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Review article

Regulatory insights into nanomedicine and gene vaccine innovation: Safety assessment, challenges, and regulatory perspectives

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ABSTRACT

This analysis explores the principal regulatory concerns linked to nanomedicines and gene vaccines, including the complexities involved and the perspectives on how to navigate them. In the realm of nanomedicines, ensuring the safety of nanomaterials is paramount due to their unique characteristics and potential interactions with biological systems. Regulatory bodies are actively formulating guidelines and standards to assess the safety and risks associated with nanomedicine products, emphasizing the need for standardized characterization techniques to accurately gauge their safety and effectiveness. Regarding gene vaccines, regulatory frameworks must be tailored to address the distinct challenges posed by genetic interventions, necessitating special considerations in safety and efficacy evaluations, particularly concerning vector design, target specificity, and long-term patient monitoring. Ethical concerns such as patient autonomy, informed consent, and privacy also demand careful attention, alongside the intricate matter of intellectual property rights, which must be balanced against the imperative of ensuring widespread access to these life-saving treatments. Collaborative efforts among regulatory bodies, researchers, patent offices, and the private sector are essential to tackle these challenges effectively, with international cooperation being especially crucial given the global scope of nanomedicine and genetic vaccine development. Striking the right balance between safeguarding intellectual properties and promoting public health is vital for fostering innovation and ensuring equitable access to these ground-breaking technologies, underscoring the significance of addressing these regulatory hurdles to fully harness the potential benefits of nanomedicine and gene vaccines for enhancing healthcare outcomes on a global scale.

Abbreviations: ADR, Adverse Drug Reactions; APCs, Antigen Presenting Cells; CNTs, Carbon Nanotubes; COVID-19, Corona Virus Disease 2019; CTAB, Cetyltrimethylammonium Bromide; CTLs, Cytotoxic T-lymphocytes; cryo-TEM, Cryo-Transmission Electron Microscopy; DNA, Deoxyribonucleic Acid; DOTAP, Dioleoyl-3-Trimethylammonium Propane; E1, E1A, E1B E2, E3, E4, Early Regions of Adeno Virus; E4ORF3, E4ORF6, Early Region and Open Reading Frames of Adeno Virus; ECM, Extracellular Matrix; EMA, European Medicines Agency; ETP, Etoposide; FAS-ligand, Ligand for Subgroup of TNF-R; FDA, Food and Drug Administration; FFP, Freedom From Progression; HPV, Human Papillomavirus; HSPGs, Heparan sulfate proteoglycans; ISGF3, Interferon-Stimulated Gene Factor 3; LLC, Lewis Lung Cancer; LNPs, Lipid Nanoparticles; LPHNs, Lipid-Polymer Hybrid Nanoparticles; MHC, Major Histocompatibility Complex; MRI, Magnetic Resonance Imaging; mRNA, Messenger Ribonucleic Acid; NF-κB, Nuclear Factor Kappa B; NLCs, Nanostructured Lipid Carriers; NSCLC, Non-Small Cell Lung Cancer; QDs, Quantum Dots; ORR, Overall Response Rate; OS, Overall Survival; PAMAM, Polyamidoamine; PDT, Photodynamic Therapies; PEI, Polyethyleneimine; PEG, Poly-(ethylene glycol); PEG-PE, Poly-(ethylene glycol)-phosphatidylethanolamine; PFS, Progression Free Survival; PHLNPs, Polymer-lipid hybrid nanoparticles; PLGA, Poly-D,L-lactide-co-glycolide; PLL, Poly(l-lysine); PTT, Photothermal Treatments; pRB, Retinoblastoma Protein; RES, Retinal Endothelial System; RFS, Recurrence-Free Survival; RNA, Ribonucleic Acid; SANS, Small Angle Neutron Scattering; SAXS, Small Angle X-Ray Scattering; SCLC, Small Cell Lung Cancer; SLNs, Solid Lipid Nanoparticles; TERT, Telomerase Reverse Transcriptase; TNF-α, Tumour Necrosis Factor.

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Statement of significance

Several biomaterials are being proposed for the development of nanovaccines, from polymeric micelles, PLGA-/PEI-/PLL-nanoparticles, solid lipid nananoparticles, cationic lipoplexes, liposomes, hybrid materials, dendrimers, carbon nanotubes, hydrogels, to quantum dots. Lipid nanoparticles (LNPs) have gained tremendous attention since the US Food and Drug Administration (FDA) approval of Pfizer and Moderna's COVID-19 vaccines, raising public awareness to the regulatory challenges associated with nanomedicines and genetic vaccines. This review provides insights into the current perspectives and potential strategies for addressing these issues, including clinical trials. By navigating these regulatory landscapes effectively, we can unlock the full potential of nanomedicine and genetic vaccines using a range of promising biomaterials towards improving healthcare outcomes worldwide.

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1. Introduction

Nanomedicines and gene vaccines hold immense potential for revolutionizing healthcare by offering innovative approaches to diagnostics, drug delivery, and disease prevention. However, their unique characteristics present significant regulatory challenges that must be addressed to ensure their safe and effective use. In recent years, nanomedicine and gene vaccines have emerged as promising approaches with the potential to revolutionize cancer therapy [1]. Gene vaccines harness the power of genetic material to stimulate immune responses against cancer cells. These innovative technologies hold immense potential to enhance patient outcomes and improve public health. Nanomedicine utilizes nanoscale materials and devices for precise drug delivery, diagnostic imaging, and targeted therapies [2]. These cutting-edge technologies hold immense promise for improving patient outcomes and addressing unmet medical needs in cancer treatment [3,4]

However, the development and utilization of nanomedicine and gene vaccines present a myriad of regulatory considerations [5]. The safety, efficacy, and ethical implications of these cutting-edge approaches necessitate robust regulatory frameworks to safeguard patient well-being and ensure equitable access to these therapies [6]. This review aims to provide a comprehensive overview of the advancements and regulatory challenges associated with nanomedicine and gene vaccines for the treatment of cancer.

The use of genetic interventions for vaccination requires the adaptation of existing regulatory frameworks to account for the unique considerations associated with this approach. Gene vaccines utilize genetic material, such as RNA and DNA, to elicit an immune response against specific antigens. Safety and efficacy evaluations of gene vaccines need to incorporate specific considerations such as vector design, target specificity, and long-term patient follow-up [7,8]. The design and delivery of the genetic payload, the choice of vectors, and the assessment of immune responses are crucial factors that influence the effectiveness and safety of gene vaccines. Ethical considerations related to patient autonomy, informed consent, and privacy must be carefully addressed in the development and deployment of gene vaccines. The use of genetic information raises ethics issues about data protection, discrimination, privacy, besides the potential unintended consequences. Informed consent processes should ensure that patients are adequately informed about the risks and benefits of gene vaccines and have the autonomy to make informed decisions about their participation [9,10].

The field of nanomedicine, encompassing nanomaterials for therapeutic and diagnostic purposes, requires rigorous safety assessment due to the distinct properties of nanomaterials and their potential biological interactions [11,12]. Regulatory agencies around the world are actively engaged in developing guidelines and standards for evaluating the safety and risk assessments of nanomedicine products. Central to this effort is the standardization of characterization techniques for nanomaterials to ensure accurate assessment of their safety, efficacy, and quality. Safety assessment of nanomaterials involves understanding their physicochemical properties, potential toxicity, and biodistribution within the body [13]. Standardization of characterization techniques for nanomaterials is a critical concern to ensure accurate assessment of their safety and efficacy. Methods, such as transmission electron microscopy, scanning electron microscopy, atomic force microscopy and dynamic light scattering, are commonly employed for characterizing nanomaterials. The development of standardized protocols and reference materials will facilitate reliable characterization and comparability of nanomedicine products across different studies and regulatory submissions [14,15].

This work highlights the importance of safety assessment of nanomaterials and nanodevices, considering their unique physicochemical properties and potential biological interactions. Standardization of characterization techniques for nanomaterials is also discussed as a crucial aspect of regulatory evaluation [16]. Furthermore, the absence of appropriate classification and regulatory pathways for nanomedicine products is examined, emphasizing the need for tailored guidelines and regulatory frameworks to accommodate the specific challenges of this field. The development of an appropriate regulatory framework to assess the safety and efficacy of gene vaccines is of paramount importance [17]. Considerations for vector design, delivery methods, target specificity, and long-term follow-up are discussed. Ethical considerations related to patient autonomy, privacy, and informed consent are also highlighted. The complex landscape of intellectual property rights in genetic vaccine regulation is addressed, emphasizing the need to balance patent protection with the goal of affordable access to these life-saving interventions.

Intellectual property rights pose another significant challenge in the regulatory landscape of gene vaccines. The complex and evolving patent landscape surrounding genetic material, particularly genes or sequences, presents uncertainties regarding the scope and validity of patents related to gene vaccines. Issues of patent ownership, licensing agreements, and fair compensation further complicate the landscape, particularly in cases where multiple parties contribute to the development of gene vaccines. The accessibility and affordability of gene vaccines can be impacted by intellectual property rights, potentially limiting competition, creating monopolies, and hindering equitable access, particularly in low-income countries or regions with limited resources [16,18]. Striking a balance between intellectual property protection and promoting public health interests necessitates collaborative efforts and alternative models, such as patent pools or patent-sharing agreements, to encourage innovation, technology transfer, and wider availability of genetic vaccine technologies [2].

