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REVIEW ARTICLE

Review: Protein Subunit and DNA Vaccines Key Research, Development & Future Directions

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ABSTRACT

Objective: To analyze the advancements, difficulties, and potential of DNA and protein subunit vaccines, with an emphasis on manufacturing capabilities, safety, and effectiveness, and to gauge their influence on upcoming vaccine studies.

Background: Purified pathogen fragments are used in protein subunit vaccines to boost immunity, whereas DNA vaccines use genetic material encoding antigens to trigger immune responses. Both offer unique opportunities and problems, but they have shown potential, especially during the COVID-19 epidemic.

Methods: A thorough literature review was carried out, with an emphasis on current research, clinical trials, and technical developments pertaining to DNA and protein subunit vaccines. Relevance, technique, and field influence were taken into consideration when choosing key papers. Articles were selected based on information on current advancements and potential future prospectives.

Results: The effectiveness of protein subunit vaccines has increased due to developments in adjuvants, protein expression, and stability. Scaling up manufacturing and high production costs are challenges. The advantages of DNA vaccines include improved efficacy because of developments in delivery technologies, such as lipid nanoparticles, rapid development and economical manufacture. The effectiveness of protein subunit vaccines has improved due to advancements in adjuvants, protein expression, and stability. Activating immunological responses, guaranteeing efficient DNA delivery, and overcoming regulatory obstacles are among the difficulties. DNA vaccines need more thorough testing, while protein subunit vaccinations have a strong safety record.

Conclusion: Both vaccination platforms offer unique benefits. Protein subunit vaccines offer proven production techniques and superior safety, whereas DNA vaccines are developed and scaled quickly. To effectively combat infectious diseases, future development necessitates overcoming current obstacles, improving delivery methods, and investigating combination techniques. Further novel research, continuous studies and clinical trials are crucial to maximize their potential in international immunization campaigns.

Keywords
DNA Vaccines, Protein Subunit Vaccines, Efficacy, Safety, Manufacturing, COVID-19, Delivery Systems, Vaccine Development.

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INTRODUCTION

In the fight against infectious illnesses, vaccines have shown to be one of the most effective public health interventions[1]. Without causing the disease, they function by boosting the immune system's ability to identify and eliminate invaders like bacteria and viruses[2]. Over the course of advancements, vaccine research has changed, and recent worldwide health emergencies, such the COVID-19 pandemic, have sped up the creation of new vaccine platforms. Protein subunit vaccines[3] and DNA vaccines[4,5] are two of the most promising strategies; both have drawn a lot of interest in the fields of immunology and vaccine research[6]. Although both DNA and protein subunit

vaccines have shown a great deal of promise[7], their methods of action, manufacturing procedures, and difficulties are very different. While DNA vaccines provide the benefits of quick creation and scalability, protein subunit vaccines typically have a longer history of safety and efficacy[8]. The need to compare these two platforms in terms of their efficacy, safety, and production scalability is growing as the need for vaccines around the world rises, particularly considering newly emerging infectious illnesses and the potential of pandemics. With an emphasis on their uses, manufacturing processes, and safety profiles, this review attempts to examine the major developments, difficulties, and potential paths forward in the creation of protein subunit and DNA vaccines.

By contrasting these two vaccination platforms, we hope to offer a thorough grasp of how they might work in tandem within the global vaccine development environment and how they might change to meet the demands of future vaccine deployment, especially in environments with limited resources. Highlighting current developments in the area, like the ongoing development of next-generation adjuvants for protein subunit vaccines and the use of innovative delivery mechanisms for DNA vaccines is needed for future advancements. Additionally, the manufacturing and regulatory obstacles that prevent both kinds of vaccines from being produced on a big scale and offer possible ways towards improvements. This review ultimately seeks to advance knowledge of how protein subunit and DNA vaccines can influence vaccine development going forward by synthesizing the current state of research and offering insight into the most successful methods for preventing infectious diseases in the twenty-first century. Table **1** providing data on efficacy of protein subunit and DNA Vaccines.

RESULT AND DISCUSSION

Protein Subunit Vaccines

Vaccines containing pure proteins or fragments obtained from infections that trigger immune responses are known as protein subunit vaccines[17]. These vaccinations frequently contain one or more antigens, which are the pathogen's proteins or other components that elicit immunity[8]. Since protein subunit vaccinations don't contain live pathogens like whole-virus or whole-bacterium vaccines do, there is a lower chance of negative reactions[18]. Protein subunit vaccines have been effectively created in recent decades to prevent diseases like influenza[19], hepatitis B[20], and the human papillomavirus (HPV)[21]. With the development of vaccines such as NVX-CoV2373 (Recombinant, Adjuvanted) COVID-19 Vaccine demonstrating encouraging outcomes[22], protein subunit vaccines have recently played a major role in the battle against COVID-19[23].

