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DNA vaccines for cancer

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TABLE OF CONTENTS

1. ABSTRACT

DNA vaccines, also referred to as genetic, plasmid or polynucleotide vaccines, represent a relatively simple and economical method to exploit gene transfer for immunization against tumor associated antigens. This review discusses the potential advantages of DNA vaccines for cancer immunotherapy as compared to conventional protein vaccines and viral vectors. The proposed mechanisms responsible for induction of immune responses following DNA-based immunization are summarized. The preclinical development of DNA vaccines and the clinical experience with DNA immunization for cancer to date are reviewed. The low toxicity associated with DNA vaccines favors its further development, but additional strategies to improve the potency of this approach are needed if it is to be successfully integrated into the clinical setting.

2. INTRODUCTION

The first cancer vaccines used proteins or cells as immunogens to elicit immune responses to tumor associated antigens. Gene transfer techniques have provided new methods for stimulating the immune response. Among the array of techniques being developed for clinical application, DNA vaccines (also termed nucleic acid, polynucleotide, -plasmid or genetic vaccines) have emerged as a novel and effective method of inducing tumor antigen-specific immune responses.

DNA vaccination relies on delivery of plasmid DNA molecules encoding an antigen of interest. There are several advantages to this mode of delivery. Perhaps most importantly, both antibody and cellular immune responses are elicited. The *in vivo* synthesis of the encoded antigen

allows the protein to be processed for presentation on the major histocompatibility class (MHC) class I complex, promoting the generation of class I restricted cytolytic T lymphocytes (CTL). CTL are known to be important mediators of the antitumor immune response and their activation against tumor antigens is critical to the success of cancer vaccine approaches. Furthermore, and in contrast to protein vaccines prepared in nonmammalian hosts, synthesis of the antigen *in vivo* allows appropriate folding and post-translational modification of the protein. DNA-based vaccines also result in extended expression of the antigen, supporting persistent antitumor immune responses.

Additional factors favoring the development of plasmid DNA-based immunization strategies include the relative ease and inexpensive nature of vaccine preparation, as well as its stability. As discussed in more detail below, DNA vaccines prepared in bacterial hosts are inherently immunostimulatory due to the presence of unmethylated CpG dinulcoetides. These sequences stimulate a nonspecific immune response that does not interfere with repeat delivery of the vaccine. This contrasts with viral-based vaccines, where pre-existing or vector-induced immune responses can strongly compromise the effectiveness of the vaccine (1,2). Safety considerations also favor polynucleotide vaccines compared to viral vaccines, because there is no risk for recombination with wild type viruses and the risk of insertional mutagenesis is low. Finally, DNA vaccines have the potential to readily deliver multiple epitopes, and even multiple antigens, in a single injection, an important consideration given the propensity of tumors to escape immune detection by antigen loss variants (3).

Despite these potential advantages and encouraging preclinical studies, DNA vaccines for cancer have thus far shown only minimal activity in the clinical setting. Many tumor antigens are not mutated, and therefore induction of an immune response to these antigens requires that the immune system be able to recognize and mount an effective response to a 'self' antigen. Initial studies suggest that this will be difficult to achieve in the setting of human cancer. Therefore, improving the potency, and thereby the clinical efficacy, of polynucleotide vaccines has become the major focus of research in the field. This review will delineate some of the approaches, currently under evaluation in preclinical models, designed to address this limitation.

3. GENE TRANSFER OF DNA FOR IMMUNIZATION

The development of nucleic acid vaccines was sparked by the observation of Wolff and colleagues that intramuscular injection of naked DNA led to the expression of the encoded gene by myofiber cells (4). Subsequent studies demonstrated the general applicability of this approach for the expression of foreign genes in a variety of species from fish (5) to nonhuman primates (6). Although an inefficient process, the transferred DNA appears to enter the myofibers via the myocyte caveolae and T tubules (7,8). The DNA is maintained in an extrachromosomal form in the nucleus, but expression can be detected for a prolonged period (9), depending on the immunogenicity of the encoded protein. Ulmer and coworkers first demonstrated the ability of intramuscular delivery of DNA encoding a viral antigen to elicit a CD8+ T cell, MHC class I-restricted immune response protective against infection, using a plasmid encoding the influenza protein nucleoprotein A (10). This study provided the rationale to develop DNA vaccines for therapy of diseases, including cancer.

Induction of cellular and humoral immune responses following delivery of DNA is not limited to intramuscular delivery. The skin is rich in antigen presenting cells (APC) such as immature Langerhans cells in the epidermis, and mature dendritic cells (DC) in the dermis. Tang and co-workers demonstrated the ability of DNA delivered to the skin to elicit a humoral immune response to the encoded gene (11). In this method, the DNA was delivered following precipitation onto gold microparticles (12). The gold particles were delivered to the skin under pressure by a ballistic delivery device. The process, commonly referred to as gene gun delivery, does not produce traumatic injury and requires much less DNA to achieve humoral immune response comparable to intramuscular delivery (13,14). Induction of effector CTL capable of mediating tumor rejection was subsequently demonstrated in a mouse model of transplantable tumors (15). Intradermal immunization can also be accomplished by injection of naked DNA or by a needle-free jet injection system delivering DNA-coated nanoparticles (16). Mucosal administration of DNA vaccines has also been explored primarily for immunization against infectious disease (17) but may also be applicable for cancer therapy (18). Finally, despite a relatively short half-life in the circulation, studies on the intrasplenic administration of a DNA vaccine (19) demonstrated that strategies to promote uptake of DNA by splenocytes following intravenous administration might lead to induction of humoral and cellular immune responses. The fact that all of these delivery routes results in antigen synthesis and induction of antigen-specific immune responses attests to the flexibility of DNA vaccination. It is important to note that these different routes of administration may lead to qualitatively different immune responses (20,21), and the relative efficacy in humans remains to be determined.