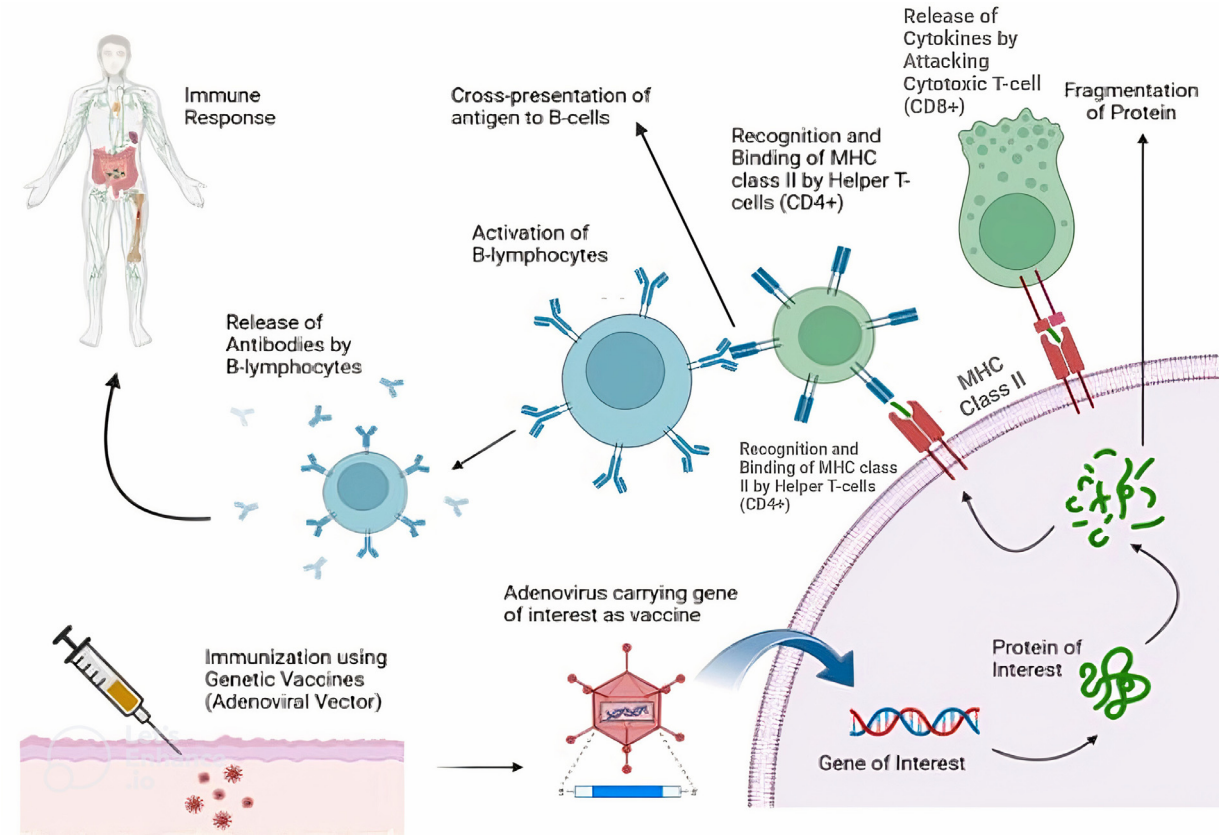

Fig. 1. Adeno virus based mechanism of polymeric, lipid and polymer-lipid nanoparticle as cancer vaccines.

Overall, this article brings together key insights from multiple sources, providing a comprehensive understanding of the advancements, challenges, and regulatory considerations in nanomedicine and gene vaccines for cancer treatment. By delving into the complexities surrounding safety assessment, standardization, classification, ethical considerations, and intellectual property, this article aims to contribute to the development of robust and effective regulatory frameworks that promote innovation while ensuring equitable access of patients to these transformative therapies and their safety.

2. Genetic therapy and immune recognition

Notably popular gene therapy carriers are adenoviruses. The adenoviral shell architecture is too tiny to allow for the appropriate packaging of additional DNA [19]. Therefore, a corresponding amount of viral genetic material needs to be deleted in order to introduce a desired gene into the adenoviral genome. Fig. 1 provides an understanding of how gene vaccines (adenoviral based) is mechanistically involved in immune recognition or stimulating an immune response.

Additionally, it is necessary to reduce the pathogenic impact of the virus [17]. Despite being significantly less virulent, the initial E1-deleted adenoviral vehicles immediately generated innate immune reactions. Vectors were altered to remove more unnecessary transcriptional areas in an effort to reduce stimulation of the immune system altogether. Vectors were produced across multiple cycles. The least immunomodulatory and allergenic of these were found in 'gutless' vectors, which had the whole adenoviral genome removed [20]. Subsequently, these viral vectors have been shown to cause a diverse range of mechanistic immune response based on the modification in the early transcript region. Investigations have shown that, deleted E1 vectors portray a decreased interferon resistance than compared to the deleted E4 viral vectors [21]. Additionally, viral vectors with deleted E2 and E3 regions play a significant role in the boosting of the immune responses and reactions. In several comparative studies, it has been proved that the deleted E1A vectors show a better status in inhibition of the immune response, by gaining resistance to interferon response due to suppression of the synthesis of interferon-stimulated gene factor 3 [ISGF3] complex. Additionally, residual amino acid products of E1A, namely, 243R and 289R show strong binding potency to vital cell cycle regulatory proteins such as the retinoblastoma protein [pRB], in-turn helping in the repression of the immune response. Furthermore, studies have also provided evidence of anti-apoptotic activity of the complex formed by the binding of E1B with E4, by obstructing the p53 transcriptional activity and host mRNA transport [21–23].

Ensuring the safety of genetic material and gene therapy is imperative for their effective clinical translation. Safety assessment strategies encompass a multifaceted approach, addressing various aspects including vector design, delivery methods, potential off-target effects, and immunogenicity [24]. Vector design optimization focuses on enhancing target specificity while minimizing cytotoxicity and immune responses. Delivery methods, ranging from viral vectors to non-viral nanoparticles, undergo rigorous evaluation to determine their efficiency, targeting capability, and potential toxicity. Assessing off-target effects involves comprehensive genomic analyses to identify unintended genetic alterations and mitigate their risks. Additionally, evaluating immunogenicity aims to minimize immune responses against the therapeutic gene product or vector components, which may compromise treatment efficacy or trigger adverse reactions [25,26]. Preclinical studies, utilizing appropriate animal models, play a crucial role in elucidating the safety profile of gene therapy interventions before clinical trials. Furthermore, continuous monitoring and long-term follow-up

of treated individuals are essential for detecting any delayed adverse events and ensuring the sustained safety and efficacy of gene therapy approaches [27,28].

Adenoviral genomic transfer into the cell genome causes transcription of both native and viral genomes, increasing the aggregate quantity of DNA in the cell and leading to the induction of cell-mediated defences [29]. The adenovirus, nevertheless, has developed specifically to overcome such immune defences. E4 peptides of the viral vectors take over the mechanisms connected to the DNA repair processes. A total of six different E4 proteins have been identified, from which E4ORF3 and E4ORF6 form complexes with the E1B protein, hence, regulating the gene expressions of the adenoviral vectors [30]. The E3 zone genes also inhibit inflammatory reactions brought on by adenoviral gene transfer. Hybrid viral vectors with the E3 region inserted reduce the maximum innate defensive reaction, allowing for long-lasting adenoviral expression of genes. Additionally, E3 proteins have specifically different functions based on the molecular weight of its respective components, E3–10.4 and 14.5 kDa inhibits the TNF-α and FAS-ligand based immune responses. Furthermore, E3-14.7 kDa and E3-19 kDa inhibits the NF-κB based immune response and presentation mechanism by the MHC class-I molecule respectively [31].

It is important to thoroughly comprehend the operational significance of adenoviral peptide involvement with immunity from within, to accomplish a successful gene therapy. Maintaining the survivability of cells and reducing inflammation are necessary for gene therapy, which calls for the continued production of therapeutic translated proteins by the transformed host cell [32]. Additionally, immune-modulating medications like cyclophosphamide, which have been demonstrated to improve the efficiency of adenoviral carriers in multiple organisms at non-toxic levels, might further decrease inflammatory conditions. The degree of inflammatory molecules that the diseased cell induces will determine the cumulative strength of the central immune activity [33]. Adenoviruses may now infiltrate a wider range of cells thanks to fibre changes, which raises the likelihood of negative side effects, particularly when systemically administered. However, to efficiently address metastatic illness or the in vivo conversion of vast numbers of cells, intravascular administration of gene carriers is crucial [34].

3. Polymeric nanomedicines for cancer vaccines

Polymerized nanomedicines maneuvered in the administration of drugs may utilize its therapeutic efficacy in restricted circumstances and potentially alter its physical as well as biochemical characteristics in response to a range of biochemical signals and detrimental alterations. By making use of the unique differences in the metabolic functions between malignant tumors and normal tissues, these nanosystems may enhance the distribution in the body and subcellular positioning of medicines [35]. Because NPs are specially picked up by the leakier vessels in tumor beds than tiny particles and are retained due to the circuitous lymphatics, they enable the preferred circulation of drugs to malignancies during cancer chemotherapy [36]. In recognition of their effective ability to load medicines and/or condensed genes, polymeric NPs play a significant role as co-delivery systems (Fig. 2). Polymers have several beneficial properties, including enhanced site-specific delivery, regulated release of medicinal substances, and protection against content degradation [37]. Additionally, PEG moieties, as well as targeting agents, can be added to the scaffolds of polymeric vehicles to increase the delivery of medication and/or gene materials to tumors. For simultaneous infusions of cytotoxic medicines or other therapeutic bioactives, a variety of polymers with various physicochemical properties have been proposed [38].

