Trial watch: DNA-based vaccines for oncological indications

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ABSTRACT
DNA-based vaccination is a promising approach to cancer immunotherapy. DNA-based vaccines specific for tumor-associated antigens (TAAs) are indeed relatively simple to produce, cost-efficient and well tolerated. However, the clinical efficacy of DNA-based vaccines for cancer therapy is considerably limited by central and peripheral tolerance. During the past decade, considerable efforts have been devoted to the development and characterization of novel DNA-based vaccines that would circumvent this obstacle.

In this setting, particular attention has been dedicated to the route of administration, expression of modified TAAs, co-expression of immunostimulatory molecules, and co-delivery of immune checkpoint blockers. Here, we review preclinical and clinical progress on DNA-based vaccines for cancer therapy.

Introduction

The development of vaccines that generate robust immune reactivity against pathogens was first described in the 18th century by the English physician Edward Jenner, who is commonly considered as the father of modern immunology. It took more than 200 years for the notion that the immune system is also able to recognize and eliminate malignant cells to become widely accepted. Thus, in the late 1990s the field of cancer immunotherapy began to expand and diversify to generate multiple approaches for (re)instating tumor-targeting immune responses, including therapeutic vaccines. The majority of immunotherapeutic agents approved so far by the US Food and Drug administration (FDA) and equivalent agencies worldwide, however, are: (1) monoclonal antibodies initially conceived to selectively inhibit trophic signaling in cancer cells, most (if not all) of which mediate therapeutic effects at least in part by triggering antibody-dependent cellular cytotoxicity (e.g., the CD20-specific agent rituximab, the ERBB2-specific agent trastuzumab) and (2) monoclonal antibodies that block immune checkpoints (e.g., the CTLA4-targeting agent ipilimumab, the PD-1-targeting agents nivolumab and pembrolizumab). In addition, one dendritic cell (DC)-based therapeutic vaccine, namely sipuleucel-T, is currently licensed for use in prostate cancer patients and no more than a few months ago chimeric antigen receptor (CAR)-expressing T cells targeting CD19 have been approved for use in children and young adults with B-cell acute lymphoblastic leukemia on second relapse.

Vaccines have been used to enhance and augment the immune response and are considered as an attractive option to cancer immunotherapy because they generally represent a relatively specific and well-tolerated intervention that (at least potentially) drives durable responses as a consequence of immunological memory.

It is now clear that the antigenic profile of malignant cells differs from that of their normal counterparts, owing to genetic and epigenetic alterations. Thus, at least theoretically, cancer vaccines can be designed to specifically target tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs).
The former are also expressed by normal cells, though in much lower amounts (which constitutes the basis for some degree of specificity), while the latter are completely absent from healthy cells as they originate from cancer-restricted mutations or from the genome of oncogenic pathogens. Even though so-called cancer-testis (CT) antigens are also expressed by the male germine and trophoblastic cells, they can also be considered as TSAs because these cells do not express sizeable amounts of MHC Class I molecules (and hence cannot present endogenous antigens). Importantly, an ideal target for cancer immunotherapy, including cancer vaccines, should be uniquely expressed by neoplastic cells, display robust antigenicity, and participate in key cellular functions to prevent the selection of malignant clones losing expression. With the intent to induce an effective immunity against cancer cells, a variety of vaccine platforms have been developed and tested, including (1) whole tumor cell vaccines, based on the administration of cancer cell lysates; (2) peptide-based vaccines, based on the direct delivery of recombinant proteins or epitopes thereof in combination with immunological adjuvants; (3) dendritic cells (DC)-based vaccines, most often based on the isolation of patient-derived DCs, their maturation ex vivo in the presence of a source of TAAs (TSAs), and their re-infusion; (4) RNA-based vaccines, based on the direct delivery of RNA molecules extracted from malignant cells or specifically encoding a single TAA (TSA); and DNA-based vaccines, based on the administration of naked plasmids or vectored TAA/TSA-coding plasmids. Naked DNA vaccines are closed circular plasmids coding for one or more TAAs (TSAs) under the control of a strong mammalian or viral promoter. Naked DNA vaccination relies on the (natural or induced by delivery) uptake of the vaccine by mammalian cells (mostly myocytes, resident DCs and monocytes) and consequent antigen expression, processing and exposure on MHC Class I molecules, potentially resulting in CD8+ T-cell priming and long-term immunity. Several routes of administration have been explored for naked DNA vaccines, including the oral, intradermal, and intranodal route. However, the intramuscular route is the most widely used since the work of Wolff and collaborators (University of Wisconsin, Madison, WI, USA), who for the first time delivered an expression vector to the mouse skeletal muscle in vivo. In order to improve plasmid uptake, the intramuscular injection of plasmid DNA is usually followed by electro-gene-transfer (EGT; also known as electroporation). Other approaches such as the so-called “gene gun”, sonoporation, hydrodynamic delivery, and liposomal formulations have also been used to boost vaccine uptake in vivo. DNA vaccination has been most successful at targeting TSAs of foreign origin. For example, several DNA vaccines specific for the oncoproteins E6 and E7 from HPV16 or HPV18 were able to generate potent cellular and humoral immunity in mice and humans. However, most malignancies arise from normal tissues and only express endogenous TAAs, which are normally covered by central or peripheral tolerance (and hence are unable to drive an immune response). Hence, several strategies have been developed to overcome tolerance and improve the immunogenicity of TAAs, including the co-expression (in cis or trans) of adjuvant-like molecules. Of note, neoplastic cells also express a large panel of so-called “tumor neoantigens”, which result from the mutational processes that accompany tumor progression. However, tumor neoantigens are specific for a single neoplasm or even cell populations thereof and rather cumbersome to identify, which implies that exploiting them for the development of vaccines is complex.

DNA-based vaccination offers several advantages as compared to other immunization techniques. The design, manufacturing and scaling-up of DNA-based vaccines are simple and relatively inexpensive. Moreover, the DNA platform is safe, stable, and well tolerated, which allows for repeated administration. Together, these characteristics favored the evaluation of multiple DNA-based vaccines in patients affected by a variety of neoplasms, as extensively discussed in previous Trial Watches dealing with this topic. Here, we present preclinical and clinical data on the development of DNA-based vaccines for oncological indications as we cover the period of time elapsing from May 2015 to October 2017.

