therefore require efficient protective delivery systems.[82,130,131] Nanocarriers must not only stabilize RNA cargoes but also promote tumor penetration, cellular uptake, endosomal escape, and controlled intracellular release.[41,59] Additional physiological and biological barriers further complicate the clinical translation of RNA nanotherapeutics in melanoma. Tumor heterogeneity, including differences in vascularization, immune infiltration, stromal composition, antigen expression, and metastatic behavior, may substantially influence nanoparticle accumulation and therapeutic responsiveness.[9,41,61] Moreover, dense extracellular matrix components, elevated interstitial fluid pressure, abnormal tumor vasculature, and immune-excluded microenvironments may limit nanoparticle penetration and intracellular RNA delivery within melanoma lesions.[40,41,80] These factors contribute to variability among preclinical and clinical outcomes and highlight the need for actively targeted, stimuli-responsive, and microenvironment-adapted RNA delivery systems.[9,61]

Safety considerations also remain critically important. Cationic and ionizable nanoparticles may induce inflammatory responses, complement activation, hepatic accumulation, and systemic toxicity following repeated administration.[55] In addition, nonspecific immune activation and off-target effects may compromise therapeutic efficacy and safety.[59] Therefore, optimization of nanoparticle composition, biodegradability, and immune compatibility remains essential for clinical application.[9,86] Manufacturing and regulatory challenges additionally complicate clinical implementation. RNA nanotherapeutics require reproducible large-scale production under good manufacturing practice (GMP) conditions, strict control of nanoparticle size and polydispersity, RNA encapsulation efficiency, sterility, storage stability, and batch-to-batch consistency.[29,82] Furthermore, personalized

mRNA vaccine approaches may present additional logistical and regulatory complexities because individualized neoantigen selection and rapid manufacturing workflows are required.[135]

Nevertheless, recent clinical successes involving lipid nanoparticle-based mRNA technologies have substantially accelerated interest in RNA nanomedicine platforms.[30,135] Clinical investigations of RNA-based melanoma therapies, including FixVac and individualized neoantigen vaccines such as mRNA-4157/V940, support the translational potential of RNA nanotherapeutics in combination with immune checkpoint blockade.[31] However, additional large-scale clinical trials and long-term safety studies remain necessary before routine clinical implementation can be achieved.[9,86]

Conclusion

Despite significant progress in melanoma treatment, particularly in the realm of immunotherapy, mortality rates remain high following metastasis to various organs. Moreover, the incidence of side effects associated with immunotherapy poses a significant challenge to its clinical application. Consequently, researchers have endeavored to reduce these side effects by integrating nanotechnology with immunotherapy, thereby enhancing its efficacy.[9] RNA-based therapies, a novel approach, offer several advantages, including heightened selectivity and reduced off-target effects, rendering them increasingly pivotal in immunotherapeutic regulation compared to traditional antibody- or protein-based modalities. RNA nanotechnology demonstrates therapeutic potential across all stages of melanoma progression. In early-stage disease, it may serve as an adjuvant strategy to prevent recurrence. In locally advanced melanoma, RNA-based nanocarriers can be engineered to deliver therapeutics directly to the tumor microenvironment, enhancing specificity and minimizing off-target effects. In metastatic settings, these platforms offer opportunities to modulate immune suppression and restore effective antitumor immunity. However, therapeutic efficacy is expected to vary depending on tumor burden and the functional status of the host immune system.[5,56] Moreover, the development and manufacturing of RNA-based therapeutics are cost-effective and expedient.[29] Additionally, nanotechnology, particularly through the utilization of nanocarriers, addresses RNA's inherent limitations such as susceptibility to degradation by nucleases. These nanoparticle-based delivery systems facilitate targeted delivery of therapeutic payloads, including high-dose RNA therapies, to specific cells or tissues. Furthermore, preclinical studies demonstrate the potential of combining RNA-mediated Nano-immunotherapy with conventional chemotherapeutics or photodynamic treatments.[114,145] However, nanoparticle recognition by the immune system may lead to clearance via renal/hepatic pathways or activation of the innate immune response, necessitating further investigation.[59]

This review article highlights the promising outcomes of employing nanomaterials for targeted delivery of RNA to tumor or immune cells, thereby eliciting an anti-tumor immune response. Moreover, the synergistic integration of RNA-based nanotechnology with immunotherapy alongside conventional treatments like chemotherapy and photodynamic therapy presents a compelling avenue for advancing cancer therapeutics, particularly in melanoma treatment. Nonetheless, the clinical utility of RNA delivery nanoplatforms in cancer treatment remains contentious, necessitating extensive experimentation and exploration. This underscores the imperative for further research endeavors aimed at elucidating the intricacies of this therapeutic modality. Future research should focus on enhancing the clinical translation of RNA nanotherapeutics by developing safer and more efficient delivery systems, improving endosomal escape strategies, and creating actively targeted nanocarriers that can penetrate immune-excluded melanoma lesions.[9,61,63] Additional priorities include optimization of personalized neoantigen mRNA vaccines, integration of RNA nanotechnology with immune checkpoint blockade and adoptive cell

therapies, and identification of predictive biomarkers for patient stratification and treatment response.[144] Furthermore, standardized safety assessment, scalable good manufacturing practice (GMP)-compliant production, and long-term toxicity evaluation remain essential for regulatory approval and broader clinical implementation.[61,146] Advances in lipid nanoparticle engineering, artificial intelligence-assisted nanocarrier design, and precision oncology approaches may further accelerate the development of next-generation RNA nanomedicines for melanoma immunotherapy.[63,135]