Despite being largely regarded as safe, protein subunit vaccinations present several difficulties. Producing and purifying recombinant proteins at a scale that satisfies demand worldwide is one of the main challenges[24]. Furthermore, adjuvants are frequently needed to improve the immune response to the antigen, and vaccine effectiveness depends on how well these adjuvants are optimized[25]. Furthermore, a crucial topic of research is finding ways to guarantee the stability of protein-based vaccines, particularly at room temperature.

Table1. Efficacy of Protein and Subunit Vaccines.

Vaccine Type	Vaccine Name	Technology	Efficacy	Adjuvant Used	Manufacturing Considerations
Protein Subunit	Nuvaxovid (NVX-CoV2373)	Recombinant protein	~90% (COVID-19)[9]	Matrix-M (Saponin-based)[9]	Requires insect-cell-based expression system[10]
	HEPLISAV-B	Recombinant Hepatitis B vaccine	~95% (Hepatitis B)[11]	CpG 1018[12] (TLR9 agonist)	Stable at 2–8°C, conventional production[13]
DNA Vaccine	ZyCoV-D	Plasmid DNA-based	~67% (COVID-19)[14]	Electroporation (Needle-free)	Rapid production, room-temperature storage[15]
	INO-4800	DNA-based	Phase 3 trials ongoing[16]	Electroporation	Easily scalable, requires electroporation devices

DNA Vaccines

DNA vaccines are a more recent and creative method[7]. To administer these vaccinations, DNA encoding particular pathogen antigens is directly injected[26]. After the DNA enters the body, host cells absorb it and utilize the genetic material to create the pathogen's antigens. Both the humoral (antibody-mediated)[27] and cellular (T-cell-mediated)[28] arms of immunity are activated when this process sets off an immune response. The ease of manufacture, the possibility of quick scale-up, and the capacity to target a variety of pathogens are some benefits of developing DNA vaccines[7,29]. Animal tests of DNA vaccines against cancer[30,31], malaria[32], and the Zika virus[33] have been completed with success. Despite these benefits, a number of issues with DNA vaccines must be resolved before they can be widely used.

The effective transfer of DNA into host cells is a significant challenge. Although DNA uptake has been enhanced recently by lipid nanoparticles (LNPs)[34] and electroporation techniques[35], more optimization is required to achieve high levels of immune response. Furthermore, research is still ongoing to determine the long-term safety and effectiveness of DNA vaccines, especially in people[36]. Furthermore, the effective role of DNA vaccines for emergency use, such ZyCoV-D for COVID-19[37], has raised hopes for their future. Table **2** below compares DNA vaccines and protein subunit vaccines in terms of effectiveness, safety, risks, production and applications.

Table 2. Comparison of DNA and Subunit Vaccines in Terms of Efficacy and Applications.

Criteria	DNA Vaccines	Protein Subunit Vaccines
Mechanism of Action	DNA encoding pathogen antigens is delivered into cells, where it triggers the production of the antigen to stimulate immunity[38].	Purified pathogen proteins or subunits are introduced to stimulate an immune response[39].
Effectiveness	Both humoral (mediated by antibodies) and cellular (mediated by T cells) immunity may be triggered. Depending on the distribution strategy (lipid nanoparticles, for example), efficacy may differ. - Emerging clinical research shows encouraging outcomes for illnesses like COVID-19 (e.g., ZyCoV-D)[40,41].	Very successful at eliciting immunological responses, particularly for illnesses with distinct antigens (e.g., HPV, Hepatitis B). A successful track record, as evidenced by the effectiveness of vaccines such as Novavax "saponin-based adjuvant" (COVID-19)[42,22].
Safety	Generally regarded as safe, these vaccinations have fewer adverse effects than live-attenuated ones. Although long-term safety evidence is still being gathered, worries about plasmid DNA immune response and genetic integration remain[29].	A long history of usage in vaccines (e.g., HPV, Hepatitis B) has led to a well-established safety profile. Injection site discomfort and moderate fever are common minor adverse effects[19].
Adjuvants	Usually needs adjuvants (such as electroporation or lipid nanoparticles) for an effective immune response. To enhance delivery and immune activation, research is still being conducted[43].	- frequently needs adjuvants such as MF59[44] (an oil-in-water emulsion adjuvant containing squalene) or aluminum salts to strengthen the immune response because protein antigens might not be enough to produce robust immunity on their own[45].
Production Complexity	Quicker and simpler to make than vaccinations using protein subunits. In vitro, DNA plasmids can be produced rapidly. Issues with DNA stability and effective delivery[46].	More costly and intricate to produce on a large scale. Involves the time-consuming and expensive process of expressing recombinant proteins in cell cells.
Manufacturing Scalability	Extremely scalable and capable of rapid production in the event of an outbreak. Economical in large-scale manufacture because of straightforward manufacturing procedures[46].	Because protein purification and quality control are required, production is slower and more expensive than with DNA vaccines[47].