4. MECHANISM OF IMMUNE RESPONSE FOLLOWING DNA IMMUNIZATION

The ability of DNA vaccines to elicit a cellular immune response paved the way for their development as a reagent for cancer immunotherapy. The mechanism(s) for induction of the immune response following immunization is still not entirely clear, but appears to involve processing of the antigen through both endogenous and exogenous pathways leading to presentation of the antigen in the context of both MHC class I and class II. DNA may transfect both target cells (for example, myocytes after intramuscular delivery) as well as resident APC. Although myocytes clearly synthesize the encoded protein, only APC are capable of delivering the costimulatory signals

necessary to effectively prime CTL. A number of studies support the central role for bone marrow-derived APC in induction of the immune response following DNA immunization (22-25). The findings suggest 'cross-presentation' of the antigen by the APC. Thus, the antigen is produced by the myocytes and transferred to the APC in such a way that the processed peptides are presented in MHC class I, allowing the APC to directly activated CTL. This contrasts with the usual situation in which proteins acquired exogenously by APC traffic into the endolysosomal pathway for degradation and presentation only by MHC class II molecules. Alternatively, or in addition, the APC themselves may be transfected by the transferred nucleic acid (26,27). The *in vivo* synthesis of the antigen in the cytoplasm of APC promotes presentation of peptide by MHC class I molecules. Presentation of the antigen in the context of both MHC class I and class II in the presence of the appropriate co-stimulatory molecules expressed by APC leads to activation of both CD4+ and CD8+ T cells, promoting both cellular and humoral immunity.

The development of mouse models in which particular aspects of the immune system have been selectively disrupted (genetic knockouts) has allowed more clear delineation of the factors critical for the induction of an effective immune response (28). Investigation of the mechanism of tumor rejection mediated by a therapeutic DNA vaccine in a transgenic mouse model of breast cancer demonstrated the coordinated role of CD4+ and CD8+ cells, antibodies, Fc receptors, perforin, interferon gamma, CD1d-restricted NKT and macrophages, with an important role for activated neutrophils, which may directly lyse cancer cells and affect tumor vasculature (29,30).

5. FACTORS INFLUENCING INDUCTION OF IMMUNE RESPONSE

A number of features of DNA vaccines influence the nature and potency of the elicited immune response. The composition of the DNA is an initial consideration for plasmid-based vaccines. The dinucleotide CpG is relatively underrepresented in the mammalian genome and areas rich in CpG are frequently methylated as a mechanism of transcriptional regulation. In contrast, DNA plasmids produced in bacterial hosts contain unmethylated CpG dinucleotides, which are recognized by the innate immune system as indicative of the presence of a pathogen (31). Specifically, the sequences are recognized by the toll-like receptor 9 (TLR9) and trigger activation of DC, macrophages, natural killer (NK) cells and NKT cells (32,33). The result is that CpG motifs, either in a plasmid or as purified oligodeoxynucleotides (ODN), are potent adjuvants and can promote Th1 type immune responses (34). CpG-ODN also have an antiapoptotic effect on both CD4+ and CD8+ T cells (35). The presence of these CpG motifs contributes significantly to the overall immunogenicity of DNA vaccines.

In addition to the composition of the nucleic acids, an important determinant of immune response is level of transgene expression. In general, increased immunogen expression augments the immune response. Hence a strong promoter is required to direct efficient transcription of the plasmid-encoded gene, and optimized polyadenylation signals and untranslated regions may contribute to enhanced transgene expression (36). The cytomegalovirus early promoter/enhancer has been widely used to drive expression of the encoded sequences and may be enhanced by the insertion of additional sequences, for example those derived from the adeno-associated virus (37).

Once an optimized vector has been developed, the route of administration may also influence the resulting immune response. As discussed in the previous section, a number of routes of DNA immunization have led to induction of cellular and humoral immune responses, but the nature of the immune response elicited by different routes of administration may be qualitatively different (20,21,38,39,40). In general, gene gun administration of DNA leads to a more Th2-like immune response, with a strong humoral component that may be less effective for cancer therapy. However, this effect can be modified by co-administration of Th1 promoting cytokines (41). The nature of the immune response can be further influenced by the vaccination dose and the schedule of administration (13,42).

The antigenicity of the encoded protein is of considerable importance in generating an effective response. The fact that most tumor antigens are 'self' represents a formidable challenge for active immunotherapy of cancer. Modifying the antigenicity of the protein or promoting its uptake by professional APC are key areas of consideration in this respect. The local cytokine milieu also plays an important role in the immune response ultimately elicited. Optimizing all of these factors so as to maximize the effectiveness of the immune response following DNA vaccination has become a major focus of investigation.

6. STRATEGIES TO ENHANCE THE IMMUNE RESPONSE

DNA vaccines have shown promise in eliciting effective CTL responses to neoantigens, but the weakly immunogenic antigens characteristic of most tumors will require cancer vaccines to be more potent if they are to be clinically useful. Thus, many studies have focused on enhancing the immune response elicited by DNA vaccines. Approaches have investigated every aspect of the vaccine, from delivery of the nucleic acid, to modification of the encoded antigen, to the perturbation of the microenvironment to maximize and tailor the immune response to a Th1 type response (Table 1). The versatility of DNA vaccines is a strength in this respect, as both the nucleic acid and the encoded antigen of interest can be readily manipulated and evaluated.

Because the process of delivery of the nucleic acid to target cells is inefficient, approaches to enhance delivery and/or increase the stability of DNA *in vivo* can

Table 1. Strategies to Enhance the Efficacy of Polynucleotide Vaccines for Cancer Therapy

Aspect of Vaccine	Intervention	References
Nucleic acid delivery	• Liposomes • PLG microparticles • Electroporation • Hydrodynamic delivery	43 44 45,46 47
Modification of the antigen to target APCs	• Fuse antigen with • CD40L, Flt-3L, CTLA4	49-51
Modification of the antigen to increase immunogencity	• Alter antigen processing • Incorporate immunogenic eptiopes • Use antigen from a different species • Codon optimization	52-54 55 67-71 56
Modification of the microenviroment	• Cytokine codelivery • Chemokine codelivery	58-60 61,62

result in higher and extended expression of the encoded antigen, increasing the magnitude of the immune response. To this end, incorporation of the nucleic acid into liposomes may protect it from endogenous nucleases and also promote uptake into cells (43). Adsorption of DNA onto the cationic microparticles composed of poly(DL-lactide-co-glycolide) (PLG) allows the slow release of the DNA and results in a more potent immune response compared to naked DNA (44). To physically enhance the transport of nucleic acids into the target cells, electroporation into skin (45) or muscle (46) has proven an effective means of increasing gene transfer efficiency. Hydrodynamic delivery of plasmid DNA may be another method to increase transfection efficiency that may be pertinent to vaccines (47). Application of these approaches in the clinical setting will require careful optimization in human subjects.