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

1. Lai M, La Rocca V, Amato R, Freer G, Pistello M. Sphingolipid/Ceramide Pathways and Autophagy in the Onset and Progression of Melanoma: Novel Therapeutic Targets and Opportunities. *International Journal of Molecular Sciences* 2019;20(14):3436. doi.org/10.3390/ijms20143436

2. Gibbs DC, Orlow I, Kanetsky PA, Luo L, Kricker A, Armstrong BK, et al. Inherited Genetic Variants Associated with Occurrence of Multiple Primary Melanoma. *Cancer Epidemiology, Biomarkers & Prevention* 2015;24(6):992-7. doi: 10.1158/1055-9965.epi-14-1426

3. Patel H, Yacoub N, Mishra R, White A, Yuan L, Alanazi S, et al. Current Advances in the Treatment of BRAF-Mutant Melanoma. *Cancers* 2020;12(2):482. DOI: 10.3390/cancers12020482

4. Beiu C, Giurcaneanu C, Grumezescu AM, Holban AM, Popa LG, Mihai MM. Nanosystems for Improved Targeted Therapies in Melanoma. *Journal of Clinical Medicine* 2020;9(2):318. DOI: 10.3390/jcm9020318

5. Lin YX, Wang Y, Blake S, Yu M, Mei L, Wang H, et al. RNA Nanotechnology-Mediated Cancer Immunotherapy. *Theranostics* 2020;10(1):281-99. doi: 10.7150/thno.35568

6. Samuel E, Moore M, Voskoboynik M, Shackleton M, Haydon A. An update on adjuvant systemic therapies in melanoma. *Melanoma Manag* 2019;6(3):Mmt28. doi: 10.2217/mmt-2019-0009

7. Asadujjaman M, Cho KH, Jang DJ, Kim JE, Jee JP. Nanotechnology in the arena of cancer immunotherapy. *Arch Pharm Res* 2020;43(1):58-79. doi: 10.1007/s12272-020-01207-4

8. Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. *Nat Rev Drug Discov* 2019;18(3):175-96. doi: 10.1038/s41573-018-0006-z

9. Bahreyni A, Mohamud Y, Luo H. Recent advancements in immunotherapy of melanoma using nanotechnology-based strategies. *Biomed Pharmacother* 2023;159:114243. doi: 10.1016/j.biopha.2023.114243

10. Caster JM, Callaghan C, Seyedin SN, Henderson K, Sun B, Wang AZ. Optimizing Advances in Nanoparticle Delivery for Cancer Immunotherapy. *Adv Drug Deliv Rev* 2019;144:3-15. doi: 10.1016/j.addr.2019.07.009

11. Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. *Nat Commun* 2018;9(1):1410. doi: 10.1038/s41467-018-03705-y

12. Zhou Z, Liu X, Zhu D, Wang Y, Zhang Z, Zhou X, et al. Nonviral cancer gene therapy: Delivery cascade and vector nanoproperty integration. *Adv Drug Deliv Rev* 2017;115:115-54. doi: 10.1016/j.addr.2017.07.021

13. Aagaard L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. *Adv Drug Deliv Rev* 2007;59(2-3):75-86. doi: 10.1016/j.addr.2007.03.005

14. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. *Nat Rev Drug Discov* 2014;13(8):622-38. doi: 10.1038/nrd4359

15. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol* 2018;19(11):1480-92. doi: 10.1016/s1470-2045(18)30700-9

16. Goldberg MS. Improving cancer immunotherapy through nanotechnology. *Nat Rev Cancer* 2019;19(10):587-602. doi: 10.1038/s41568-019-0186-9

17. Herzberg B, Fisher DE. Metastatic melanoma and immunotherapy. *Clin Immunol* 2016;172:105-10. doi: 10.1016/j.clim.2016.07.006

18. Linck RDM, Costa RLP, Garicochea B. Cancer immunology and melanoma immunotherapy. *An Bras Dermatol* 2017;92(6):830-5. doi: 10.1590/abd1806-4841.201756511

19. Franklin C, Livingstone E, Roesch A, Schilling B, Schadendorf D. Immunotherapy in melanoma: Recent advances and future directions. *Eur J Surg Oncol* 2017;43(3):604-11. doi: 10.1016/j.ejso.2016.07.145