Cold Chain Requirement	Usually needs to be kept cold, although new formulations are making stability better. DNA vaccine stability at room temperature is still being worked out[48].	Protein stability frequently requires stringent cold chain management (e.g., 2–8°C), however new developments in stabilizers and adjuvants are enhancing storage[49].
Regulatory Approval Status	Certain DNA vaccines, including ZyCoV-D, have been approved for emergency use. Many DNA vaccines still need full regulatory approval[15].	Numerous protein subunit vaccines, such as those for HPV and Hepatitis B, have received international licensure and approval. Vaccines against the COVID-19 protein component, such as Novavax (saponin-based adjuvant), are approved for emergency use[50].
Applications	It can be applied to a variety of illnesses, including as genetic disorders, cancer immunotherapy, and viral infections (such Zika and COVID-19). Ideal for quick reactions to emerging pandemics[51].	Hepatitis, influenza, HPV, and COVID-19 are among the illnesses for which it is effective against known antigens. It is also used for routine vaccination against chronic infections[52].
Side Effects and Potential Risks	DNA vaccines are generally well-tolerated, with mild adverse effects such as injection site reactions, fever, and fatigue[53]. However, there are concerns regarding potential genomic integration, the risk of inducing autoimmunity and long-term persistence of plasmid DNA[54]. Theoretical risks include the possibility of anti-DNA antibodies, although clinical evidence is still inconclusive[55].	Protein subunit vaccines have a firm safety profile but might induce mild to moderate side effects such as local pain, fever, and exhaustion[56]. Some protein-based vaccines require strong adjuvants, which can increase reactogenicity, leading to heightened immune responses or inflammatory reactions in some cases[57]. There is also a potential risk of allergic reactions to certain protein components[58].

Table 3. Types of Adjuvants.

Adjuvant Type	Mechanism of Action	Effectiveness & Application	Safety Profile
Aluminum-Based Adjuvants	Forms a depot at the injection site, prolongs antigen exposure, activates immune cells[64].	Used in DTP, Hepatitis A & B, HPV vaccines[64].	Local site reactions, mild fever.
Emulsion-Based Adjuvants	Oil-in-water emulsion enhances antigen uptake by APCs[65].	Used in influenza vaccines, improves antibody response.	Mild systemic reactions (fatigue, muscle pain), rare allergic reactions[65].
Toll-Like Receptor (TLR) Agonists	Activates TLR4 (MPL) or TLR9 (CpG), enhances innate and adaptive immune response[66].	Used in HPV, hepatitis B, and malaria vaccines[66].	Low risk of autoimmunity, mild systemic effects.
Saponin-Based Adjuvants	Stimulates dendritic cells, enhances cellular immunity[67].	Used in malaria and shingles vaccines[67].	Transient pain at the injection site, fever.
Virosomes & Nanoparticles	Mimics virus-like particles, enhances antigen presentation and immune activation[63].	Used in COVID-19 (NVX-CoV2373) and hepatitis A vaccines.	Mild local and systemic reactions, well-tolerated.

Future Directions for Protein Subunit Vaccines

The efficacy, safety, and scalability of vaccine platforms can now be improved because to the quick developments in vaccine technology[59]. Protein subunit vaccines have already demonstrated their effectiveness against various infectious diseases. However, there is room for improvement in several key areas to maximize their potential.

Development of More Effective Adjuvants

Protein antigens alone may not elicit significant immune responses, so adjuvants are frequently included in subunit vaccinations to supplement the immune response[60]. The goal of future research is to create less reactive and more effective next-generation adjuvants[61]. Adjuvants based on saponins, such as Matrix-M (a saponin-based adjuvant derived from the bark of the Quillaja saponaria tree), which

is a component of Novavax's COVID-19 vaccine, have the potential to improve humoral and cellular responses[62]. In clinical trial phase 3, conducted in the United Kingdom, Novavax's COVID-19 vaccine candidate, NVX-CoV2373, has demonstrated an overall effectiveness of 89.7% against COVID-19[9]. Adjuvants based on nanoparticles may provide offer longer-lasting immune activation and more precise delivery[63]. Table **3** above provides details about some types of vaccine adjuvants, their mechanism of action and applications.