The ease of manipulation of recombinant DNA allows the encoded antigen to be readily altered in ways that may enhance immunogenicity, and possible manipulations in this respect are numerous and varied. Since uptake and appropriate presentation of the antigen is critical to induction of an effective immune response, several groups have modified encoded antigens to target them to for more efficient uptake by profession APC (48). Antigens fused to either CD40 ligand (49) the extracellular domain of the Fms-like tyrosine kinase-3 (flt-3) ligand (50), or cytotoxic T-lymphocyte antigen 4 (CTLA4) (51) are examples in which the receptor for each ligand is found on surface of DC, targeting the antigen to these cells for an enhanced immune response. Within the cell, the encoded antigen can be modified to promote degradation via the endosomal / lysosomal pathway (52,53) as a means to enhance antigen presentation by MHC class II and increase CD4+ T cell responses. In a similar approach targeting a different pathway, proteolytic processing of the encoded antigen can be promoted by fusing it with sequences directing its ubiquitination (54). Incorporation of heterologous immunogenic sequences, such as a tetanus toxin CTL epitope, into a tumor antigen resulted in rapid CTL induction against the tumor antigen with protection against tumor challenge (55). For human papilloma virus (HPV)-based cancers such as cervical cancer, codon optimization of the viral antigen gene has proven an effective means of increasing protein expression in mammalian cells and enhancing immune response (56).

Another approach to enhance immunogenicity of DNA vaccines is the co-delivery of DNA encoding cytokines, based on the rationale that a more potent immune response will be elicited if the antigen is presented in a favorable cytokine milieu. To this end, cytokines promoting a Th1 type response, including GM-CSF, IFN-gamma, IL-2, IL-12, and IL-18 have been extensively evaluated in preclinical models of infectious disease (57) and cancer (58-60), and have demonstrated the ability of this approach to favorably influence the nature and magnitude of immune response. Based on the rationale that more efficient delivery of the antigen to APC will enhance immune responsiveness, chemokines have been used to attract APC to the site of antigen synthesis. This has been accomplished either by fusion of the antigen to inflammatory chemokines (61) or by co-delivery of the antigen with chemokines (62). Additional strategies include co-delivery of anti-apoptotic genes to enhance the survival of DNA transfected DC (63) and co-administration of the antigen-encoding DNA with a soluble lymphocyte activating gene-3 (LAG3) protein as a means to promote cross-presentation of the antigen (64). *In vivo* expansion of DC to enhance immune responsiveness has been directed by delivery of a plasmid encoding the flt-3 ligand (65). This approach can be used in combination with convention peptide vaccines to enhance cellular immune response (66).

The concept of cross-species homologous immunization, also called xenogenic or orthologous immunization, has proven to be an effective method of breaking tolerance to weakly immunogenic tumor associated antigens. This strategy uses a tumor antigen gene derived from a different species than the vaccine recipient to induce a cross-reactive immune response to the host autologous protein. For multiple proteins studied to date, the foreign species ortholog displays enhanced immunogenicity as compared to the autologous or self antigen. This approach leads to immunity that cross-reacts with, and breaks tolerance to, the self antigen. Orthologous immunization has been used successfully in animal models to induce anti-tumor immune responses against either endogenous tumor antigens (67-70) or tumor promoting factors (71). Initial clinical studies in prostate cancer using

a protein/DC vaccine demonstrated induction of immune response to the self antigen, suggesting the potential utility of this approach in the clinical setting (72).

The ease of preparation and lack of vector-directed immune response associated with DNA vaccines have led to its incorporation into a variety of heterologous prime and boost strategies, which have proven more efficacious than DNA immunization alone in a variety of preclinical models. These have included the use of DNA vaccines in combination with other genetic vectors (73,74) or with proteins, for example, adsorbed to PLG microparticles (75). Although such approaches will be somewhat more complicated to bring to the clinic, the increased potency of combination vaccines may override this consideration.

7. CLINICAL EXPERIENCE WITH DNA VACCINES

While induction of both T and B cell responses to foreign antigens has been convincingly demonstrated in humans with respect to foreign antigens relevant to infectious disease (76-79) effective application of DNA vaccines for the treatment of cancer has thus far been less successful. Induction of an effective antitumor immune response to weakly immunogenic tumor antigens represents a considerable challenge and, to date, the clinical experience with DNA vaccines has met with mixed results. Clinical studies have supported the general safety and low toxicity of the vectors, but the potency of the immune response has been disappointing and antitumor efficacy has proven elusive.

Several human clinical trials for cancer have been completed to date. Direct intramuscular delivery of DNA encoding a cloned tumor antigen (carcinoembryonic antigen, CEA) has been reported for advanced stage colon cancer (80). Patients were immunized with a plasmid expressing both CEA and, as a control, hepatitis B surface antigen. Although protective levels of antibodies recognizing the hepatitis protein were detected in some patients, there was limited evidence of an immune response directed against CEA. Rosenberg and colleagues reported similar findings using a plasmid DNA encoding the melanoma antigen gp100 in a phase I clinical trial for patients with metastatic melanoma (81). In this trial of 22 patients immunized either intramuscularly or intradermally, no evidence of gp100-specific immune responses were detected, although one patient exhibited a partial response. The authors concluded that no significant clinical or immunological response was generated. This contrasts with previous clinical trials involving the gp100 antigen delivered as a transgene in a fowlpox-based vaccine or as peptides, and emphasizes the need for strategies to enhance immune response to plasmid DNA vaccines. An alternative route of delivery, intranodal, was evaluated in twenty-six patients with advanced melanoma (82). Infusion of a plasmid DNA encoding tyrosinase epitopes resulted in the induction of an antigen-specific immune response in eleven patients. No clinical responses were seen in this trial, but overall survival was unexpectedly long. A plasmid DNA encoding prostate specific antigen (PSA) was delivered with the cytokines GM-CSF and IL-2 in patients with hormone-refractory prostate cancer (83). Cellular and humoral immune responses to PSA were detected in two of three patients in the highest dose cohort, and a decrease in slope of PSA levels was noted in these patients, as well.