Preclinical studies – Highlights

Twenty-five new designs for DNA-based vaccine have been evaluated in preclinical settings since the publication of the latest Trial Watch dealing with this topic. In this scenario, the efficacy of DNA-based vaccines is commonly assessed by: (1) the robustness of cellular and humoral tumor-targeting immune responses elicited by vaccination, and (2) the impact of vaccination on the growth of neoplastic lesions and the survival of the host. Several methods to enhance the efficacy of DNA-based vaccines, including the co-delivery of adjuvants or adjuvant-like molecules, immune checkpoint blockers (ICBs), chemotheraphy, as well as radiotherapy have been tested with encouraging results. Importantly, alternative routes of administration and dosages have also been shown to enhance the efficacy and decrease the toxicity of DNA-based vaccines for cancer.

Provinciali et al. (from the Advanced Technology Center for Aging Research, Ancona, Italy) investigated dosage, efficacy and toxicity of prophylactic immunizations with a vaccine encoding Erb-B2 receptor tyrosine kinase 2 (ERBB2; best known as HER-2) in a spontaneous mammary tumor model. The study demonstrated that recall vaccinations spaced by 1.5 months provide superior protection against tumor development as compared to vaccinations spaced by 3 or 6 months. Such a robust protective immunity was associated with improved production of ERBB2-specific antibodies (and isotype switching to IgG2a), expansion of circulating CD4+ T cells, and increased cytotoxicity of ERBB2-specific cytotoxic T lymphocytes (CTLs) in vivo. In another study, a plasmid encoding C-C motif chemokine ligand 4 (CCL4), which naturally reproduces several aspects of human breast cancer. In particular, Gibson et al. (from Wayne State University, Detroit,
MI, USA) vaccinated healthy cats with a plasmid coding for heterologous (xenogeneic) or mutated feline ERBB2, demonstrating that vaccination can break immune tolerance and induce antibodies with distinct specificity.126

Fibroblast activation protein alpha (FAP) is a serine peptidase integral to the plasma membrane and overexpressed by cancer-associated fibroblasts (CAFs).127–129 Because high intratumoral levels of FAPs are associated with poor disease outcome in patients with breast cancer,130,131 FAP has recently attracted interest as a potential target for multiple forms of immunotherapy,9 including DNA-based vaccination. In this setting, the CpVR-FAP plasmid (coding for a FAP variant with the point mutation S624A, for increased safety) was administered as both a prophylactic and therapeutic intervention against mouse mammary carcinoma 4T1 cells. Vaccination consistently inhibited tumor growth as it elicited FAP-specific T cell-dependent immunity.132 In a different study, a FAP-coding construct was delivered by a recombinant adenovirus,133 with the specific aim of enhancing FAP-specific cellular immunity. This strategy promoted the abundant secretion of the immunosuppressive cytokine interleukin 10 (IL10),134 which could be overcome by co-administering low-dose cyclophosphamide, resulting in robust CAF-specific immunity, reduced FAP expression and enhanced survival rates.135 These results suggest that IL10 inhibitors may represent a good strategy to bypass vaccine-driven immunosuppression, at least in some settings.

One method to boost antigen presentation is to introduce specific point mutations in the sequence of the antigenic epitope, resulting in improved binding to MHC Class I molecules or the T-cell receptor (TCR).136 Along these lines, the antitumor activity of a plasmid encoding mutated SSX family member 2 (SSX2; a TAA relevant for prostate carcinoma)9,137–139 was demonstrated in mice bearing an HLA-A2-expressing neoplasm engineered to express SSX2. Contrary to predictions, this DNA-based vaccine had limited immunostimulatory properties and poor efficacy, which was associated with increased expression of the immunosuppressive receptor programmed cell death 10 (IL10),134–136 which could be overcome by co-administering low-dose cyclophosphamide, resulting in robust CAF-specific immunity, reduced FAP expression and enhanced survival rates.137 These results suggest that IL10 inhibitors may represent a good strategy to bypass vaccine-driven immunosuppression, at least in some settings.

A key step for vaccination to elicit robust immunity is the uptake and presentation of the target antigen by professional antigen-presenting cells (APCs).8,145–147 and efforts are being made to improve this aspect for DNA-based vaccination. Xue et al. (from the University of Nottingham, Nottingham, UK) tested a DNA-based vaccine in which 16 epitopes from human cancer/testis antigen 1B (CTAG1B; best known as NY-ESO-1)53,148 were inserted in the complementarity-determining region (CDR) of a human IgG1 (a so-called “Immunobody”8)149,150 Vaccination drove the expansion of antigen-specific T cells with higher avidity and affinity than T cells derived from a peptide-based vaccine, and efficiently controlled the progression of mouse B16 melanoma cells engineered to express human NY-ESO-1 in a T cell-dependent manner.102 Two other strategies have recently been used to increase antigen delivery to APCs: (1) fusion with C-C motif chemokine ligand 20 (CCL20),151,152 based on the high expression levels of the CCL20 receptor C-C motif chemokine receptor 6 (CCR6) by DCs;133 and (2) fusion with surfactant protein D (SFTPD) and CD40 ligand (CD40LG),153,154 based on the spontaneous ability of SFTPD to multimerize and the high expression levels of CD40 on DCs.155 Both these approaches boosted the immunogenicity and therapeutic potential of a DNA-based vaccine targeting premelanosome protein (PMEL; best known as gp100)155 in mice bearing B16-F10 melanoma cells. As an alternative strategy, target antigens have been fused with proteins expressed on the surface of Gram-negative bacteria (which are commonly taken up by APCs).158 The potential of such approach has recently been demonstrated by Mei and collaborators (from the Institute of Blood and Marrow Transplantation, Suzhou, Jiangsu, China), who designed a DNA-based vaccine co-encoding MHC Class I epitopes from melan-A (MLANA), tyrosinase related protein 1 (TYRP1) and dopachrome tautomerase (DCT; also known as TYRP2) together with the autotransporter element adhesin involved in diffuse adherence 1 (AIDA-1).159,160 Intranasal delivery of this preparation resulted in the expansion of melanoma-specific CD4+ and CD8+ T cells that robustly controlled tumor progression in mice.161 Along similar lines, the administration of an attenuated strain of Salmonella spp. containing a plasmid coding for MYCN proto-oncogene, bHLH transcription factor (MYCN) showed remarkable antitumor efficacy in a mouse model of neuroblastoma.162