3.1. Polymeric micelles

Different polymers with amphiphilic qualities have been used to create micelles throughout the past few decades for non-polar

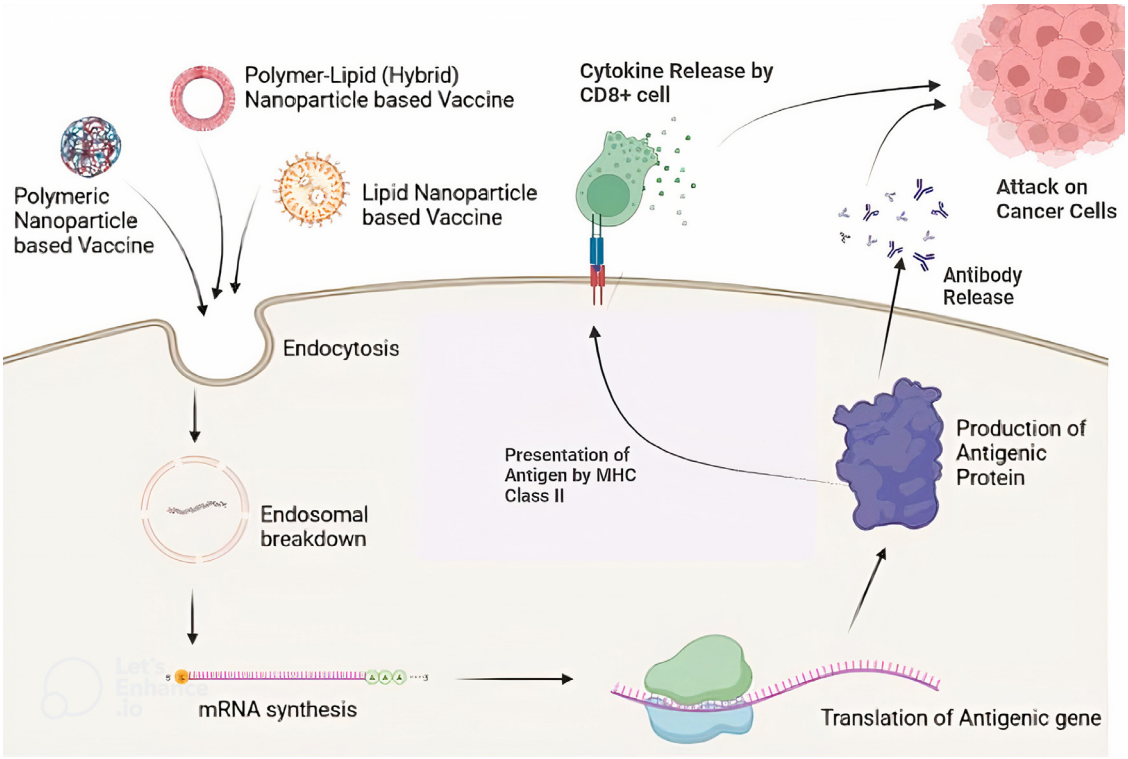

Fig. 2. Mechanism of genetic vaccine and its role in immune response activation.

drug delivery applications. Amphiphilic polymers, which self-assemble to produce vesicles with a diameter in the nanometer range, are used to create micelles, as was previously mentioned [39]. Due to their nano-size, micelles are able to cross the damaged endothelial cell junction and finally assemble in the tumor microenvironment. Since extravasation of nanosized particles is size-dependent, polymeric micelles are more effectively internalized than other nano-sized vesicles like liposomes and solid lipid nanoparticles [40]. The water-friendly poly(ethylene glycol) (PEG) covering may also prevent the vesicles from being recognized by the retinal endothelial system (RES), thus avoiding their clearance in the liver and spleen. Utilizing ligand-attached amphiphilic polymers, the surface of micelles may be readily changed, and can therefore be employed for active targeting [41]. Several amphiphilic molecules, such as poly(ethylene glycol)-phosphatidylethanolamine (PEG-PE), PEG-PPO-PEG co-polymer, PEG-amino acidic substances, and PEG-carbonates, have been used to create micelles [42]. The connecting of PE with the water-friendly PEG is one of several possible ways to create amphiphilic polymers by mixing hydrophobic as well as hydrophilic blocks, and it only requires a single conjugation phase between amine- or acidic terminal PE. The synthesis has good lipophilic-hydrophilic equilibrium, chemical durability, biological compatibility, and sustainability. It also has a manageable structural control [43].

3.2. Poly-d,l-lactide-co-glycolide (PLGA)-based nanoparticles

Long-circulating PLGA polymeric NPs were available in the late middle of the 1990s, opening up tremendous possibilities for the delivery of pharmaceuticals. The biodistribution, biocompatibility, and biodegradability of PLGA polymer were authorized by the United States Food and Drug Administration (FDA). The Krebs cycle's substrates, oligomers, and D, l-lactic, and glycolic acid monomers, are produced during the breakdown of PLGA by an autocatalytic breakage of ester linkages by spontaneous hydrolysis [44]. For the therapy of osteosarcoma, combinatorial chemotherapy is used, which includes co-loading PLGA nanoparticles along with PTX as well as etoposide (ETP). The nanoparticles underwent a release study after various physicochemical and biological characteristics were evaluated. The findings revealed no evidence of early burst release, and both anticancer drugs had sustained release profiles from the nanoparticles [45]. The hybrid drug-loaded PLGA NPs demonstrated considerable absorption in MG63 cells as well as an improved duration and concentration-related anticancer efficacy in MG63 and Saos-2 tumor cells. Additionally, the outcomes demonstrated that loaded nanoparticles had an improved therapeutic index and a stronger inhibitory impact, both of which would be extremely helpful during systemic cancer therapy [46].

3.3. Polyethyleneimine (PEI)-based nanoparticles

Primary, secondary, and tertiary amino acid groups are present in the cationic polymer polyethyleneimine (PEI). Both linear and branching compounds can produce PEI polymers. PEI is an important cationic polyamine that has been widely used as a potent medication as well as a gene nanocarrier [47]. The cationic PEI could gather the nucleic acids containing negative charge through electrostatic interactions, much like non-viral delivery systems. According to some theories, PEI-based vectors' high transfection effectiveness results from their ability to prevent trafficking to lysosomes that break down cells. Heparan sulfate proteoglycans (HSPGs), which serve as the interface molecules for PEI on the cell's surfaces, are necessary for the extent of PEI absorption by cells [48].

3.4. Poly(l-lysine) (PLL)-based nanoparticles

The polycation poly(l-lysine) (PLL) is often used to deliver both the medication and the gene. Concerning molecular weight as well as secondary structure components, the PLL constitutes a peptide with exceptional structural accuracy. Because it contains amine groups that aid in cell adherence, it functions as a non-viral transfection tool for the transfer of genes and DNA condensation [49]. Molecules, such as asialoglycoprotein N-glutaryl phosphatidyl ethanolamine, tyrosine-amide-triantennary oligosaccharide, fusogenic peptides, transferrin and antibodies, were covalently linked to PLL to overcome the cytotoxicity concerns of this polymer and to make it usable as a delivery system. It is however unknown whether particles made from PLL may be absorbed by cells [50].

4. Lipid nanomedicines for cancer vaccines

Lipid nanoparticles (LNPs) have gained tremendous attention since the US Food and Drug Administration (FDA) approval of Pfizer and Moderna's COVID-19 vaccines [51,52]. The advantage of the use of LPNs vaccines are various, include that this nanotechnology can be easily produced on a large scale, LPNs are self-adjuvant and customizable [53].

Lipid-based nanomedicines have emerged as promising tools in the field of cancer vaccines due to their unique properties, which include biocompatibility, biodegradability, and the ability to encapsulate and deliver nucleic acids, antigens, and adjuvants [50,54]. Notable instances of clinical translation encompass liposomes, lipid nanoparticles (LNPs), and cationic lipoplexes [55,56]. Lipid nanoparticles (LNPs) offer versatile platforms for the development of effective cancer vaccines by providing controlled delivery, protection of cargo, and efficient immune activation. Lipid-based nanoparticles (LNPs) are now a promising tool for delivering genetic material in non-viral gene therapy [54,57]. Historically, cationic lipids, such as DOTAP and CTAB have introduced in formulation development, along with other lipids, to form complexes that can store and transport nucleic acids [58,59]. However, these systems have drawbacks, including toxicity and immune activation caused by their permanent positive charge. The discovery of ionizable cationic lipids, such as DLin-MC3-DM, has revolutionized LNP technology [56]. These lipids have the ability to remain positively charged at acidic pH, facilitating efficient nucleic acid trapping, while becoming neutral under physiological conditions. This property allows for reduced toxicity, enhanced interaction with therapeutic nucleic acids, and improved release of the genetic cargo into the cytoplasm. Recent studies demonstrates the impact of complexing lipids on the successful delivery of messenger RNA to human skin, highlighting the potential of LNP technology in treating skin issues [60]. Furthermore, certain lipoplexes composed of quaternary ammonium-based lipids have shown promise as cancer immunotherapy vaccines by inducing a specific immune response [61–63]. Clinical trials have been conducted using RNA-lipoplexes to target melanoma-associated antigens, with promising results regarding immune response and tumour regression [64]. Rahimi et al. (2023) described the use of lipid-based nanoparticles loaded with siRNA in a clinical trial to target PLK1 in patients with advanced solid tumours, showing both efficacy and safety [65].

Lipid nanomedicines used in cancer vaccines typically consist of a lipid bilayer surrounding an aqueous core, where the vaccine cargo is encapsulated. The lipid bilayer provides stability, protects the encapsulated cargo, and facilitates cellular uptake and endosomal escape. Targeting ligands (e.g., antibodies, peptides) can be used to modify the surface of LNPs, to enhance their specificity and uptake by antigen-presenting cells (APCs) [66]. Upon administration, LNPs interact with immune cells in the tumour microenvironment or draining lymph nodes. The LNPs are taken up by APCs,

such as dendritic cells, macrophages, or B cells, through receptor-mediated phagocytosis or endocytosis. The encapsulated vaccine cargo, including nucleic acids encoding tumour antigens or adjuvants, is released, and processed within the APCs. Nucleic acids are translated into antigenic proteins, which are presented on the cell surface by the major histocompatibility complex (MHC), initiating an immune response [66,67].