Levy and co-worker evaluated the immunogenicity of a plasmid DNA vaccine for patients with B-cell lymphoma (84). Previous clinical studies using proteins representing tumor-specific immunoglobulin idiotype for active immunization have demonstrated clinical benefit for immunized patients (85, 86); however preparation of patient-specific protein vaccines is laborious and not feasible for widespread application. DNA vaccination offers the advantage of comparatively rapid and inexpensive preparation. Patients were immunized with a DNA vaccine encoding a chimeric molecule consisting of the patient-specific idiotype fused to the IgG2a and k mouse immunoglobulin constant region chains. Cohorts of patients were immunized intramuscularly and intradermally using the Biojector needle free delivery device, with or without the addition of plasmid DNA encoding GM-CSF. In all cohorts, most patients generated an immune response to the murine immunoglobulin carrier protein, demonstrating that the encoded protein was produced and was capable of eliciting an immune response. Induction of an immune response to the tumor-specific Id portion of the encoded gene was detected, albeit at a lower frequency. The clinical efficacy of the vaccine was difficult to determine, given prior and concurrent chemotherapy administered to patients, and lack of an unvaccinated control group. Nevertheless, the lack of toxicity and induction of detectable immune response supports further development of this vaccine approach.

It should be noted that these clinical trials were performed in the setting of advanced disease, where induction of an immune response may not be optimal. Nevertheless, collectively the experience with naked DNA transfer in humans for cancer immunotherapy suggests that first generation plasmid DNA vaccines will not be sufficient to elicit a clinically effective immune response against nonmutated self antigens. Translation of the most promising strategies outlined in table 1 into the clinic may address the limitations of current methods.

Two Phase I trials of DNA vaccines directed against human papilloma virus (HPV)-related malignancies have been reported. Immunotherapy of HPV-related malignancies has the advantage that malignant cells express foreign viral antigens. Plasmid DNA encoding MHC class I-restricted peptide epitopes from HPV16 E7 protein was encapsulated in a biodegradable polymer microparticles (PLG) and delivered i.m. (87). This therapeutic trial for individuals with dysplasia led to increased T cell responses as detected by ELISPOT assay in 10 of 12 patients, and partial histological responses in some subjects in the higher dose groups. Use of the same reagent delivered s.c. or i.m. to women with cervical intraepithelial neoplasia led to detectable HPV E7 immune response in most patients

(73%), and complete histological responses in 33% (88). No serious vaccine related adverse events were reported. These studies suggest that DNA vaccines directed at HPV antigens may have a role in management of HPV-related malignancies.

8. PERSPECTIVES

The pace of tumor antigen identification has accelerated rapidly in the past few years (89) and will likely increase as new techniques such as expression profiling (90, 91), SEREX (92), and proteomic analysis (93) lead to the identification of new potential targets for active immunotherapy. The use of DNA vaccines in preclinical models provides a relatively rapid means of evaluating the potential utility of these candidate antigens in mediating tumor rejection. In addition to traditional tumor associated antigens, DNA vaccines may also find a role in vaccines strategies directed against the tumor vasculature (94, 95). Studies of DNA vaccines in the area of infectious disease will continue to be valuable in developing novel strategies that can be incorporated into cancer vaccines. A recent clinical study targeting infectious disease (malaria) suggests that diversified prime and boost immunization strategies employing DNA in combination with other modes of vaccination can potentiate the immune response in the humans (96). Although definitive clinical evidence of the efficacy of DNA vaccines in cancer therapy remains to be demonstrated, there is reason to be optimistic about their potential in the management of a wide variety of malignancies. As a relatively nontoxic therapy, DNA immunization may ultimately find its clinical application as an adjuvant in setting of minimal residual disease, where it may be useful in preventing disease recurrence. Eventually, use of DNA vaccines may extend to the cancer prevention. The notable advantages of DNA immunization and its proven safety thus far in clinical studies provides a sound basis for their continued development and eventual incorporation into the management of malignant disease.

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10. REFERENCES

1. Rosenberg S. A., Y. Zhai, J. C. Yang, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, S. L. Topalian, N. P. Restifo, C. A. Seipp, J. H. Einhorn, B. Roberts & D. E. White: Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. *J Natl Cancer Inst* 90, 1894-1900 (1998)

2. Conry R. M., M. B. Khazaeli, M. N. Saleh, K. O. Allen, D. L. Barlow, S. E. Moore, D. Craig, R. B. Arani, J. Schlom & A. F. LoBuglio: Phase I trial of a recombinant vaccinia virus encoding carcinoembryonic antigen in metastatic adenocarcinoma: comparison of intradermal versus subcutaneous administration. *Clin Cancer Res* 5, 2330-2337 (1999)

3. Dunn G. P., A. T. Bruce, H. Ikeda, L. J. Old & R. D. Schreiber: Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 3, 991-998 (2002)

4. Wolff J. A, R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani & P. L. Felgner: Direct gene transfer into mouse muscle *in vivo. Science* 247, 1465-1468 (1990)

5. Hansen E., K. Fernandes, G. Goldspink, P. Butterworth, P. K. Umeda & K. C. Chang: Strong expression of foreign genes following direct injection into fish muscle. *FEBS Lett* 290, 73-76 (1991)

6. Jiao S., P. Williams, R. K. Berg, B. A. Hodgeman, L. Liu, G. Repetto & J. A. Wolff: Direct gene transfer into nonhuman primate myofibers *in vivo. Hum Gene Ther* 3, 21-33 (1992)

7. Danko I. & J. A. Wolff: Direct gene transfer into muscle. *Vaccine* 12, 1499-1502 (1994)

8. Wolff J. A., M. E. Dowty, S. Jiao, G. Repetto, R. K. Berg, J. J. Ludtke, P. Williams & D. B. Slautterback: Expression of naked plasmids by cultured myotubes and entry of plasmids into T tubules and caveolae of mammalian skeletal muscle. *J Cell Sci* 103, 1249-1259 (1992)

9. Wolff J. A., J. J. Ludtke, G. Acsadi, P. Williams & A. Jani: Long-term persistence of plasmid DNA and foreign gene expression in mouse muscle. *Hum Mol Genet* 1, 363-369 (1992)

10. Ulmer J. B., J. J. Donnelly, S. E. Parker, G. H. Rhodes, P. L. Felgner, V. J. Dwarki, S. H. Gromkowski, R. R. Deck, C. M. DeWitt, A. Friedman, L. A. Hawe, K. R. Leander, D. Martinez, H. C. Perry, J. W. Shiver, D. L. Montgomery & M. A. Liu: Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 259, 1745-1749 (1993)

