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ADVANCING DNA VACCINES: UNLOCKING POTENTIAL, OVERCOMING CHALLENGES, AND SHAPING THE FUTURE OF IMMUNIZATION

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Abstract

DNA vaccines have emerged as a groundbreaking approach in immunization, providing notable benefits such as safety, stability, and rapid production. This review focuses on recent advancements in DNA vaccine technology, particularly in combatting infectious diseases like chikungunya virus (CHIKV) and Trypanosoma cruzi. Employing innovative delivery methods, such as electroporation, enhances the efficacy of synthetic DNA plasmids encoding monoclonal antibodies (dMAb), resulting in rapid antibody production and effective neutralization of various CHIKV strains. The synergistic combination of dMAb with traditional DNA vaccines demonstrates superior performance, indicating potential for both immediate and long-lasting immunity. Ongoing clinical trials highlight DNA vaccine advancements in oncology and HPV, using novel delivery systems, immunogenic epitopes, and combination therapies to boost therapeutic efficacy. Additionally, the DNA-prime/protein-boost vaccination strategy has shown significant long-term T cell responses against T. cruzi, achieving up to 85% vaccine efficacy while substantially reducing parasite burdens over time. This underscores the critical role of prime-boost regimens in fostering robust immune memory. The review also highlights the advantages of DNA vaccines in pandemic preparedness, emphasizing their swift adaptability to emerging pathogens and stability under diverse storage conditions. Notable innovations, such as SynCon DNA vaccines, which maintain efficacy for extended periods, reinforce their viability for deployment during outbreaks. This study emphasizes ongoing research aimed at addressing the challenges faced by DNA vaccines, paving the way for broader applications in both preventive and therapeutic strategies against a wide array of diseases.

Keywords: *Chikungunya virus, DNA vaccines, Electroporation, Immunization, Monoclonal antibodies, Pandemic preparedness, Prime-boost strategy, SynCon DNA vaccines, Trypanosoma cruzi*

INTRODUCTION

DNA vaccines have emerged as a revolutionary immunization approach, particularly highlighted during the COVID-19 pandemic. They work by delivering genetic material encoding antigens, which prompts the host's cells to generate specific immune responses. Recent studies in clinical and preclinical settings have showcased their promising efficacy and immunogenicity across various trials. Although challenges related to human immunogenicity persist, advancements in delivery systems and the incorporation of molecular adjuvants are enhancing their effectiveness. These innovations are crucial for overcoming barriers and improving vaccine response rates (1-3). This overview will explore the latest developments, case studies, and future perspectives of DNA vaccines in the prevention and treatment of infectious diseases, underscoring their potential to transform public health strategies and improve global vaccine accessibility.

HISTORY AND BACKGROUND

DNA vaccines, introduced in the early 1990s, marked a significant advancement in immunization by using plasmid DNA to trigger immune responses. The approval of ZyCovD in 2021 for COVID-19 highlighted their stability and effectiveness in eliciting strong immune reactions. Early challenges, including variable gene expression, have been mitigated through improved delivery systems and viral vectors shown in Table I. Currently, DNA vaccines are being tested in clinical trials for conditions like HIV, influenza, and

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various cancers, with ongoing research aimed at developing personalized therapies and enhancing adjuvants (4).

In the field of cancer immunotherapy, vaccines utilize the immune system to target malignant cells. Recent advancements feature DNA and RNA vaccines that express tumor-specific antigens, allowing for tailored treatments that differentiate between healthy and cancerous tissues (5). Despite encouraging trial outcomes, challenges persist, such as the diversity of tumor antigens and the potential for recurrence (6).

Table I. This table shows how DNA/RNA vaccines began, how they work, and how future improvements could make them powerful tools against tough diseases

History	Principles	Future aspects
Introduced in early 1990s	Use DNA/RNA to produce antigens inside the body	Self-replicating RNA vaccines enhance immune response
Early success in animal models	Targeted delivery to muscle or skin tissues	Potential for fighting cancer and chronic infections
Limited clinical success initially	Use of plasmids, adjuvants, co-stimulatory molecules to boost response	Better delivery methods and stronger immunogenicity with replicase-based vectors

MECHANISM OF ACTION (CELLULAR AND MOLECULAR)

DNA vaccines deliver genes encoding antigens, triggering host cells to produce and present these antigens, activating both cellular and humoral immune responses via specific pathways and mechanisms (7). The mechanism of DNA vaccines requires further molecular understanding, particularly regarding antigen presentation through MHC I and II pathways. Recent studies reveal that DNA vaccines, via their double-stranded structure, activate TBK1-dependent immune signaling pathways, bypassing Toll-like receptors. This influences antigen presentation by dendritic cells, which is vital for initiating CD4+ and CD8+ T cell responses. Further research into these mechanisms will enhance DNA vaccine efficacy and safety (8).

DNA vaccine technology has advanced significantly over the past two decades, yet low immunogenicity remains a challenge. Strategies to enhance effectiveness include novel plasmid vectors, optimized codons, electroporation, viral vector boosters, and adjuvants, alongside understanding host responses to improve immunogenicity (9).

This review assesses the principal SARS-CoV-2 vaccines, evaluating their mechanisms of action, efficacy, safety, storage, dosing strategies, and the impact of variants and alternative therapies like monoclonal antibodies (10). DNA vaccines work by introducing plasmid DNA encoding target antigens into cells, leading to antigen expression, which stimulates both cellular and humoral immune responses against pathogens (11).