4.1. Liposomes

Liposomes were the pioneering lipid-based nanomedicines to make their way into clinical applications. Today, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved over a dozen liposome products [55]. Liposomes were first discovered in the 1960s. These spherical vesicles composed mainly of phospholipids are categorized according to the number of layers, preparation method and size. As phospholipids are amphiphilic compounds containing a hydrophobic tail linked to a hydrophilic head, they confer to liposomes the encapsulation of hydrosoluble molecules inside and liposoluble molecules in the lipid bilayer [68]. Additionally, liposomes are biocompatible, biodegradable, improve product stability and reduce toxicity. In addition, liposomes can be negatively or positively charged [62,69-71]. Hydration of the lipid film is one of the main methods of preparing liposomes, but these can also be made by supercritical fluid, ultrasonication, extrusion, among others [69]. They can load hydrophilic drugs or nucleic acids within the aqueous core and hydrophobic drugs inside the lipid bilayers. Liposomes offer flexibility in terms of size, charge, and surface modifications, allowing for efficient delivery of vaccine components and enhanced immune responses. They have been extensively studied for cancer vaccines, including mRNA-based vaccines. Liposomes have shown promise as vehicles for cancer vaccines. They can encapsulate tumor-associated antigens or genetic material encoding these antigens, enhancing their delivery to antigen-presenting cells (APCs) and promoting immune recognition [56,67,72]. Liposomes can be designed to incorporate adjuvants that stimulate immune responses, such as toll-like receptor agonists, further boosting vaccine efficacy. One challenge is identifying the optimal combination of antigens and adjuvants to elicit robust and specific antitumor immune responses [55]. Additionally, enhancing the uptake and presentation of liposomal antigens by APCs to activate T cells efficiently is a key consideration. Further optimization is required to balance antigen release kinetics, ensuring sustained presentation while avoiding immune tolerance. Liposomes can encapsulate and deliver genetic material, such as DNA or mRNA, to target cells. The liposomal membrane protects the genetic material from degradation, facilitating its entry into cells for gene expression [56].

Liposomes can incorporate tumor-associated antigens, enabling their presentation to immune cells. This promotes the activation of antigen-specific immune responses, i.e., generation of cytotoxic T lymphocytes (CTLs) and the production of tumor-specific antibodies. They can be formulated to include immune-stimulating molecules or adjuvants, enhancing the immune response against cancer cells. Adjuvants can help stimulate dendritic cells, enhance antigen presentation, and promote immune cell activation [73]. Liposomes can be modified with antibodies or other ligands to target specific cancer cells or tissues. Such targeted delivery approach improves the efficiency of gene expression in cancer cells, minimizing off-target effects and maximizing therapeutic efficiency. However, some of the challenges include achieving the desired properties of liposomes, such as size, stability, and encapsulation efficiency, requires careful formulation optimization [66]. Liposome characteristics can impact gene delivery efficiency, immune response, and toxicity, necessitating thorough characterization and refinement. The choice of adjuvants or immune-stimulating molecules incorporated into liposomes plays a key role in shaping the immune response. Selecting appropriate adjuvants and optimizing their concentrations and combinations is essential to elicit potent and targeted anti-tumor immune responses [54,73]. Also, efficient endosomal escape of genetic material from liposomes is crucial for successful gene expression. Strategies to enhance endosomal disruption, such as pH-sensitive or fusogenic liposomes, need to be explored to maximize gene delivery and expression. Liposome-based gene delivery systems must be thoroughly evaluated for potential toxicity and immunogenicity. Liposome composition, surface charge, and size can influence interactions with immune cells and organs, necessitating comprehensive preclinical and clinical studies to ensure their efficacy and safety. Large-scale production of liposomes with consistent quality, batch-to-batch reproducibility, and stability is a key challenge. Optimizing manufacturing processes, scalability, and quality control measures are crucial for the clinical translation of liposome-based gene therapy [62].

In general, the liposomes most commonly used in cancer vaccines are cationic liposomes, while neutral and anionic liposomes are less commonly used. This is because cationic liposomes have immunogenic properties, they are an efficient transport for antigens and a local deposit is formed at the injection site which improves cellular uptake, thereby eliciting antitumour immune responses through time-dependent uptake of APCs and antigen delivery (called as the depot effect). Moreover, the absorption process of cationic liposomes in cells is due to a non-specific binding between the positively charged liposome and the negatively charged cell membrane, which confers a high antigen delivery [74]. Table 1 summarizes examples of works describing the use of liposomes in cancer vaccines.

After entering the human body, nanovaccines are recognised by natural killers (NK) cells, which play an important role in anticancer immunity. In a recent study, involving a nanovaccine with cationic liposomes containing an antigen legumain peptide and a recombinant protein of trichosanthin, obtained eminent results, showing a potent antitumor activity *in vitro* for B16-F10 melanoma, intracranial LLC xenograft, Lewis lung cancer (LLC) and CT-26 colon cancer. It was evaluated by subcutaneous administration in mice, and proved to be a promising vaccine against these types of cancer, as it increased the numbers of immunostimulatory cytokines (e.g., IFN-γ, IL-12 and TNF-α), as well as triggered immunity by T cells, remodeling the cancer microenvironment [78].

4.2. Lipid-Based nanoparticles

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are classical examples of lipid nanoparticles that have been explored for cancer vaccines. SLNs and NLCs are solid or semi-solid particles composed of biodegradable lipids. These nanoparticles can encapsulate hydrophobic drugs or nucleic acids and provide controlled release properties, stability, and protection of cargo during circulation. SLNs and NLCs have shown promise in delivering various cancer vaccine components, including antigens, adjuvants, and immunostimulatory nucleic acids [54,79].

The role of lipid components is crucial for the structure and function of LNPs when developed for vaccines and gene therapy. LNPs are used as carriers for nucleic acid cargo, such as mRNA or siRNA, and the composition of lipids determines their stability, delivery efficiency, and biological effects [73]. Multiple studies have explored the impact of lipid composition on the structure of LNP. Analysis by cryo-transmission electron microscopy (cryo-TEM) demonstrated that small modifications of the molecular structure of a single lipid component, such as cholesterol derivatives, can significantly influence the overall LNP structure [54,60,67,72]. LNPs, based either as a multilamellar structure or as a lamellar

Table 1
Examples of articles on liposome-based cancer vaccines.

Nanovaccines	Administration route	Encapsulated compounds	Type of cancer	References
mRNA vaccines with positively charged protamine liposomes	Intranasal	Cytokeratin 19 (CK19)	Lung cancer	[71]
Cationic Liposomal gp100 nanovaccine	Intravenous	Liposomal Epacadostat	Melanoma	[75]
Cationic Liposomal gp100 nanovaccine	Intraperitoneal in mice	CpG-ODN with anti PD-1 monoclonal antibody (mAb)	Melanoma	[76]
Cationic Liposomal gp100 nanovaccine	Subcutaneous in mice	anti PD-1 monoclonal antibody(mAb)	Melanoma	[74]
Nanoliposomal (with around 128 and 168 nm)	Subcutaneous in mice	Vascular endothelial growth factor receptor-2 (VEGFR-2)	Melanoma	[77]
Cationic Liposome-encapsulated technology	Subcutaneous in mice	Antigen legumain peptide and recombinant protein (termed rTL) of trichosanthin	Melanoma, Intracranial, Colon and Lewis Lung cancer	[78]

lipid phase, have shown capacity for enhanced gene transfection. SAXS (small-angle X-ray scattering) has been utilized to characterize LNPs to obtain their size, characterize their structure, and to disclose the presence of ordered lipid packing and cholesterol crystallites. These techniques have helped identify different populations of LNPs and the localization of RNA within the formulation [63,80,81]. Thionine staining in cryo-TEM has shown that RNA tends to localize at the outer layer of LNPs or in aqueous compartments surrounded by lipid shells. In terms of lipid distribution, SANS (small-angle neutron scattering) studies have indicated that specific lipid components exhibit distinct spatial arrangements within LNPs. DSPC lipids primarily reside at the LNP surface, while cholesterol is displaced both in the core and at the surface of the particles [73,82].

Overall, these studies highlight the significant impact of lipid composition on LNP structure and function. Small changes in lipid components can influence the overall LNP morphology, gene transfection efficiency, and spatial distribution of cargo. Understanding the relationship between lipid composition, LNP structure, and biological effects is crucial for the rational design and optimization of LNPs for vaccines and gene therapy applications [73].

4.3. Cationic lipid nanoparticles

Cationic lipid nanoparticles, or lipoplexes, are formed by complexing cationic lipids with nucleic acids through electrostatic interactions. Cationic lipids promote the cellular uptake and endosomal escape of nucleic acids, enabling efficient transfection and translation of encoded antigens. Lipoplexes have been used for the delivery of cancer vaccines based on nucleic acids, including plasmid DNA and mRNA vaccines [57].

During the early 1990s, significant progress was achieved in the field of lipid-based nanoparticles for mRNA delivery with the development of pH-responsive cationic lipids [56]. These atypical lipids exhibited a unique property wherein their net charge could be altered in response to the surrounding pH conditions. Specifically, under acidic pH, these lipids acquired a net positive charge, enabling the encapsulation of mRNA within lipid nanoparticles [16]. This pH-dependent feature has proven crucial for achieving efficient mRNA delivery. One notable example of these pH-responsive cationic lipids is DLin-MC3-DMA (MC3), which possesses an optimized pKa value of 6.44 [54,60]. This specific lipid has been widely employed for RNA delivery and has demonstrated exceptional efficacy in protein replacement therapy for genetic diseases (e.g., Friedreich's ataxia). Studies using MC3-based lipid nanoparticles encapsulating mRNA encoding proteins like luciferase and frataxin have shown high levels of protein expression in dorsal root ganglion neurons when administered via intrathecal injection in mice. Nevertheless, these systems encounter important shortcomings, such as the risk of inducing toxicity and immune activation due to their inherent permanent positive charge [57,79].