NF-κB is known to orchestrate the transcriptional remodeling of innate and adaptive immune responses upon antigen recognition.163,164 Based on this rationale, Gálvez-Cancino and colleagues (from the Genetic Immunotherapy Laboratory, Santiago, Chile) performed intradermal injections of a DNA-based vaccine co-encoding TYRP2 and a small-hairpin RNA (shRNA) silencing the NF-κB repressor, NFκB inhibitor alpha (NFKBIA; best known as IκBα).165,166 De-repressing NF-κB promoted the migration of mature dural DCs to the site of vaccination and consequent activation of CD8+ T cells, which controlled the growth of B16-F10 melanomas.167

Thalmensi and collaborators (from Invercysis, Paris, France) conceived a DNA-based vaccine that encodes an inactive form of telomerase reverse transcriptase (TERT; also known as hTERT),168–171 which is overexpressed in more than 85% of human malignancies.65 Although such a vaccine (INVAC-1) is already being tested in the clinics (see below), it remains under rigorous preclinical investigation. Thus, the intradermal administration of INVAC-1 by electroporation was shown to generate a broad and strong T1/2-polarized TERT-specific cellular response in mice with different genetic backgrounds, and was proven effective at increasing the survival rate of 50% in an aggressive model of spontaneous murine sarcoma.65
Additional vaccination strategies have been examined in preclinical models of prostate and colorectal cancer. For example, Olson and colleagues (from the University of Wisconsin, Madison, WI, USA) tested a GMP-grade plasmid encoding the ligand-binding domain of androgen receptor (AR). This preparation was administered together with colony stimulating factor 2 (CSF2; best known as GMCSF) to mice bearing prostate carcinomas, resulting in the elicitation of effective AR-specific cellular immune responses that has a beneficial effect on survival in the absence of major toxicities. Cross et al. (from the University of Melbourne, Melbourne, VIC, Australia) conceived a DNA-based vaccine encoding MYB proto-oncogene, transcription factor (MYC) flanked by the p2 and p30 universal epitopes of the tetanus toxoid (employed as adjuvants), which controlled the growth of mouse colorectal CT26 and MC38 cancer cells when applied prophylactically in combination with low-dose cyclophosphamide and an anti-PD-1 antibody, respectively. Moreover, Xiang and collaborators (from Thomas Jefferson University, Philadelphia, PA, USA) developed a guanylate cyclase C (GUCY2C)-targeting heterologous immunization protocol consisting in a prime/boost regimen with a DNA-based vaccine and an adenosvirus (both encoding GUCY2C), which effectively controlled the progression of CT26 colorectal carcinomas in the absence of autoimmune toxicity. Interestingly, this approach resulted in enhanced TCR avidity as compared to the administration of the naked plasmid or adenosvirus as standalone intervention, correlating with improved antineoplastic effects.

Since the publication of the latest Trial Watch dealing with DNA-based vaccination, a number of preclinical studies focusing on HPV-specific vaccines have been published. HPV16, HPV18 and HPV58 are the most common HPV serotypes associated with an increased risk for viral carcinogenesis, and are responsible for about 55% of all cases of cervical carcinoma, as well as for close to half of vaginal, vulvar and penile cancers worldwide. Lee et al. (from the Konkuk University, Seoul, Republic of Korea) compared the commercial peptide-based vaccine Cervarix® with AcHERV-HPV – a recombinant baculovirus encoding the human endogenous retrovirus (HERV)-envelope protein, used as nano-carrier, and the L1 proteins of HPV16, HPV18, and HPV58 – in therapeutic (as opposed to prophylactic) settings. L1 antigens have been widely included in cancer vaccines due to their ability to generate neutralizing antibodies with potent antitumor effects. Therapeutic vaccination with AcHERV-HPV promoted increased levels of L1-specific CTLs as compared to the administration of Cervarix®, and efficiently delayed tumor growth. Ma and colleagues (from the Johns Hopkins Medical Institutions, Baltimore, MD, USA) characterized the immune response and antitumor effects driven by the intramuscular injection of a pcDNA3-based vector encoding E6 from HPV18, followed by electroporation. This approach resulted in robust CD8 T cell responses specific for E6, and generally restricted to H-2Kb, which inhibited the growth of mouse cervical carcinoma TC-1 cells (which express both E6 and E7).

The endoplasmic reticulum (ER) chaperone calreticulin (CALR) can be harnessed to boost the efficacy of DNA-based vaccines by favoring intracellular antigen processing and presentation. Sun et al. (from Yale University School of Medicine, New Haven, CT, USA) evaluated the immunogenicity and efficacy of a DNA-based vaccine co-encoding E7 and CALR administered via different routes with or without electroporation. Interestingly, intravaginal vaccination followed by electroporation generated the most potent therapeutic effects against an orthotopic E7-expressing tumor model when compared to other delivery routes. Likewise, Perez-Trujillo and colleagues (from Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico) described a potent therapeutic effect from a DNA-based vaccine encoding the E6 and E7 HPV16 flanked by the ER import and retention signals of CALR. Such a therapeutic effect was comparable to that obtained with human full-length CALR, providing a safe and potent method to boost antitumor immune responses. A different approach to enhance antigen presentation involves TNF superfamily member 13b (TNFSF13B; also known as BAFF). Wu et al. (from Mackay Memorial Hospital, New Taipei City, Taiwan) fused E7 from HPV16 with TNFSF13B to enhance the antigen presentation in the context of MHC Class I molecules and evaluated antitumor activity. This construct generated robust E7-specific CD8 T cell immunity, which retarded the growth of TC-1 cells in vivo, and prolonged the survival of tumor-bearing mice. Recently, the efficacy of a plasmid encoding a fusion of signal peptide (Sig), a variant of E7 from HPV16 modified at residues 24 and 27 to abrogate its transforming potential and chaperone protein DnAK (dnaK; best known as HSP70) from Mycobacterium tuberculosis (pNGVL4a-Sig/E7(detox)/HSP70) followed by a single boost with a vaccinia virus expressing E6 and E7 (TA-HPV) was monitored in mice bearing TC-1 cells. The cervicovaginal administration of this vaccine improved tumor infiltration with E7-specific CD8 T cells, increased their expression of α4β7 integrins and C-C motif chemokine receptor 9 (CCR9) (which is required for T-cell homing), and ameliorated tumor control.

