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Analysis of physical and biological delivery systems for DNA cancer vaccines and their translation to clinical development

DNA cancer vaccines as an approach in tumor immunotherapy are still being investigated in preclinical and clinical settings. Nevertheless, only a small number of clinical studies have been published so far and are still active. The investigated vaccines show a relatively stable expression in *in-vitro* transfected cells and may be favorable for developing an immunologic memory in patients. Therefore, DNA vaccines could be suitable as a prophylactic or therapeutic approach against cancer. Due to the low efficiency of these vaccines, the administration technique plays an important role in the vaccine design and its efficacy. These DNA cancer vaccine delivery systems include physical, biological, and non-biological techniques. Although the pre-clinical studies show promising results in the application of the different delivery systems, further studies in clinical trials have not yet been successfully proven.

Keywords: Vaccines, DNA, Oncology, Immunotherapy, Vaccination, Delivery

Introduction

Despite current advances in oncology, cancer is still a major leading cause of death and a severe public health problem worldwide. Therefore, new therapeutic options are warranted and in this context, immunotherapy is a potential option for cancer patients. Although a large variety of tumors arise from tissues and express endogenous antigens, these are not recognized by the body's immune system which is a natural mechanism to prevent autoimmunity. Regulatory T cells also down-regulate the immune functions of lymphocytes which can recognize these endogenous antigens within the tumor cells. These mechanisms of central and peripheral immune tolerance limit the efficacy of DNA vaccines.

The employment of DNA cancer vaccines as a novel approach in tumor immunotherapy shows promising results in pre-clinical trials. Two types of DNA vaccines can be distinguished: the prophylactic cancer vaccines against human papillomavirus (HPV) and the therapeutic cancer vaccines which are currently under investigation in clinical trials. These vaccines include viral/bacterial vector vaccines, nucleic acid vaccines (DNA, RNA), protein/peptide vaccines, and whole cell vaccines [1,2].

DNA Vaccines

Normally DNA plasmids used as vaccines consist of two components: the transcrip-

tional unit which is driven by a cytomegalovirus promoter and an immune-stimulatory sequences unit to enable the immune response [3]. DNA vaccines contain genes encoding selected proteins of target organisms which do not trigger their pathogenic ability.

DNA vaccination leads to humoral and cellular responses [1,2] and aims to elicit a CD8+ cytotoxic T lymphocyte (CTL) activation. There are several strategies to enhance these responses which lead to a higher antigen expression on dendritic cells (DC) and a higher interaction between DC and T cells.

In recent years many tumor-associated antigens (TAA) were investigated as possible targets for cancer immunotherapy. Several antigens have been used as targets and have shown positive effects which add supporting evidence for the eventual success of vaccine use.

The major problem using these as possible vaccines was the method of the delivery system used in the experiments and trials conducted. The proper delivery system can enhance the efficacy of DNA cancer vaccines [1].

The main obstacles observed by the administration of DNA vaccines are the low levels of antigen production, the inefficient cellular delivery of the plasmids and the insufficient stimulation of the innate immune system [4]. Therefore, the

DNA vaccines must be specially designed to enhance their efficacy through the antigen design, vector system, plasmid dose, timing of the administered dose, adjuvants, and the delivery system [1].

The skin as the main barrier and administration route for DNA vaccines has been used for successful administration. The overall immunogenicity of DNA vaccines is related to the high prevalence of antigen-presenting cells (APC) in the skin as Langerhans cells in the epidermis and DCs in the dermis [5,6]. The skin itself is built up of multiple layers with characteristic resident and transient subsets of immune cells. The epidermis layer (0.05–0.2 mm) is built up of epithelial cells, Langerhans cells, melanocytes, and Merkel cells. The underlying dermis (1.5–3 mm) consists of collagen fibers and macrophages, mast cells, Langerhans cells, and DCs which belong to the adaptive and innate immune system. The APCs are essential for processing antigens penetrating the epidermis which trigger an activation of the immune system or a tolerance to self-antigens. The delivery of vaccines to the epidermis or dermis resulted in superior immune responses compared to other anatomical sites [7].