11. Tang D. C., M. DeVit & S. A. Johnston: Genetic immunization is a simple method for eliciting an immune response. *Nature* 356, 152-154 (1992)

12. Williams R. S., S. A. Johnston, M. Riedy, M. J. DeVit, S. G. McElligott, & J. C. Sanford: Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. *Proc Natl Acad Sci USA* 88, 2726-2730 (1991)

13. Barry M. A. & S. A. Johnston: Biological features of genetic immunization. *Vaccine* 15, 788-1791 (1997)

14. Fynan E. F., R. G. Webster, D. H. Fuller, J. R. Haynes, J. C. Santoro & H. L. Robinson: DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proc Natl Acad Sci USA* 90, 11478-11482 (1993)

15. Ross H. M., L. W. Weber, S. Wang, G. Piskun, R. Dyall, P. Song, Y. Takechi, J. Nikolic-Zugic, A. N. Houghton & J. J. Lewis: Priming for T-cell-mediated rejection of established tumors by cutaneous DNA immunization. *Clin Cancer Res* 3, 2191-2196 (1997)

16. Cui Z., L. Baizer & R. J. Mumper: Intradermal immunization with novel plasmid DNA-coated nanoparticles via a needle-free injection device. *J Biotechnol* 102, 105-115 (2003)

17. Eriksson K. & J. Holmgren: Recent advances in mucosal vaccines and adjuvants. *Curr Opin Immunol* 14, 666-672 (2002)

18. Rocha-Zavaleta L., J. E. Alejandre & A. Garcia-Carranca: Parenteral and oral immunization with a plasmid DNA expressing the human papillomavirus 16-L1 gene induces systemic and mucosal antibodies and cytotoxic T lymphocyte responses. *J Med Virol* 66, 86-95 (2002)

19. White S. A., A. F. LoBuglio, R. B. Arani, J. F. Pike, S. E. Moore, D. L. Barlow & R. M. Conry: Induction of anti-tumor immunity by intrasplenic administration of a carcinoembryonic antigen DNA vaccine. *J Gene Med* 2, 135-140 (2000)

20. Pertmer T. M., T. R. Roberts & J. R. Haynes: Influenza virus nucleoprotein-specific immunoglobulin G subclass and cytokine responses elicited by DNA vaccination are dependent on the route of vector DNA delivery. *J Virol* 70, 6119-6125 (1996)

21. Feltquate D. M., S. Heaney, R. G. Webster & H. L. Robinson: Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. *J Immunol* 158, 2278-2284 (1997)

22. Doe B., M. Selby, S. Barnett, J. Baenziger & C. M. Walker: Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells. *Proc Natl Acad Sci USA* 93, 8578-8583 (1996)

23. Iwasaki A., C. A. Torres, P. S. Ohashi, H. L. Robinson & B. H. Barber: The dominant role of bone marrow-derived cells in CTL induction following plasmid DNA immunization at different sites. *J Immunol* 159, 11-14 (1997)

24. Fu T. M., J. B. Ulmer, M. J. Caulfield, R. R. Deck, A. Friedman, S. Wang, X. Liu, J. J. Donnelly & M. A. Liu: Priming of cytotoxic T lymphocytes by DNA vaccines: requirement for professional antigen presenting cells and evidence for antigen transfer from myocytes. *Mol Med* 3, 362-371 (1997)

25. Corr M., A. von Damm, D. J. Lee & H. Tighe: *In vivo* priming by DNA injection occurs predominantly by antigen transfer. *J Immunol* 163, 4721-4727 (1999)

26. Condon C., S. C. Watkins, C. M. Celluzzi, K. Thompson & L. D. Falo, Jr.: DNA-based immunization by *in vivo* transfection of dendritic cells. *Nat Med* 2, 1122-1128 (1996)

27. Casares S., K. Inaba, T. D. Brumeanu, R. M. Steinman & C. A. Bona: Antigen presentation by dendritic cells after immunization with DNA encoding a major histocompatibility complex class II-restricted viral epitope. *J Exp Med* 186, 1481-1486 (1997)

28. Song K., Y. Chang & G. J. Prud'homme: IL-12 plasmid-enhanced DNA vaccination against carcinoembryonic antigen (CEA) studied in immune-gene knockout mice. *Gene Ther* 7, 1527-1535 (2000)

29. Dranoff G: Coordinated tumor immunity. *J Clin Invest* 111, 1116-1118 (2003)

30. Curcio C., E. Di Carlo, R. Clynes, M. J. Smyth, K. Boggio, E. Quaglino, M. Spadaro, M. P. Colombo, A. Amici, P. L. Lollini, P. Musiani & G. Forni: Nonredundant roles of antibody, cytokines, and perforin in the eradication of established Her-2/neu carcinomas. *J Clin Invest* 111, 1161-1170 (2003)

31. Klinman D. M., A. K. Yi, S. L. Beaucage, J. Conover & A. M. Krieg: CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. *Proc Natl Acad Sci USA* 93, 2879-2883 (1996)

32. Ashkar A. A. & K. L. Rosenthal: Toll-like receptor 9, CpG DNA and innate immunity. *Curr Mol Med* 2, 545-556 (2002)

33. Agrawal S. & E. R. Kandimalla: Medicinal chemistry and therapeutic potential of CpG DNA. *Trends Mol Med* 8, 114-121 (2002)

34. Dalpke A., S. Zimmermann & K. Heeg: Immunopharmacology of CpG DNA. *Biol Chem* 383, 1491-1500 (2002)

35. Davila E., M. G. Velez, C. J. Heppelmann & E. Celis: Creating space: an antigen-independent, CpG-induced peripheral expansion of naïve and memory T lymphocytes in a full T-cell compartment. *Blood* 100, 2537-2545 (2002)

36. Zinckgraf J. W. & L. K. Silbart: Modulating gene expression using DNA vaccines with different 3'-UTRs influences antibody titer, seroconversion and cytokine profiles. *Vaccine* 21, 1640-1649 (2003)

37. Xin K. Q., T. Ooki, N. Jounai, H. Mizukami, K. Hamajima, Y. Kojima, K. Ohba, Y. Toda, S. Hirai, D. M. Klinman, K. Ozawa & K. Okuda: A DNA vaccine containing inverted terminal repeats from adeno-associated virus increases immunity to HIV. *J Gene Med* 5, 438-445 (2003)