Blocking tumor angiogenesis has also recently emerged as an important approach for DNA-based vaccination. In this setting, Gao et al. (from Beijing LuHe Hospital, Beijing, China) designed a DNA-based vaccine encoding E6 and E7 from HPV16 fused with kinase insert domain receptor (KDR, best known as VEGFR2) for simultaneously targeting malignant cells and the endothelial tumor compartment. This vaccine was more efficient at controlling the growth of TC-1 tumors in vivo than vaccines targeting E6 and E7 or VEGFR2 alone. Finally, Ahrends et al. (from the Netherlands Cancer Institute- Amsterdam, The Netherlands) described a mechanism by which CD4 T cells can be harnessed to improve CD8 T cell responses upon intradermal DNA immunization via CD27 signaling. In particular, this group used HELP-E7SH DNA-based vaccines (which contain elements that enforce ER localization as well as several other immunostimulatory sequences) to generate an effective immune reaction against E7 from HPV16. Importantly, the combination of HELP-E7SH, an immunostimulatory antibody operating as CD27 agonist and an ICB targeting PD-1 resulted in complete TC-1 tumor eradication.

Taken together, these studies exemplify the current trends on the preclinical development of DNA-based vaccines for cancer therapy.
Completed clinical trials – Highlights

Since the publication of the latest Trial Watch dealing with this topic (May 2015), the results of multiple clinical studies assessing the safety and efficacy of DNA-based vaccination in cancer patients have been disseminated in international, peer-reviewed scientific journals (source: http://www.ncbi.nlm.nih.gov/pubmed) or presented at American Society of Clinical Oncology (ASCO) Annual Meetings (source: http://meetinglibrary.asco.org).

McNeel and collaborators (from the University of Wisconsin, Madison, WI, USA) previously demonstrated that the intradermal co-administration of a plasmid encoding full-length human acid phosphatase, prostate (ACP) and recombinant GM-CSF can elicit an ACP-specific T cell response in prostate cancer patients (NCT00582140). The same group followed-up this study with a hitherto ongoing Phase II clinical trial aimed at evaluating if prolonged immunization on a fixed or personalized schedule would render ACP-specific responses persistent (NCT00849121). The study was designed to explore two vaccination protocols: six immunizations at 2-week intervals for all patients, followed by booster injections over a 2-year timespan every 12 weeks (arm 1) or every 2, 4 or 12 weeks according to real-time immunomonitoring (arm 2). Both safety and the presence of T1-polarized antigen-specific T cells were evaluated. An early report on 16 patients demonstrated that multiple vaccine recalls were well tolerated, with no severe adverse events (AEs) observed. However, the personalized immunization schedule did not increase the frequency of patients developing effector and memory T cell responses. In an extended cohort of 38 patients, the team has recently reported that the presence of ACP-specific bystander CTLA4^CD8^ T cells constitutes a predictive marker of response. This observation is being evaluated prospectively as the trial continues. In a separate clinical trial, McNeel and colleagues assessed the immunological and clinical efficacy of the same DNA-based vaccine and pembrolizumab, administered simultaneously or sequentially, in patients with castration-resistant metastatic prostate cancer (NCT02499835). This study, which is still recruiting patients, will ultimately enroll 32 patients randomly assigned to three escalating intradermal doses of INVAC-1 vaccine investigated dose-limiting toxicities, immunostimulatory properties and antitumor activity of single-agent INVAC-1 (NCT02301754). This Phase I study was conducted in a cohort of 20 patients with refractory/progressive solid tumors, which were allocated to three escalating intradermal doses of INVAC-1: 100 μg (n = 3), 400 μg (n = 3), 800 μg (n = 14). No dose-limiting toxicities or treatment-related AEs were documented, and clinical benefits including disease stabilization were experienced by 12 patients. Interestingly, interferon gamma (IFNG)-producing TERT-specific CD8^+ and CD4^+ T cell immune response with antineoplastic activity. The first clinical trial on this vaccine investigated dose-limiting toxicities, immunostimulatory properties and antitumor activity of single-agent INVAC-1 (NCT02301754). 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immunogenicity of VGX-3100 in patients with HPV16/18-positive Grade 2/3 cervical intraepithelial neoplasia (CIN). In a Phase I/II trial, Patel et al. (from the University of Nottingham, Nottingham, UK) evaluated the performance of an ImmunoBody® referred as SCIB1 in advanced melanoma patients (NCT01138410). SCIB1 encodes an human IgG1 encompassing (at the CDR level) four T-cell epitopes of DCT and gp100. In particular, SCIB1 harbors two CD8+ T cell epitopes restricted to HLA-A*0201 (DCT180–188 and gp100178–186) and two CD4+ T cell epitopes, one restricted to HLA-DR4 (gp10044–59) and one restricted to HLA-DR7, HLA-DR53 and HLA-DQ6 (gp100174–190). It targets DCs in vivo via their high affinity Fcy receptor. In this context, 32 patients were immunized with SCIB1 >3 times by intramuscular injection plus electroporation at week 1, 3, 6, 12 and 24 post-screening. In the first part of the trial, 9 patients with stage III/IV melanoma received escalating doses of SCIB1, namely, 0.4, 2 or 4 mg (n = 3 per dose; Part 1, Cohorts 1–3). In the second part of the trial, an expansion cohort of 16 patients with fully resected advanced melanoma were given the selected dose of 4 mg (n = 14 new patients constituting Cohort 1 of Part 2, plus 2 patients recruited from Part 1 after surgery). Due to lack of toxicity, nine additional participants were administered a higher dose of 8 mg: five with Stage IV melanoma (Part 1, Cohort 4) and four with resected tumor at the entry (Part 2, Cohort 2). Even at 8 mg, SCIB1 remained safe and well tolerated with no serious AEs reported. All patients that received 0.4 mg progressed, with a median survival of 7 months. Out of the 6 patients of the initial cohorts treated with 2–4 mg of SCIB1, 4 were still alive with a median survival time of 26 months. One objective response was documented in one patient harboring multiple lesions, all of which regressed or disappeared (besides one that was resected). All 16 subjects with surgically removed tumors were alive at follow-up, with a median survival time of 29.5 months from study entry and a PFS of 75%. Interestingly, 70% (10/14) of melanoma patients from Part 1 (Cohorts 1–4), as well as 100% (16/16) of tumor-resected patients from Part 2 (Cohorts 1 and 2) mounted T cell reactivity against vaccine-encoded epitopes. Ex vivo immunological analyses revealed that tumor-specific T cells exceeded 2% of blood lymphocytes in patients receiving 8 mg SCIB1. Altogether, these results suggest that SCIB1 may confer therapeutic benefits to melanoma patients with no toxicity.