4.4. Ionizable lipid-based nanomedicines

Ionizable lipids are lipids that can undergo pH-dependent charge conversion. They are designed to be neutral at the physiological pH but turn into positively charged under acidic environment of endosomes or lysosomes within cells. This charge conversion enables efficient endosomal escape of the lipid nanoparticles and facilitates the release of encapsulated cargo, such as nucleic acids or drugs, into the cytoplasm. Ionizable lipid-based nanomedicine has gained attention for its ability to overcome the endosomal entrapment barrier and enhance the intracellular delivery of therapeutics. The exploration of lipid-like materials, known as lipidoids, has further expanded the possibilities of ionizable lipid design. Lipidoids are characterized by the presence of highly hydrophobic side chains and protonable tertiary-amino groups, both of which play crucial roles in determining transfection efficiency [73,81].

Experimental observations have indicated that the chemical structure of the lipid tail greatly influences the efficacy of lipid-based nanoparticles. For instance, longer and unsaturated alkyl tails have been found to enhance mRNA delivery efficiency. Innovative modifications to lipid structures have also demonstrated significant advancements in mRNA delivery [73]. For instance, the introduction of alkyne and ester groups into the hydrophobic tail of MC3-based lipids has not only improved their tolerability but also enhanced transfection efficiency. The incorporation of unsaturated alkyne groups in the non-polar tail of ionizable lipids has been found to enhance fusogenicity with the endosomal membrane, facilitating efficient endosomal escape and subsequent release of mRNA into the cytosol [54]. Furthermore, the combination of different ionizable lipids has shown promise in the development of more efficient delivery systems. Co-formulation of MC3-based lipids with cKK-E12 pH-responsive cationic lipids has synergistically enhanced mRNA delivery into hepatocytes. The addition of cKK-E12-based lipids has improved protein binding and serum stability of the nanoparticles, further optimizing their performance [50,57,73]. The potential of ionizable lipid-based nanoparticles for mRNA-based immunotherapies in chemotherapy has also been explored. These nanoparticles have been utilized for the delivery of antigen-encoding mRNA, resulting in the induction of antigen-specific CD8+ *T* cells and significant suppression of tumor growth in animal models. Modified mRNA encoding bispecific antibodies has been successfully loaded into hybrid polymer/lipid-based formulations, effectively impairing tumor growth in preclinical studies [82,83].

Overall, the advancements in lipid-based nanoparticles and ionizable lipids have greatly contributed to the field of mRNA delivery. These innovations in lipid design, tail modifications, and co-formulation strategies have improved transfection efficiency, endosomal escape, and therapeutic outcomes. The potential applications of these technologies extend beyond genetic diseases,

holding promise for the development of mRNA-based therapies for a wide range of medical conditions, including cancer [80]. Table 2 summarizes the main lipid nanomedicines for cancer vaccines and the main outcomes.

5. Hybrid (lipid-polymeric) cancer vaccines

The lipid bilayer membrane of liposomes, which are spherical fat bubbles, is made up of both water-soluble and lipophilic lipid molecules. Phospholipids, either native or developed, constitute the lipid membrane. In addition to cholesterol, phospholipids are another lipid that is employed to produce NPs with greater fluidity, which increases the biostability as well as porosity inside the human body [85]. Additionally, by adding polyethylene glycol, also known as PEG, to the surface of the liposomes to create PEGylated or concealed liposomes, their circulation duration can be increased. Both hydrophilic and hydrophobic drugs are encapsulated into lipophilic along with lipophobic compartments in liposomes, which creates a flexible surface with a variety of ligands that are designed that actively destroy cancer cells [86]. The liposomes are capable of transferring specific drugs and are produced in huge quantities for use in cancer research (Fig. 2). They additionally exhibit biocompatibility as well as biodegradability. Polymer-lipid hybrid nanoparticles (PHLNPs), combine the benefits of liposomes and polymeric NPs. Due to its ability to transcend beyond the drawbacks of lipids and polymers, this hybrid system has a lot of potential for application in nanomedicine [87]. Regarding PLHNPs, a one-stage and a two-stage synthetic method are often two separate options. In the one-stage method, the hydrophobic drug and either the unloaded polymeric core or both are dissolved in a suitable solvent before the created lipid shell along with lipid-PEG is combined alongside the drug-loaded polymer core as well as aqueous lipid solution in a single pot [88]. This mixture is then self-assembled and nanoprecipitated. The polymer core, as well as the lipid shell, are initially made separately in the two-stage method, and they are then combined to create PLHNPs. In line with variations in their lipid alongside polymer structures, PLHNPs are categorized [89]. The solidity and durability of the polymers increase both overall particulate stability and the drug delivery kinetics in each hybrid structure. Lipids, however, frequently increase medication loading and boost particle biocompatibility. As a result, the improved hybrid NPs frequently show stronger and more persistent in vivo activity [90]. The boundaries of liposomes and organically recyclable polymeric nanoparticles led to the development of an inventive drug delivery system, so-called lipid-polymer hybrid nanoparticles (LPHNs), with improved profile when compared to nanoparticles and liposomes alone. The chemical relationship between the drug and the polymer, the drug's solubility, the rate of polymer breakdown, and the size of the particles are some of the elements that affect the profile of drug release of the PLHNs [20]. The medicine is released from the PLHNs, which are physically enclosed, by drug diffusion and polymer erosion. The release of medications that were chemically encapsulated was dependent on the linker's hydrolysis (the connection between the polymer chain and the drug). The dynamics of the release of the drug are improved by the uniform dispersion of the molecule of the drug in nanoparticles, which significantly reduces the consequences of burst release [91].

6. Miscellaneous nanomedicines

6.1. Dendrimers

The new-generation nanoparticles used in the nanosize ranged dendritic medicines delivering system have demonstrated promising effects for treatments for the administration of chemotherapeutic drugs and as a diagnostic tool. Dendrimers are an ideal nanocarrier for targeted drug delivery because of their characteristics including monodispersibility, surface modification, and a greater volume of interior perforations [92]. Dendrimer-based nanocarriers' primary characteristics include acting as drug delivery vehicles for increased absorption of anti-cancer drugs in cancer cells. Increased the permeability of the tumor vasculature to macromolecules and the decreased rate of cancer cell export constitute the effects of the EPR mechanism [93]. Polyamidoamine (PAMAM)-based dendrimers were conjugated with folic acid to be loaded with methotrexate were found ideal for EPR effect [94]. These conjugated dendrimers consist of particles 50 nm and exhibit higher cell-surface absorption of drugs in cancer cells as shown by confocal microscopy. Dendrimeric nanocarriers are developed as diagnostic tools for MRI and as contrasting agents in boron neutron capture treatment thanks to advancements in therapeutic nanomedicine [95].

6.2. Carbon nanotubes

Because of their unique characteristics and structure (e.g., sizable surface areas, high aspect ratios, a wealth of surface chemical features, and size equilibrium on the nanoscale), carbon nanotubes (CNTs) are receiving a great deal of attention from the biomedical field, since their discovery in 1991 by Sumio Iijima [96]. CNTs are particularly appealing for the delivery of drugs and biomolecules and are being exploited to carry proteins, genes, and anticancer drugs by surface functionalization [97]. To obliterate cancer cells directly, they have also been utilized as facilitators for photothermal treatment (PTT) and photodynamic therapies (PDT). CNTs are currently focusing on the capacity for anticancer drug delivery. Their distinctive needle-like structures, which may be functionalized for absorption or covalently connect to a wide range of drugs and internalize into the target cell, may be to blame for this [98]. Furthermore, due to the well-established reliability of vesicle-based carriers, especially liposomes, many researchers have given up on studying CNTs for the therapy of numerous disorders besides cancer [99]. It has been discovered that siRNA-CNTs can stimulate apoptosis in the aimed tumor by suppressing cyclinA expression. Similar results have been shown, for example, when functionalized SWNTs have been coupled to siRNAs against the telomerase reverse transcriptase (TERT) [100]. The desired gene has been effectively silenced, and both in vivo and in vitro tumor development was suppressed by utilizing murine tumor cell lines and a mouse model [101].

6.3. Hydrogels

In addition to being much more effective at modifying the cancer microenvironment than active and passive targeting, hydrogels' prolonged or triggered drug release makes them an attractive option for targeted, localized treatment, regardless of the tumor blood supply and microvasculature structures [102]. In short, changes in hormone, protein, and structural component levels stabilize and activate hypoxia-inducible factors, which lead to changes in stiffness and rigidity over time. These, in turn, encourage intratumoral hypoxia and control the production of a protein called matrix metalloproteinase, which breaks down the extracellular matrix (ECM) and alters its structural makeup [103]. These are among just a handful of the changes that take place while cancer develops. Hydrogels may additionally deliver multiple therapeutic substances at the same time. Owing to the heterogeneous character of tumors and the presence of cancer cells at various cell division or development phases, a single treatment may not be able to eliminate all cancer cells [104]. A potential approach to combat medication resistance and lower the risk of tumor metastasis is the si-

Table 2
Summary of main lipid-based nanomedicines for cancer vaccines, main outcomes and challenges.

