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Personalized Neoantigen DNA Cancer Vaccines: Current Status and Future Perspectives

Nadia Viborg[1], Daniela Kleine-Kohlbrecher[1], Birgitte Rønø[1,*]

[1]Evaxion Biotech, Dr. Neergaards Vej 5F, 2970 Hørsholm, Denmark

*Correspondence should be addressed to Birgitte Rønø, br@evaxion-biotech.com

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Abstract

Tumor mutation-derived neoantigens are considered promising targets for cancer immunotherapy. Personalized vaccines have emerged as an approach to deliver neoantigens and thereby trigger the induction of specific T-cell responses that can find and eliminate tumor cells based on the cell-surface presence of neoantigens. To this end, several neoantigen vaccine formats have provided encouraging results in clinical trials, resulting in neoantigen immunogenicity and clinical benefit. DNA offers a versatile and safe platform to deliver neoantigens and immune stimulants in a single entity through vaccination. Herein, we provide an overview of how DNA vaccines are being used as a means to deliver personalized neoantigens to cancer patients. We summarize the developments in DNA vaccine formulation and delivery technologies that contribute to elicit robust immune responses after vaccination. We outline the main results from central preclinical and clinical investigations, showing that neoantigen DNA vaccines induce a specific immune response directed against tumor neoantigens. Lastly, we discuss the opportunities and challenges for neoantigen DNA vaccines as an individualized approach to immunotherapy of cancer.

Keywords: Cancer immunology, DNA vaccine, Immunomodulation, Immunotherapy, Neoantigens, Personalized cancer vaccines, Tumor immunology, Vaccines

Introduction

The advent of immunotherapy has markedly reshaped the treatment landscape in oncology, with immunotherapies being part of first-line treatment for various types of cancers [1]. As such, several immunotherapies have been linked to durable clinical responses and sometimes even long-term remission in patients with advanced cancers. This includes immune checkpoint inhibitors (ICIs), including anti-programmed cell death 1 antibody (anti-PD-1 antibody), chimeric antigen receptor T cells, adoptive cell therapy with tumor infiltrating lymphocytes, and therapeutic vaccines that aim to utilize and educate the patient's own immune system to recognize and eliminate cancerous cells [2–5]. Several cancer immunotherapies with favorable clinical outcomes have been associated with immune recognition of tumor mutation-derived neoantigens (NeoAgs) [6–10] which are in theory ideal immune-targets due to tumor-restricted expression and hence avoidance of central tolerance along with limited risk of autoimmune-related adverse effects [11,12]. Personalized cancer vaccines (PCVs) with NeoAgs are considered a promising and potent approach to cancer care, by designing vaccines that generate or amplify T-cell responses tailored to the tumor and the immune system of each individual patient. Therefore, NeoAg PCVs have been assessed for efficacy in a multitude of preclinical and early clinical studies for the last decade. This has been enabled in part by advances in next generation sequencing (NGS) techniques together with bioinformatics progresses in NeoAg identification and prioritization from tumor sequencing data. To this end, a range of vaccine platforms have been assessed to deliver NeoAgs in several cancer types, such as nucleic acids (encompassing both DNA and RNA), peptides, and cell-based platforms - each posing its own advantages and challenges. Consensus in the field is that it is beneficial to include multiple NeoAgs in each PCV to target the heterogenous tumor broadly, hence circumventing potential immune evasion, and to mitigate the fact that far from all NeoAgs being immunogenic [13,14].

In this article, we will summarize the developments, possibilities, and challenges in using DNA as a format for NeoAg PCVs.