Axaligimogene filolisibac (also known as ADXS11-001 or AXAL; from Advaxis, Inc., Princeton, NJ, USA) is a vectored DNA vaccine based on live, irreversibly attenuated, Listeria monocytogenes bioengineered to secrete E7 from HPV16 fused with the non-hemolytic fragment of listeriolysin O (LLO). This fusion protein induces adaptive antitumor immunity as it reduces immunosuppression within the tumor microenvironment. A single-arm, 2-stage, Phase II study (NCT01266460, currently listed as active but not recruiting), evaluated the safety and efficacy of axaligimogene filolisibac as a standalone therapy in women affected with persistent or recurrent metastatic cervical carcinoma. In this setting, axaligimogene filolisibac (1 × 10⁷ colony forming units) was administered intravenously over 30 minutes on day 1 and every 28 days, until disease progression or unacceptable toxicity. Stage 1 results from 26 patients were presented at the 2016 ASCO Annual Meeting. Enrolled participants had ≥1 prior line of chemotherapy or antiangiogenic therapy with bevacizumab, a
monoclonal antibody that neutralized vascular endothelial growth factor (VEGF). Axalimogene filolisbac was well tolerated, 12-month OS was 38.5% (n = 10) with a median OS of 7.7 months. One subject experienced an unconfirmed partial response, while nine had disease stabilization. Moreover, preliminary Stage 2 results showed an extended 12-months OS (55.6%; 10/18 patients) for individuals receiving 3 doses of the vaccine, which is consistent with a robust survival benefit for this patient population. Another study evaluated the performance of axalimogene filolisbac in combination with standard chemoradiation (5-fluorouracil and mitomycin C plus 30 fractions of 1.8 Gy) in patients with HPV+ anal cancers (NCT01671488). The vaccine was well tolerated and 89% of patients who concluded treatment were disease-free at a median follow-up of 34 months. To date, the study is listed as active but not recruiting, and – to the best of our knowledge – additional results have not been posted. Of note, a study testing the safety and efficacy of axalimogene filolisbac in women with cervical carcinoma (NCT01116245) has recently been terminated owing to lack of enrollment.

Trimble et al. (from Johns Hopkins University School of Medicine, Baltimore, MD, USA) are investigating the safety and efficacy of two doses of pNGVL4a-Sig/E7(detox)/HSP70 at day 1 and 29 followed by one boost with escalating doses of TA-HPV at day 57 (see above) in patients with HPV16+ Grade 3 CIN. Vaccination was either administered alone or in combination with topical imiquimod as an adjuvant (NCT00788164). This dose-escalation, Phase I clinical trial is currently recruiting participants as it aims for a total of 48 patients. Even though the final results have not been released yet, preliminary data demonstrate that intramuscular vaccination can elicit systemic HPV-specific CD8+ T cell responses strongly associated with (1) immune infiltration of the stromal and epithelial compartment; (2) increased expression of C-X-C motif chemokine receptor 3 (CXCR3), T-box 21 (TBX21; best known as T-bet) and interferon beta 1 (IFNB1), and (3) tumor regression (at least in some patients). In a different study, Trimble and colleagues evaluated the safety and efficacy of pNGVL4a-CRT-E7(detox) administered at 0, 4 and 8 weeks either intradermally, intramuscularly, or intraleesionally to patients with HPV16-associated Grade 2/3 CIN (NCT00988559). pNGVL4a-CRT-E7(detox) encodes the detoxified form of HPV16 E7 fused to CALR, which aids antigen delivery to DCs. While the study is still recruiting participants, preliminary results on 32 patients documented vaccine-related Grade 1 or less AEs in 22 (69%) individuals, and histological regression in 8 (30%) of the patients. Intraleisional administration of the vaccine resulted in higher intraepithelial CD8+ T cell infiltrates as compared to other delivery routes. The authors concluded that pNGVL4a-CRT-E7(detox) is well-tolerated and potentially triggers a clinically meaningful immune robust immune response when administered to the cervix.

A randomized, double-blind, placebo-controlled, Phase Ib/II/III trial assessed the efficacy and safety of first-line chemotherapy (optionally combined with bevacizumab) plus a recombinant modified vaccinia virus strain Ankara (TG4010) carrying coding sequences for human mucin 1 (MUC1) and IL2 (NCT01383148). MUC1 is a tumor-associated antigen expressed by many solid tumors, including non-small cell lung carcinoma (NSCLC). This approach was evaluated in Stage IV NSCLC patients randomly allocated (1:1) to receive either subcutaneous injections of 10^6 plaque-forming units of TG4010 or placebo from the beginning of chemotherapy every week for 6 weeks, and then every 3 weeks until progression. The study documented no serious treatment-related AEs and an improved PFS relative to placebo plus chemotherapy. Moreover, the so-called “TrPAL” biomarker (baseline values of CD16+CD56+CD69+ lymphocytes) was potentially predictive of TG4010 efficacy in this setting.

