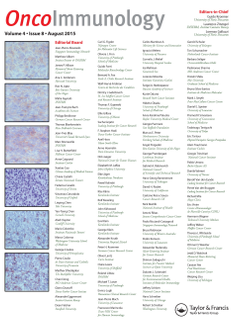

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Trial Watch
DNA vaccines for cancer therapy

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Keywords: cross-presentation, dendritic cells, electroporation, *Listeria monocytogenes*, mucosal immunity, *Saccharomyces cerevisiae*

Abbreviations: ADT, androgen deprivation therapy; CEA, carcinoembryonic antigen; DC, dendritic cell; EBV, Epstein-Barr virus; FDA, Food and Drug Administration; HPV, human papillomavirus; ICOS, inducible T-cell co-stimulator; LTB, *E. coli* heat labile toxin, B subunit; MAMA, mammaglobin A; PSA, prostate specific antigen; PSMA, prostate-specific membrane antigen; TAA, tumor-associated antigen; TERT, telomerase reverse transcriptase; VRP, virus-like replicon particle

During the past 2 decades, the possibility that preparations capable of eliciting tumor-specific immune responses would mediate robust therapeutic effects in cancer patients has received renovated interest. In this context, several approaches to vaccinate cancer patients against their own malignancies have been conceived, including the administration of DNA constructs coding for one or more tumor-associated antigens (TAAs). Such DNA-based vaccines conceptually differ from other types of gene therapy in that they are not devised to directly kill cancer cells or sensitize them to the cytotoxic activity of a drug, but rather to elicit a tumor-specific immune response. In spite of an intense wave of preclinical development, the introduction of this immunotherapeutic paradigm into the clinical practice is facing difficulties. Indeed, while most DNA-based anticancer vaccines are well tolerated by cancer patients, they often fail to generate therapeutically relevant clinical responses. In this Trial Watch, we discuss the latest advances on the use of DNA-based vaccines in cancer therapy, discussing the literature that has been produced around this topic during the last 13 months as well as clinical studies that have been launched in the same time frame to assess the actual therapeutic potential of this intervention.

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Introduction

The possibility to employ vaccination as a therapeutic means against cancer was first proposed at the end of the 19th century by the German physician Paul Ehrlich (1854–1915) and the American surgeon William Bradley Coley (1862–1936),[1] but the interest in this approach quickly declined thereafter.[2] It's only with the late 1990s that renovated enthusiasm has gathered around the use of vaccines in cancer therapy, at least in part owing to 2 conceptual advances: (1) Polly Matzinger's danger theory, proposing that the immune system does not simply react against non-self constituents, but rather respond to situations of danger (irrespective of their origin),[3] and (2) the discovery of antigens that are expressed preferentially, when not exclusively, by malignant cells.[4]

Thus, starting with the late 1990s, significant efforts have been dedicated at the development of preparations that would actively elicit a tumor-associated antigen (TAA)-specific immune response, including dendritic cell (DC)-based,[5-7] purified component-based,[8-10] and DNA-based vaccines.[11] The latter de facto consist in TAA-encoding circularized DNA constructs that are administered to cancer patients (most often via the intramuscular route) either in the form of naked DNA or by means of a suitable vector.[11-14] Of note, DNA-based vaccines should be conceptually differentiated from other forms of gene therapy in that (1) they do not directly target diseased (malignant) cells, as it is the case of constructs that code for cytotoxic proteins,[15-25] or enzymes that can transform an inactive chemical into a toxic drug;[26-31] and (2) they do not boost the immune system in a relatively unspecific fashion,

like constructs encoding immunostimulatory cytokines or ligands for co-stimulatory T-cell receptors.[19,32-55] Although their efficiency strictly relies on the achievement of high transfection rates and efficient TAA presentation in vivo (see below), DNA-based vaccines have several advantages as compared with their DC-based and purified component-based counterparts, including (but limited to) those provided by genetic engineering. We have discussed these advantages in our latest Trial Watch dealing with the use of DNA-based vaccines in cancer therapy, which has been published in the April 2013 issue of *OncoImmunology*.[56]

DNA-based vaccines are expected to enter tissue-resident antigen-presenting cells and/or myocytes, resulting in (1) local TAA synthesis; (2) presentation of TAA-derived peptides to naïve T cells; and (3) the consequent activation of potentially therapeutic TAA-specific $CD8^+$ T cell-dependent and/or humoral immune responses.[56,57] Several vectors have been evaluated for their ability to promote efficient TAA-specific immune responses upon the administration of DNA-based vaccines. As it stands, naked constructs remain the preferred form of DNA-based vaccines for clinical applications.[11-14] Viral vectors generally yield improved rates of transduction and stable TAA expression,[58-61] but are associated with several disadvantages, including the facts that (1) they can elicit potent neutralizing humoral responses (driven by packaging proteins); (2) they are relatively expensive; (3) some viral genomes cannot be engineered to bear large transgenes; and (4) some vectors have been associated with a non-negligible risk of insertional mutagenesis.[58,59,62] Along similar lines, prokaryotic as well as eukaryotic vehicles are advantageous as they are compatible with oral administration, are capable of eliciting mucosal immune responses (which are generally considered superior than intramuscular ones), and endogenously express potent immunostimulatory factors (e.g., lipopolysaccharide, bacterial DNA),[63-65] yet their large-scale application to clinical settings appears premature at this stage.[11-14]