A Brief History of DNA Vaccines

In brief, classical DNA vaccines are generated by inserting genes or DNA sequences encoding antigens into plasmids, the inserts being controlled by a powerful promotor, such as the CMV promoter. This allows for transcription and translation of the insert into proteins by cells that have taken up the plasmid. DNA has been explored as a vaccine delivery platform since the first pioneering description that the injection of plasmid DNA into a mouse muscle could result in local expression of the delivered gene product [15]. This was followed by publications of how DNA plasmid vaccination had the ability to lead to both humoral and T-cell immune responses against viral antigens in preclinical models [16,17]. Hereafter several DNA vaccines were tested in larger animals and human studies, showing that DNA was well tolerated and safe, and harbored excellent stability along with self-adjuvanting capabilities [18]. But despite encouraging results from preclinical studies, DNA vaccines elicited inadequate immunogenicity in initial human clinical trials, first with plasmid DNA formulations delivering antigens from infectious diseases and later also tumor associated antigens in melanoma and colorectal cancer (as reviewed in detail by others [19-22]). However, several DNA vaccines have since been approved for use in veterinary medicine [23], and continued developments have been explored to increase the immunogenicity of DNA vaccines in humans. Recently, nucleic acid vaccines have gained momentum, after the global efforts during the COVID-19 pandemic led to the regulatory approval of different mRNA vaccines for human use, but also the first ever approval of a DNA vaccine in humans, namely ZyCoV-D in India [24]. The studies with DNA vaccines have highlighted no safety concerns, and DNA vaccines are continuously being tested in clinical trials for a range of infectious disease indications and cancers, incorporating novel delivery technologies, adjuvants, and antigens trying to overcome the challenges of low immunogenicity observed in the early clinical trials [25].

DNA Vaccine Formulation and Delivery Methods

Though it is well-described that DNA harbors self-adjuvating properties, activating the innate DNA sensing machinery of mammalian cells [26] as described in the paragraph above the early clinical trials with DNA vaccines in humans presented limitations in the induced immunogenicity after immunization, and hence lack of robust efficacy. Therefore, efforts have since been ongoing to formulate DNA to improve uptake by target cells such as myocytes at the injection site and antigen presenting cells (APCs) along with ensuring sufficient expression of the encoded antigens, all with the purpose of priming a potent and antigen-specific immune response after DNA vaccination [27–29]. These improvements will be outlined in the following section.

Carriers

The uptake of plasmid DNA can be improved by varying vaccine co-formulations such as carriers, nanoparticles and polymers, often with a cationic charge [30,31], proposed to work on several axes: protection of DNA from degradation *in vivo*, promotion of cellular entry (in part due to the cationic charge) and immune stimulatory effects, either as adjuvants or by steering delivery to e.g. APCs [32]. The approved mRNA vaccines against COVID-19 have been formulated with lipid nanoparticles (LNPs), and there are also descriptions of DNA vaccines that benefit from LNP formulation, with a 10-fold increased antigen expression resulting from a 10-fold lower dosage of LNP-delivered DNA compared to naked DNA [33]. This indicates that the use of a fitting type of carrier can improve DNA vaccine efficacy and that this is a promising approach for further optimization.

Molecular adjuvants

Another approach to improve immunogenicity after DNA vaccination is to encode molecular adjuvants such as cytokines or chemokines into either the same DNA plasmid as the antigen payload or a separate DNA plasmid that will be co-administered. Here, DNA has inherent advantages, offering a versatile vaccine platform that enables co-expression of immune modulators and vaccine antigens thereby enhancing immunogenicity. Different types of molecular adjuvants in DNA vaccines have been investigated in the form of cytokines, chemokines, co-stimulatory molecules, and ligands for pathogen recognition receptors [34]. There are several descriptions of enhancing the immune response via plasmids encoding interleukin (IL)-12 also demonstrated in the setting of a liposome-encapsulated NeoAg DNA vaccine in a mouse model of melanoma, where plasmid co-expression of IL-12 led to higher expression of the NeoAg payload by APCs and a superior anti-tumor efficacy [35]. Also, granulocyte-macrophage colony stimulating factor (GM-CSF) [36], IL-6 and TNF-alpha [37] have been applied as molecular adjuvants to modulate the plasmid DNA immune response upon vaccination. Another approach that enhances DNA vaccine immunogenicity has been observed from encoding APC binding molecules into the DNA plasmid, such as chemokine (C-C motif) ligand 3 (CCL3) [38] and CCL19 [39], the latter describing the improved *in vivo* efficacy of plasmid DNA encoded neoepitopes in a murine model of colon carcinoma resulting from the CCL19-targeted approach. The adjuvants investigated in clinical and pre-clinical settings demonstrate that the use of molecular adjuvants enhances immunogenicity of DNA PCV´s and thereby vaccine efficacy and that this approach has promising clinical value.