TYPES OF DNA VACCINES

Monovalent DNA vaccines are designed to target a single pathogen serotype, inducing robust immune responses. They offer advantages in production and stability, facilitating effective immunization against specific infectious diseases like dengue (12). In aquaculture, DNA vaccines represent a significant advancement by utilizing plasmids that encode specific pathogen antigens. Delivery methods like electroporation or microinjection stimulate robust immune responses, prompting both humoral and cellular immunity (13). For dengue, the DNA vaccine pVAX1-D1ME showed promise by expressing the prME protein of dengue virus 1, inducing strong immune responses in mice (Table II) (12). In brucellosis research, multivalent DNA vaccines elicited robust immune responses but conferred limited protection against *Brucella abortus* (14).

Table II. Immunological response profile

Results	Details
Immune Response	Robust humoral and cellular responses
Measured Immune Markers	Increased IgM, IgG, and IFN-γ levels
Response Type	Th1-dominated response

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The study on multivalent DNA vaccines for brucellosis revealed robust immune responses, characterized by increased IgM, IgG, and IFN-γ levels, but offered limited protection against *B. abortus* 2308. The response was predominantly Th1-dominated (Table III).

Table III. Types of DNA Vaccines: monovalent, multivalent, and dengue-specific

Vaccines	Subtypes	Description
Monovalent DNA Vaccines	pSagF	Targets a specific antigen to induce protective immune responses
	pSagG	Focused approach with demonstrated survival rates against specific pathogens
	pSagI	Stimulates specific antibody production and enhances immune gene expression
		Survival Rates in Japanese Flounder**: 78% (pSagF), 65% (pSagG), 76%
Multivalent DNA Vaccines	pV273-sod	Incorporates multiple antigens to elicit a broader immune response
	pV278-sod	Targets additional Brucella abortus antigens for enhanced protection.
	pV273-278-sod	Combined approach targeting multiple antigens from Brucella abortus
		Immune Response in BALB/c Mice: Increased IgM, IgG, IFN-γ; limited protection against *B. abortus* 2308
Dengue DNA Vaccines	pVAX1-D1ME	Targets Dengue Virus serotype 1, expressing the prME protein; induces protection against lethal challenges
	pVAX1-D2ME	Targets Dengue Virus serotype 2; used in combination for a balanced immune response

**The table outlines DNA vaccine subtypes, highlighting monovalent vaccines for specific antigens, multivalent vaccines for broader responses, and dengue vaccines targeting serotypes for effective immune protection against pathogens*

APPLICATIONS OF DNA VACCINES

EXPLORING THE POTENTIAL OF DNA VACCINES IN CANCER THERAPY

DNA vaccines, particularly DNA and mRNA vaccines, have emerged as promising tools in cancer therapy by encoding tumor-associated antigens (TAs) that stimulate immune responses against cancer cells. DNA vaccines, including DNA and mRNA, offer promising cancer therapy by encoding tumor-associated antigens to stimulate immune responses. Challenges such as suitable antigens and immunosuppressive tumor environments remain, but ongoing research aims to improve their efficacy for broader applications (15).

SUMMARY OF ZIKA VIRUS DNA VACCINE TRIALS

Fig. 1 compares binding antibody responses to Zika virus for 1 mg and 2 mg doses over time. It shows that the 2 mg dose typically leads to a stronger immune response. Antibody levels may peak at certain times before declining, helping inform future vaccination strategies.

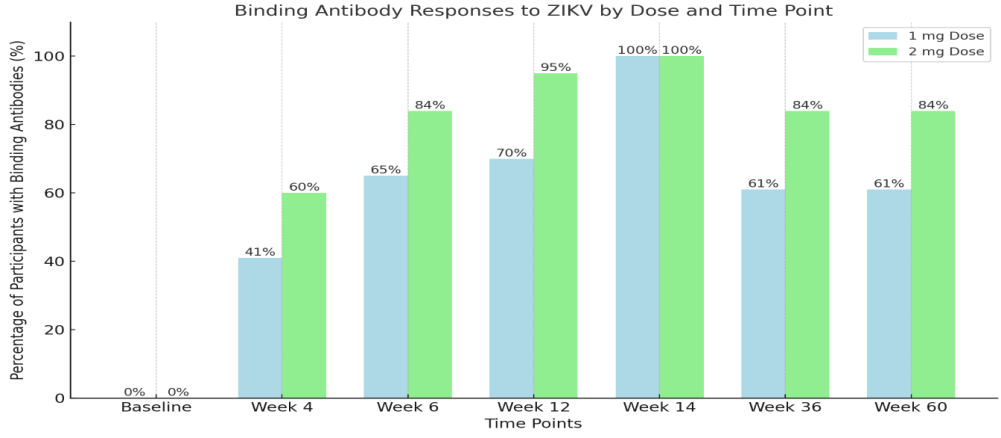

Fig. 1. Comparative analysis of zika virus antibody responses: 1 mg vs 2 mg doses

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Two phase 1 clinical trials (VRC 319 and VRC 320) evaluated Zika virus DNA vaccines in 125 adults, confirming safety and immunogenicity, particularly for VRC5283, which advanced to phase 2 efficacy testing (16). The Zika virus (ZIKV), linked to congenital defects and neurologic issues, lacks approved vaccines. The synthetic DNA vaccine GLS-5700 showed safety and immunogenicity in the ZIKA-001 phase 1 trial (17).