The first results of pre-clinical and clinical trials showed that the efficiency of DNA cancer vaccines must be enhanced
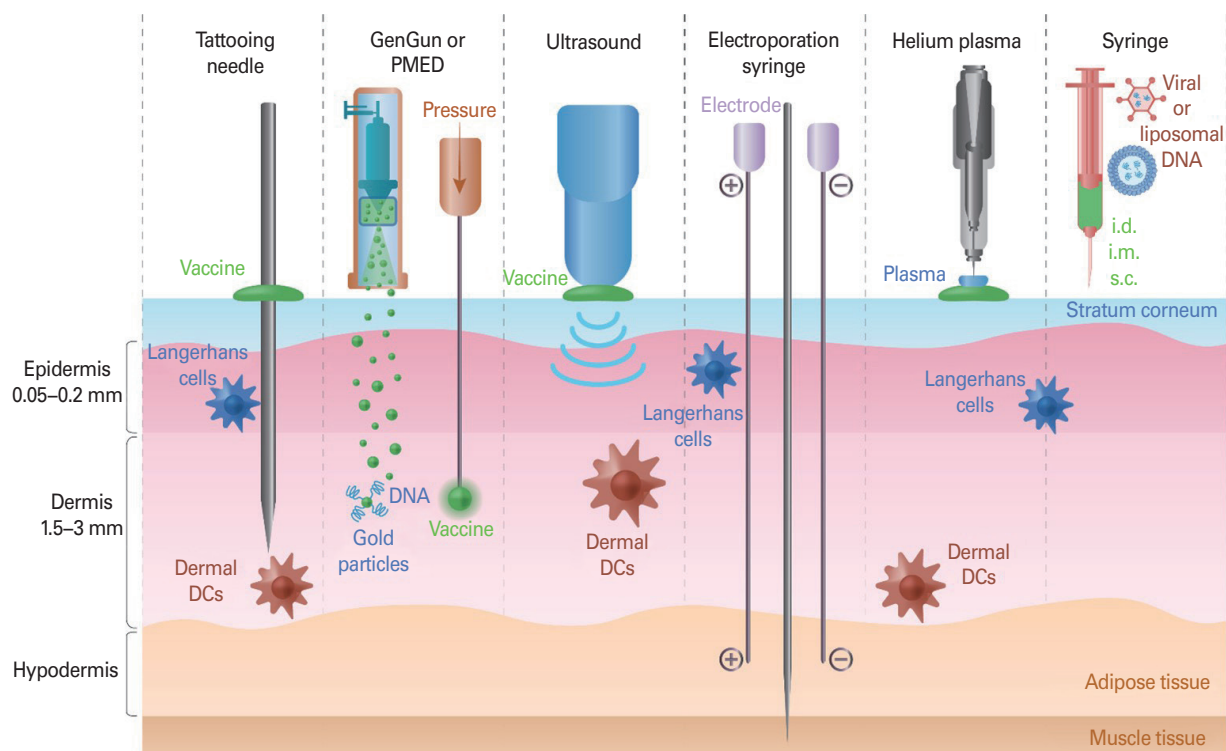

Fig. 1. Delivery systems of DNA cancer vaccines targeting different skin compartments. PMED, particle-mediated epidermal delivery; i.d., intradermal; i.m., intramuscular; s.c., subcutaneous; DC, dendritic cell.

through different delivery systems. Without these delivery systems an injection of naked DNA into local tissues resulted in a rapid degradation by nucleases and clearance by phagocytes [4]. Therefore, these DNA constructs must often be applied in higher doses due to their limited ability to transmigrate through the cell membranes if the membranes of the target cells have not been destabilized. Although higher doses of plasmid do not always result in an increased efficacy or immunogenicity [1,3,4]. Hence, the delivery of these molecules applied together with chemical or physical forces can cause a transient membrane destabilization and so often enable lower doses to be utilized. Consequently, there is a necessity for current research to examine the effectiveness of the various delivery systems: the physical, the biological, and non-biological.

The physical delivery systems include DNA-tattooing, gene gun, ultrasound, electroporation, and contact independent helium plasma. Within this group, the different routes of administration and immunization normally combine a physical delivery and chemical formulations with micro/nano-particles to target APCs. The biological systems include viral vectors and the non-biological systems comprise polysaccharides/polymers, liposomes, and cationic peptides [1]. The different DNA cancer vaccine delivery systems are displayed in Fig. 1 and encompass also the delivery to the different skin compartments including epidermal, dermal, and hypodermal layer.