Physical delivery methods

Some physical methods have been applied to successfully improve cellular uptake of DNA vaccines, which will allow for induction of potent immune responses and enhanced efficacy. The use of electroporation (EP) following the injection of DNA momentarily increases the permeability of cell membranes, allowing DNA to enter the cytoplasm and then reach the cell nucleus, reported to increase transfection rates by 100-1000-fold [19]. It is speculated that the benefits of EP in DNA vaccination can also be attributed to local tissue damage and activation of damage associated molecular patterns (DAMPs), acting as adjuvants. Another approach to a physical delivery method is needle-free injectors, such as jet injectors, that apply high pressure and high velocity to deliver the vaccine into the tissue leading to higher uptake and expression, along with an inflammatory response [40]. Such a needle-free injector device has been applied in the clinic to facilitate DNA vaccine delivery of personalized NeoAgs and human papillomavirus (HPV) derived antigens for HPV-positive cancers [41–43], as well as the approved DNA vaccine for COVID-19; ZyCoV-D [24]. The combined increase in cellular uptake of DNA and the creation of a perceptive immune environment for increased signaling is believed to enhance antigen immunogenicity.

Route of immunization

DNA vaccines are mainly delivered via intramuscular or intradermal routes, and both can be facilitated by jet-injection or by EP [30]. The most commonly applied route of DNA immunization in mice and humans has been intramuscular injection. Here, vaccine DNA is delivered to muscle tissue, where it can initially transfect local myocytes, and later APCs that are recruited due to inflammatory signals from local tissue damage (as described above). Immunization via the intradermal route will allow transfection of local residing APCs in the dermis and might therefore require lower doses of DNA to effectively raise immune responses, but it has been described that intradermal delivery can be challenging and need specialized equipment [44]. There are mixed reports on pros and cons of the delivery routes for DNA, but a recent study with NeoAg DNA vaccines in mice highlighted an improved ability to delay tumor growth via intramuscular rather than intradermal delivery, perhaps owing to qualitative differences in the induced immune response [45]. So far there are not sufficient data available for final conclusions, but current results highlight that further exploration of immunization routes that fit to the type of vaccine modality offers opportunities to enhance DNA PCV function.