DNA VACCINES FOR VIRAL DISEASES

A DNA vaccine targeting the SARS-CoV-2 spike glycoprotein (S protein) demonstrated strong humoral and cellular immune responses following intramuscular administration with an alum adjuvant. Observed responses included increased antibody levels, IFNγ expression, and IgG2b production, promoting Th1-type immunity. Neutralization assays confirmed antibody effectiveness, and the vaccine showed protective effects in hamsters, indicating promise for human trials (18). DNA vaccines also show potential in aquaculture by encoding antigens for humoral and cell-mediated immunity, offering cost advantages over traditional vaccines (19).

Evaluations in rhesus macaques showed neutralizing antibodies similar to recovered individuals (20). A vaccine encoding the full-length S protein notably reduced respiratory tract viral loads post-challenge, highlighting DNA vaccines' potential to induce robust immunity and advance vaccine innovation (21).

DNA VACCINE FOR INFECTIOUS DISEASE

This review explores DNA vaccine advancements, focusing on stability, low costs, internalization mechanisms, immunogenicity challenges, and recombinant technology's role in development. Table IV summarizes various methods in engineered DNA vaccines, highlighting advancements in stability, safety, and immunogenicity. Key results demonstrate improved uptake efficiency and enhanced immune responses through recombinant technologies and effective adjuvant use (22).

Table IV. Recombinant DNA vaccines: Improving uptake efficiency and immune responses

Methods	Results
Engineering DNA plasmids	Enhanced stability and safety compared to traditional vaccines
Recombinant technology	Improved amino acid and DNA-based vaccines with controlled virulence
Non-viral gene delivery systems	Increased uptake efficiency and reduced endolysosomal degradation
Use of adjuvants	Boosted immunogenicity and extended duration of protection.
Internalization via endocytosis	Effective antigen presentation through both MHC I and MHC II pathways
Plasmid engineering	Controlled gene expression and enhanced immune responses

DNA vaccines for viral diseases and tumor antigens both leverage in vivo expression of antigens, inducing both humoral and cell-mediated immunity. While tumor vaccines focus on targeting cancer-specific antigens, viral vaccines aim to prevent or treat infections by targeting viral antigens (23). Electroporation uses electrical fields to temporarily open cell membranes for DNA and drug delivery, boosting gene uptake up to 1000-fold with minimal damage. It's effective across many tissues and has shown success in cancer therapy, vaccines, and gene therapy, including in human trials (24).

DNA VACCINES AND IMMUNITY AGAINST INFECTIOUS DISEASES

The development of DNA vaccines represents a breakthrough in immunization, effectively stimulating cytotoxic T-lymphocyte responses against viral infections and enhancing immunity where traditional vaccines fall short, offering promising adjunctive therapeutic options (25). DNA vaccines for influenza offer a promising alternative to traditional methods, enhancing immunogenicity through rapid production, dual immune responses, and advancements in antigen design and delivery, improving protection against seasonal and pandemic strains (26).

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DNA VACCINES IN AQUATIC DISEASE PREVENTION

DNA vaccines, like Clynav for salmon pancreas disease, show promise in inducing protective immune responses in fish. This review highlights immune profiling post-DNA vaccination, aiming to improve vaccine efficacy and targeted design (27).

DNA VACCINES FOR ALLERGY

Allergen-specific immunotherapy, delivered via subcutaneous injections or sublingual methods, effectively treats type I allergies but often faces patient hesitance due to treatment duration and side effects. An innovative approach involves preventive intervention in young children using genetic vaccines, such as plasmid DNA and mRNA. These vaccines promote T helper 1 (TH1) and regulatory responses to counteract allergic T helper 2 (TH2) reactions. With enhanced safety and purity, mRNA vaccines show promise for long-lasting protection against type I allergies (28).

DNA-VACCINES COMBINATIONS

Combinations of DNA vaccines with other platforms, such as mRNA, viral vectors, and protein subunits, enhance immunogenicity. These strategies can improve immune responses, providing a synergistic effect for better protection against diseases (29). Combining DNA vaccines with viral vectors and protein-based approaches enhances immune responses in HIV vaccine development, fostering protective antibody and T-cell responses against the virus (30). Combination therapy involving DNA vaccines with treatments like radiotherapy, chemotherapy, and immune checkpoint inhibitors enhances antitumor responses, reduces immune tolerance, and improves overall efficacy in cancer immunotherapy strategies (31).

NANOTECHNOLOGY IN DNA VACCINES

NANOTECHNOLOGY IN DNA VACCINE DELIVERY

Nanotechnology has advanced DNA vaccine delivery by improving stability, uptake, and targeting of DNA vaccines to immune cells. This approach tackles key issues like limited immunogenicity and poor cellular uptake seen in traditional DNA vaccines, showing potential for strong immune responses against various diseases (32).

ENHANCING IMMUNE ACTIVATION WITH NANOPARTICLES

Using nanoparticles allows encapsulation or adsorption of DNA, stabilizing and protecting it from degradation. These engineered particles resemble viral structures, promoting efficient antigen presentation and enhancing both humoral and cellular immune responses in DNA vaccines (33).

IMPROVING MRNA VACCINE EFFICACY

The mRNA vaccines, including those for COVID-19, have transformed infectious disease responses by prompting cells to produce immune-stimulating proteins. However, effective mucosal delivery remains challenging. Nanotechnology can improve delivery across mucosal barriers, bolstering immunity for respiratory infections (34)..