38. Hanke T., V. C. Neumann, T. J. Blanchard, P. Sweeney, A. V. Hill, G. L. Smith & A. McMichael: Effective induction of HIV-specific CTL by multi-epitope using gene gun in a combined vaccination regime. *Vaccine* 17, 589-596 (1999)

39. Smith B. F., H. J. Baker, D. T. Curiel, W. Jiang & R. M. Conry: Humoral and cellular immune responses of dogs immunized with a nucleic acid vaccine encoding human carcinoembryonic antigen. *Gene Ther* 5, 865-868 (1998)

40. Ito K., K. Ito, N. Shinohara & S. Kato: (2003) DNA immunization via intramuscular and intradermal routes using a gene gun provides different magnitudes and durations on immune response. *Mol Immunol* 39, 847-854 (2003)

41. Tuting T., A. Gambotto, P. D. Robbins, W. J. Storkus & A. B. DeLeo: Co-delivery of T helper 1-biasing cytokine genes enhances the efficacy of gene gun immunization of mice: studies with the model tumor antigen beta-galactosidase and the BALB/c Meth A p53 tumor-specific antigen. *Gene Ther* 6, 629-636 (1999)

42. Leitner W. W., M. C. Seguin, W. R. Ballou, J. P. Seitz, A. M. Schultz, M. J. Sheehy & J. A. Lyon: Immune responses induced by intramuscular or gene gun injection of protective deoxyribonucleic acid vaccines that express the circumsporozoite protein from Plasmodium berghei malaria parasites. *J Immunol* 159, 6112-6119 (1997)

43. Gregoriadis G., A. Bacon, W. Caparros-Wanderley & B. McCormack: A role for liposomes in genetic vaccination. *Vaccine* 20, Suppl 5:B1-9 (2002)

44. Singh M., M. Briones, G. Ott & D. O'Hagan: Cationic microparticles: A potent delivery system for DNA vaccines. *Proc Natl Acad Sci USA* 97, 811-816 (2000)

45. Drabick J. J., J. Glasspool-Malone, A. King & R. W. Malone: Cutaneous transfection and immune responses to intradermal nucleic acid vaccination are significantly enhanced by in vivo electropermeabilization. *Mol Ther* 3, 249-255 (2001)

46. Paster W., M. Zehetner, M. Kalat, S. Schuller & T. Schweighoffer: In vivo plasmid DNA electroporation generates exceptionally high levels of epitope-specific CD8+ T-cell responses. *Gene Ther* 10, 717-724 (2003)

47. Chen H.-W., Y.-P. Lee, Y.-F. Chung, Y.C. Shih, J.-P Tsai, M.-H. Tao, & C.-C Ting. Inducing long-term survival with lasting anti-tumor immunity in treating B-cell lymphoma by a combined cell-based and hydrodynamic plasmid-encoding IL-12 gene therapy. *Internal Immunol* 15:427-435 (2003)

48. Hung C. F. & T. C. Wu: Improving DNA vaccine potency via modification of professional antigen presenting cells. *Curr Opin Mol Ther* 5, 20-24 (2003)

49. Xiang R., F. J. Primus, J. M. Ruehlmann, A. G. Niethammer, S. Silletti, H. N. Lode, C. S. Dolman, S. D. Gillies & R. A. Reisfeld: A dual-function DNA vaccine encoding carcinoembryonic antigen and CD40 ligand trimer induces T cell-mediated protective immunity against colon cancer in carcinoembryonic antigen-transgenic mice. *J Immunol* 167, 4560-4565 (2001)

50. Hung C. F., K. F. Hsu, W. F. Cheng, C. Y. Chai, L. He, M. Ling & T. C. Wu: Enhancement of DNA vaccine potency by linkage of antigen gene to a gene encoding the extracellular domain of Fms-like tyrosine kinase 3-ligand. *Cancer Res* 61, 1080-1088 (2001)

51. Boyle J. S., J. L. Brady & A. M. Lew: Enhanced responses to a DNA vaccine encoding a fusion antigen that is directed to sites of immune induction. *Nature* 392, 408-411 (1998)

52. Su Z., J. Vieweg, A. Z. Weizer, P. Dahm, D. Yancey, V. Turaga, J. Higgins, D. Boczkowski, E. Gilboa & J. Dannull: Enhanced induction of telomerase-specific CD4(+) T cells using dendritic cells transfected with RNA encoding a chimeric gene product. *Cancer Res* 62, 5041-5048 (2002)

53. Ji H., T. L. Wang, C. H. Chen, S. I. Pai, C. F. Hung, K. Y. Lin, R. J. Kurman, D. M. Pardoll & T. C. Wu: Targeting human papillomavirus type 16 E7 to the endosomal/lysosomal compartment enhances the antitumor immunity of DNA vaccines against murine human papillomavirus type 16 E7-expressing tumors. *Hum Gene Ther* 10, 2727-2740 (1999)

54. Xiang R., H. N. Lode, T. H. Chao, J. M. Ruehlmann, C. S. Dolman, F. Rodriguez, J. L. Whitton, W. W. Overwijk, N. P. Restifo & R. A. Reisfeld: An autologous oral DNA vaccine protects against murine melanoma. *Proc Natl Acad Sci USA* 97, 5492-5497 (2000)

55. Rice J., S. Buchan & F. K. Stevenson: Critical components of a DNA fusion vaccine able to induce protective cytotoxic T cells against a single epitope of a tumor antigen. *J Immunol* 169, 3908-3918 (2002)

56. Cid-Arregui A., V. Juarez & H. zur Hausen: A synthetic E7 gene of human papillomavirus type 16 that yields enhanced expression of the protein in mammalian cells and is useful for DNA immunization studies. *J Virol* 77, 4928-4937 (2003)

57. Scheerlinck J. P., G. Casey, P. McWaters, J. Kelly, D. Woollard, M. W. Lightowlers, J. M. Tennent & P. J. Chaplin: The immune response to a DNA vaccine can be modulated by co-delivery of cytokine genes using a DNA prime-protein boost strategy. *Vaccine* 19, 4053-60 (2001)