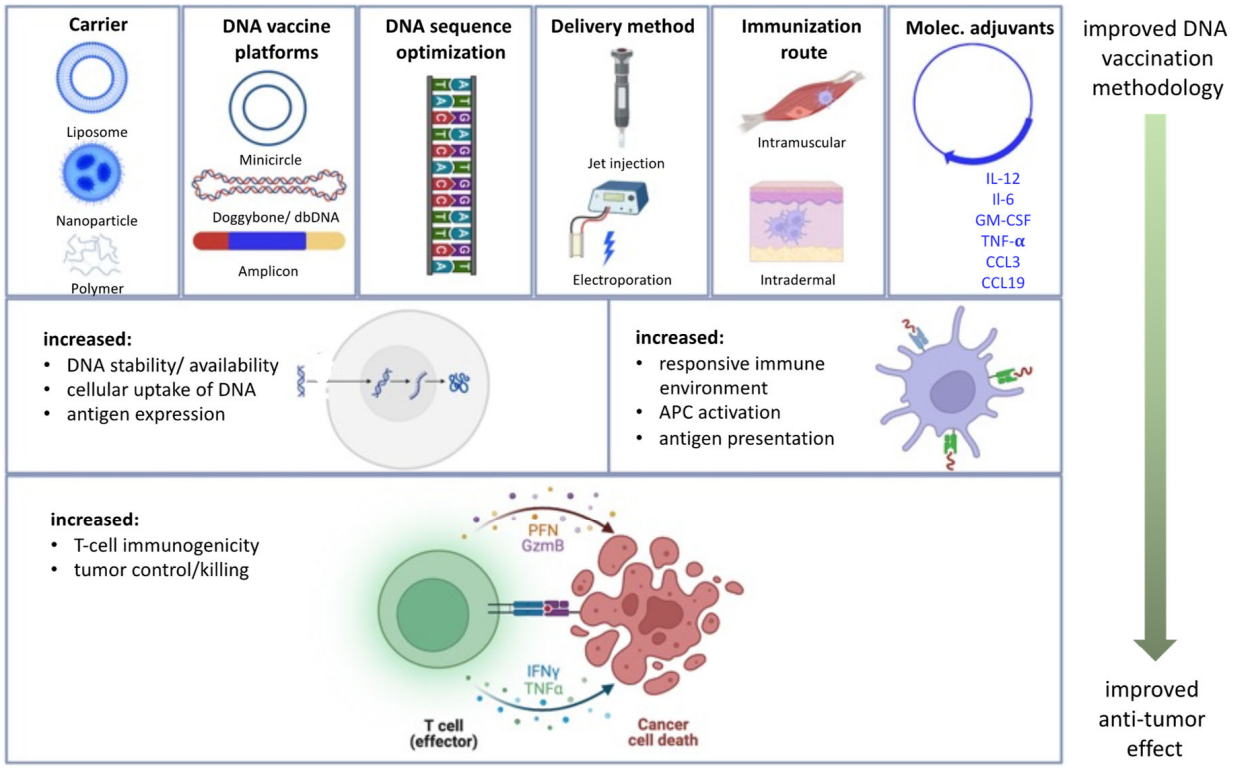

Figure 1. Schematic showing optimization approaches to increase DNA vaccine efficacy to improve potential anti-tumor effects of the vaccine therapy. The illustration was created with BioRender.com.

Emerging DNA vaccine platforms

Limitations to traditional plasmid DNA vectors include a high potential to be silenced *in vivo* and degradation by intracellular nucleases. Innovative approaches have emerged as alternatives that address some of these drawbacks to the traditional DNA plasmids. Some are produced via cell-free processes, thereby removing the remnants from bacterial production, including synthetic, minimal, linear, double-stranded DNA, such as DNA amplicons, the product of polymerase chain reaction [46] and Doggybone or dbDNA, produced via rapid enzymatic manufacturing [47]. Others approaches work by removing the bacterial backbone components, such as DNA minicircles or mini-intronic plasmids (MIPs), has been shown able to overcome silencing and increase expression of the insert [48].

Sequence optimization

Sequence optimization is often carried out by bioinformatic tools and described as a pivotal step in the design of DNA vaccines to improve immunogenicity [25] also assessed by the authors in NeoAg PCVs [49]. Codon optimization (i.e. utilizing the codons that are preferred by the host organism) and other sequence optimization of e.g. GC content, repeats, secondary RNA structures and TATA boxes of DNA plasmids can be utilized to increase plasmid manufacturability and enhance the expression of the delivered antigens after vaccination. Some plasmids have the incorporation of immune stimulatory sequences such as CpG motifs in the plasmid backbone as is the case for the pTVG4 plasmid, adding to increase the innate immune signaling via toll like receptor 9 engagement [50].

Improvement of the DNA plasmid modalities together with optimization of the DNA sequences seem to be productive methods to secure sufficient antigen expression and consequently immunogenic signal transduction.

In summary, the described formulations, delivery methods, and sequence modifications for DNA vaccines can be used both to increase the immunogenicity towards the delivered antigens following vaccination but also acts as immune milieu modulators to qualitatively direct the immune response in a wanted direction.