Pre-clinical Studies

Physical delivery systems
The immune response that results after DNA vaccination is influenced by its method of delivery. These routes include an intradermal, intramuscular, intranasal, or a subcutaneous administration.

DNA-tattooing
The DNA-tattooing technique is similar to the technique that was used for the smallpox vaccination. This technique is directly applied to the skin (intradermal tattooing) and shows a higher efficiency (gene expression) than an intradermal injection or gene gun administration of a DNA vaccine [8]. Furthermore, minor mechanical injuries such as hemorrhage, necrosis and inflammation were observed. The gene expression was only identified in the epidermal and dermal layers of the skin.

Pokorna et al. [8] have shown in preclinical trials, that a DNA vaccine against the HPV-16 antigen administered through an intradermal tattoo cannot be enhanced by mo-

lecular adjuvants (cardiotoxin or granulocyte-macrophage colony-stimulating factor [GM-CSF] DNA co-delivery). Interestingly, the adjuvants enhanced the efficacy by an intramuscular injection of the DNA vaccine [8]. A needle injection typically induced a T helper type 1 response [1,8].

Gene gun and particle-mediated epidermal delivery
The administration of a DNA vaccine through gene gun (particle-mediated) represents a non-viral method to transfer genes into cells. This technique was developed for *in-vitro* applications as a biolistic device. Furthermore, this application allows an intradermal administration of DNA-coated gold particles through a helium-driven gene gun. This technique generates high numbers of CTLs compared to intramuscular needle injections or the Biojector system (Biojector 2000; Bioject Inc., Portland, OR, USA), which administers the DNA in a solution in high-pressure needle-free jet injection as a particle-mediated epidermal delivery (PMED) [9].

These delivery systems can directly target the APC (Langerhans cells) under the skin, which then are able to migrate through the lymphatic system to drain lymph nodes and prime naïve T cells [2]. Furthermore, muscle cells are also a possible target of these administration methods.

Since muscle cells are not able to prime the immune response to an antigen directly, these cells can release soluble antigen into the surrounding fluid which can be taken up by APCs [3]. This form of intramuscular immunization of a secreted form of an antigen also generates higher CTL responses and it is possible to deliver multiple genes simultaneously [3,9].

The preclinical DNA vaccination development was made using the gene gun technology to administer cancer DNA vaccines as particle mediated epidermal delivery. With these systems, a smaller amount of vaccination DNA was required to elicit a response *in-vivo* and higher levels of specific antibody responses besides the high levels of specific T lymphocytes were observed, compared to other methods [2]. The gene gun administration of a specific DNA vaccine triggers a T helper type 2 response [1,8].

Ultrasound
The ultrasound technique to deliver a DNA vaccine into the specific target cell disrupts the cell membranes making them permeable and hence enables the incorporation of the DNA-plasmid. Preclinical studies have shown that the administration of a vaccine by ultrasound resulted in a 10-fold greater

immune response compared with subcutaneous injection [10]. Furthermore, vaccine administration by ultrasound resulted in an activation of Langerhans cells. The exact mechanism behind this is still not understood. Novel mannose-modified carriers compared to the normal lipofection technique undergoing ultrasound showed a higher gene expression *in-vivo* [11,12].

Electroporation

The electroporation technique was developed as a laboratory tool to transfect target cells with foreign DNA. Here, electrical pulses create micro-pores in the cell membrane and cause destabilization [1]. The technique shows promising results in animal trials but the transition to the clinic is still in progress. The electroporation enhances humoral and cellular responses. In addition, a decrease of the injection volume, number of applications and prolonged immunological effects were observed, maintaining the same high efficacy [13].

A conventional intramuscular injection resulted in a suboptimal immunogenicity even by a high plasmid concentration. The administration of a DNA vaccine into a target tissue followed by an electrical pulse (electroporation) increased the uptake of the DNA [2]. Best et al. [2] have shown that this technique, using the luciferase gene, resulted in higher levels of circulating protein and therefore an increased bioluminescence signal compared to an intramuscular injection.