Improving Vaccine Stability

Cold chain storage is frequently necessary for protein subunit vaccinations to remain stable, which makes distribution difficult in environments with limited resources[68]. The goal of future studies is to use stabilizers and freeze-drying methods to increase the thermostability of protein antigens[69]. For example, formulations that include protein encapsulation in nanoparticles or sugars like trehalose may assist increase stability in ambient circumstances[70].

Scaling Up Production

Recombinant protein expression in mammalian or insect cells is one of the intricate and expensive procedures used in the creation of protein subunit vaccines. Utilizing plant-based expression systems or cell-free protein synthesis are two examples of bioprocess engineering advancements that could lower costs and boost scalability[71]. These techniques are especially pertinent for quick reactions to outbreaks and pandemics.

Future Directions for DNA Vaccines

Because of their quick development cycle and capacity to elicit humoral and cellular immunity, DNA vaccines have become a potential platform[72]. In the future, the following domains will be crucial to their progress.

Personalized Medicine

The capacity of DNA vaccines to encode specific antigens has enormous promise for customized therapy, especially in the treatment of cancer[73]. Tumor-specific antigens can be encoded into custom DNA vaccines, allowing for accurate cancer cell targeting[74]. Field of personalized medicine is expected to advance because of the advancements in genomic technology and AI-driven antigen design[75,76].

Optimizing Delivery Methods

DNA transport into host cells and its efficiency is still challenging[77]. Future studies will concentrate on improving delivery methods like viral vectors, electroporation, and lipid nanoparticles[78]. Cutting-edge techniques like polymer-based carriers[79] and microneedle patches may also increase delivery effectiveness and lessen the necessity for invasive techniques[80].

Potential Use in Cancer Vaccines

Because DNA vaccines stimulate both innate and adaptive immunity, they have demonstrated potential in cancer immunotherapy[81]. DNA vaccines that encode checkpoint inhibitors or neoantigens, for instance, have shown promise in preclinical cancer models[82]. To assess their efficacy in treating human diseases like lung cancer and melanoma, clinical trials are still being conducted[73].

Combination Approaches

By utilizing each platform's advantages, combining DNA and protein subunit vaccines may have synergistic effects.

Broader Protection

Protein subunit vaccinations can serve as boosters to improve antibody titers and long-term memory responses[83], whereas DNA vaccines can activate the immune system by encoding antigens[84]. This strategy may offer more comprehensive defense against various pathogen strains or variations.

Higher Efficacy

By more successfully promoting humoral and cellular immunity, combining the two platforms may increase efficacy[85]. For example, subunit vaccinations produce potent antibody responses[86], but DNA vaccines can stimulate powerful T-cell responses[38]. A combination like this might be very helpful for diseases like HIV, malaria, or newly discovered zoonotic viruses[87].

Rapid Pandemic Response

When it comes to pandemics, DNA vaccines can be quickly created and manufactured for initial distribution, and as production is scaled up, protein subunit vaccines can be used as boosters[88]. This two-pronged strategy could maximize vaccination coverage and guarantee long-term protection in populations.

CONCLUSION

Vaccinations have transformed public health by successfully preventing infectious diseases. The continuous developments in DNA and protein subunit vaccines represents a crucial area in immunology and biotechnology. Improved production methods, increased stability, and next-generation adjuvants all contribute to proven efficacy against a variety of viral illnesses, including hepatitis B, influenza, and HPV. The expensive production procedures, stability in non-cold-chain environments, and dependence on adjuvants are among the difficulties. DNA vaccines are a desirable alternative due to their quick design, simplicity in manufacturing, and capacity to elicit humoral and cellular immunity. Regulatory permission for widespread use, long-term safety concerns, and distribution mechanism optimization are some of the current obstacles. By utilizing the advantages of both platforms, the synergistic application of DNA and protein subunit vaccines provides increased protection and enhanced efficacy.