Snook and colleagues (from the Thomas Jefferson University, Philadelphia, PA, USA) reported the results of a single-arm, open-label, Phase I study conducted to evaluate safety, tolerability and efficacy of a recombinant adenoviral vaccine (Ad5-GUCY2C-PADRE) in colorectal carcinoma patients (NCT01972737). This vaccine encodes GUCY2C fused to the pan DR epitope (PADRE), a universal CD4+ T cell epitope that mediates consistent immunostimulatory functions. Ten surgically-resected, node-negative, Stage I/II colorectal carcinoma patients were treated with a single intramuscular injection of the vaccine (10^11 viral particles). In line with preclinical findings, vaccination induced GUCY2C-specific humoral and CD8+ T cell responses. Interestingly, specific responses occurred only in subjects with low titers of adenovirus-neutralizing antibodies at the time of vaccination. Overall, the vaccine was well tolerated, with only 30–40% of patients experiencing mild AEs such as short-lived injection site pain/swelling, body aches and chills.

PROSTAVAC-V/TRICOM and PROSTAVAC-F/TRICOM consist of attenuated recombinant vaccinia and fowlpox viruses, respectively, encoding PSA and a triad of co-stimulatory molecules, namely CD58 (best known as LFA-3), CD80, and intercellular adhesion molecule 1 (ICAM1). These vectored DNA-based vaccines have been tested in combination with flutamide (a nonsteroidal pure antiandrogen) in a Phase II trial enrolling patients with prostate cancer (NCT00450463). In particular, patients with rising PSA received flutamide orally 3 times daily, alone or in combination with subcutaneous PROSTAVAC-V/TRICOM on day 1 and PROSTAVAC-F/TRICOM on day 29. In the absence of disease progression, treatment was repeated every 4 weeks. This study is listed as completed but, to the best of our knowledge, results have not yet been disseminated. Along similar lines, a dose-finding Phase II study testing safety, feasibility and immunogenicity of escalating doses of pVAXrPSA53l (a naked DNA-based vaccine encoding PSA from Macaca mulatta) administered every 4 weeks intradermally followed by electroporation to patients with prostate cancer (NCT00859729) is listed as completed by official sources, but results are not publicly available.

Taken together, these clinical observations suggest that naked and vectored DNA-based are generally well tolerated by patients with a variety of tumors, and – when administered in combination with immunostimulatory interventions that overcome tolerance – induce humoral and cellular immune responses associated with clinical benefit (at least in some patients).
Recently initiated clinical trials

Twenty-one clinical studies have been opened since the publication of the latest Trial Watch dealing with this topic (May 2015), with no reported results to date (sources clincaltrials.gov, https://www.ncbi.nlm.nih.gov/pubmed, and https://www.isrctn.com/). Efforts are being focused on exploring the safety, efficacy and routes of administration of vaccines targeting the following antigens: (1) E6 or E7 from HPV16 and HPV18 (11 studies), (2) TERT, (3) mesothelin (MSLN), (4) members of the carcinoembryonic antigen (CEA) family, (5) idiotype antigens, and (6) patient-specific neoantigens (study). Seven different naked molecules and two vectored vaccines are being tested in 10 and 11 clinical trials, respectively (Table 1 and Table 2).

GX-188E (from Genexine, Seoul, South Korea) is a therapeutic DNA vaccine encoding E6 and E7 fused with the fms related tyrosine kinase 3 ligand (FLT3L), a potent immunostimulatory molecule. GX-188E has been tested in patients with Grade 3 CIN since 2012 in the context of two completed Phase I (NCT01634503) and Phase II (NCT02139267) clinical trials, with a focus on safety profile, optimal dose and efficacy. Two prospective follow-up studies (NCT02100085 and NCT02411019) are currently observing patients receiving 1 mg, 2 mg or 4 mg GX-188E in the original studies to determine recurrence and long-term safety. To date, the results of these studies have not been released. In addition, a double-blind, placebo-controlled, multi-center, Phase II clinical trial has been initiated to determine the therapeutic profile of GX-188E in HPV patients with biopsy-proven Grade 2 or 3 CIN. This trial will enroll 134 patients and randomly assign them to receiving either 3 intramuscular injections of GX-188E (1 mg) – followed by electroporation – at 0, 4 and 12 weeks, or placebo (NCT02596243). Furthermore, a randomized, open-label study is currently recruiting an estimated 50 HPV+ patients with Grade 3 CIN to test the safety and efficacy of GX-188E in combination with the intravaginal application of GX-I7 (recombinant human IL7) for 4 times, or imiquimod for 8 times (NCT03206138). The studies will assess the ratio of patients experiencing histopathological regression of cervical lesions (primary objective), cytological changes of the cervix and HPV clearance (secondary objectives).

An open-label, Phase I clinical study that has recently opened at the Abramson Cancer Center (Philadelphia, PA, USA) aims at testing the safety, tolerability and immunological properties of INO-1400, a naked DNA-based vaccine encoding TERT (NCT02960594, previously NCT02327468). This trial will enroll an estimate of 54 patients with solid tumors who are at high risk of relapse but show no evidence of disease after surgery and standard-of-care therapy. Subjects will be allocated to 1 of the 6 experimental arms testing two doses of INO-1400 (2 or 8 mg), either alone or in combination with INO-9012 (0.5 or 2 mg). Plasmids will be injected intramuscularly followed by electroporation every 4 weeks for a total of 4 treatments. The primary objective of the study is to evaluate AEs including injection site reactions and changes in laboratory parameters as compared to baseline. The secondary objective is to determine the immunogenicity of immunization, based on the assessment of antigen-specific cellular and humoral responses. Time-to-progression will also be evaluated (Table 1).