20. Furue M, Ito T, Wada N, Wada M, Kadono T, Uchi H. Melanoma and Immune Checkpoint Inhibitors. *Curr Oncol Rep* 2018;20(3):29. doi: 10.1007/s11912-018-0676-z

21. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *J Clin Oncol* 2015;33(25):2780-8. doi: 10.1200/jco.2014.58.3377

22. Kohlhapp FJ, Kaufman HL. Molecular Pathways: Mechanism of Action for Talimogene Laherparepvec, a New Oncolytic Virus Immunotherapy. *Clin Cancer Res* 2016;22(5):1048-54. doi: 10.1158/1078-0432.ccr-15-2667

23. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* 2017;168(4):707-23. doi: 10.1016/j.cell.2017.01.017

24. O'Donnell JS, Long GV, Scolyer RA, Teng MWL, Smyth MJ. Resistance to PD1/PDL1 checkpoint inhibition. *Cancer Treatment Reviews* 2017;52:71-81. doi: 10.1016/j.ctrv.2016.11.007

25. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. *British Journal of Cancer* 2018;118(1):9-16. doi: 10.1038/bjc.2017.434

26. Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nature Reviews Immunology* 2020;20(1):25-39. doi: 10.1038/s41577-019-0218-4

27. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *New England Journal of Medicine* 2016;375(9):819-29. doi: 10.1056/NEJMoa1604958

28. Sade-Feldman M, Jiao YJ, Chen JH, Rooney MS, Barzily-Rokni M, Eliane J-P, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nature Communications* 2017;8(1):1136. doi: 10.1038/s41467-017-01062-w

29. Shin H, Park SJ, Yim Y, Kim J, Choi C, Won C, et al. Recent advances in RNA therapeutics and RNA delivery systems based on nanoparticles. *Advanced Therapeutics* 2018;1(7):1800065. doi: 10.1002/adtp.201800065

30. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* 2018;17(4):261-79. doi: 10.1038/nrd.2017.243

31. 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

32. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN Promotes Resistance to T Cell–Mediated Immunotherapy. *Cancer Discovery* 2016;6(2):202-16. doi: 10.1158/2159-8290.cd-15-0283

33. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* 2015;523(7559):231-5. doi: 10.1038/nature14404

34. Sioud M. Releasing the Immune System Brakes Using siRNAs Enhances Cancer Immunotherapy. *Cancers* 2019;11(2):176. DOI: 10.3390/cancers11020176

35. Qian Y, Qiao S, Dai Y, Xu G, Dai B, Lu L, et al. Molecular-Targeted Immunotherapeutic Strategy for Melanoma via Dual-Targeting Nanoparticles Delivering Small Interfering RNA to Tumor-Associated Macrophages. *ACS Nano* 2017;11(9):9536-49. doi: 10.1021/acsnano.7b05465

36. Wang Q, Lin W, Tang X, Li S, Guo L, Lin Y, et al. The Roles of microRNAs in Regulating the Expression of PD-1/PD-L1 Immune Checkpoint. *International Journal of Molecular Sciences* 2017;18(12):2540. DOI: 10.3390/ijms18122540

37. Grenda A, Krawczyk P. New Dancing Couple: PD-L1 and MicroRNA. *Scand J Immunol* 2017;86(3):130-4. doi: 10.1111/sji.12577

38. Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, et al. Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. *Nano Letters* 2017;17(3):1326-35. doi: 10.1021/acs.nanolett.6b03329

39. Grenda A, Krawczyk P. New Dancing Couple: PD-L1 and MicroRNA. *Scandinavian Journal of Immunology* 2017;86(3):130-4. doi: 10.1111/sji.12577

40. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology* 2015;33(9):941-51. doi: 10.1038/nbt.3330

41. Sindhwani S, Syed AM, Ngai J, Kingston BR, Maiorino L, Rothschild J, et al. The entry of nanoparticles into solid tumours. *Nature Materials* 2020;19(5):566-75. doi: 10.1038/s41563-019-0566-2

42. Yang T, Zhai J, Hu D, Yang R, Wang G, Li Y, et al. "Targeting Design" of Nanoparticles in Tumor Therapy. *Pharmaceutics* 2022;14(9). doi: 10.3390/pharmaceutics14091919

43. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Adv Drug Deliv Rev* 2013;65(1):71-9. doi: 10.1016/j.addr.2012.10.002

44. Yang X, Lai C, Liu A, Hou X, Tang Z, Mo F, et al. Anti-Tumor Activity of Mannose-CpG-Oligodeoxynucleotides-Conjugated and Hepatoma Lysate-Loaded Nanoliposomes for Targeting Dendritic Cells In Vivo. *J Biomed Nanotechnol* 2019;15(5):1018-32. doi: 10.1166/jbn.2019.2755