58. Conry R. M., G. Widera, A. F. LoBuglio, J. T. Fuller, S. E. Moore, D. L. Barlow, J. Turner, N. S. Yang & D. T. Curiel: Selected strategies to augment polynucleotide immunization. *Gene Ther* 3, 67-74 (1996)

59. Irvine K. R., J. B. Rao, S. A. Rosenberg & N. P. Restifo: Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. *J Immunol* 156, 238-245 (1996)

60. Kim J. J., J. S. Yang, K. Dang, K.H. Manson, & D. B. Weiner. Engineering enhancement of immune responses to DNA-based vaccines in a prostate cancer model in rhesus macaques through the use of cytokine adjuvants. *Clin Cancer Res* 7:882s-889s (2001)

61. Biragyn A., M. Surenhu, D. Yang, P. A. Ruffini, B. A. Haines, E. Klyushnenkova, J. J. Oppenheim & L. W. Kwak: Mediators of innate immunity that target immature, but not mature, dendritic cells induce antitumor immunity when genetically fused with nonimmunogenic tumor antigens. *J Immunol* 167, 6644-6653 (2001)

62. Kim J. J., J. S. Yang, T. Dentchev, K. Dang & D. B. Weiner: Chemokine gene adjuvants can modulate immune responses induced by DNA vaccines. *J Interferon Cytokine Res* 20, 487-498 (2000)

63. Kim T. W., C. F. Hung, M. Ling, J. Juang, L. He, J. M. Hardwick, S. Kumar and T. C. Wu: Enhancing DNA vaccine potency by coadministration of DNA encoding antiapoptotic proteins. *J Clin Invest* 112, 109-117 (2003)

64. Cappello P., F. Triebel, M. Iezzi, C. Caorsi, E. Quaglino, P. L. Lollini, A. Amici, E. Di Carlo, P. Musiani, M. Giovarelli & G. Forni: LAG-3 Enables DNA vaccination to persistently prevent mammary carcinogenesis in HER-2/neu transgenic BALB/c Mice. *Cancer Res* 63, 2518-25 (2003)

65. He Y., A. A. Pimenov, J. V. Nayak, J. Plowey, L. D. Falo, Jr. & L. Huang: Intravenous injection of naked DNA encoding secreted flt3 ligand dramatically increases the number of dendritic cells and natural killer cells in vivo. *Hum Gene Ther* 11, 547-554 (2002)

66. Fong C. L. & K. M. Hui: Generation of potent and specific cellular immune responses via in vivo stimulation of dendritic cells by pNGVL3-hFLex plasmid DNA and immunogenic peptides. *Gene Ther* 9, 1127-1138 (2002)

67. Weber L. W., W. B. Bowne, J. D. Wolchok, R. Srinivasan, J. Qin, Y. Moroi, R. Clynes, P. Song, J. J. Lewis & A. N. Houghton: Tumor immunity and autoimmunity induced by immunization with homologous DNA. *J Clin Invest* 102, 1258-1264 (1998)

68. Su J. M., Y. Q. Wei, L. Tian, X. Zhao, L. Yang, Q. M. He, Y. Wang, Y. Lu, Y. Wu, F. Liu, J. Y. Liu, J. L. Yang, Y. Y. Lou, B. Hu, T. Niu, Y. J. Wen, F. Xiao, H. X. Deng, J. Li & B. Kan: Active immunogene therapy of cancer with vaccine on the basis of chicken homologous matrix metalloproteinase-2. *Cancer Res* 63, 600-607 (2003)

69. Hawkins W. G., J. S. Gold, N. E. Blachere, W. B. Bowne, A. Hoos, J. J. Lewis & A. N. Houghton: Xenogeneic DNA immunization in melanoma models for minimal residual disease. *J Surg Res* 102, 137-143 (2002)

70. Bergman P. J., J. McKnight, A. Novosad, S. Charney, J. Farrelly, D. Craft, M. Wulderk, Y. Jeffers, M. Sadelain, A. E. Hohenhaus, N. Segal, P. Gregor, M. Engelhorn, I. Riviere, A. N. Houghton & J. D. Wolchok: Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. *Clin Cancer Res* 9, 1284-1290 (2003)

71. Wei Y.-Q., M.-J. Huang, L. Yang, X. Zhao, L. Tian, Y. Lu, J.- M. Shu, C.-J. Lu, T. Niu, B. Kang, Y.-Q. Mao, F. Liu, Y.-J. Wen, S. Lei, F. Luo, L.-Q. Zhou, F. Peng, Y. Jiang, J.-Y. Liu, H. Zhou, Q.-R. Wang, Q.-M. He, F. Xiao, Y.-Y. Lou, X.-J. Xie, Q. Li, Y. Wu, Z.-Y. Ding, B. Hu, M. Hu & W. Zhang: Immunogene therapy of tumors with vaccine based upon *Xenopus* homologous vascular endothelial growth factor as a model antigen. *Proc Natl Acad Sci USA* 98, 11545-11550 (2001)

72. Fong L., D. Brockstedt, C. Benike, J. K. Breen, G. Strang, C. L. Ruegg & E. G. Engleman: Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. *J Immunol* 167, 7150-7156 (2001)

73. Woodberry T., J. Gardner, S. L. Elliott, S. Leyrer, D. M. Purdie, P. Chaplin & A. Suhrbier: Prime boost vaccination strategies: CD8 T cell numbers, protection, and Th1 bias. *J Immunol* 170, 2599-2604 (2003)

74. Pasquini S., S. Peralta, E. Missiaglia, L. Carta & N. R. Lemoine: Prime-boost vaccines encoding an intracellular idiotype/GM-CSF fusion protein induce protective cell-mediated immunity in murine pre-B cell leukemia. *Gene Ther* 9, 503-510 (2002)

75. Otten G., M. Schaefer, C. Greer, M. Calderon-Cacia, D. Coit, J. Kazzaz, A. Medina-Selby, M. Selby, M. Singh, M. Ugozzoli, J. Zur Megede, S. W. Barnett, D. O'Hagan, J. Donnelly & J. Ulmer: Induction of broad and potent anti-human immunodeficiency virus immune responses in rhesus macaques by priming with a DNA vaccine and boosting with protein-adsorbed polylactide coglycolide microparticles. *J Virol* 77, 6087-6092 (2003)