NANOPARTICLE-BASED PLATFORMS FOR DNA VACCINE DELIVERY

Nanoparticles like PLGA and liposomes are promising for delivering DNA vaccines. They enhance immune response modulation, offering targeted solutions against infectious diseases and autoimmune disorders (35).

STRATEGIES TO IMPROVE IMMUNOGENICITY AND POTENCY OF DNA VACCINES

ENHANCING IMMUNOGENICITY OF DNA VACCINES

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The study presents a novel nanoparticle-in-microsphere delivery system for DNA vaccines, enhancing immunogenicity by ensuring stable, sustained release and eliciting strong humoral and cellular immune responses in mice (36).

ROUTE OF IMMUNIZATION

DNA vaccines can be administered via various routes, including intramuscular, intradermal, and oral. Each route influences immunogenicity and immune system priming, affecting overall vaccine efficacy and clinical outcomes (37). Different routes of DNA vaccine delivery offer unique benefits, including high efficacy (Intramuscular), non-invasiveness (Oral), reduced pain (Subcutaneous), and improved patient compliance (Transdermal) (Table VI).

Table VI. Routes of DNA vaccine delivery: benefits and advantages

Route of delivery	Benefits
Intramuscular (IM)	High efficacy in protein expression; strong immune response, including CTL activation.
Oral	Non-invasive; suitable for mass immunization; enhances mucosal immunity.
Subcutaneous (SC)	Less painful than IM; effective for systemic immunity.
Transdermal	Painless, improves patient compliance; enhances local immune response.

IN VITRO AND IN VIVO EVALUATION

IN VITRO AND IN VIVO EVALUATIONS OF SYNTHETIC DNA VACCINES AGAINST ZIKA VIRUS

The Zika virus (ZIKV) poses a global health risk, linked to severe birth defects and Guillain-Barré syndrome, with no licensed vaccines available. Recent studies on a synthetic DNA vaccine targeting ZIKV's pre-membrane and envelope proteins show promising immune responses in vitro and robust protection in animal models, highlighting its potential for clinical use (38).

IN VIVO AND IN VITRO EVALUATION OF DNA VACCINES

Evaluating DNA vaccines involves both in vitro and in vivo methodologies to assess immunogenicity and efficacy. In vitro studies measure antigen expression in transfected cells, guiding candidate selection. In vivo evaluations in animal models assess humoral and cellular immune responses, critical for understanding protective effects and clinical applications in cancer immunotherapy (39).

IN VIVO EVALUATIONS

DNA vaccines were tested in mice and rhesus macaques using various delivery routes (IM, ID, gene gun, etc.). Immune responses were assessed by ELISA (anti-HBs antibodies, IgG1/IgG2a) and CTL assays. IM delivery induced strong Th1 (IgG2a) responses, while gene gun favored Th2 (IgG1). In macaques, response strength varied with dose and schedule, highlighting the need for species-specific optimization of DNA vaccine strategies (40).

In vivo studies show TBK1 is essential for both innate and adaptive immune responses to DNA vaccines. TBK1-deficient mice had reduced B and T cell responses, acting through a TLR-independent pathway. Bone marrow transfer confirmed TBK1's key role in hematopoietic cells for activating antigen-specific immunity (41).

SAFETY AND EFFICACY OF DNA VACCINES

DNA vaccines rely on TBK1, not traditional TLR pathways, to trigger immune responses. Their plasmid DNA activates TBK1-dependent signaling, producing type I interferons and enhancing antigen presentation. This is key for strong $CD4^+$ and $CD8^+$ T cell responses. Without TBK1, effective immunity from DNA vaccines is lost, highlighting its crucial role in vaccine efficacy (42).

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This review explores the advantages of biomaterial-based delivery systems, specifically micro- and nanoparticles, in improving DNA vaccine efficacy. These innovative strategies enhance immunogenicity and ensure effective immune responses against infectious diseases through optimal delivery and administration routes (43).

One study shows that CuMVTT-VLPs, due to their nano-size (~30 nm), quickly enter the lymphatic system. However, combining them with the larger MCT adjuvant (~5 μm) creates a local depot, prolonging immune exposure. In a preclinical B16F10p33 melanoma model, the CuMVTT-p33 nano-vaccine formulated with MCT significantly boosted specific T cell responses. Notably, MCT performed as well as B-type CpGs and outperformed Alum in enhancing CD8+ T cells, p33-specific responses, and tumor protection (44).

CLINICAL TRIALS

DNA vaccines for melanoma induce both humoral and cellular immune responses by encoding tumor-specific antigens. These vaccines stimulate both humoral (antibody-mediated) and cellular (T cell-mediated) immune responses, enhancing tumor recognition and destruction. They offer advantages such as enhanced stability, minimal side effects, and improved delivery strategies, making them a promising approach for effective cancer immunotherapy (45). DNA vaccines, like INO-4800, deliver plasmids encoding SARS-CoV-2 spike proteins, inducing strong humoral and cellular responses. They're stable, rapidly adaptable to emerging variants like Omicron, and effective in preclinical and early clinical trials, offering promise for next-gen COVID-19 protection (46). DNA vaccines against Zika virus (ZIKV) use prM and E proteins to induce strong immune responses. They are stable, easy to produce, cost-effective, and show promise for long-term protection against ZIKV infection (47).