Lipid Nanomedicine	Lipid Components	Application and Outcome	Secondary outcomes	Challenges	References
Liposomes	Phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine) Cholesterol, sphingolipids	Encapsulation of tumor antigens and adjuvants Enhanced immune response	- Liposomes can incorporate tumor-associated antigens, enabling their presentation to immune cells. This promotes the activation of antigen-specific immune responses, such as the production of tumor-specific antibodies and generation of cytotoxic T lymphocytes (CTLs)	- Optimal combination of antigens and adjuvants - Efficient uptake and presentation by APCs	[55,56,60]
	Targeting ligands (e.g., antibodies, peptides)	Targeted delivery to specific cancer cells		- Achieving desired properties (size, stability, efficiency)	
	Cationic lipids (e.g., DOTAP, DOPE)	Encapsulation and delivery of genetic material		- Endosomal escape for successful gene expression	
Cationic Lipid Nanoparticles (Lipoplexes)	Cationic lipids (e.g., DOTAP, DODAP, DC–Chol) Helper lipids (e.g., DOPE) pH-sensitive lipids (e.g., DMPE, DMPC)	Efficient transfection and translation of antigens Delivery of nucleic acid-based cancer vaccines pH-responsive lipids for mRNA delivery	- 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) conjunction with e.g., phospholipids and cholesterol, for the delivery of therapeutic genes to cells in melanoma - lipoplex (DOTAP/DOPE) are effectively accumulated in the spleen and promotes mRNA delivery to dendritic cells - DLin-MC3-DMA (MC3) exceptional efficacy in protein replacement therapy for gene treatment of diseases such as Friedreich's ataxia	- Potential toxicity and immune activation	[16,54,60,73]
Ionizable Lipid-based Nanomedicines	Ionizable lipids (e.g., DSPC, DOPE, DODMA) Helper lipids (e.g., DOPE, cholesterol) Ionizable lipids (e.g., DSPC, DMPE) Cationic lipids, helper lipids, ionizable lipids	pH-dependent charge conversion for endosomal escape Enhanced intracellular delivery of therapeutics Optimization of lipidoid design for transfection RNA delivery for mRNA-based immunotherapies	- The addition of cKK-E12-based lipids has been found to improve serum stability and protein binding of the nanoparticles, further optimizing their performance. - Substituting DSPC, a common helper lipid in siRNA formulations, with DOPE has been found to significantly enhance mRNA delivery efficacy	- Lipid tail structure and optimization - Manufacturing scalability and quality control - Selecting appropriate adjuvants and combinations - Ensuring safety and efficacy through evaluation	[50,55–57,73,79]
Lipid Nanoparticles (SLNs and NLCs)	Solid lipids (e.g., stearic acid, glycerol monostearate) Phospholipids, solid lipids Co-surfactants (e.g., Tween, Poloxamer)	Controlled release and stability of cargo Encapsulation of cancer vaccine components Delivery of nucleic acids and hydrophobic drugs	- Long unsaturated alkyl tails enhance mRNA delivery efficiency - The percentage of cholesterol in the formulation exerts a significant impact on intracellular gene delivery. The use of cholesterol analogues in the formulation, such as C-24 alkyl phytosterols, has been shown to enhance mRNA delivery - Multilamellar or faceted structure, lamellar lipid phase enhanced gene transfection	- Lipid composition for optimal structure and function - Understanding the impact of lipid composition - Rational design and optimization for effective use	[16,50,54,57,60,72,73,80,83,84]

Abbreviations: DNA: deoxyribonucleic acid, RNA: ribonucleic acid, DSPC: 1,2-distearoyl-sn–glycero-3-phosphocholine, DMPE: 1,2-Bis(dimethylphosphino)ethane, DOPE: dioleoyl phosphatidylethanolamine, DODMA: 1,2-Dioleyloxy-3-dimethylaminopropane, DMPC: 1,2-dimyristoyl-sn–glycero-3-phosphocholine, DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane, DODAP: 1,2-dioleoyl-3-dimethylammonium-propane, APC: antigen-presenting cell.

multaneous administration of various therapeutic drugs that have diverse molecular targets. This strategy could successfully enhance the course of therapy and lessen side effects associated with the use of a very life-threatening medicine [105]. Another potentially clever approach for targeted cancer treatment is the functionalization of hydrogels with tumor-targeting ligands as well as nanodevices which are sensitive to certain tumor features. For instance, relative to healthy cells, the majority of cancer cell type's upregulate the glycosylphosphatidylinositol receptor known as the folate receptor. Folate-conjugated nano-gels can improve the intracellular absorption of nanocarriers through receptor-mediated endocytosis by targeting excessively expressed receptors on the surface of cancer cells [106].

6.4. Quantum dots [QDs]

The treatment of cancer has already benefited significantly from recent advances in QD technology. The detection and diagnosis of cancer depend primarily on molecular biomarkers. If a collection of molecular markers can be measured and statistically discriminated between cancerous cells and normal cells, biomarker tests may be helpful for cancer screening and diagnosis [107]. Because cancer markers are frequently present in extremely low quantities, approaches with low detection limits are needed. For active tumor targeting, this might be accomplished by conjugating quantum dots with immunoglobulin aptamers, oligonucleotides, or proteins that are specific to the target. Additionally, bioconjugated QDs may be used to precisely estimate the therapeutic effect of molecular target treatment for any given patient [108]. Bioconjugated QDs may be anticipated to give significant details on the expression levels of tumor markers on cancerous cells before and the following targeted therapy as an imaging approach for the over-expression of tumor markers, which will directly impact the patient's future care [109]. The simultaneous detection of several biomarkers for cancer diagnosis and treatment utilizing multiplexed sensing using QDs with adjustable emission spectra is also promising. For the development of multi-functional nanoparticles for concurrent drug administration and imaging, QDs offer a flexible nanoscale scaffold [110]. It is preferable to use single-QD molecules for in vivo applications because their intermediate size lowers renal clearance and reticuloendothelial system absorption, prolonging blood circulation time, and boosting delivery effectiveness [111]. Additionally, QDs that include water-soluble capping stabilizers such as mercaptoacetic acid, mercapto-ethylamine, and polyethylene glycol polymer are prepared to conjugate with medicinal molecules by covalent bonding or electrostatic contact [112].

7. Fabrication methods of nanovaccines

These vaccines, designed at the nanoscale, offer several advantages over traditional vaccine formulations, including improved antigen presentation, controlled release kinetics, and the potential for co-delivery of adjuvants or immunomodulatory agents. Various fabrication techniques are employed to engineer nanovaccines, each offering unique advantages and challenges [113].

One prevalent method is the use of nanocarriers such as liposomes, polymeric nanoparticles, and virus-like particles (VLPs). Liposomes, composed of lipid bilayers, can encapsulate antigens within their aqueous core or lipid membrane, providing protection against degradation and facilitating antigen uptake by antigen-presenting cells (APCs) [114]. Polymeric nanoparticles offer versatility in terms of size, surface modification, and payload encapsulation, enabling tailored vaccine formulations. VLPs mimic the structure of viruses without the genetic material, eliciting robust immune responses due to their repetitive antigen display and intrinsic immunogenicity [115,116].

Another approach involves the conjugation of antigens onto nanomaterials, such as gold nanoparticles or carbon nanotubes, using chemical or physical methods. Surface conjugation allows for precise control over antigen density and orientation, potentially enhancing immune recognition and activation. Additionally, the unique physicochemical properties of nanomaterials can serve as adjuvants, stimulating innate immune responses and augmenting vaccine efficacy [117,118]. In recent years, advances in nucleic acid-based vaccines have spurred the development of nanovaccine platforms for the delivery of DNA or mRNA constructs. These vaccines typically utilize lipid nanoparticles or polymer-based carriers to protect nucleic acids from degradation and facilitate cellular uptake. mRNA vaccines, in particular, have gained prominence due to their rapid development timelines and potential for inducing both humoral and cellular immune responses [119].

Furthermore, emerging strategies such as self-assembled nanoparticles and biomimetic nanovaccines offer innovative approaches to vaccine fabrication. Self-assembled nanoparticles leverage non-covalent interactions to spontaneously form nanostructures, simplifying manufacturing processes and enabling precise control over particle size and composition [120]. Biomimetic nanovaccines, inspired by natural immune processes, incorporate components such as cell membranes or virus-derived proteins to enhance antigen presentation and immune recognition. Despite the progress in nanovaccine fabrication, several challenges remain. These include optimizing vaccine formulation parameters, ensuring vaccine stability during storage and transportation, and addressing safety concerns associated with nanomaterials. Moreover, scalability and manufacturing reproducibility are critical considerations for clinical translation [121,122].

8. In-Vitro and in-vivo evaluation techniques

The assessment of biomaterials in nanovaccines involves a comprehensive combination of in vitro and in vivo techniques aimed at evaluating their safety, efficacy, and immunogenicity. In vitro evaluations encompass a series of assays designed to scrutinize the impact of nanovaccine components on cellular systems [123]. Cell viability assays, such as the MTT assay and Alamar Blue assay, provide insights into the potential cytotoxicity of biomaterials, while cellular uptake studies, employing techniques like flow cytometry and confocal microscopy, elucidate the internalization of nanovaccine components by immune cells. Cytokine release assays, including ELISA and Luminex assays, serve to quantify the immunomodulatory effects of nanovaccines by measuring the production of key cytokines [124,125]. Hemocompatibility studies assess the compatibility of nanovaccines with blood components, utilizing hemolysis assays and coagulation studies. Intracellular trafficking studies, employing fluorescence and electron microscopy, offer a detailed understanding of the fate and subcellular localization of nanovaccine components. Antigen presentation assays, such as dendritic cell maturation assays and T cell activation assays, gauge the nanovaccine's ability to stimulate immune responses [123,126].

In vivo evaluation encompasses biodistribution studies to determine the distribution of nanovaccine components in the body, often using radiolabelling or near-infrared imaging. Immunogenicity assessments, including antibody titration assays and T cell proliferation assays, gauge the ability of nanovaccines to induce a robust immune response [127,128]. Toxicity studies, involving histopathology and blood chemistry analysis, assess potential adverse effects. Vaccine efficacy studies involve challenging animals with the target pathogen and quantifying protection through viral load measurements. Pharmacokinetic studies investigate the kinetics of nanovaccine components in the body, utilizing blood sampling and bioanalytical assays [129,130]. Long-term safety and persistence assessments involve longitudinal studies and repeated-dose toxicity eval-

uations to ensure the sustained safety and efficacy of nanovaccines over time. These diverse techniques collectively provide a comprehensive understanding of the biomaterials in nanovaccines, addressing safety, efficacy, and immunogenicity considerations [128].