Combination tactics may be crucial in combating pandemics, especially if DNA vaccines and protein subunit boosters are quickly implemented. By enabling targeted responses to both endemic and emerging infectious illnesses, these developments highlight the potential to completely transform vaccine development. DNA and protein subunit vaccines are complimentary strategies that have the potential to revolutionize vaccine development worldwide. The scientific community can better prepare for the changing landscape of infectious illnesses in the twenty-first century by funding cutting-edge research, resolving current obstacles, and encouraging collaboration between different platforms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Shukla VV, Shah RC. Vaccinations in primary care. Indian J Pediatr. 2018;85(12):1118-27.
2. Canouï E, Launay O. History and principles of vaccination. Rev Mal Respir. 2019;36(1):74-81.
3. 3.Chavda VP, Ghali E, Balar PC, Chauhan SC, Tiwari N, Shukla S, *et al*. Protein subunit vaccines: Promising frontiers against COVID-19. J Control Release. 2024;366:761-82.
4. Reimann J, Schirmbeck R. DNA vaccines. Vox Sang. 2000;78 Suppl 2:57-60.
5. Tiptiri-Kourpeti A, Spyridopoulou K, Pappa A, Chlichlia K. DNA vaccines to attack cancer: Strategies for improving immunogenicity and efficacy. Pharmacol Ther. 2016;165:32-49.
6. Coirada FC, Fernandes ER, Mello LR, Schuch V, Soares Campos G, Braconi CT, *et al*. Heterologous DNA prime- subunit protein boost with chikungunya virus E2 induces neutralizing antibodies and cellular-mediated immunity. Int J Mol Sci. 2023;24(13).
7. Pagliari S, Dema B, Sanchez-Martinez A, Montalvo Zurbia-Flores G, Rollier CS. DNA vaccines: history, molecular mechanisms and future perspectives. J Mol Biol. 2023;435(23):168297.
8. Tsoras AN, Champion JA. Protein and peptide biomaterials for engineered subunit vaccines and immunotherapeutic applications. Annu Rev Chem Biomol Eng. 2019;10:337-59.
9. Heath PT, Galiza EP, Baxter DN, Boffito M, Browne D, Burns F, *et al*. Safety and efficacy of NVX-CoV2373 Covid-19 Vaccine. New Eng J Med. 2021;385(13):1172-83.
10. Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, *et al*. Phase 1–2 Trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. New Eng J Med. 2020;383(24):2320-32.
11. Schillie S, Harris A, Link-Gelles R, Romero J, Ward J, Nelson N. Recommendations of the advisory committee on immunization practices for use of a hepatitis B vaccine with a novel adjuvant. MMWR Morb Mortal Wkly Rep. 2018;67(15):455-8.
12. Lee GH, Lim SG. CpG-Adjuvanted Hepatitis B Vaccine (HEPLISAV-B®) Update. Expert Rev Vaccin. 2021;20(5):487-95.
13. Splawn LM, Bailey CA, Medina JP, Cho JC. Heplisav-B vaccination for the prevention of hepatitis B virus infection in adults in the United States. Drugs Today (Barc). 2018;54(7):399-405.
14. Momin T, Kansagra K, Patel H, Sharma S, Sharma B, Patel J, *et al*. Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical

study by intradermal route in healthy subjects in India. E Clin Med. 2021;38:101020.