ADVANTAGES OF DNA VACCINES

DNA vaccines exhibit a strong safety record with minimal side effects. They are stable at room temperature, simplifying storage and transport. Quick design and production process enable swift responses to emerging diseases. Effective against various pathogens and can be tailored for specific diseases (48).

CHALLENGES AND LIMITATIONS

DNA vaccines for avian infectious bronchitis face limitations, including variable immunogenicity compared to live attenuated vaccines, challenges in effective delivery mechanisms, regulatory hurdles delaying licensing, safety concerns regarding integration into the host genome, and production costs affecting economic viability for poultry farmers (Table VII) (49).

mRNA vaccines offer several advantages over DNA vaccines, making them a more effective platform in many cases. Unlike DNA vaccines, mRNA vaccines function directly in the cytoplasm without needing to enter the nucleus, resulting in more efficient protein expression and a stronger immune response. They also eliminate the risk of genomic integration, enhancing safety. Additionally, mRNA vaccines can be rapidly and safely produced using cell-free systems, allowing for quicker responses to emerging pathogens. A prominent example is the success of mRNA-based COVID-19 vaccines, which demonstrated high efficacy and adaptability during the pandemic (50).

Table VII. Comparison of DNA vaccines vs traditional vaccines (Regulatory Focus – FDA & EMA)

Immunogenicity	Lower; often needs adjuvants or prime-boost strategies	Strong, well-established immunity
FDA Regulation	CBER; 2007 guidance: preclinical focus—biodistribution, persistence, safety	Standard biologic license application process
EMA Regulation	Not defined as gene therapy; follows general vaccine guidelines	Covered under CPMP/465/95 and product-specific directives
Preclinical Requirements	Detailed: local/systemic toxicity, immunogenicity, autoimmunity risk	Standard: toxicity and efficacy in relevant animal models
Use in Humans	Undergoing Phase 1/2 trials; no market authorization	Widely used globally
Target Diseases	Emerging infections (HIV, Zika, Ebola, COVID-19)	Established (measles, polio, influenza,

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		tetanus, etc.)
Production	Rapid, cell-free synthesis	Biologic cultures (cells, eggs); slower, complex
Environmental Risk	Considered genetically modified organism (GMO); requires environmental assessment	Usually not GMO; less environmental oversight

DNA vaccines offer a novel approach, focusing on genetic material to induce immunity, while traditional vaccines use whole pathogens or their components. Regulatory pathways for both differ, with DNA vaccines in early stages (51).

ADVANCEMENTS IN DNA VACCINES

Recent advancements in DNA vaccines have shown promising potential in preventing infectious diseases and treating cancers. Innovations in delivery methods, such as electroporation, have enhanced immune responses. Clinical trials demonstrate efficacy against diseases like COVID-19 and Zika virus. Additionally, DNA vaccines offer safety and stability advantages, enabling rapid development and scalability in response to emerging health threats (52).

KEY ADVANCEMENTS IN DNA VACCINES

DNA vaccines allow easy modification of gene sequences for targeted immunization against various diseases. They can elicit both humoral and cellular immune responses. Generally more stable and safer than conventional vaccines. Current immunogenicity may be limited. Ongoing studies focus on improving effectiveness through diverse delivery methods, polymer-based carriers, and adjuvants (53). Recent advancements in DNA vaccines for cancer immunotherapy focus on enhancing efficacy through new delivery routes, immunomodulatory signals, modified prime-boost protocols, and checkpoint inhibition to stimulate robust immune responses against tumor antigens (54).

ADVANCEMENTS IN DNA VACCINES FOR CANCER

Clinical trials indicate DNA vaccines are well tolerated and safe. Immunogenicity: Enhanced antigen expression is linked to better immune responses. New methods are needed to trigger immunity against weakly immunogenic tumor antigens. Developments include tissue-specific elements, codon optimization, and targeting to improve vaccine efficacy. Genetic factors and cytokines are explored to boost immune responses (55).

THE PROMISE OF DNA VACCINES

DNA vaccines present an attractive option due to their ease of manufacture and ambient stability. However, effective delivery to immune cells remains a challenge. Ongoing research seeks to improve targeting, paving the way for future human applications (56). It highlights that DNA vaccines have demonstrated a good safety profile in clinical trials, with no significant adverse effects reported. However, the article does not specifically address concerns related to oncogenesis or the potential risks of DNA vaccines in inducing cancer. Therefore, based on the information provided in this article, DNA vaccines appear to be safe in the context of oncogenesis, but further studies are needed to fully assess their long-term safety and potential risks (57).

RECENT ADVANCES IN THERAPEUTIC DNA CANCER VACCINES

Clinical trials show DNA cancer vaccines are well-tolerated. Early results reveal modest therapeutic outcomes due to tumor-driven immunosuppression and identifying optimal antigens. Integrating with other therapies to strengthen immunity. Research targets overcoming limitations through preclinical and clinical analysis. Identifying critical points for advancing DNA vaccines in standard cancer care (57).

METHODS

The construction of DNA vaccines involves a systematic approach to produce plasmid DNA that encodes specific target antigens. The process begins with the amplification of the gene of interest using polymerase chain reaction (PCR), ensuring that there is an adequate quantity of DNA for further

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manipulation (58). This amplified gene is then cloned into an appropriate expression vector, The plasmid expression vector plays a central role in DNA vaccines by serving as the delivery vehicle for the antigen-encoding gene into host cells. Once inside the cells, the plasmid utilizes a eukaryotic promoter to drive transcription of the inserted transgene, leading to the production of the target antigen. This antigen is then processed and presented by the host's immune system, triggering both humoral and cellular immune responses (59) which includes critical regulatory elements such as promoters and enhancers, necessary for efficient gene expression within host cells (60).