45. Sacko K, Thangavel K, Shoyele SA. Codelivery of Genistein and miRNA-29b to A549 Cells Using Aptamer-Hybrid Nanoparticle Bioconjugates. *Nanomaterials (Basel)* 2019;9(7). doi: 10.3390/nano9071052

46. Zhang LX, Xie XX, Liu DQ, Xu ZP, Liu RT. Efficient co-delivery of neo-epitopes using dispersion-stable layered double hydroxide nanoparticles for enhanced melanoma immunotherapy. *Biomaterials* 2018;174:54-66. doi: 10.1016/j.biomaterials.2018.05.015

47. Thomas SN, Vokali E, Lund AW, Hubbell JA, Swartz MA. Targeting the tumor-draining lymph node with adjuvanted nanoparticles reshapes the anti-tumor immune response. *Biomaterials* 2014;35(2):814-24. doi: 10.1016/j.biomaterials.2013.10.003

48. Irvine DJ, Hanson MC, Rakhra K, Tokatlian T. Synthetic Nanoparticles for Vaccines and Immunotherapy. *Chem Rev* 2015;115(19):11109-46. doi: 10.1021/acs.chemrev.5b00109

49. Correia-Pinto JF, Csaba N, Alonso MJ. Vaccine delivery carriers: insights and future perspectives. *Int J Pharm* 2013;440(1):27-38. doi: 10.1016/j.ijpharm.2012.04.047

50. Jia Y, Omri A, Krishnan L, McCluskie MJ. Potential applications of nanoparticles in cancer immunotherapy. *Hum Vaccin Immunother* 2017;13(1):63-74. doi: 10.1080/21645515.2016.1245251

51. Park W, Heo YJ, Han DK. New opportunities for nanoparticles in cancer immunotherapy. *Biomater Res* 2018;22:24. doi: 10.1186/s40824-018-0133-y

52. Agarwal R, Singh V, Jurney P, Shi L, Sreenivasan SV, Roy K. Mammalian cells preferentially internalize hydrogel nanodiscs over nanorods and use shape-specific uptake mechanisms. *Proc Natl Acad Sci U S A* 2013;110(43):17247-52. doi: 10.1073/pnas.1305000110

53. Carboni E, Tschudi K, Nam J, Lu X, Ma AW. Particle margination and its implications on intravenous anticancer drug delivery. *AAPS PharmSciTech* 2014;15(3):762-71. doi: 10.1208/s12249-014-0099-6

54. Foged C, Brodin B, Frokjaer S, Sundblad A. Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. *Int J Pharm* 2005;298(2):315-22. doi: 10.1016/j.ijpharm.2005.03.035

55. Buabeid MA, Arafa EA, Murtaza G. Emerging Prospects for Nanoparticle-Enabled Cancer Immunotherapy. *J Immunol Res* 2020;2020:9624532. doi: 10.1155/2020/9624532

56. Zhang R, Billingsley MM, Mitchell MJ. Biomaterials for vaccine-based cancer immunotherapy. *J Control Release* 2018;292:256-76. doi: 10.1016/j.jconrel.2018.10.008

57. Yue ZG, Wei W, Lv PP, Yue H, Wang LY, Su ZG, et al. Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles. *Biomacromolecules* 2011;12(7):2440-6. doi: 10.1021/bm101482r

58. Abd Elwakil MM, Khalil IA, Elewa YHA, Kusumoto K, Sato Y, Shobaki N, et al. Lung-Endothelium-Targeted Nanoparticles Based on a pH-Sensitive Lipid and the GALA Peptide Enable Robust Gene Silencing and the Regression of Metastatic Lung Cancer. *Advanced Functional Materials* 2019;29(18):1807677. doi: 10.1002/adfm.201807677

59. Kanasty R, Dorkin JR, Vegas A, Anderson D. Delivery materials for siRNA therapeutics. *Nat Mater* 2013;12(11):967-77. doi: 10.1038/nmat3765

60. Sui Y, Hou X, Zhang J, Hong X, Wang H, Xiao Y, et al. Lipid nanoparticle-mediated targeted mRNA delivery and its application in cancer therapy. *J Mater Chem B* 2025;13(33):10085-117. doi: 10.1039/d5tb01556a

61. Estapé Senti M, García Del Valle L, Schiffelers RM. mRNA delivery systems for cancer immunotherapy: Lipid nanoparticles and beyond. *Adv Drug Deliv Rev* 2024;206:115190. doi: 10.1016/j.addr.2024.115190

62. Farooq MA, Johnston APR, Trevaskis NL. Impact of nanoparticle properties on immune cell interactions in the lymph node. *Acta Biomater* 2025;193:65-82. doi: 10.1016/j.actbio.2024.12.039

63. Shan Z, Liu F. Opportunities, obstacles and challenges of nano-immunotherapy in melanoma. *Front Immunol* 2025;16:1611423. doi: 10.3389/fimmu.2025.1611423