Preclinical Investigations of NeoAg DNA Vaccines

There is a growing body of literature with investigations in mouse models supporting the notion that NeoAgs can be used to generate cancer vaccines that are immunogenic and safe. Pioneering work by Castle et al. [51], Yadav et al. [52], Gubin et al. [53], and Kreiter et al. [54] paved the way and provided the earliest descriptions of screening the tumor "mutanomes" for immunogenicity and finding NeoAgs that could be utilized in vaccines to prevent or delay tumor growth across several murine cancer models. The studies described in the publications all assessed NeoAg vaccines formulated either as peptides with adjuvant polyinosinic–polycytidylic acid (poly I:C) or as poly-epitope encoding mRNA-based vaccines. In recent years several publications have substantiated that NeoAgs delivered as a DNA vaccine can induce anti-tumor immunity in mouse models of cancer. Duperret et al. [55] and Aurisicchio et al. [56] displayed how EP-facilitated delivery of multiple NeoAgs encoded in a plasmid DNA could delay tumor growth and lead to improved survival of mice in several tumor models. A follow-up publication by Bhojnagarwala et al. [57] with DNA and EP claimed that delivery of a large payload (up to 40 NeoAgs and shuffling the order of the antigens around) in a single plasmid did not affect the immunogenicity of the individual antigens, hence there was no positional bias or evident immunodominance from the large NeoAg payload. Tondini et al. [58] utilized plasmid DNA formulated with cationic lipoplexes to induce partial tumor control and improved survival via therapeutic NeoAg immunization when in combination with ICI therapy. Another example is Li et al. [59] where the results show potent tumor control after gene gun-facilitated immunization with plasmid DNA encoding NeoAgs in a murine model of breast cancer, when combined with ICI therapy. The authors have published a study that showed how delivery of NeoAgs encoded in plasmid DNA formulated with cationic polymers could prevent tumor growth in murine models of colon carcinoma and melanoma [49]. We measured a high magnitude and broad, NeoAg-specific $CD4^+$ and $CD8^+$ T-cell response in blood, spleens and tumors of vaccinated mice and found that both T-cell subsets contributed to the anti-tumor immune response. The NeoAg-specific immune responses were long-lived and efficacious, as a single prophylactic dose of the NeoAg DNA vaccine could prevent growth of a subcutaneous tumor in approximately 40% of vaccinated mice when challenged with cancer cells more than 200 days after the single dose of DNA vaccination. We further found a favorable effect of combining a low dose of the NeoAg DNA vaccine (i.e. non-protective dose) with anti-PD-1 ICI treatment, significantly reducing tumor burden and prolonging survival in combination treated mice, compared to the effect of the DNA vaccine or the anti-PD-1 as monotherapies.

The authors and colleagues have furthermore published a follow-up study that demonstrated how the addition of an APC-targeting molecule (chemokine CCL19, as described briefly in the prior paragraph) to a NeoAg DNA vaccine led to improved efficacy compared to NeoAg DNA without APC targeting [39]. This was evident from observations of APC-targeted NeoAg DNA effectively preventing subcutaneous tumor growth at a five-fold lower dose than the NeoAg DNA without APC targeting. There was a clear association between the ability to prevent tumor growth and the amount of NeoAg specific $CD8^+$ T cells present in circulation. Furthermore, APC-targeted NeoAg DNA resulted in four times higher magnitude of NeoAg specific $CD4^+$ and $CD8^+$ T cells in splenocytes compared to non-targeted NeoAg DNA, thus

highlighting the improved immunogenicity of the targeted approach. The study lastly described how the addition of EP-assisted delivery of APC-targeted NeoAg DNA vaccine could elicit abrogation of tumor growth in 38% of vaccinated mice, in an early therapeutic setting, i.e. where mice were vaccinated after the subcutaneous inoculation of tumor cells.

Though direct translatability from preclinical models to humans can be debated, there have been and are still many valuable insights to gain from the murine investigations into NeoAg DNA vaccines, that are not as simple to address in clinical trials. This encompasses the mode of action and effects of changing parameters such as dose, route, delivery technology and number of encoded NeoAgs in the vaccine.

Personalized NeoAg DNA Vaccines for Cancer Patients

A plethora of clinical trials with NeoAg PCVs have been and are currently being conducted. Particularly three early clinical studies in melanoma patients provided the proof of concept for NeoAg PCVs based on different delivery modalities: NeoAg-loaded dendritic cells [60], NeoAg-encoding mRNA [61] and neopeptide pools adjuvanted by polyinosinic–polycytidylic acid complexed with poly-L-lysine (poly-ICLC) [62]. These first-in-human studies provided evidence that it is feasible to design, manufacture and deliver NeoAg PCVs that are well-tolerated and immunogenic in melanoma patients. The studies delivered NeoAg PCVs in combination with ICI therapy and provided some early evidence of clinical efficacy, though patient cohorts were small, and the studies did not include control arms with e.g. ICI monotherapy for comparison. Since then, a multitude of clinical studies have investigated the use of NeoAg PCVs via different vaccine formats and in other cancer indications, such as lung cancer, bladder cancer, glioblastoma, and pancreatic cancer [63-66]. Importantly, the KEYNOTE-942 study has recently announced groundbreaking results and the first robust proof of clinical benefit from NeoAg PCVs in a randomized Phase 2b clinical trial in adjuvant melanoma (NCT03897881), comparing to the standard-of-care anti-PD-1 therapy alone. The study reported significantly improved relapse free survival and clinically meaningful reduced risk of death in high-risk, fully resected melanoma patients after treatment with a NeoAg mRNA PCV in combination with anti-PD-1 therapy, compared to anti-PD-1 therapy alone [67]. These findings are now being investigated further in Phase 3 studies of melanoma and lung cancer (NCT05933577 and NCT06077760). This adds to strengthen the promise of NeoAg PCVs and ameliorates the fact that patient-tailored vaccines are significantly more expensive and time-consuming to manufacture than off-the-shelf therapies, why the observations of clinical efficacy make these vaccines "worth" the wait, extra efforts and costs of personalization.