The creation of DNA vaccines involves the introduction of genes encoding target antigens into plasmids, which are then delivered into host cells via methods like injection or gene guns. Once inside, the cells express the antigens, eliciting immune responses through MHC class I and II pathways, enhancing both humoral and cellular immunity.

The methods outlined include gene amplification via PCR to obtain target antigen genes, cloning these genes into plasmid vectors for expression, and introducing plasmids into bacteria for replication. Following this, high-purity plasmid DNA is isolated from bacterial cultures and formulated with adjuvants to enhance the immune response (Table VIII).

Table VIII. Plasmid DNA vaccine production: a methodological overview

Method	Description
Gene amplification (PCR)	Amplifies target antigen genes to obtain sufficient quantities.
Cloning into expression vectors	Inserts genes into plasmid vectors for proper antigen expression.
Bacterial transformation	Introduces plasmids into bacteria to replicate DNA.
Plasmid purification	Isolates high-purity plasmid DNA from bacterial cultures.
Formulation with adjuvants	Combines purified DNA with agents to boost immune response.

CURRENT RESEARCH AND CLINICAL TRIALS

Clinical Trial Summary of Andes Virus DNA Vaccine (61), has been shown in Table VII. This summary is derived from a Phase 1 clinical trial that evaluated the safety and immunogenicity of a novel Andes virus DNA vaccine, indicating its potential as a candidate for preventing hantavirus pulmonary syndrome (HPS) (Table IX).

Table IX. Phase 1 trial results: novel andes virus DNA vaccine shows promise against HPS

Category	Details
Background	Andes virus (ANDV) causes hantavirus pulmonary syndrome (HPS)
Trial Design	Phase 1, double-blind, dose-escalation trial with 48 healthy adults
Groups	1. Cohorts 1 & 2: 2 mg DNA; 3 doses (days 1, 29, 169) or 4 doses (days 1, 29, 57, 169) 2. Cohorts 3 & 4: 4 mg DNA; same dosing schedules
Safety	98% experienced local/systemic adverse events (AEs); most were mild or moderate
Immunogenicity	First-in-human trial shows promising safety and immunogenicity for the ANDV DNA vaccine

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Table X. Clinical trial results: evaluating the efficacy, immunogenicity, and safety of the DNA vaccine

Study/trial	Vaccine name	Type	Phase	Participants	Efficacy (%)	Immunogenicity	Key findings
Study 1	INO-4800	DNA vaccine	Phase 1	120	83.8	Strong humoral response	Safe and well-tolerated, good immune response observed.
Study 2	ZyCoV-D	DNA vaccine	Phase 2/3	28,000	66.6	T-cell and B-cell activation	Demonstrated safety and efficacy in diverse population.
Study 3	pVAX1-SARS-CoV-2	DNA vaccine	Phase 1	100	72.5	Robust neutralizing antibodies	Induced strong immune responses with minimal side effects.
Study 4	VGX-3100	DNA vaccine	Phase 2	400	70.0	High levels of IgG antibodies	Showed good safety profile and immunogenicity in older adults.

Key Points: Efficacy: Percentage of participants showing protective immune response; Immunogenicity: Measurement of the vaccine's ability to provoke an immune response; Safety: Generally well-tolerated with mild side effects reported (61).

CLINICAL AND PRECLINICAL ADVANCES IN DNA VACCINE EFFICACY

In the last two decades, DNA vaccines have demonstrated potential in clinical and preclinical trials for infections and cancer. Despite their stability and low production costs, poor immunogenicity in humans remains a challenge. Enhancements focus on optimizing delivery, using nano-carriers, targeting antigen-presenting cells, and incorporating molecular adjuvants for improved responses (58).

PRECLINICAL TRIALS OF DNA VACCINES

Preclinical trials are essential for assessing DNA vaccines' safety and effectiveness prior to human testing. Animal studies reveal that delivery methods impact immune responses, with needle injections favoring Th1 responses and gene guns promoting Th2 responses. Ongoing research aims to optimize formulations and explore genetic adjuvants to enhance efficacy and safety in humans (62).

CASE STUDIES

SUMMARY OF ZIKA VIRUS VACCINE TRIALS

Two Phase 1 clinical trials (VRC 319 and VRC 320) evaluated the safety and immunogenicity of DNA vaccines VRC5288 and VRC5283 for zika virus. Both vaccines showed a favorable safety profile, with VRC5283 eliciting significant immune responses, especially with split dosing (16).

Two vaccine delivery methods, standard injection (VRC5288) and needle-free injection (VRC5283), demonstrated safety and mild/moderate effects. However, VRC5283 showed a more significant immune response (Table XI).

Table XI. Comparative analysis of VRC5288 and VRC5283 vaccines

Vaccine	Administration	Safety	Immnogenecity
VRC5288	Standard Injection	Safe, mild/moderate effects	Moderate immune response
VRC5283	Needle-free Injection	Safe, mild/moderate effects	Significant immune response

. *DNA VACCINES FOR COVID-19*

The COVID-19 pandemic spurred the rapid development of DNA vaccines, which provide strong cellular immune responses, enhanced safety, and straightforward production. They prompt host cells to

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produce SARS-CoV-2 proteins, generating neutralizing antibodies. Current trials show DNA vaccines, like Zydus's EUA vaccine, exhibit strong immune responses and improved safety profiles (3).