The electrical pulses also up-regulated pro-inflammatory cytokines and recruited monocytes and phagocytes to the site of application/electroporation. This led to an enhancement of the antigen presentation to the immune system and elicited higher specific responses to the antigen [2]. The Tri-Grid delivery system (Ichor Medical Systems Inc., San Diego, CA, USA) is mainly used in preclinical and clinical trials and consists of an electrode array with disposable 30G needles to administer the DNA vaccine and a pulse generator. In the TriGrid system, an injection needle is surrounded by four electroporation needles.

Contact-independent helium plasma

Helium plasma as a low power and cold (non-thermal) plasma source has been used in the past for the surface modification in order to enhance their biocompatibility. In recent years, different plasma sources were investigated for the direct treatment of biological samples including cells and bacteria. In this context, the effects of these non-thermal plasmas were previously used for sterilization of medical devices. In addition, the plasma ap-

plication led to biochemical modifications within cells. The advantage of this method is the contactless application of vaccines which decouples the application device from the patient. Interestingly, it is possible to disrupt the cell membrane and generate pores which allow an enhanced delivery of transfection agents or plasmids. The delivery of DNA by plasma discharge exposure still remains as a novel application technique and has to be further tested as a delivery system for DNA vaccines. Connolly et al. [14] have shown that the delivery of a human immunodeficiency virus gp120 plasmid vaccine with helium plasma in a mouse model induced an increased humoral and cellular antigen specific immune response. The highest response observed led to a 19-fold higher antigen titer than the plasmid delivery alone. Furthermore, the helium plasma delivery of the plasmid induced a 17-fold increase of antigen specific CD8+ T cells [14].

Biological delivery systems

Biological carriers as a DNA vaccine delivery system are viruses that evolved naturally to infect cells and insert their specific genetic material into the cell genome. This option is a commonly used tool in the laboratory or gene therapy in preclinical trials [1].

Viral

The main purpose of viral delivery systems is to target host cells directly and insert genetic material with a high efficacy. These viral vectors are modified to eliminate their toxicity in terms of safety and immunogenicity but retain their high efficiency [15].

Due to their application *in-vitro* and *in-vivo*, viral vectors can be used to create a sustained expression in gene therapy for gene dysfunction. Furthermore, a short gene expression is sufficient for cancer gene therapy. The promising results and the knowledge about viral vectors led to the development of different viral strategies including retrovirus, adenovirus, herpes simplex virus, adeno-associated virus, and pox virus [15-17].

The advantage of these systems is that they produce the antigen in its native conformation which facilitates the antigen entry into the major histocompatibility complex (MHC) class I processing pathway to elicit a CTL response [16]. Problems occur in boost regimens because the immune system responds to the immunogenicity of the vector and forms an anti-vector immunity.

Bridle et al. [18] have shown a synergistic effect by a combi-

nation of different oncolytic viruses. An antitumor response to the administered vesicular stomatitis virus (VSV) was shown to be weaker than the actual anti-VSV response. To gain a better antitumor immune response, a combination of an initial injection of VSV with an injection of a different virus was administered in hope of attaining the original anti-VSV response [18].

Interestingly, the VSV antigen triggered a more tumor-specific CD8+ T cell response with an increased cytokine and granzyme production leading to a higher cytotoxicity. Furthermore, the unspecific integration of DNA into the human genome is a main problem of the viral gene therapy and might cause severe change in the genome of the cells itself [1,16].

Conry et al. [17] showed that a recombinant vaccinia virus-carcinoembryonic antigen (rV-CEA) could be used to target the CEA in metastatic adenocarcinoma by intradermal needle injection, subcutaneous jet injection, or dermal scarification.

The viral vector was able to target APCs directly and present the CEA peptides to T lymphocytes in an MHC-I and MHC-II manner. Local inflammation and pruritis was observed at the site of inoculation [17,19]. Using viral vectors to deliver CEA and TAA as a recombinant protein was more immunogenic, than the administration of the peptide and adjuvants [16-21].