15. Dey A, Chozhavel Rajanathan TM, Chandra H, Pericherla HPR, Kumar S, Choonia HS, *et al*. Immunogenic potential of DNA vaccine candidate, ZyCoV-D against SARS-CoV-2 in animal models. Vaccin. 2021;39(30):4108-16.
16. Tebas P, Yang S, Boyer JD, Reuschel EL, Patel A, Christensen-Quick A, *et al*. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, Phase 1 clinical trial. E Clin Med. 2021;31:100689.
17. Zhang Y, Xu JC, Hu ZD, Fan XY. Advances in protein subunit vaccines against tuberculosis. Front Immunol. 2023;14:1238586.
18. 18. Karauzum H, Adhikari RP, Sarwar J, Devi VS, Abaandou L, Haudenschild C, *et al*. Structurally designed attenuated subunit vaccines for S. aureus LukS-PV and LukF-PV confer protection in a mouse bacteremia model. PLoS One. 2013;8(6):e65384.
19. Zhang Y, Gao J, Xu W, Huo X, Wang J, Xu Y, *et al*. Advances in protein subunit vaccines against H1N1/09 influenza. Front Immunol. 2024;15:1499754.
20. Gattinger P, Kratzer B, Sehgal ANA, Ohradanova-Repic A, Gebetsberger L, Tajti G, *et al*. Vaccine based on recombinant fusion protein combining hepatitis B Virus PreS with SARS-CoV-2 Wild-Type- and omicron-derived receptor binding domain strongly induces omicron-neutralizing antibodies in a murine model. Vaccine (Basel). 2024;12(3).
21. Liu R, He X, Bao W, Li Z. Enhancement of HPV therapeutic peptide-based vaccine efficacy through combination therapies and improved delivery strategies: A review. Hum Vaccin Immunother. 2024;20(1):2396710.
22. Sheng WH, Lin PH, Cheng YC, Wu YY, Hsieh MJ, Yang HC, *et al*. Immunogenicity and safety of heterologous booster with protein-based COVID-19 vaccine (NVX-CoV2373) in healthy adults: A comparative analysis with mRNA vaccines. J Formos Med Assoc. 2024;123(3):340-6.
23. Heath PT, Galiza EP, Baxter DN, Boffito M, Browne D, Burns F, *et al*. Safety and Efficacy of NVX-CoV2373 Covid-19 Vaccine. N Engl J Med. 2021;385(13):1172-83.
24. Peyret H, Steele JFC, Jung JW, Thuenemann EC, Meshcheriakova Y, Lomonossoff GP. Producing Vaccines against enveloped viruses in plants: making the impossible, difficult. Vaccines (Basel). 2021;9(7).
25. Bashiri S, Koirala P, Toth I, Skwarczynski M. Carbohydrate Immune adjuvants in subunit vaccines. Pharmaceutics. 2020;12(10).
26. Ghaffarifar F. Plasmid DNA vaccines: where are we now? Drugs Today (Barc). 2018;54(5):315-33.
27. Karlsson I, Borggren M, Nielsen J, Christensen D, Williams J, Fomsgaard A. Increased humoral immunity by DNA vaccination using an α-tocopherol-based adjuvant. Hum Vaccin Immunother. 2017;13(8):1823-30.
28. Gurunathan S, Wu CY, Freidag BL, Seder RA. DNA vaccines: a key for inducing long-term cellular immunity. Curr Opin Immunol. 2000;12(4):442-7.
29. 29. Liu MA. DNA vaccines: an historical perspective and view to the future. Immunol Rev. 2011;239(1):62-84.
30. Jahanafrooz Z, Baradaran B, Mosafer J, Hashemzaei M, Rezaei T, Mokhtarzadeh A, *et al*. Comparison of DNA and mRNA vaccines against cancer. Drug Discov Today. 2020;25(3):552-60.
31. Tang J, Li M, Zhao C, Shen D, Liu L, Zhang X, *et al*. Therapeutic DNA Vaccines against HPV-related malignancies: promising leads from clinical trials. Viruses. 2022;14(2).
32. Gardner MJ, Doolan DL, Hedstrom RC, Wang R, Sedegah M, Gramzinski RA, *et al*. DNA vaccines against malaria: immunogenicity and protection in a rodent model. J Pharm Sci. 1996;85(12):1294-300.
33. Roth C, Pitard B, Levillayer L, Lay S, Vo HTM, Cantaert T, *et al*. Zika virus T-cell based 704/DNA vaccine promotes protection from Zika virus infection in the absence of neutralizing antibodies. PLoS Negl Trop Dis. 2024;18(10):e0012601.
34. Maeki M, Uno S, Sugiura K, Sato Y, Fujioka Y, Ishida A, *et al*. Development of polymer-lipid hybrid nanoparticles for large-sized plasmid DNA Transfection. ACS Appl Mater Interfaces. 2024;16(2):2110-9.
35. Lambricht L, Lopes A, Kos S, Sersa G, Préat V, Vandermeulen G. Clinical potential of electroporation for gene therapy and DNA vaccine delivery. Expert Opin Drug Deliv. 2016;13(2):295-310.
36. Geoghegan S, O'Callaghan KP, Offit PA. Vaccine safety: myths and misinformation. Front Microbiol. 2020;11:372.
37. Baghban R, Ghasemian A, Mahmoodi S. Nucleic acid-based vaccine platforms against the coronavirus disease 19 (COVID-19). Arch Microbiol. 2023;205(4):150.
38. Herrada AA, Rojas-Colonelli N, González-Figueroa P, Roco J, Oyarce C, Ligtenberg MA, *et al*. Harnessing DNA-induced immune responses for improving cancer vaccines. Hum Vaccin Immunother. 2012;8(11):1682-93.
39. Hou Y, Chen M, Bian Y, Zheng X, Tong R, Sun X. Advanced subunit vaccine delivery technologies: From vaccine cascade obstacles to design strategies. Acta Pharm Sin B. 2023;13(8):3321-38.