64. Fereidouni M, Roointanpour Y, Movahed H, Taghipour A, Vafafar A, Mofazzal Jahromi MA. Advances in the systemic administration of nanotechnology-mediated drug delivery systems in melanoma treatment: a systematic review. *Discover Applied Sciences* 2025;7(6):512. doi: 10.1007/s42452-025-06998-z

65. Musetti S, Huang L. Nanoparticle-Mediated Remodeling of the Tumor Microenvironment to Enhance Immunotherapy. *ACS Nano* 2018;12(12):11740-55. doi: 10.1021/acsnano.8b05893

66. Zang X, Zhang X, Zhao X, Hu H, Qiao M, Deng Y, et al. Targeted Delivery of miRNA 155 to Tumor Associated Macrophages for Tumor Immunotherapy. *Mol Pharm* 2019;16(4):1714-22. doi: 10.1021/acs.molpharmaceut.9b00065

67. Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. *Nat Mater* 2013;12(11):991-1003. doi: 10.1038/nmat3776

68. Lou J, Zhang L, Zheng G. Advancing Cancer Immunotherapies with Nanotechnology. *Advanced Therapeutics* 2019;2(4):1800128. doi: 10.1002/adtp.201800128

69. Cai L, Xu J, Yang Z, Tong R, Dong Z, Wang C, et al. Engineered biomaterials for cancer immunotherapy. *MedComm (2020)* 2020;1(1):35-46. doi: 10.1002/mco2.8

70. Zhu Y, Xue J, Chen W, Bai S, Zheng T, He C, et al. Albumin-biomineralized nanoparticles to synergize phototherapy and immunotherapy against melanoma. *J Control Release* 2020;322:300-11. doi: 10.1016/j.jconrel.2020.03.045

71. Zhang Q, Guo X, Cheng Y, Chudal L, Pandey NK, Zhang J, et al. Use of copper-cysteamine nanoparticles to simultaneously enable radiotherapy, oxidative therapy and immunotherapy for melanoma treatment. *Signal Transduct Target Ther* 2020;5(1):58. doi: 10.1038/s41392-020-0156-4

72. Xie YQ, Arik H, Wei L, Zheng Y, Suh H, Irvine DJ, et al. Redox-responsive interleukin-2 nanogel specifically and safely promotes the proliferation and memory precursor differentiation of tumor-reactive T-cells. *Biomater Sci* 2019;7(4):1345-57. doi: 10.1039/c8bm01556b

73. Trombino S, Cassano R. Special Issue on Designing Hydrogels for Controlled Drug Delivery: Guest Editors' Introduction. *Pharmaceutics* 2020;12(1). doi: 10.3390/pharmaceutics12010057

74. Tang L, Zheng Y, Mabardi L, Irvine DJ. T lymphocyte engineering with cytokine nanogels for enhanced cancer immunotherapy. *Journal for ImmunoTherapy of Cancer* 2015;3(2):P54. doi: 10.1186/2051-1426-3-S2-P54

75. Lv Q, He C, Quan F, Yu S, Chen X. DOX/IL-2/IFN-γ co-loaded thermo-sensitive polypeptide hydrogel for efficient melanoma treatment. *Bioact Mater* 2018;3(1):118-28. doi: 10.1016/j.bioactmat.2017.08.003

76. Zhang Y, Li N, Suh H, Irvine DJ. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. *Nat Commun* 2018;9(1):6. doi: 10.1038/s41467-017-02251-3

77. Guo X, Huang L. Recent advances in nonviral vectors for gene delivery. *Acc Chem Res* 2012;45(7):971-9. doi: 10.1021/ar200151m

78. Wang Y, Lin YX, Qiao ZY, An HW, Qiao SL, Wang L, et al. Self-assembled autophagy-inducing polymeric nanoparticles for breast cancer interference in-vivo. *Adv Mater* 2015;27(16):2627-34. doi: 10.1002/adma.201405926

79. Loh XJ, Lee TC, Dou Q, Deen GR. Utilising inorganic nanocarriers for gene delivery. *Biomater Sci* 2016;4(1):70-86. doi: 10.1039/c5bm00277j

80. Shen J, Zhang W, Qi R, Mao ZW, Shen H. Engineering functional inorganic-organic hybrid systems: advances in siRNA therapeutics. *Chem Soc Rev* 2018;47(6):1969-95. doi: 10.1039/c7cs00479f

81. Pecot CV, Calin GA, Coleman RL, Lopez-Berestein G, Sood AK. RNA interference in the clinic: challenges and future directions. *Nat Rev Cancer* 2011;11(1):59-67. doi: 10.1038/nrc2966

82. Xiong Q, Lee GY, Ding J, Li W, Shi J. Biomedical applications of mRNA nanomedicine. *Nano Res* 2018;11(10):5281-309. doi: 10.1007/s12274-018-2146-1

83. Islam MA, Xu Y, Tao W, Ubellacker JM, Lim M, Aum D, et al. Restoration of tumour-growth suppression in vivo via systemic nanoparticle-mediated delivery of PTEN mRNA. *Nat Biomed Eng* 2018;2(11):850-64. doi: 10.1038/s41551-018-0284-0