COVID-EVAX: A CASE STUDY IN DNA VACCINES FOR COVID-19

The Table XII highlights the essential findings related to the COVID-eVax DNA vaccine (63).

Table XII. COVID-eVax DNA vaccine: safety, immunogenicity, and efficacy results

Vaccines type	Results
COVID-eVax (DNA vaccine targeting SARS-CoV-2 RBD)	Well tolerated; induced binding antibodies and T cells in up to 90% of participants at the highest dose; did not induce neutralizing antibodies; robust T cell-mediated immune response (Th1) observed.

FUTURE PERSPECTIVES

DNA VACCINES: A NEXT-GENERATION APPROACH

DNA vaccines, including DNA and mRNA formulations, offer a novel immunization strategy that stimulates strong cellular and humoral immunity, akin to live attenuated vaccines. Despite challenges in delivering fragile nucleic acids, nanoparticles (NPs) enhance stability and delivery, enabling simultaneous administration of multiple constructs for effective vaccination (64). DNA vaccines show great potential by inducing robust humoral and cellular immune responses. Recent advancements in vector design, molecular adjuvants, and delivery methods significantly enhance their immunogenicity and safety profiles (65). The review highlights the potential of DNA vaccines as a novel approach for chronic hepatitis B treatment. It emphasizes the need for innovative strategies to enhance immunogenicity and improve clinical outcomes in HBV patients (66).

PERSONALIZED DNA VACCINES: A PROMISING FRONTIER

Personalized DNA vaccines leverage tumor-specific mutations to create tailored immunotherapies, enhancing the immune response against unique cancer neoepitopes. Recent clinical trials demonstrate their feasibility, safety, and potential for effective cancer treatment (67).

INNOVATIONS IN CANCER VACCINE DEVELOPMENT

Therapeutic cancer vaccines activate CD8+ and CD4+ T cells to boost anti-tumor immunity. Emerging trials explore mRNA, DNA, and peptide-based strategies, refining delivery and dosing for effective immune responses in solid tumors (68).

PERSONALIZED DNA VACCINES AND IMMUNOGENETIC VARIABILITY

Vaccines are a promising disease prevention strategy, but their effectiveness varies due to individual genetic differences. Genetic polymorphisms can influence immune responses, affecting vaccine protection. Advances in vaccine technology underscore the need for personalized approaches, utilizing DNA biobanking and immunoprofiling to optimize immunogenicity and enhance efficacy in personalized healthcare (69).

REGULATORY PATHWAYS AND ACCEPTANCE

REGULATORY CONSIDERATIONS FOR DNA VACCINES

All DNA vaccines undergo testing in appropriate animal models to establish immunogenicity and safety, adhering to national guidelines. Follow regulations established by authorities, such as the USDA and EU directives on animal welfare. DNA vaccines intended for human use must undergo a clinical development program that includes trials approved by regulatory authorities and ethics committees. Participants in human trials must provide informed consent, understanding the study's purpose, potential risks, and their right to withdraw at any time. After demonstrating safety and efficacy, a comprehensive MAA (Marketing Authorization Application) must be submitted to the national competent authority for review. Conduct an environmental risk assessment to evaluate the potential effects of genetically modified

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organisms (GMOs). Monitor for unintended immune responses and safety concerns even after the vaccine is approved for commercial use (70).

LONG-TERM PROTECTION AND BOOSTER REGIMENS: INSIGHTS FROM DNA VACCINE RESEARCH

This study introduces electroporation-mediated delivery of synthetic DNA plasmids encoding a monoclonal antibody (dMAb) to enhance immunity against chikungunya virus (CHIKV). dMAb rapidly produced protective antibodies, neutralized diverse CHIKV strains, and, when combined with a DNA vaccine, provided immediate and long-term protection, improving responses to viral outbreaks (71).

Electroporation-mediated delivery of synthetic DNA plasmids encoding dMAb resulted in rapid antibody production and effective neutralization of diverse CHIKV strains. When combined with DNA vaccines, dMAb demonstrated superior performance compared to traditional antibody therapies, highlighting its potential for enhanced protection against CHIKV (Table XIII).

Table XIII. Novel approach to chikv protection: electroporation-mediated dMAb delivery

Methods	Results
Electroporation-mediated delivery	Effective delivery of synthetic DNA plasmids encoding dMAb
Intramuscular injection of dMAb	Rapid production of protective antibodies.
Neutralization assays	dMAb neutralized diverse CHIKV strains
Combination with DNA vaccine	
Comparison with traditional vaccination	dMAb outperformed conventional antibody therapies

The study demonstrates that a DNA-prime/protein-boost (D/P) vaccine effectively induces long-lasting T cell immunity against *Trypanosoma cruzi* infection. Notably, the vaccine maintains robust CD4+ and CD8+ T cell responses for up to 180 days post-vaccination, leading to significantly reduced parasite burdens compared to unvaccinated controls. The introduction of booster immunizations enhances protective efficacy by increasing the quantity of parasite-specific T cells, highlighting the potential of this strategy to improve long-term vaccine effectiveness against *T. cruzi* (72).

Table XIV summarizes the results of the DNA-prime/protein-boost vaccine against *Trypanosoma cruzi*. The DNA-prime/protein-boost strategy significantly enhanced immune responses and reduced parasite burden, achieving 85% efficacy and sustained immunity.