40. Mucker EM, Brocato RL, Principe LM, Kim RK, Zeng X, Smith JM, *et al*. SARS-CoV-2 Doggybone DNA vaccine produces cross-variant neutralizing antibodies and is protective in a COVID-19 animal model. Vaccines. 2022;10(7):1104.
41. Ura T, Takeuchi M, Kawagoe T, Mizuki N, Okuda K, Shimada M. Current vaccine platforms in enhancing t-cell response. Vaccines (Basel). 2022;10(8).
42. Elbahrawy A, Atalla H, Alboraie M, Alwassief A, Madian A, El Fayoumie M, *et al*. Recent advances in protective vaccines against hepatitis viruses: a narrative review. Viruses. 2023;15(1).
43. Suschak JJ, Williams JA, Schmaljohn CS. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity. Hum Vaccin Immunother. 2017;13(12):2837-48.
44. Sahly HE. MF59™ as a vaccine adjuvant: a review of safety and immunogenicity. Expert Rev Vaccines. 2010;9(10):1135-41.
45. Facciolà A, Visalli G, Laganà A, Di Pietro A. An overview of vaccine adjuvants: current evidence and future perspectives. Vaccines (Basel). 2022;10(5).
46. Cid R, Bolívar J. Platforms for production of protein-based vaccines: from classical to next-generation strategies. Biomolecules. 2021;11(8).
47. Lee J, Arun Kumar S, Jhan YY, Bishop CJ. Engineering DNA vaccines against infectious diseases. Acta Biomater. 2018;80:31-47.
48. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? Nat Rev Genet. 2008;9(10):776-88.
49. Kumru OS, Joshi SB, Smith DE, Middaugh CR, Prusik T, Volkin DB. Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies. Biologicals. 2014;42(5):237-59.
50. Qian G, Gao C, Zhang M, Chen Y, Xie L. A review of protein-based COVID-19 vaccines: from monovalent to multivalent formulations. Vaccines (Basel). 2024;12(6).
51. Shyr ZA, Cheng YS, Lo DC, Zheng W. Drug combination therapy for emerging viral diseases. Drug Discov Today. 2021;26(10):2367-76.
52. Tortellini E, Fosso Ngangue YC, Dominelli F, Guardiani M, Falvino C, Mengoni F, *et al*. Immunogenicity and efficacy of vaccination in people living with human immunodeficiency virus. Viruses. 2023;15(9).
53. Yaamika H, Muralidas D, Elumalai K. Review of adverse events associated with COVID-19 vaccines, highlighting their frequencies and reported cases. J Taibah Univ Med Sci. 2023;18(6):1646-61.
54. Acevedo-Whitehouse K, Bruno R. Potential health risks of mRNA-based vaccine therapy: A hypothesis. Med Hypotheses. 2023;171:111015.
55. Schalk JA, Mooi FR, Berbers GA, van Aerts LA, Ovelgönne H, Kimman TG. Preclinical and clinical safety studies on DNA vaccines. Hum Vaccin. 2006;2(2):45-53.
56. Heidary M, Kaviar VH, Shirani M, Ghanavati R, Motahar M, Sholeh M, *et al*. A Comprehensive review of the protein subunit vaccines against COVID-19. Front Microbiol. 2022;13:927306.
57. Díaz-Dinamarca DA, Salazar ML, Castillo BN, Manubens A, Vasquez AE, Salazar F, *et al*. Protein-based adjuvants for vaccines as immunomodulators of the innate and adaptive immune response: current knowledge, challenges, and future opportunities. Pharmaceut. 2022;14(8).
58. Hung SI, Preclaro IAC, Chung WH, Wang CW. Immediate hypersensitivity reactions induced by COVID-19 vaccines: current trends, potential mechanisms and prevention strategies. Biomed. 2022;10(6).
59. Ghattas M, Dwivedi G, Lavertu M, Alameh MG. Vaccine technologies and platforms for infectious diseases: current progress, challenges, and opportunities. Vaccines (Basel). 2021;9(12).
60. Berger S, Zeyn Y, Wagner E, Bros M. New insights for the development of efficient DNA vaccines. Microb Biotechnol. 2024;17(11):e70053.
61. Cui Y, Ho M, Hu Y, Shi Y. Vaccine adjuvants: current status, research and development, licensing, and future opportunities. J Mater Chem B. 2024;12(17):4118-37.
62. Ahlberg V, Hjertner B, Wallgren P, Hellman S, Lövgren Bengtsson K, Fossum C. Innate immune responses induced by the saponin adjuvant Matrix-M in specific pathogen free pigs. Vet Res. 2017;48(1):30.
63. Filipić B, Pantelić I, Nikolić I, Majhen D, Stojić-Vukanić Z, Savić S, *et al*. Nanoparticle-based adjuvants and delivery systems for modern vaccines. Vaccines (Basel). 2023;11(7).
64. Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. Immunol Cell Biol. 2004;82(5):488-96.
65. O'Hagan D, Tsai T, Brito L. Emulsion based vaccine adjuvants. Human vaccines & immunother. 2013;9.
66. Jeon D, Hill E, McNeel DG. Toll-like receptor agonists as cancer vaccine adjuvants. Hum Vaccin Immunother. 2024;20(1):2297453.
67. Sun HX, Xie Y, Ye YP. Advances in saponin-based adjuvants. Vaccine. 2009;27(12):1787-96.
68. Talbot A, de Koning-Ward TF, Layton D. Left out in the cold - inequity in infectious disease control due to cold chain disparity. Vaccine. 2025;45:126648.
69. Thakral S, Sonje J, Munjal B, Suryanarayanan R. Stabilizers and their interaction with formulation