84. Xu X, Wu J, Liu Y, Saw PE, Tao W, Yu M, et al. Multifunctional Envelope-Type siRNA Delivery Nanoparticle Platform for Prostate Cancer Therapy. *ACS Nano* 2017;11(3):2618-27. doi: 10.1021/acsnano.6b07195

85. Liu Y, Ji X, Tong WWL, Askhatova D, Yang T, Cheng H, et al. Engineering Multifunctional RNAi Nanomedicine To Concurrently Target Cancer Hallmarks for Combinatorial Therapy. *Angew Chem Int Ed Engl* 2018;57(6):1510-3. doi: 10.1002/anie.201710144

86. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer* 2017;17(1):20-37. doi: 10.1038/nrc.2016.108

87. Xu X, Wu J, Liu S, Saw PE, Tao W, Li Y, et al. Redox-Responsive Nanoparticle-Mediated Systemic RNAi for Effective Cancer Therapy. *Small* 2018;14(41):e1802565. doi: 10.1002/smll.201802565

88. Singha K, Namgung R, Kim WJ. Polymers in small-interfering RNA delivery. *Nucleic Acid Ther* 2011;21(3):133-47. doi: 10.1089/nat.2011.0293

89. Samal SK, Dash M, Van Vlierberghe S, Kaplan DL, Chiellini E, van Blitterswijk C, et al. Cationic polymers and their therapeutic potential. *Chem Soc Rev* 2012;41(21):7147-94. doi: 10.1039/c2cs35094g

90. Samanta A, Banerjee S, Liu Y. DNA nanotechnology for nanophotonic applications. *Nanoscale* 2015;7(6):2210-20. doi: 10.1039/c4nr06283c

91. Stulz E. DNA architectonics: towards the next generation of bio-inspired materials. *Chemistry* 2012;18(15):4456-69. doi: 10.1002/chem.201102908

92. van den Boorn JG, Dassler J, Coch C, Schlee M, Hartmann G. Exosomes as nucleic acid nanocarriers. *Adv Drug Deliv Rev* 2013;65(3):331-5. doi: 10.1016/j.addr.2012.06.011

93. Evans BC, Nelson CE, Yu SS, Beavers KR, Kim AJ, Li H, et al. Ex vivo red blood cell hemolysis assay for the evaluation of pH-responsive endosomolytic agents for cytosolic delivery of biomacromolecular drugs. *J Vis Exp* 2013(73):e50166. doi: 10.3791/50166

94. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. *Biochim Biophys Acta* 2014;1846(1):75-87. doi: 10.1016/j.bbcan.2014.04.005

95. Zhang P, Liu G, Chen X. Nanobiotechnology: Cell Membrane-Based Delivery Systems. *Nano Today* 2017;13:7-9. doi: 10.1016/j.nantod.2016.10.008

96. Popat A, Hartono SB, Stahr F, Liu J, Qiao SZ, Qing Max Lu G. Mesoporous silica nanoparticles for bioadsorption, enzyme immobilisation, and delivery carriers. *Nanoscale* 2011;3(7):2801-18. doi: 10.1039/c1nr10224a

97. Hom C, Lu J, Liong M, Luo H, Li Z, Zink JI, et al. Mesoporous silica nanoparticles facilitate delivery of siRNA to shutdown signaling pathways in mammalian cells. *Small* 2010;6(11):1185-90. doi: 10.1002/smll.200901966

98. Lee T, Yagati AK, Pi F, Sharma A, Choi JW, Guo P. Construction of RNA-Quantum Dot Chimera for Nanoscale Resistive Biomemory Application. *ACS Nano* 2015;9(7):6675-82. doi: 10.1021/acsnano.5b03269

99. He C, Lu K, Liu D, Lin W. Nanoscale metal-organic frameworks for the co-delivery of cisplatin and pooled siRNAs to enhance therapeutic efficacy in drug-resistant ovarian cancer cells. *J Am Chem Soc* 2014;136(14):5181-4. doi: 10.1021/ja4098862

100. Ke W, Afonin KA. Exosomes as natural delivery carriers for programmable therapeutic nucleic acid nanoparticles (NANPs). *Adv Drug Deliv Rev* 2021;176:113835. doi: 10.1016/j.addr.2021.113835

101. Lener T, Gimona M, Aigner L, Börger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J Extracell Vesicles* 2015;4:30087. doi: 10.3402/jev.v4.30087

102. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001;411(6836):494-8. doi: 10.1038/35078107

103. Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature* 2004;431(7006):343-9. doi: 10.1038/nature02873

104. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev* 2001;15(2):188-200. doi: 10.1101/gad.862301

105. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136(2):215-33. doi: 10.1016/j.cell.2009.01.002