9. Clinical trials

A Phase 1 clinical trial (NCT03546361) trial is currently ongoing to evaluate gene therapy-based vaccine in Non-Small Cell Lung Cancer (NSCLC). The vaccine being investigated is the CCL21-Gene Modified Dendritic Cell Vaccine, administered in combination with a PD-1 immune checkpoint inhibitor, pembrolizumab. The primary outcomes of this trial are to determine the maximum tolerated dose of the vaccine and evaluate the Overall Response Rate (ORR). The ORR reflects the proportion of patients experiencing a significant reduction in tumour size. Additionally, the trial examines secondary outcomes such as the incidence of Adverse Drug Reactions (ADRs) and the assessment of biomarkers to identify potential predictive factors for treatment response [131]. Another clinical trial (NCT05242965), is also an ongoing trial on NSCLC patients. It evaluates the CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine along with Sargramostim. The trial focuses on immune response and tumour-infiltrating lymphocytes. By measuring immune responses and clinical outcomes, such as ORR, progression-free survival (PFS), and overall survival (OS), this trial aims to provide valuable insights into the efficacy of combining gene-based vaccines with immunotherapy [132]. NCT03164772 is a completed Phase 1/2 trial investigating the BI 1361849 mRNA Vaccine in combination with Durvalumab and Tremelimumab in NSCLC patients. This trial focused on unspecific cancer types. The primary outcome was the assessment of treatment-related ADRs. Additionally, secondary outcomes included PFS, Best ORR, Duration of Response (DOR), and OS were also assessed. PFS measured the duration of time during which the disease does not progress, while ORR reflected the proportion of patients experiencing a significant reduction in tumour size. DOR indicates how long these responses are sustained [133].

Another clinical trial NCT02775292 investigated a NY-ESO-1(157-165) which is a peptide-pulsed Autologous Dendritic Cell Vaccine in combination with Aldesleukin, Nivolumab, Cyclophosphamide, and Fludarabine in solid tumours. This is a completed Phase 1 trial that focused on solid tumours. The primary outcomes of this trial were to assess the incidence of ADRs and the determination of the maximum tolerated dose. Furthermore, the trial assessed the feasibility of the treatment and analysed the persistence of transgenic cells in the body [134]. NCT05354323 investigates the NECVAX-NEO1 oral DNA vaccine in solid tumours. Primarily the assessment of ADRs was carried out. Whereas secondary outcomes included ORR, PFS, time to progression, OS, and immune response. By evaluating these endpoints, this trial aims to determine the safety profile, efficacy, and immunogenicity of the NECVAX-NEO1 vaccine [135].

NCT03532217 is a completed Phase 1 trial that investigated a Neoantigen DNA vaccine in combination with Nivolumab and Ipilimumab in prostate cancer. The primary objectives of this trial were to assess the safety and tolerability of the vaccine regimen. Additionally, the trial aimed to evaluate the immune response generated by the vaccine. The primary outcomes of this trial included safety, ensuring that the vaccine was well-tolerated by patients. The secondary outcomes encompassed measuring the immune response elicited by the vaccine, such as the activation of immune cells and the production of specific antibodies. The trial also evaluated several other endpoints as secondary outcomes. These included Freedom from Progression (FFP), Milestone Survival (a measure of survival at specific time points), Radiographic Progression (measuring tumour growth on imaging), and PSA responses (changes in prostate-specific antigen levels). These measures provide insights into the efficacy of the vaccine in controlling disease progression and overall survival [136].

An ongoing phase 2 clinical trial (NCT04397003) investigates a Neoantigen DNA vaccine in combination with a PD-L1 immune checkpoint inhibitor, durvalumab in Small Cell Lung Cancer (SCLC). The primary objectives of this trial are to assess the safety and tolerability of the vaccine and evaluate its feasibility. The trial also aims to analyse secondary outcomes, including PFS, OS, DOR, and Response Conversion Rate. These measures provide insight into the effectiveness of the vaccine in controlling tumour growth, prolonging survival, and inducing tumour response [137]. In another ongoing clinical trial, NCT05455658 investigates a CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine in combination with Sargramostim in Triple Negative Breast Cancer. The primary outcome of this trial is the evaluation of the cellular immune response induced by the vaccine. This involves assessing the activation and proliferation of immune cells specifically targeting tumour cells. The trial also examines secondary outcomes, including Recurrence-Free Survival (RFS), the incidence of ADRs, and the kinetics of the vaccine response. RFS measures the length of time during which the disease does not recur [138].

NCT03655756 is a completed phase 1 clinical trial to evaluate IFx-Hu2.0 (plasmid DNA) vaccine in Cutaneous Melanoma. The primary outcome of this trial was to assess the occurrence of Serious ADRs associated with the vaccine. This trial aimed to determine the safety profile of the vaccine and identify any potential severe adverse events. Additionally, the trial investigated the induction of an antitumor immune response, indicating the ability of the vaccine to activate the immune system against melanoma cells [139]. Another ongoing clinical trial NCT05192460 mainly focuses on Gastric, Liver, and Oesophageal cancer. It investigates the efficacy and safety of the PGV002 mRNA Vaccine. The primary outcome of this trial is the assessment of ADR incidence associated with the vaccine. The trial also evaluates secondary outcomes, including ORR and the concentration of cytokines and lymphocytes in the blood. ORR reflects the proportion of patients experiencing a significant reduction in tumour size [140]. Lastly, NCT03162224 is a phase 1/2 completed trial investigating the MEDI0457, an HPV DNA vaccine, in combination with a PD-L1 immune checkpoint inhibitor, durvalumab in Head and Neck Cancer. The trial aimed to evaluate the safety and efficacy of this combination therapy for the treatment of patients with head and neck cancer associated with human papillomavirus (HPV) infection [141]. In summary, these clinical trials aim to explore various vaccines and interventions in the treatment of different types of cancers. They assess safety, immune response, tumour response rates, survival outcomes, and the incidence of adverse events. The results of these trials will provide valuable insights into the efficacy and safety of revolutionary vaccine approaches and combinations with immunotherapies for cancer treatment. Table 3 summarizes clinical trials in various phases for gene therapy vaccines in different cancers.

10. Regulatory issues

Regulatory issues surrounding nanomedicines and gene vaccines are complex and of paramount importance due to their potential impact on public health and the unique characteristics of these innovative technologies [142]. Nanomedicine involves the use of nanoscale materials and devices for medical applications, while gene vaccines utilize genetic material to stimulate immune responses. Both fields hold tremendous prospects for advancing healthcare, combating diseases, and improving patient outcomes, but they also present challenges related to safety, efficacy, and ethical considerations, which calls for the need for appropriate

Table 3
Summary of clinical trials in various phases for gene therapy vaccines in different cancers.

NCT number	Cancer	Phase	Status	Vaccine	Intervention	Primary outcomes	Secondary outcomes	References
NCT03546361	NSCLC	1	Ongoing	CCL21-Gene Modified Dendritic Cell Vaccine	Pembrolizumab	Maximum tolerated dose, ORR	ADR incidence, Biomarker assessment	[131]
NCT05242965		2	Ongoing	CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine	Sargramostim	Percentage of CD8+ TIL, Incidence of ADRs	Immune response, Vaccine induced T cell traffic, ORR, PFS, OS	[132]
NCT03164772		1/2	Completed	BI 1361849 mRNA Vaccine	Durvalumab/ Tremelimumab	Treatment-related ADRs	PFS, Beast ORR, DOR, OS	[133]
NCT02775292	Solid tumours	1	Completed	NY-ESO-1(157-165) Peptide-pulsed Autologous Dendritic Cell Vaccine	Aldesleukin, Nivolumab, Cyclophosphamide, Fludarabine	ADR incidence, Maximum tolerated dose	Feasibility analysis, Transgenic cell persistence	[134]
NCT05354323		1	Ongoing	NECVAX-NEO1 (oral DNA vaccine)		ADRs	ORR, PFS, TTP, OS, immune response	[135]
NCT03532217	Prostate cancer	1	Completed	Neoantigen DNA vaccine	Nivolumab/ Ipilimumab	Safety and tolerability, Immune response,	FFS, Milestone survival, Radiographic progression, PSA responses,	[136]
NCT04397003	SCLC	2	Ongoing	Neoantigen DNA vaccine	Durvalumab	Safety and tolerability, Feasibility analysis	PFS, OS, DOR, Response conversion rate	[137]
NCT05455658	Triple-negative breast cancer	2	Ongoing	CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine	Sargramostim	Cellular immune response	RFS, ADR incidence, kinetics	[138]
NCT03655756	Cutaneous melanoma	1	Completed	IFx-Hu2.0 (plasmid DNA)		Serious ADRs	Antitumour response induction	[139]
NCT05192460	Gastric/Liver/Oesophageal cancer		Ongoing	PGV002 mRNA Vaccine		ADR incidence, ORR	PFS, cytokine/ lymphocyte concentration	[140]
NCT03162224	Head and neck cancer	1/2	Completed	MEDI0457 (HPV DNA vaccine)	Durvalumab	Treatment-related ADRs, Objective response	DCR, PFS, OS, antidrug antibodies	[141]

Abbreviations: ADR: adverse drug reactions, DNA: deoxyribonucleic acid, DCR: disease control rate, DOR: duration of response, FFS: freedom from progression, NSCLC: non-small cell lung cancer, ORR: overall response rate, OS: overall survival, PFS: progression-free survival, PSA: prostate-specific antigen, RFS: recurrence-free survival, RNA: ribonucleic acid, SCLC: small cell lung cancer, TIL: tumour infiltrating lymphocytes, TTP: time to progression.

regulations [143]. In this response, we will delve into some of the key regulatory issues associated with nanomedicine and gene vaccines.