Besides the delivery of DNA into target cells triggering an immune response, viral systems, especially genetically engineered oncolytic viruses seem promising. These oncolytic viruses can kill infected cancer and associated endothelial cells via direct oncolysis and targeting of the tumor vasculature. In addition, these viruses can be armed with immunostimulatory genes as GM-CSF and combined with histone deacetylase inhibitors to inhibit the innate immunity to further promote infection and the spread of the viral particles. Also, the combination with cyclophosphamide induced immunogenic cell death and depleted Tregs, which led to an enhanced T cell infiltration into the tumor tissue [22,23].

Non-biological systems

Non-biological DNA delivery systems are able to evade immunogenic responses that are often associated with viral vectors and therefore are further being investigated as promising delivery systems. Furthermore, these delivery systems are also able to enter APCs by different pathways and are capable to modulate an immune response to the encoded antigen and trigger a T helper 1 type immune response.

Non-biological systems for the delivery of DNA vaccines can be distinguished into 2 types based on the nature of their formulation. These are polymeric delivery systems and liposomal delivery systems. Polymeric delivery systems or cationic polymers are often used for gene delivery. These systems form complexes easily with the anionic DNA molecules generating a cationic polyplex which interacts with the negatively charged cell surface to improve DNA uptake. Polymers used for the polyplexes include polyethylenimine, chitosans, and dendrimers. Furthermore, liposomes are often used as DNA drug delivery systems by entrapping the DNA inside the aqueous core or by integration of these into the phospholipid lamellae [15,24-27].

To further enhance DNA cancer vaccines that are currently under investigation, potential new delivery systems are under research in order to enhance the immune response to the encoded gene of interest. These systems include the delivery of the DNA via tattooing needle, gene gun or PMED, electroporation, helium plasma, and viral or liposomal delivery via syringe. In general, these methods allow the delivery of DNA vaccines into the epidermal, dermal, and hypodermal compartments of the skin by providing an enhanced delivery and uptake by APCs compared to conventional delivery via needle. After the administration of the DNA vaccine through the different delivery systems, the transcription of the encoded gene is initiated in transfected APCs or somatic cells. It is followed by the production in the cytoplasm and the formation of foreign antigens. After the expression in APCs and somatic cells, these antigens are taken up by APCs and processed to small peptides which can be displayed via MHC I or II molecules and activate CD8 CTLs and CD4+ T helper cells, respectively.

Clinical Trials Using DNA Vaccine Delivery Systems in Cancer Patients

In the last decade the investigation of DNA cancer vaccines and their delivery systems has led to promising results in pre-clinical trials. A high efficiency of these vaccines can be shown in *in-vitro* and animal studies, but the translation into clinically proven therapeutic drugs must undergo further investigation to find the best possible delivery system. Several clinical phase I trials investigated the safety of DNA cancer vaccines and their delivery systems.

Developing and applying new delivery systems is one of the key factors to enhance clinical responses in cancer patients undergoing DNA vaccination. Since the injection of naked DNA into specific tissue results in a local expression of