76. Wang R., D. L. Doolan, T. P. Le, R. C. Hedstrom, K. M. Coonan, Y. Charoenvit, T. R. Jones, P. Hobart, M. Margalith, J. Ng, W. R. Weiss, M. Sedegah, C. de Taisne, J. A. Norman, & S. L. Hoffman: Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science* 28, 476-480 (1998)

77. Roy M. J., M. S. Wu, L. J. Barr, J. T. Fuller, L. G. Tussey, S. Speller, J. Culp, J. K. Burkholder, W. F. Swain, R. M. Dixon, G. Widera, R. Vessey, A. King, G. Ogg, A. Gallimore, J. R. Haynes & D. Heydenburg Fuller: Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine* 19, 764-778 (2000)

78. MacGregor R. R., R. Ginsberg, K. E. Ugen, Y. Baine, C. U. Kang, X. M. Tu, T. Higgins, D. B. Weiner & J. D. Boyer: T-cell responses induced in normal volunteers immunized with a DNA-based vaccine containing HIV-1 env and rev. *AIDS* 16, 2137-2143 (2002)

79. Calarota S., G. Bratt, S. Nordlund, J. Hinkula, A. C. Leandersson, E. Sandstrom & B. Wahren: Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. *Lancet* 351, 1320-1325 (1998)

80. Conry R. M., D. T. Curiel, T. V. Strong, S. E. Moore, K. O. Allen, D. L. Barlow, D. R. Shaw & A. F. LoBuglio: Safety and immunogenicity of a DNA vaccine encoding carcinoembryonic antigen and hepatitis B surface antigen in colorectal carcinoma patients. *Clin Cancer Res* 8, 2782-2787 (2002)

81. Rosenberg S. A., J. C. Yang, R. M. Sherry, P. Hwu, S. Topalian, D. J. Schwartzentruber, N. P. Restifo, L. R. Haworth, C. A. Seipp, L. J. Freezer, K. E. Morton, S. A. Mavroukakis & D. E. White: Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. *Hum Gene Ther* 14, 709-714 (2003)

82. Tagawa S. T., P. Lee, J. Snively, W. Boswell, S. Ounpraseuth, S. Lee, B. Hickingbottom, J. Smith, D. Johnson, & J.S. Weber. Phase I study of intranodal delivery of a plasmid DNA vaccine for patients with Stage IV melanoma. *Cancer* 98, 144-154 (2003)

83. Pavlenko, M., A. K. Ross, A. Lundqvist, A. Palmborg, A.M. Miller, V. Ozenci, B. Bergman, L. Egevad, M. Hellstrom, R. Kiessling, G. Masucci, P. Wersall, S. Nilsson, & P. Pisa. A phase I trial of DNA vaccination with a plasmid expressing prostate-specific antigen in patients with hormone-refractory prostate cancer. *Br J Cancer* 91, 688-694 (2004)

84. Timmerman J. M., G. Singh, G. Hermanson, P. Hobart, D. K. Czerwinski, B. Taidi, R. Rajapaksa, C. B. Caspar, A. Van Beckhoven & R. Levy: Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. *Cancer Res* 62, 5845-5852 (2002)

85. Timmerman J. M. & R. Levy: The history of the development of vaccines for the treatment of lymphoma. *Clin Lymphoma* 1, 129-139 (2000)

86. Bendandi M., C. D. Gocke, C. B. Kobrin, F. A. Benko, L. A. Sternas, R. Pennington, T. M. Watson, C. W. Reynolds, B. L. Gause, P. L. Duffey, E. S. Jaffe, S. P. Creekmore, D. L. Longo & L. W. Kwak: Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat Med* 5, 1171-1177 (1999)

87. Klencke B., M. Matijevic, R. G. Urban, J. L. Lathey, M. L. Hedley, M. Berry, J. Thatcher, V. Weinberg, J. Wilson, T. Darragh, N. Jay, M. Da Costa & J. M. Palefsky: Encapsulated plasmid DNA treatment for human papillomavirus 16-associated anal dysplasia: a Phase I study of ZYC101. *Clin Cancer Res* 8, 1028-1037 (2002)

88. Sheets E. E., R. G. Urban, C. P. Crum, M. L. Hedley, J. A. Politch, M. A. Gold, L. I. Muderspach, G. A. Cole & P. A. Crowley-Nowick: Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. *Am J Obstet Gynecol* 188, 916-926 (2003)

89. Stevanovic S.: Identification of tumour-associated T-cell epitopes for vaccine development. *Nat Rev Cancer* 2, 514-520 (2002)

90. Nelson P. S.: Identifying immunotherapeutic targets for prostate carcinoma through the analysis of gene expression profiles. *Ann NY Acad Sci* 975, 232-46 (2002)

91. Schultze J. L. & R. H. Vonderheide: From cancer genomics to cancer immunotherapy: toward second-generation tumor antigens. *Trends Immunol* 22, 516-523 (2001)

92. Chen Y. T.: Cancer vaccine: identification of human tumor antigens by SEREX. *Cancer J* 6, Suppl 3, S208-217 (2000)

93. Naour F. L., F. Brichory, L. Beretta & S. M. Hanash: Identification of tumor-associated antigens using proteomics. *Technol Cancer Res Treat* 1, 257-262 (2002)

94. Niethammer A. G., R. Xiang, J. C. Becker, H. Wodrich, U. Pertl, G. Karsten, B. P. Eliceiri & R. A. Reisfeld: A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med* 8, 1369-1375 (2002)

95. Xiang R., N. Mizutani, Y. Lou, C. Chiodoni, H. Zhou, M. Mizutani, Y. Ba, J.C. Becker, & R. A. Reisfeld: A DNA vaccine targeting survivin combines apoptosis with suppression of angiogenesis in lung tumor eradication. *Cancer Res* 65, 553-561, (2005)

96. McConkey S. J., W. H. Reece, V. S. Moorthy, D. Webster, S. Dunachie, G. Butcher, J. M. Vuola, T. J. Blanchard, P. Gothard, K. Watkins, C. M. Hannan, S. Everaere, K. Brown, K. E. Kester, J. Cummings, J. Williams, D. G. Heppner, A. Pathan, K. Flanagan, N. Arulanantham, M. T. Roberts, M. Roy, G. L. Smith, J. Schneider, T. Peto, R. E. Sinden, S. C. Gilbert & A. V. Hill: Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat Med* 9, 729-735 (2003)

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