Table XIV. Vaccine efficacy and immunogenicity: unvaccinated control vs. DNA prime + protein boost

Parameter	Unvaccinated control	Dna prime + protein boost
CD4+ T Cell Response	Low	High
CD8+ T Cell Response	Low	High
Parasite Burden (Day 30)	High	Low
Parasite Burden (Day 180)	High	Low
Long-term Immunity	Absent	Sustained
Vaccine Efficacy	0 %	85%

ENHANCING IMMUNITY THROUGH PRIME-BOOST VACCINATION STRATEGIES

Prime-boost vaccination strategies enhance long-term immunity by combining different vaccine modalities, with heterologous approaches often outperforming homologous ones in immunogenicity (73).

Protein-based adjuvants enhance vaccine efficacy by activating innate immune pathways and promoting cytokine production. These adjuvants stimulate immune receptors like Toll-like receptors and the NLRC4 inflammasome, leading to stronger immune responses. While the mechanisms behind adjuvant action are well explained, the comparison of immune responses from DNA vaccines alone versus those combined with protein or adjuvant boosters is not directly addressed. Therefore, while the article provides valuable insight into adjuvant function, it does not specifically compare their impact on DNA vaccine performance (74).

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LONG-TERM IMMUNE RESPONSES INDUCED BY DNA VACCINES

DNA vaccines targeting hantaviruses offer long-term immune protection through enhanced cellular and humoral responses. The lysosome-associated membrane protein (LAMP) strategy improves MHC class II antigen presentation, fostering sustained immunological memory. Repeated immunization promotes durable antibody production and robust T-cell activation, demonstrating the potential for lasting immunity against viral pathogens (75).

LONG-TERM EFFICACY OF PVAX-LAMP/GN VACCINE

The recombinant DNA vaccine pVAX-LAMP/Gn demonstrates promising long-term immunity against Hemorrhagic fever with renal syndrome (HFRS) caused by Hantavirus. Evaluated through robust immune assays, it establishes memory responses and significantly enhances protective immunity. These findings indicate its potential as a viable candidate for future vaccination strategies, warranting further exploration into booster dose requirements for sustained protection (76).

ROLE IN PANDEMIC PREPAREDNESS

ADVANCEMENTS IN DNA VACCINES: A RAPID RESPONSE TO PANDEMIC CHALLENGES

DNA vaccines offer a promising rapid response to pandemics, addressing the limitations of egg-based vaccines highlighted during the 2009 swine flu outbreak. Their benefits include temperature stability, cost-effectiveness, and rapid production. Targeting influenza antigens enhances immunogenicity, with single-dose DNA vaccines inducing strong humoral and cytotoxic T-cell responses for future preparedness (77).

DNA vaccines are pivotal in pandemic preparedness, offering rapid development, thermostability, and cost-effectiveness. Their ability to quickly adapt to emerging pathogens, coupled with strong safety profiles and immune responses, positions them as viable options for future outbreaks. Successful implementations, like ZyCoV-D for SARS-CoV-2, further illustrate their potential in enhancing global vaccination strategies.(78)They also show promise against avian influenza viruses, inducing strong immune responses while providing advantages such as stability and safety.(79)Targeting CD8+ T cells, these vaccines enhance cytotoxic responses crucial for viral infections like influenza.(80)Encoding bivalent fusion proteins targeting hemagglutinin allows rapid production, potentially within a month during pandemics (81).

ISSUES FOR RESOLVING PLASMID DNA VACCINES

DNA vaccines offer a promising alternative to conventional vaccines by stimulating both humoral and cellular immune responses against intracellular pathogens. Enhancing their immunogenicity involves optimizing plasmid vector backbones, employing diverse delivery methods, exploring alternative administration routes, and incorporating adjuvants, crucial for advancing their clinical applications and overcoming current challenges (37).

DNA VACCINES STORAGE STABILITY

SynCon DNA vaccines, including INO-4201 and INO-4202, maintain stability at room temperature and 2–8°C for extended periods, with INO-4202 remaining stable for up to 36 months at 4°C, facilitating efficient storage and deployment during outbreaks (82).

CONCLUSION

In conclusion, DNA vaccines represent a significant advancement in the field of immunization, especially in the wake of the COVID-19 pandemic. Their ability to elicit strong immune responses through genetic material delivery has been validated in various studies. While challenges regarding human immunogenicity remain, ongoing innovations in delivery methods and molecular adjuvants hold the promise of improving vaccine efficacy. As research continues to evolve, DNA vaccines have the potential to play a pivotal role in the prevention and treatment of infectious diseases, offering a versatile and effective

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tool for enhancing global health and combating emerging pathogens, Including DNA vaccines have shown great promise over the past two decades as a safe, stable, and easily engineered platform for immunization. By inducing strong cellular and humoral immune responses, they offer potential for both prophylactic and therapeutic applications. Ongoing research supports their use against infectious diseases and cancers, marking a significant advancement in molecular vaccinology and immunotherapy

Authors' contribution:

IH Abstract, Conclusion, Review of literature and Case studies; ZS Tables and Figures; UM References cross check; MN Citations, Cross check; AS Data analysis; SLA Drafting of the Entire document, Topic selection, Review of Literature, Case Studies, Editing and Final Compilation; AM Conceptualiztion, Data collection and Proof reading; MSS Manuscript writing, Data collection and Formatting; SB Data Collection; AR Data collection.

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