Table 1. DNA cancer vaccines in clinical trials

Condition	Target gene	Delivery system	Single daily dose	Schedule	Adjuvants/ conditioning	No. of patients	No. of clinical response (%)	Comments	References
Metastatic adenocarcinoma	CEA	Viral, i.d.	1×10^7 pfu	2 in 4 weeks	No	10	1 (10)	No humoral or cellular response	Conry et al. [17]
Metastatic adenocarcinoma	CEA	Viral (Biojector)	1×10^7 pfu	2 in 4 weeks	No	10	3 (30)	No humoral or cellular response	Conry et al. [17]
Stage II melanoma	Tyrosinase	Viral, i.m./s.c.	5×10^8 pfu	3 in 4 weeks	No	20	0	Humoral and cellular responses against the carrier	Meyer et al. [32]
Stage II–IV melanoma	Xenogenic gp100	Gene gun	4 µg	8 in 4 months	No	17	6 (35)	No side effects in 6, 3 NED, 3 PD	Ginsberg et al. [31]
Stage II–IV melanoma	Xenogenic gp100	i.m. (Biojector)	2,000 µg	8 in 4 months	No	17	2 (12)	No side effects in 10, 2 immune responses but POD	Ginsberg et al. [31]
Melanoma	gp100	Gene gun	0.25 µg	2 in 3 weeks	No	6	1 (17)	3 PD, 1 SD, 2 NED (were NED at enrolment)	Cassaday et al. [29]
Melanoma	gp100	Gene gun	0.25 µg	2 in 3 weeks	GM-CSF (DNA)	6	1 (17)	3 PD, 1 SD, 2 NED (were NED at enrolment)	Cassaday et al. [29]
Adenocarcinoma	CEA	i.d. (Biojector)	2,000 µg	3 in 6 weeks	GM-CSF, cyclophosphamide prior to vaccination	5	4/5	Enrolment after tumor resection, 1 dead, 5 NED	Staff et al. [30]
Adenocarcinoma	CEA	i.m. (Biojector)	8,000 µg	3 in 6 weeks	GM-CSF, cyclophosphamide prior to vaccination	5	4 (80)	Enrollment after tumor resection, 1 recurrence, 5 NED	Staff et al. [30]
Metastatic adenocarcinoma	CEA/HBsAg	i.m.	0.1/0.3/ 1.0/2.0 mg (1× or 3×)	3-week interval	No	17	5 (29)	In 4 cellular responses; no humoral response	Conry et al. [33]
Stage IV melanoma	Tyrosinase	i.n. (needle)	200/400/ 800 µg	Every 14 days/ 4 cycles	No	26	0	11/26 showed immune responses, long survival	Tagawa et al. [34]
Stage II–IV prostate cancer	PSA	i.m.+i.d.	100/300/ 900 µg	5 in 4 weeks	GM-CSF/IL-2	9	3 (33)	2/3 showed immune responses in the 900-µg group	Pavlenko et al. [35]

CEA, carcinoembryonic antigen; i.d., intradermal; i.m., intramuscular; s.c., subcutaneous; NED, no evidence of disease; PD, progressive disease; POD, progression of disease; GM-CSF, granulocyte-macrophage colony-stimulating factor; SD, stable disease; HBsAg, hepatitis B surface antigen; i.n., intranasal; PSA, prostate-specific antigen; IL-2, interleukin-2.

the administered gene, these delivery systems still must be improved to further enhance this expression [28]. The comparison of different delivery systems in clinical trials is shown in Table 1 and discussed in the following text.

Physical Delivery System

Gene gun and particle-mediated epidermal delivery
The gene gun technique showed a slightly higher response at a lower DNA dose which was used for the vaccination. Cassaday et al. [29] analyzed the delivery of the gp100 gene via the gene gun in 12 melanoma patients. Six patients also received a GM-CSF complementary DNA vaccination as an adjuvant therapy. No dose limiting toxicity was observed and patients showed local skin reactions at the site of vaccination. Each cohort showed stable disease in one patient. The study had to be ended after a viral DNA sequence was found in the

backbone plasmid [29].

A clinical study by Staff et al. [30] investigating the efficiency of the Bioject system administered intradermal and intramuscular in patients with surgically removed adenocarcinoma showed promising results. The target in this study was CEA and the patients were enrolled after resection of the primary tumor. No evidence of macroscopic disease was found. Both cohorts of the study also received GM-CSF as an adjuvant treatment. The DNA dose in the intramuscular vaccination cohort was 4 times higher compared to the intradermal patient group. All patients also received cyclophosphamide prior to the vaccination to deplete regulatory T cells. In eight patients, no evidence of disease was observed after vaccination in the follow-up (cohort A: 72 weeks, cohort B: 104 weeks). One patient (cohort A) died of urine bladder cancer that was diagnosed 72 weeks after the start of vaccination. Another patient (cohort B) showed recurrence of the disease [30]. This

study shows an interesting option for immunotherapy with DNA vaccines. The data suggest that the vaccination of patients after resection of the primary tumor and also the depletion of regulatory T cells which play an important role in the suppression of the immune system, is a possible approach.

Ginsberg et al. [31] investigated the immunologic response against xenogenic (murine origin) gp100 in stage II–IV melanoma patients via the gene gun and Biojector technique. Patients underwent eight immunizations in total. The Bioject and gene gun DNA delivery was tolerated well, with 10 patients in the Bioject cohort and six patients in the gene gun cohort, showing no side effects. One patient undergoing the DNA delivery via Bioject showed an increase in gp100 specific CD8+ T lymphocytes compared to three patients in the gene gun group [31]. No correlation between the observed immune responses and the clinical outcome of the patients can be drawn.