106. Bartel DP. Metazoan MicroRNAs. *Cell* 2018;173(1):20-51. doi: 10.1016/j.cell.2018.03.006

107. Tan L, Sun X. Recent advances in mRNA vaccine delivery. *Nano Research* 2018;11(10):5338-54. doi: 10.1007/s12274-018-2091-z

108. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* 2016;3:16011. doi: 10.1038/mto.2016.11

109. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013;369(2):134-44. doi: 10.1056/NEJMoa1305133

110. Anfossi S, Babayan A, Pantel K, Calin GA. Clinical utility of circulating non-coding RNAs - an update. *Nat Rev Clin Oncol* 2018;15(9):541-63. doi: 10.1038/s41571-018-0035-x

111. Ghafouri-Fard S, Ghafouri-Fard S. siRNA and cancer immunotherapy. *Immunotherapy* 2012;4(9):907-17. doi: 10.2217/imt.12.87

112. Aleku M, Schulz P, Keil O, Santel A, Schaeper U, Dieckhoff B, et al. Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression. *Cancer Res* 2008;68(23):9788-98. doi: 10.1158/0008-5472.can-08-2428

113. Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Pérez-Gracia JL, et al. Cytokines in clinical cancer immunotherapy. *Br J Cancer* 2019;120(1):6-15. doi: 10.1038/s41416-018-0328-y

114. Wang D, Wang T, Liu J, Yu H, Jiao S, Feng B, et al. Acid-Activatable Versatile Micelleplexes for PD-L1 Blockade-Enhanced Cancer Photodynamic Immunotherapy. *Nano Lett* 2016;16(9):5503-13. doi: 10.1021/acs.nanolett.6b01994

115. Dai L, Li K, Li M, Zhao X, Luo Z, Lu L, et al. Size/Charge Changeable Acidity-Responsive Micelleplex for Photodynamic-Improved PD-L1 Immunotherapy with Enhanced Tumor Penetration. *Advanced Functional Materials* 2018;28(18):1707249. doi: 10.1002/adfm.201707249

116. Vasievich EA, Huang L. The suppressive tumor microenvironment: a challenge in cancer immunotherapy. *Mol Pharm* 2011;8(3):635-41. doi: 10.1021/mp1004228

117. Wang Y, Lin YX, Qiao SL, Wang J, Wang H. Progress in Tumor-Associated Macrophages: From Bench to Bedside. *Adv Biosyst* 2019;3(2):e1800232. doi: 10.1002/adbi.201800232

118. Sioud M. Releasing the Immune System Brakes Using siRNAs Enhances Cancer Immunotherapy. *Cancers (Basel)* 2019;11(2). doi: 10.3390/cancers11020176

119. Conroy H, Galvin KC, Higgins SC, Mills KH. Gene silencing of TGF-β1 enhances antitumor immunity induced with a dendritic cell vaccine by reducing tumor-associated regulatory T cells. *Cancer Immunol Immunother* 2012;61(3):425-31. doi: 10.1007/s00262-011-1188-y

120. Zheng X, Koropatnick J, Chen D, Velenosi T, Ling H, Zhang X, et al. Silencing IDO in dendritic cells: a novel approach to enhance cancer immunotherapy in a murine breast cancer model. *Int J Cancer* 2013;132(4):967-77. doi: 10.1002/ijc.27710

121. Heo MB, Cho MY, Lim YT. Polymer nanoparticles for enhanced immune response: combined delivery of tumor antigen and small interference RNA for immunosuppressive gene to dendritic cells. *Acta Biomater* 2014;10(5):2169-76. doi: 10.1016/j.actbio.2013.12.050

122. Denaro N, Merlano MC, Lo Nigro C. Long noncoding RNAs as regulators of cancer immunity. *Mol Oncol* 2019;13(1):61-73. doi: 10.1002/1878-0261.12413

123. Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM. MicroRNA expression and function in cancer. *Trends Mol Med* 2006;12(12):580-7. doi: 10.1016/j.molmed.2006.10.006

124. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007;302(1):1-12. doi: 10.1016/j.ydbio.2006.08.028

125. Mirzaei H, Masoudifar A, Sahebkar A, Zare N, Sadri Nahand J, Rashidi B, et al. MicroRNA: A novel target of curcumin in cancer therapy. *J Cell Physiol* 2018;233(4):3004-15. doi: 10.1002/jcp.26055

126. Hosseinahli N, Aghapour M, Duijf PHG, Baradaran B. Treating cancer with microRNA replacement therapy: A literature review. *J Cell Physiol* 2018;233(8):5574-88. doi: 10.1002/jcp.26514

127. Lu LF, Liston A. MicroRNA in the immune system, microRNA as an immune system. *Immunology* 2009;127(3):291-8. doi: 10.1111/j.1365-2567.2009.03092.x

128. Wang Q, Lin W, Tang X, Li S, Guo L, Lin Y, et al. The Roles of microRNAs in Regulating the Expression of PD-1/PD-L1 Immune Checkpoint. *Int J Mol Sci* 2017;18(12). doi: 10.3390/ijms18122540