DNA-based formats of NeoAg PCV delivery are also being tested in early clinical trials (see **Table 1**). To this end, Phase 1/2a clinical trials with NeoAg DNA PCV (in combination with anti-PD-1) from companies Geneos Therapeutics, Nykode and the authors at Evaxion Biotech have been presented at conferences and in press releases during the last couple of years.

Geneos Therapeutics have an ongoing, fully enrolled clinical trial in hepatocellular carcinoma (HCC) patients (NCT04251117) applying EP-facilitated delivery of personalized NeoAgs encoded in plasmid DNA ("GNOS-PV02", containing up to 40 NeoAgs) formulated together with an IL-12-encoding plasmid, that works as an adjuvant [68]. Interim data from Geneos Therapeutics reported the PCV as safe and well-tolerated, leading to clinical responses in 8 out of 34 HCC patients, and the detection of primarily $CD8^+$ T cells specific to NeoAgs after vaccination [69,70].

Nykode have presented safety and immunogenicity data from their basket trial (NCT03548467) with jet injector-assisted delivery of APC-targeted NeoAg DNA PCV ("VB10.

Table 1. DNA based NeoAg PCV in Phase1/2a clinical trials.

Personalized NeoAg DNA vaccine	DNA Modality	Administration method	Indication	Sponsor	Identifier/ Reference
GNOS-PV02	up to 40 NeoAg in plasmid DNA + IL-12 encoding plasmid	EP	HCC	Geneos Therapeutics	NCT04251117 [68-70]
VB10.NEO	up to 20 NeoAg in a plasmid containing CCL3 as APC targeting domain	JetInjector	locally advanced and metastatic tumors (melanoma, NSCLC, CRCC, bladder cancer, HNSCC)	Nykode	NCT03548467 NCT05018273 [42,71]
EVX-02	13 NeoAg in DNA plasmid	needle-injection with cationic polymer and JetInjector	adjuvant melanoma	Evaxion Biotech	NCT0445503 [41,72]

NEO", containing up to 20 NeoAgs) to treat melanoma, non-small cell lung cancer (NSCLC), clear renal cell carcinoma, bladder cancer or squamous cell carcinoma of head and neck (HNSCC) [42,71]. The data presented by Nykode showed that all patients mounted T-cell responses to a broad selection of vaccine NeoAgs and that these T-cell responses were long-lasting. Furthermore, the data highlighted an increase in the breadth and magnitude of the NeoAg T-cell immune response resulting from multiple vaccinations and the Nykode DNA vaccine was generally well-tolerated.

Evaxion Biotech have communicated safety and immunogenicity data evaluating NeoAg DNA PCV ("EVX-02", containing 13 NeoAgs) in a trial of adjuvant melanoma (NCT04455503) [41]. The trial contained two study arms; (1) standard needle-delivery of plasmid DNA with cationic polymers (i.e. poloxamers), or (2) jet injector-assisted delivery of plasmid DNA. The 10 melanoma patients that received the full vaccine dosing schedule were relapse free at their last assessment (5 patients in each study arm) and the vaccine was found to be safe and well-tolerated, with only mild adverse events related to the DNA vaccine. All patients displayed long-lasting NeoAg-specific immune responses after DNA PCV, contributed to by both $CD4^+$ and $CD8^+$ T cells, albeit in general a higher magnitude of $CD8^+$ T cell responses was observed compared to $CD4^+$ T cell responses. Immune analyses with individual NeoAgs revealed that on average 50% of the delivered vaccine NeoAgs elicited a specific T cell response in patients. It was reported that Evaxion Biotech's proprietary AI immunology™ model PIONEER™, which was used to design the NeoAg PCVs for each patient, had assigned significantly higher predicted quality scores to the NeoAgs that were immunogenic compared to those that were not [72].