Meyer et al. [32] targeted tyrosinase with a viral vector in melanoma patients. The modified vaccinia virus Ankara (MVA) human tyrosinase was injected intradermal and subcutaneous. At the site of application, a local inflammation was observed. Furthermore, the vaccination showed a strong MVA antibody response. There was also no detectable humoral or cellular response to the tyrosinase antigen [32]. Viral DNA vaccine delivery systems can just be used once or twice, since further vaccinations will be neutralized, due to the high immunogenicity of the viral vector [3]. Modulating of the viral vector which encodes the same target gene, after each immunization (in a prime boost regimen) could lead to an enhanced efficacy in clinical trials.

Ultrasound

The ultrasound delivery system has not been used in clinical trials. This technique as a delivery system in a clinical trial (NCT00849524) was only implemented to verify the correct position of the needle for an intranodal delivery of the DNA vaccine [1].

Needle and electroporation

Conry et al. [17] showed in a second study a combination of the CEA gene and the gene encoding for the hepatitis B surface antigen (HBsAg) as an intramuscular (needle) vaccination in metastatic adenocarcinoma patients. The HBsAg was included as a positive control to monitor the immune responses of the patients. In this study 17 patients were enrolled and four showed a cellular response to CEA and no humoral response was observed. The HBsAg induced hu-

moral and cellular responses. In addition, stable disease was observed in five patients [33].

Tagawa et al. [34] investigated intranodal (needle) delivery of a DNA vaccine containing the tyrosinase gene in stage IV melanoma patients using a pump to apply the vaccine. Twenty-six patients were enrolled. No dose limiting toxicities were measured. Patients showed inflammation at the site of injection. There was no clinical response in the vaccinated patients and 11 showed immune responses to tyrosinase specific CD8+ T lymphocytes. Interestingly, a long median survival of 15.2 months was observed. The normal median survival in stage IV melanoma patients is 7 to 9 months [34].

Pavlenko et al. [35] showed the delivery of the DNA vaccine pVAX/prostate-specific antigen (PSA) containing the PSA gene in stage II–IV prostate cancer patients by a combination of an intramuscular and intradermal administration (needle). Nine patients were enrolled in this study and in combination with the DNA vaccination they also received a GM-CSF and interleukin-2 (IL-2) treatment. No dose limiting toxicity was observed. Local inflammation at the site of injection developed in combination with GM-CSF and systemic toxicities were detected after IL-2 administration. Clinical responses to the vaccination were observed in three patients. Two patients showed immune responses in the high dose group receiving 900 µg of DNA vaccine [35].

Contact-independent helium plasma

The delivery of DNA cancer vaccines through contact-independent helium plasma has not yet been investigated in clinical trials.

Biological delivery system

DNA therapeutics for gene therapy was already approved for head and neck cancer using a biological adenoviral delivery system (Gendicine; SIBIONO, Shenzhen, China) in 2004. This therapeutic was used to insert the p53 suppressor gene into tumor cells [21]. In contrary, DNA cancer vaccines are specially designed to elicit humoral and/or cellular immune responses against a specific antigen. Interestingly, in clinical trials using cancer vaccines, monoclonal antibodies or cytokines, a response or stable disease was observed after an increase in the tumor burden. Several clinical studies were ended, and patients received no further treatment because of this incidence in their clinical results [36].

Conry et al. [17] analyzed the toxicity and efficacy of a viral DNA delivery system in adenocarcinoma cancer patients. The

vector was a rV-CEA containing the carcinoembryonic antigen gene which was injected intradermal (needle) and subcutaneous using the Biojector system. Patients showed inflammation at the site of injection. Both techniques were tolerated well. No humoral or cellular response was measured concerning CEA. The vaccinia virus induced a strong response due to its high immunogenicity. A clinical response was observed in one patient receiving the intradermal injection compared to the subcutaneous injection, where three patients had shown a clinical response [17].