A multi-center, open-label Phase Ib/IIa study has been initiated to evaluate the safety and efficacy of INO-3112 in combination with the anti-PD-L1 antibody durvalumab (NCT03162224). Fifty patients with recurrent/metastatic HPV-associated HNSCC with no curative treatments options will be vaccinated intramuscularly with subsequent electroporation. Safety profile will be determined based on the rate of patients experiencing AEs, changes in laboratory parameters, and variations in the electrocardiogram as compared to baseline. Efficacy will be assessed by the number of patients who develop anti-drug antibodies, objective response rates, PFS and OS. Of note, INO-3112 is well tolerated in patients with solid tumors at higher doses compared to lower doses (NCT03162224). INO-3112 is generally well tolerated in patients with head and neck cancer at higher doses (NCT03162224).

A recent clinical trial aimed at testing the safety and efficacy of INO-9012 in combination with the anti-PD-L1 antibody durvalumab (NCT02960594). Fifteen patients with recurrent/metastatic HPV-associated HNSCC with no curative treatments options were vaccinated intramuscularly with subsequent electroporation. Safety profile was determined based on the rate of patients experiencing AEs, changes in laboratory parameters, and variations in the electrocardiogram as compared to baseline. Efficacy will be assessed by the number of patients who develop anti-drug antibodies, objective response rates, PFS and OS. Of note, INO-9012 is generally well tolerated in patients with solid tumors at higher doses compared to lower doses (NCT03162224). INO-9012 is well tolerated in patients with head and neck cancer at higher doses (NCT03162224).

Table 1. Recently initiated clinical trials testing naked DNA-based vaccines in cancer patients.

| Vaccine | TAA(s) | Co-encoded molecules | Indication | Phase | Status | Estimated enrollment | Delivery | Co-therapy | Ref |
|---------|--------|----------------------|------------|-------|--------|----------------------|----------|------------|-----|
| GX-188E | E6/E7  | FLT3L                | CIN        | I     | Active, not recruiting | 6         | Intramuscular (electroporation) | None | NCT02100085 |
|         |        |                      | CIN        | I     | Recruiting        | 50        | Intramuscular (electroporation) | None | NCT03206138 |
|         |        |                      | CIN        | II    | Active, not recruiting | 67        | Intramuscular (electroporation) | None | NCT02411019 |
|         |        |                      | CIN        | II    | Active, not recruiting | 134       | Intramuscular (electroporation) | None | NCT02596243 |
| INO-1400| TERT   | None                 | Solid tumors | I    | Recruiting        | 54        | Intramuscular (electroporation) | INO-9012 | NCT02960594 |
| INO-3112| E6/E7  | None                 | Head and neck cancer | Ib/IIa| Not yet recruiting | 50        | Intramuscular (electroporation) | INO-9012 and durvalumab | NCT03162224 |
| —       | Tumor neoantigens | None | Cervical carcinoma | II    | Withdrawn        | 0         | Intramuscular (electroporation) | INO-9012 and cisplatin durvalumab | NCT02501278 |
| —       | Idiotype PVXCP or CCL20 | B-cell lymphoma | None | Recruiting | 30        | Intramuscular (PEI) | None | ISRCTN31090206 |
| —       | MSLN and tumor neoantigens | None | Pancreatic cancer | None | Recruiting | 15        | Intramuscular (electroporation) | None | NCT03122106 |

Abbreviations: CIN, cervical intraepithelial neoplasia; PEI, polylethyleneimine; TAA; tumor-associated antigen, TNBC, triple-negative breast cancer.

*Initiated between 2015, May 1st and 2017, October 1st.
a Phase II clinical trial testing INO-3112 in women with cervical carcinoma (NCT02501278) opened in late 2015 but was withdrawn in 2016 because the sponsor was no longer able to support the study (Table 1).

Idiotype DNA vaccination (which targets rearranged immunoglobulins)\textsuperscript{256,257} in lymphoma patients is safe and tolerable, but generally mediates limited immunogenicity.\textsuperscript{92,258} Such strategy is currently being tested in a non-randomized, open-label Phase I clinical trial that involves two DNA vaccines encoding a patient-specific idiotype fused with an immunostimulatory sequence consisting of either potato virus X coat protein (PVXCP) or CCL20 (ISRCTN31090206).\textsuperscript{259} The scFv-PVXCP or CCL20-scFv vaccines are delivered intramuscularly upon complexation with polyethylenimine (PEI), which facilitates transfection \textit{in vivo}. A maximum of 30 patients will receive one round of vaccination consisting of 3 monthly injections of either vaccine. If no peripheral anti-idiotypic response is detected, a second cycle of vaccination will be administered. The study will evaluate safety and tolerability as well as cellular and humoral responses. Finally, clinical responses will be evaluated by monitoring minimal residual disease (molecularly) and tumor dissemination (radiographically) (Table 1).

An open-label, single-institution, randomized, Phase I clinical trial will enroll an estimate of 24 patients with Stage II/III triple-negative breast cancer,\textsuperscript{260,261} who will receive standard-of-care therapy followed by 6 intramuscular vaccinations targeting patient-specific tumor neoantigens (on days 1, 29\pm7, 57\pm7, 85\pm7, 113\pm7, and 141\pm7) alone, or in combination with durvalumab\textsuperscript{10} beginning at day 57 (NCT03199040). In addition, an open-label Phase I study using a similar approach will initially recruit 15 pancreatic cancer patients at 2 locations (Johns Hopkins School of Medicine, Baltimore, MD, USA and Washington University School of Medicine, St. Louis, MO, USA) to evaluate a personalized polypeptide DNA-based vaccine directed against patient-specific tumor neoantigens and MSLN\textsuperscript{262} (NCT03122106). In this study, patients undergoing surgical resection and adjuvant chemotherapy will receive 6 intramuscular immunization (at week 1, 5, 9, 13, 17, and 21) followed by electroporation with the TDS-IM system (Ichor Medical Systems, Inc., San Diego, CA, USA). The primary objective of these two studies is to evaluate safety by monitoring the appearance and severity of AEs and variations in laboratory parameters as compared to baseline. The secondary objective is to monitor the extent and quality of the immune response driven by vaccination (Table 1).