components in frozen and freeze-dried protein formulations. Adv Drug Deliv Rev. 2021;173:1-19.

70. Giri J, Li WJ, Tuan RS, Cicerone MT. Stabilization of proteins by nanoencapsulation in sugar-glass for tissue engineering and drug delivery applications. Adv Mater. 2011;23(42):4861-7.
71. Brookwell A, Oza JP, Caschera F. Biotechnology Applications of Cell-Free Expression Systems. Life (Basel). 2021;11(12).
72. Lu B, Lim JM, Yu B, Song S, Neeli P, Sobhani N, *et al*. The next-generation DNA vaccine platforms and delivery systems: advances, challenges and prospects. Front Immunol. 2024;15:1332939.
73. Pandya A, Shah Y, Kothari N, Postwala H, Shah A, Parekh P, *et al*. The future of cancer immunotherapy: DNA vaccines leading the way. Med Oncol. 2023;40(7):200.
74. Buonaguro L, Tagliamonte M. Selecting target antigens for cancer vaccine development. Vaccines (Basel). 2020;8(4).
75. Schork NJ. Artificial intelligence and personalized medicine. Cancer Treat Res. 2019;178:265-83.
76. Ho D, Quake SR, McCabe ERB, Chng WJ, Chow EK, Ding X, *et al*. Enabling technologies for personalized and precision medicine. Trends Biotechnol. 2020;38(5):497-518.
77. Bai S, Luo H, Tong H, Wu Y. Application and technical challenges in design, cloning, and transfer of large DNA. Bioeng (Basel). 2023;10(12).
78. Taghdiri M, Mussolino C. Viral and non-viral systems to deliver gene therapeutics to clinical targets. Int J Mol Sci. 2024;25(13).
79. Hazarika G, Jadhav SV, Ingole PG. Exploring the potential of polymeric membranes in cutting-edge chemical and biomedical applications: A review. Materials Today Commun. 2024;39:109022.
80. Nazary Abrbekoh F, Salimi L, Saghati S, Amini H, Fathi Karkan S, Moharamzadeh K, *et al*. Application of microneedle patches for drug delivery; doorstep to novel therapies. J Tissue Eng. 2022;13:20417314221085390.
81. Pagliari S, Dema B, Sanchez-Martinez A, Montalvo Zurbia-Flores G, Rollier CS. DNA vaccines: history, molecular mechanisms and future perspectives. J Mol Biol. 2023;435(23):168297.
82. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. Signal Trans Target Ther. 2023;8(1):9.
83. Song Y, Wang J, Yang Z, He Q, Bao C, Xie Y, *et al*. Heterologous booster vaccination enhances antibody responses to SARS-CoV-2 by improving Tfh function and increasing B-cell clonotype SHM frequency. Front Immunol. 2024;15:1406138.
84. Davis HL, McCluskie MJ. DNA vaccines for viral diseases. Microbes Infect. 1999;1(1):7-21.
85. Kim WS, Zhi Y, Guo H, Byun EB, Lim JH, Seo HS. Promotion of cellular and humoral immunity against foot-and-mouth disease virus by immunization with virus-like particles encapsulated in monophosphoryl lipid a and liposomes. Vaccines (Basel). 2020;8(4).
86. Oli AN, Rowaiye AB. Chapter 2 - Vaccine types and reverse vaccinology. In: Ashfield R, Oli AN, Esimone C, Anagu L, editors. Vaccinology and methods in vaccine research: Academic Press; 2022. p. 31-55.
87. Babaeimarzangou SS, Zaker H, Soleimannezhadbari E, Gamchi NS, Kazeminia M, Tarighi S, *et al*. Vaccine development for zoonotic viral diseases caused by positive-sense single-stranded RNA viruses belonging to the coronaviridae and togaviridae families (Review). Exp Ther Med. 2023;25(1):42.
88. Kozak M, Hu J. DNA vaccines: their formulations, engineering and delivery. Vaccines (Basel). 2024;12(1).