Non-biological delivery system

Not all DNA cancer vaccine delivery systems (non-biological) have been utilized in associated clinical trials, although they have shown promising results in preclinical trials [15,21,24-26]. Especially, the utilization of DNA vaccines and non-biological delivery systems for the prevention of infectious diseases have been shown and almost 100 phase I and II clinical trials have confirmed the safety of these DNA vaccines and their delivery systems in humans [27].

Discussion

Clinical trials investigating different DNA vaccine delivery systems are still ongoing. DNA vaccines have already demonstrated promising results as effective immunotherapeutic strategies against different types of cancer. Investigating different types of delivery in a clinical setting allows to further enhance the potential responses of the immune system to the tumor. Nevertheless, till now only a small number of clinical trials have been published and are still marked as active or active, not recruiting on clinicaltrials.gov [37,38].

The DNA tattooing technique showed interestingly a higher efficiency compared to intradermal injection or gene gun administration in preclinical trials. The results of clinical phase I trials (pDermat, melanoma patients) are still not published [8,39].

The electroporation delivery system induces high humoral and cellular responses in preclinical trials, due to its up-regulation of inflammatory cytokines and recruitment of macrophages which also enhances the antigen presentation to the immune system [2,13,40-42]. Because of these indications, the use as a possible delivery system in DNA cancer vaccine administration is becoming popular [41]. Clinical trials are still under investigation.

Viral delivery systems are often used in DNA cancer vacci-

nation trials. The major problem in these trials is the immunogenicity of the viral vector itself which induces responses in a humoral and cellular manner. Modulating the viral vector by changing the administered virus (still containing the same DNA target gene) might be a possible solution to elicit an immunological response to the actual target [17,32].

The most promising results in a clinical study were observed by Staff et al. [30] with 80% of the patients reaching no evidence of disease till the end of the study. Unfortunately, the specific humoral and cellular responses to the CEA antigen were not investigated in this trial [31].

The application of different DNA vaccine delivery systems in cancer patients still has to be optimized to elicit high immune responses. Some of the results are promising (Staff et al. [30]), but many of the clinical trials conducted, presented only the safety of the administration of these delivery systems and failed to demonstrate how delivery systems could enhance the efficacy of DNA cancer vaccines. A successful translation from pre-clinical DNA cancer vaccine delivery systems to clinical application has not yet occurred. Furthermore, recent research has not been concluded so that the clinical data resulting has not yet been published and clinical phase II and III trials are just commencing [30].

The administration of DNA plasmid vaccines through contact-independent helium plasma might be a promising novel option in vaccine technology. Especially the involvement of physical methods which include a contact between the application device and the patients' tissue often results in a transient subject discomfort. To overcome these limitations of contact-dependent delivery, a helium plasma source might be beneficial in order to decrease the discomfort and elicit higher immune reactions.

In addition, the design of clinical trials has a major impact on the outcome. Especially regarding the end point of such trials in cancer immunotherapy. Hoos et al. [43] has suggested three novel endpoints, since the results from T cell immune response assays are highly variable. There has to be a harmonization of assays to minimize this variability. Immunotherapies induce novel patterns of antitumor responses and are not captured by the World Health Organization criteria. Thirdly, survival curves in randomized immunotherapy trials can show a delayed separation, which can impact the study results [43].

Several techniques to enhance the immunogenicity of DNA cancer vaccines have been developed, although further improvements are needed to increase antitumor immunity by circumventing immune tolerance and the immunosuppres-

sive networks in the tumor microenvironment.

In recent years, it became evident that the gut microbiome has also a direct impact on cancer progression and response to therapy. The influence of the gut microbiome on immunotherapy has been shown in several preclinical and observational studies. Especially, a potential gut microbiome modulation via antibiotics, pro-biotics or fecal transplantation are currently conducted in combination with immunotherapies [44] and might be able to increase the efficacy of tumor immunotherapies and DNA cancer vaccines in the future.

In conclusion, DNA cancer vaccines play a significant role in tumor immunotherapy. Further investigation in the vaccine design, administration technique and the role of the gut microbiome to optimize the efficiency as well as to reduce the negative side-effects, would provide a beneficial and essential advancement in oncological research and clinical outcome for cancer patients.

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