A number of studies focused on vectored DNA-based vaccines have been recently initiated with no results disclosed to date. For instance, GI-6207 is a promising agent based on a heat-killed \textit{Saccharomyces cerevisiae} strain genetically modified to express CEA. Previous clinical studies determined that GI-6207 is safe and mediated some degree of efficacy in patients with CEA-expressing carcinomas.\textsuperscript{263} These encouraging results have stimulated the initiation of six new clinical trials aimed at evaluating the safety and efficacy of GI-6207 administered in the context of a combination therapy to TNBC (NCT03175666), urothelial carcinoma (NCT03197571), HNSCC (NCT03169764), colorectal carcinoma (NCT03169777), NSCLC (NCT03169738) and melanoma (NCT03167177) patients progressing on chemotherapy or immunotherapy with ICBS. Safety and efficacy, as determined by the incidence of AEs and objective response rate are the objectives of all these studies (Table 2).

Axalimogene filolisbac has been tested for the treatment of a number of HPV-related cancers (see above).\textsuperscript{218–220,264} Recently, five distinct clinical trials have been initiated to test axalimogene filolisbac as a standalone immunotherapeutic interventions or in combination with ICBS in patients affected by various neoplasms. A double-blind, randomized, Phase III study currently recruiting participants has been designed to enroll an estimated 450 patients with advanced cervical cancer at high risk of recurrence after cisplatin-based therapy\textsuperscript{265} (NCT02053604). The study will determine safety and efficacy of the vaccine as compared to placebo. A two-arm Phase I/II study is investigating the therapeutic profile of addition of axalimogene filolisbac plus durvalumab in patients with cervical carcinoma and HNSCC (NCT02291055). Moreover, axalimogene filolisbac is being tested in combination with pemetrexed (a folate antimetabolite)\textsuperscript{266–268} in patients with NSCLC (NCT02531854), as a standalone therapy in patients with anorectal cancer (NCT02399813), and prior to robotic surgery in subjects with HPV\textsuperscript{+} oropharyngeal squamous cell carcinoma (NCT02002182) (Table 2).

In summary, some naked and vectored DNA-based vaccines are being intensively scrutinized for the clinical potential.

### Table 2. Recently initiated clinical trials testing vectored DNA vaccines in cancer patients\textsuperscript{2}

| Vaccine                  | TAA(s) | Co-encoded molecules | Indication                     | Phase     | Status                       | Estimated enrollment | Delivery | Co-therapy | Ref                  |
|--------------------------|--------|----------------------|--------------------------------|-----------|------------------------------|----------------------|----------|------------|---------------------|
| Axalimogene filolisbac   | E6/E7  | LLO                  | Anorectal carcinoma            | II        | Not yet recruiting           | 124                  | Intravenous | None       | NCT02399813         |
|                          |        |                      | Cervical carcinoma             | III       | Recruiting                  | 450                  | Intravenous | None       | NCT02853604         |
|                          |        |                      | Head and neck cancer           | II        | Recruiting                  | 30                   | Intravenous | None       | NCT02002182         |
|                          |        |                      | HPV\textsuperscript{+} tumors  | I/I       | Recruiting                  | 66                   | Intravenous | Durvalumab   | NCT02291055         |
|                          |        |                      | NSCLC                          | II        | Not yet recruiting           | 124                  | Intravenous | Pemetrexed    | NCT02531854         |
| GI-6207                  | CEA    | None                 | Colorectal carcinoma           | II        | Not yet recruiting           | 79                   | Subcutaneous | Multiple    | NCT03169777         |
|                          |        |                      | Head and neck cancer           | II        | Not yet recruiting           | 113                  | Subcutaneous | Multiple    | NCT03169764         |
|                          |        |                      | Melanoma                       | II        | Not yet recruiting           | 67                   | Subcutaneous | Multiple    | NCT03167177         |
|                          |        |                      | NSCLC                          | II        | Not yet recruiting           | 85                   | Subcutaneous | Multiple    | NCT03169738         |
|                          |        |                      | TNBC                           | I/I       | Not yet recruiting           | 79                   | Subcutaneous | Multiple    | NCT03175666         |
|                          |        |                      | Urothelial carcinoma           | I/I       | Not yet recruiting           | 113                  | Subcutaneous | Multiple    | NCT03197571         |

Abbreviations: CEA, carcinoembryonic antigen; HPV, human papillomavirus; LLO, listeriolysin O; NSCLC, non-small cell lung carcinoma; TAA, tumor-associated antigen; TNBC, triple-negative breast cancer.

\textsuperscript{2}Initiated between 2015, May 1\textsuperscript{4} and 2017, October 1\textsuperscript{1}
The targeted activation of the immune system by cancer vaccines has been a long-lasting goal in cancer immunotherapy, yet this approach is insufficient to eradicate established malignancies. The works discussed herein demonstrate that DNA-based vaccines have a very favorable safety profile, both as single agents and combined with adjuvants. Moreover, most of the immunization strategies tested so far are able to elicit cellular and humoral antigen-specific immune responses, as they overcome central and peripheral tolerance mechanisms. Nevertheless, the local and systemic immunosuppression established by progressing tumors robustly control (and hence limit the clinical efficacy of) vaccine-driven immunity. Novel combinatorial approaches that unleash the full clinical potential of DNA-based vaccines are urgently awaited.

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Abbreviations

AE adverse event
APC antigen-presenting cell
AML acute myeloid leukemia
CAF cancer-associated fibroblast
CDR complementarity-determining region
CEA carcinoembryonic antigen
CIN cervical intraepithelial neoplasia
CML chronic myeloid leukemia
CT cancer-testis
CTL cytotoxic T lymphocyte
DC dendritic cell
DOM domain of tetanus toxoid
ER endoplasmic reticulum
HNSCC head and neck squamous cell carcinoma
HPV human papillomavirus
ICB immune checkpoint blocker
IL interleukin
NSCLC non-small cell lung carcinoma
OS overall survival
PADRE pan-DR epitope
PFS progression-free survival
TAA tumor-associated antigen
TCR T cell receptor
TLR Toll-like receptor
TNBC triple-negative breast cancer
TSA tumor-specific antigen

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