129. Parayath NN, Parikh A, Amiji MM. Repolarization of Tumor-Associated Macrophages in a Genetically Engineered Nonsmall Cell Lung Cancer Model by Intraperitoneal Administration of Hyaluronic Acid-Based Nanoparticles Encapsulating MicroRNA-125b. *Nano Lett* 2018;18(6):3571-9. doi: 10.1021/acs.nanolett.8b00689

130. Yamamoto A, Kormann M, Rosenecker J, Rudolph C. Current prospects for mRNA gene delivery. *Eur J Pharm Biopharm* 2009;71(3):484-9. doi: 10.1016/j.ejpb.2008.09.016

131. Islam MA, Reesor EK, Xu Y, Zope HR, Zetter BR, Shi J. Biomaterials for mRNA delivery. *Biomater Sci* 2015;3(12):1519-33. doi: 10.1039/c5bm00198f

132. Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. *Mol Ther* 2019;27(4):757-72. doi: 10.1016/j.ymthe.2019.01.020

133. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. *Ther Deliv* 2016;7(5):319-34. doi: 10.4155/tde-2016-0006

134. Weiss R, Scheiblhofer S, Roesler E, Weinberger E, Thalhamer J. mRNA vaccination as a safe approach for specific protection from type I allergy. *Expert Rev Vaccines* 2012;11(1):55-67. doi: 10.1586/erv.11.168

135. Li Y, Wang M, Peng X, Yang Y, Chen Q, Liu J, et al. mRNA vaccine in cancer therapy: Current advance and future outlook. *Clin Transl Med* 2023;13(8):e1384. doi: 10.1002/ctm2.1384

136. Tang X, Rao J, Yin S, Wei J, Xia C, Li M, et al. PD-L1 knockdown via hybrid micelle promotes paclitaxel induced Cancer-Immunity Cycle for melanoma treatment. *Eur J Pharm Sci* 2019;127:161-74. doi: 10.1016/j.ejps.2018.10.021

137. Gulla SK, Kotcherlakota R, Nimushakavi S, Nimmu NV, Khalid S, Patra CR, et al. Au-CGKRK Nanoconjugates for Combating Cancer through T-Cell-Driven Therapeutic RNA Interference. *ACS Omega* 2018;3(8):8663-76. doi: 10.1021/acsomega.8b01051

138. Wang Y, Xu Z, Guo S, Zhang L, Sharma A, Robertson GP, et al. Intravenous delivery of siRNA targeting CD47 effectively inhibits melanoma tumor growth and lung metastasis. *Mol Ther* 2013;21(10):1919-29. doi: 10.1038/mt.2013.135

139. Li SY, Liu Y, Xu CF, Shen S, Sun R, Du XJ, et al. Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation. *J Control Release* 2016;231:17-28. doi: 10.1016/j.jconrel.2016.01.044

140. Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, et al. Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. *Nano Lett* 2017;17(3):1326-35. doi: 10.1021/acs.nanolett.6b03329

141. Shah PD, Huang AC, Xu X, Orlowski R, Amaravadi RK, Schuchter LM, et al. Phase I Trial of Autologous RNA-electroporated cMET-directed CAR T Cells Administered Intravenously in Patients with Melanoma and Breast Carcinoma. *Cancer Res Commun* 2023;3(5):821-9. doi: 10.1158/2767-9764.crc-22-0486

142. Miller M, Sahin U, Derhovanessian E, Kloke B, Simon P, Bukur V, et al. IVAC MUTANOME: A first-in-human phase I clinical trial targeting individual mutant neoantigens for the treatment of melanoma. *Annals of Oncology* 2017;28:xi1-xi2. DOI:10.1158/1538-7445.AM2016-CT022

143. Dannull J, Haley NR, Archer G, Nair S, Boczkowski D, Harper M, et al. Melanoma immunotherapy using mature DCs expressing the constitutive proteasome. *J Clin Invest* 2013;123(7):3135-45. doi: 10.1172/jci67544

144. Weber JS, Carlino MS, Khattak A, Meniawy T, Ansstas G, Taylor MH, et al. Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma

(KEYNOTE-942): a randomised, phase 2b study. *Lancet* 2024;403(10427):632-44. doi: 10.1016/s0140-6736(23)02268-7

145. Van Woensel M, Mathivet T, Wauthoz N, Rosière R, Garg AD, Agostinis P, et al. Sensitization of glioblastoma tumor micro-environment to chemo- and immunotherapy by Galectin-1 intranasal knock-down strategy. *Sci Rep* 2017;7(1):1217. doi: 10.1038/s41598-017-01279-1

146. Huang T, Peng L, Han Y, Wang D, He X, Wang J, et al. Lipid nanoparticle-based mRNA vaccines in cancers: Current advances and future prospects. *Front Immunol* 2022;13:922301. doi: 10.3389/fimmu.2022.922301