Nanomedicine is a multidisciplinary field that utilizes nanotechnology for medical applications. It involves the design, development, and application of nanoscale materials and devices for the diagnosis, treatment, and prevention of diseases. It also encompasses a wide range of applications, including drug delivery systems, diagnostic tools, and therapeutic agents [144]. One of the primary regulatory concerns in nanomedicine is the safety assessment of nanomaterials and nanodevices. Nanomaterials exhibit unique physicochemical properties due to their small size, which can result in different biological interactions compared to conventional materials. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have been working to establish guidelines for evaluating the safety and risk assessments of nanomedicine products. These guidelines focus on the characterization, toxicity, and environmental impact of nanomaterials, as well as the need for appropriate preclinical and clinical studies to assess their safety and efficacy [145]. Standardization of characterization techniques for nanomaterials is another critical regulatory issue. The diverse nature of nanomaterials and their properties poses a challenge in developing universally applicable characterization methods. There is a rising requirement for the development and adoption of standardized protocols for characterizing nanomaterials, including their physicochemical properties, stability, and potential for aggregation or transformation. Standardized characterization methods will enable accurate assessments of the safety and efficacy of nanomedicine products and facilitate regulatory approval processes [146]. Another regulatory issue in nanomedicines is the absence of appropriate classification and regulatory pathways for nanomedicine products. Traditional regulatory frameworks may not adequately address the unique properties and challenges associated with nanomedicine. Regulatory agencies are actively working to develop specific guidelines and regulatory pathways tailored to nanomedicine products. For instance, the FDA has issued guidance documents on nanotechnology-related applications, outlining specific considerations for safety and efficacy evaluations of nanomedicine products [147].

On the other hand, gene vaccines, such as DNA or RNA-based vaccines, utilize genetic material to elicit immune responses which in turn helps in the prevention or remission of diseases. The regulatory landscape for gene vaccines is shaped by advancements in genomics, gene therapy, and gene editing technologies. One of the key challenges is the development of an appropriate regulatory framework for evaluating the safety and efficacy of gene vaccines. These frameworks need to consider the unique aspects of genetic interventions, including the potential for long-term effects due to the introduction of genetic modifications. Regulatory agencies have been working to establish guidelines for assessing the safety and efficacy of gene vaccines. This includes considerations for vector design, delivery methods, target specificity, and long-term follow-up of patients [148]. For example, the FDA has developed specific guidelines for evaluating the safety and effectiveness of gene therapy products, including gene vaccines. These guidelines emphasize the need for rigorous preclinical and clinical studies to evaluate the potential risks and benefits of gene vaccines [8]. Ethical considerations are also a prominent regulatory issue surrounding gene vaccines. Genetic interventions raise questions about patient autonomy, privacy, informed consent, and the potential for unintended consequences. Regulatory agencies must ensure that ethical considerations are adequately addressed in the development, approval, and use of gene vaccines. Collaboration with bioethicists, patient advocacy groups, and other stakeholders is crucial to ensure ethical decision-making and societal acceptance of these technologies [10].

Intellectual property rights are another significant aspect of genetic vaccine regulation. Companies and researchers involved in developing gene vaccines often seek patents to protect their inventions. However, the patent landscape for gene vaccines is complex and can pose challenges [149]. In many jurisdictions including the United States and Europe, the patentability of genetic material, particularly genes or sequences, has been subject to debates and legal decisions. The criteria for patent eligibility may vary, and it often requires a demonstration of originality, non-obviousness, and industrial applicability. This complexity can lead to uncertainty regarding the scope and validity of patents related to gene vaccines [150]. Issues of patent ownership and licensing are also associated with gene-based vaccines. The development of gene vaccines is often involved multiple parties, including researchers, institutions, and commercial entities. Determining the ownership of genetic vaccine patents can be complicated, especially when multiple contributors are involved in the development process. This can result in disputes over patent rights, licensing agreements, and fair compensation for the use of patented technologies [151].

Furthermore, the accessibility and affordability of gene vaccines can be impacted by intellectual property rights. Patents granted for gene vaccines can create monopolies and limit competition, leading to higher prices and restricted access to these life-saving interventions. This raises concerns about equitable access to gene vaccines, especially in low-income countries or regions with limited resources. Balancing intellectual property rights with the goal of public health and ensuring affordable access to gene vaccines is an ongoing challenge for regulators and policymakers [152]. In recent years, there have been calls for alternative models to address these challenges. Open innovation models, such as patent pools or patent-sharing agreements, have been proposed as ways to promote collaboration, encourage technology transfer, and facilitate access to genetic vaccine technologies. These models aim to strike a balance between protecting intellectual property rights and promoting the wider availability of gene vaccines for the benefit of public health [153].

Additionally, regulatory agencies play a crucial role in navigating intellectual property issues related to gene vaccines. They need to ensure that patent claims and licensing agreements do not hinder innovation, competition, or access to these important medical interventions. Regulatory frameworks should encourage transparency, disclosure of relevant patent information, and fair licensing practices to promote the development and availability of gene vaccines. Collaboration between regulatory agencies, patent offices, research institutions, and the private sector is essential to address the complex intellectual property landscape surrounding gene vaccines [154]. International cooperation is particularly crucial, as genetic vaccine research and development are often global endeavours. Harmonizing patent laws, streamlining patent examination processes, and promoting information sharing can help address intellectual property challenges and facilitate the development and dissemination of gene vaccines [155].

In a nutshell, intellectual property rights pose significant regulatory challenges in the development, accessibility, and affordability of gene vaccines. Balancing the need for patent protection to ensure equitable access to these life-saving interventions is essential. Collaborative efforts between regulatory agencies, researchers, patent offices, and the private sector are necessary to navigate the complexities of intellectual property in genetic vaccine regulation and foster innovation while promoting global health [156]. Thus, strict and well-formed regulations are required to achieve a uniform utilization of gene-based vaccines in cancer management.

11. Conclusions

Currently cancer is the second leading cause of death in the world, and is a global problem to be investigated. The traditional interventions used are chemotherapy, radiotherapy, and surgery to remove the cancer. However, all these therapies cause several problems and compromise patients compliance, making the creation of cancer vaccines an urgent demand. Several clinical studies are currently in progress. Recent studies have shown that therapeutic cancer vaccines can take up to 3 weeks to induce immune responses at a level sufficient to inhibit cancer growth. However, during this period, the tumour can grow significantly and get out of control, even if high immunity is promoted. Cancer vaccines are based on compounds used in immunotherapy, but monotherapy with these vaccines has not demonstrated efficacy in clinical trials. In order to overcome this barrier, nanomedicine-based research has been initiated, which can improve the effectiveness and efficacy of cancer vaccines, increase the duration and half-life time of vaccines. Additionally, nanomedicine presents minimal toxicity, which represents a promising technology. Cancer vaccines induce tumour reduction due to triggering specific T-cell responses against tumour neoantigens, however this step can be improved with the use of nanocarriers. In order to boost the anti-tumor immune response and overcome immune suppression in the tumor microenvironment, lipid nanomedicines can be engineered to deliver both immune checkpoint inhibitors and cancer antigens. In general, nanovaccines present a size of 50–250 nm, and have demonstrated greater efficacy in terms of prolonged circulation, tissue targeting, and preferential uptake by professional antigen presenting cells. The size and shape of nanoparticles strongly influence their efficacy, influence the biodistribution, as well as the choice of route of administration and injection site. Nanovaccines that are smaller than 50 nm are transferred more rapidly across the skin barrier. Moreover, they are more likely to accumulate in the lymph nodes.

In conclusion, this review highlights the significant potential of nanomedicine in revolutionizing the field of healthcare, particularly in targeted drug delivery and cancer treatment. Nanocarriers such as liposomes, polymer-lipid hybrid nanoparticles, dendrimers, carbon nanotubes, hydrogels, and quantum dots have demonstrated unique properties that make them superior candidates for delivering therapeutic agents. One of the key advantages of nanomedicine is its ability to enhance drug stability and controlled release. By encapsulating drugs within nanocarriers, researchers can protect them from degradation, allowing for improved shelf life and efficacy. Moreover, the controlled release properties of nanocarriers enable sustained drug delivery, ensuring a continuous therapeutic effect while minimizing the frequency of administration. Another important aspect of nanomedicine is its ability to enhance tumor targeting. Through the use of targeting ligands or surface modifications, nanocarriers can specifically recognize and bind to cancer cells, thereby increasing drug accumulation at the tumor site and reducing systemic toxicity. Compared to traditional chemotherapy, this focused strategy has significant promise for enhancing treatment outcomes and decreasing adverse effects.

This article also shed light on the potential applications of nanomedicine beyond traditional chemotherapy. Gene-based vaccines and DNA vaccines represent innovative approaches that utilize nanocarriers to deliver genetic material for therapeutic purposes. These strategies have shown promise in cancer immunotherapy by activating the patient's immune system to target and destroy cancer cells specifically. Additionally, the combination of different therapeutic modalities (e.g., chemotherapy and immunotherapy) can be facilitated by nanocarriers, allowing for synergistic effects and improved treatment efficacy. While the field of nanomedicine has made remarkable progress, it is important to acknowledge the challenges that still need to be addressed. The safety and long-term effects of nanomaterials on the human body require thorough investigation to ensure their clinical translation. Standardized protocols for the synthesis, characterization, and evaluation of nanocarriers must be established to facilitate regulatory approval and clinical adoption. The collective findings shown demonstrate the immense potential of nanomedicine in revolutionizing cancer therapy and targeted drug delivery. Continued research and development efforts in nanotechnology hold the promise of providing more effective, personalized, and less toxic treatment options for patients in the future. With ongoing advancements, nanomedicine has the potential to significantly improve patient outcomes, enhance quality of life, and shape the future of healthcare.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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