Shared among the described NeoAg DNA PCVs in early clinical trials is the feasibility and proof-of-concept that DNA offers a versatile and relevant vaccine platform, suitable for delivery of multiple NeoAgs in a single formulation. The DNA PCVs were reported to have a good safety and tolerability profile, which is in line with prior DNA vaccine clinical studies delivering antigens from infectious diseases and tumor associated antigens [22]. Furthermore, the NeoAg DNA PCV platforms were all reported to induce both $CD4^+$ and $CD8^+$ T cell responses, though mostly $CD8^+$ T cells. This differs from observations of NeoAg T-cell responses resulting from peptide and mRNA vaccines in clinical trials, which have predominantly been $CD8^+$ T cell driven [61,73-75]. Both T cell subsets can contribute to immunotherapy of cancer; while cytotoxic $CD8^+$ T cells can mediate direct killing of cancer cells, the helper $CD4^+$ T cells also play a multi-faceted role in directing and building sustained anti-tumor immune responses [76,77]. Most of the described clinical trials were conducted in small, single-arm studies with few participants without control groups and with short follow-up times thus limiting evaluation of clinical efficacy and the vaccine attributed effect in combination studies. Furthermore, the trials mainly demonstrated safety, feasibility and pharmacodynamic effects of the tested PCVs. These findings from early clinical trial do not prove so far clinical efficacy of DNA PCV therapies but it warrants further studies with larger cohorts and randomized design to properly assess clinical efficacy in comparison to standard-of-care therapy.

Advances in immune monitoring and precision biomarkers will offer growing opportunities to understand the immunological response to NeoAg DNA PCVs along with effects of changing e.g. the DNA dose, vaccination schedule and delivery modality. This knowledge can be leveraged to improve PCVs and gain benefit from their full potential.

Perspectives

NeoAg PCV is undergoing rapid development; after early testing and smaller feasibility studies NeoAg vaccines have now reached broad clinical investigation in Phase 2 and 3 studies. Varying vaccine formats and delivery technologies have signified that the approach is feasible and safe, and the recently published pivotal data from KEYNOTE-942 with clinical efficacy in melanoma patients corroborates the rationale and potential of NeoAg PCVs to improve the clinical outcome for cancer patients. The consensus in the field is that NeoAg PCVs constitute treatment options for cancer patients packed with both opportunities and challenges [78]. As described in this article, DNA offers a versatile platform to deliver NeoAg PCVs in clinical studies, able to elicit NeoAg-specific T cells and early evidence of clinical efficacy along with a good safety profile. DNA as a vaccine format has multiple advantages such as its intrinsic immune stimulatory properties, high stability at room temperature, and capacity to encode lengthy genetic sequences such as molecular adjuvants and NeoAgs simultaneously in a single formulation [25]. Several improvements in DNA vaccine formulation and delivery are being applied to increase cellular uptake, enhance translation, induce a supportive immune environment and thereby strengthen NeoAg immunogenicity after vaccination. These improvements contribute to mitigate the historically described shortcomings with poor immunogenicity following DNA vaccination. Compared to NeoAg delivered as mRNA, DNA offers better stability and does not require cold chain for transport and storage of vaccines. Conversely, mRNA has the benefit of needing only to reach the cell cytoplasm and not all the way to the nucleus, as is the case for DNA, before encoded antigens can be expressed.

Considerable outstanding questions remain to be addressed for NeoAg PCVs to reach their full potential, for DNA and other vaccine modalities alike. We need to gain a better understanding of the optimal immunization route, dose, and immunization schedule of DNA PCVs to improve clinical efficacy. Furthermore, efforts to reduce the time and cost of manufacturing NeoAg PCVs will be imperative to be able to make a clinical difference for cancer patients with advanced disease, where the therapeutic window is short.

Continued investigations into the immunocorrelates and biomarkers that characterize the successful (and the non-successful) clinical response to NeoAg PCVs will contribute to the development of better PCVs for immunotherapy of cancer. Here, the immune monitoring for NeoAg specific T-cell responses following PCVs will lead to an increased understanding of the rules that govern what constitutes a good NeoAg, which we can feed back to bioinformatic selection algorithms and vaccine design to obtain an improved target selection of clinically relevant NeoAgs in the future.

Conflicts of Interest

NV, DKK, and BR are employees at Evaxion Biotech.

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