Consistent efforts have also been dedicated at the identification of the delivery method that would allow for optimal immune responses to DNA-based vaccines.[11,12,14,66,67] In this context, it soon turned out that simple intramuscular injections generally result in poor TAA-specific immune responses, an effect that has been attributed to the limited hydrostatic pressure generated by standard injection volumes.[68] Several methods have been proposed as an alternative to intramuscular injections, including gene gun-mediated delivery,[69,70] jet injection,[71,72] and tattooing,[73] all of which involve the transdermal route, as well as oral administration,[74-76] and electroporation.[69,77-79] Electroporation, i.e., the intramuscular delivery of naked DNA immediately followed by the application of an innocuous electrical stimulus, nowadays stands out as the delivery method of choice for DNA-based vaccines.[80-83] This reflects the facts that electroporation (1) is associated with high transfection rates (irrespective of injection volume),[68,79,83] (2) causes some extent of local tissue injury, resulting in the release of damage-associated molecular patterns (DAMPs) that operate as endogenous immunostimulants,[84-86] and (3) may be perceived as uncomfortable yet is associated with no significant toxicities even when employed repetitively over several vaccination sessions.[11,14]

The efficacy of all anticancer vaccines, including DNA-based preparations, obviously depends to a large extent on the TAA of choice. For illustrative purposes, TAAs can be classified into 4 mutually exclusive categories: (1) viral TAAs, which are by definition non-self but can be shared by different neoplasms caused by the same virus; (2) unique TAAs, which originate from cancer cell-specific mutations and hence are not shared by distinct tumors, not even of the same type; (3) idiotypic TAAs, another type of tumor-specific TAAs that reflect the rearrangement of transmembrane immunoglobulins expressed by clonal B-cell neoplasms; and (4) shared TAAs, which are also expressed by one or more healthy tissues (though to a limited extent). So-called cancer-testis antigens constitute bona fide shared TAAs, as they are expressed by both malignant and germline cells.[87,88] Interestingly, efficient DNA-based vaccines that target TAAs from each of these classes have been constructed.[56]

In spite of a significant amount of encouraging clinical results,[56] no DNA-based vaccines are currently licensed by the US Food and Drug Administration (FDA) for use in cancer patients, neither as a prophylactic nor as a therapeutic intervention (source http://www.fda.gov). Conversely, 3 of such preparations have been approved for veterinary use.[89-92] Interestingly, one of these DNA-based vaccines, which is employed for the treatment of canine melanoma, relies on a xenogenous TAA (i.e., human tyrosinase).[92]

One year ago, in the April issue of *OncoImmunology*, we summarized the scientific rationale behind the use of DNA-based vaccines in cancer therapy and discussed recent clinical trials evaluating the safety and efficacy of this approach.[56] Here, we present the latest advances on the clinical development of tumor-targeting DNA-based vaccines.

Update on the Development of Anticancer Vaccines

Since the submission of the latest Trial Watch dealing with this topic,[56] i.e., February 2013, the results of only 7 clinical trials testing the safety, immunogenicity and/or therapeutic activity of DNA-based anticancer vaccines have been published in peer-reviewed scientific journals (**Table 1**).[93-98] Of these studies, 3 tested naked DNA preparations,[93-95] 3 relied on viral or eukaryotic vectors,[96-98] and 1 tested an heterologous prime-boost approach. In same period, 6 clinical trials have been launched to test the safety and therapeutic potential of DNA-based anticancer vaccines in cancer patients (source http://www.clinicaltrials.gov).

Eriksson and colleagues reported the results of a Phase I study involving the intradermal administration of a plasmid encoding kallikrein-related peptidase 3 (KLK3, best known as prostate specific antigen, PSA)[99,100] from *Macaca mulatta* (Rhesus macaque) to 15 patients with biochemical manifestations of relapsing prostate cancer who had previously been subjected to a 1 mo course of androgen deprivation therapy (ADT)[101-103] (NCT00859729).[94] In this setting, the vaccine was administered by electroporation

Table 1. Recent clinical trials testing the therapeutic profile of DNA-based vaccines in cancer patients*

Indication(s)	Phase	Status	TAA(s)	Co-encoded molecule(s)	Co-therapy	Vector	Delivery	Ref.
Bladder carcinoma	II	Recruiting	CEA MUC1	CD80, CD57, ICAM1	BCG	Vaccinia virus (prime) Fowlpox virus (boost)	s.c.	NCT02015104
Breast carcinoma	I	Recruiting	MAMA	-	-	n.a.	i.m.	95
Colorectal carcinoma	I	Recruiting	CEA	-	-	Alphavirus-derived VRP	i.m.	NCT01890213
			GUCY2C	PADRE	-	Adenovirus serotype 5	i.m.	NCT01972737
Medullary thyroid cancer	II	Recruiting	CEA	-	-	*Saccharomyces cerevisiae*	s.c.	NCT01856920
Nasopharyngeal cancer	I	Recruiting	EBNA1 LMP2	-	-	*MVA*	i.d.	NCT01800071
	Ib	Recruiting	EBNA1 LMP2	-	-	MVA	i.d.	97
Oropharyngeal cancer	I	Recruiting	HPV-16 E7	-	-	*Listeria monocytogenes*	i.v.	NCT02002182
Prostate cancer	I	Completed	PSA	-	-	pVAX-based plasmid	i.d. + EP	94
			PSMA	-	-	Alphavirus-derived VRP	s.c.	98
	II	Recruiting	PSA	CD80, CD57, ICAM1	Enzalutamide	Vaccinia virus (prime) Fowlpox virus (boost)	s.c.	NCT01867333
								NCT01875250
Solid tumors	I	Completed	CEA HER2	LTB	-	pV1J-based plasmids	i.m. + EP	93
						pV1J-based plasmids (prime) Adenovirus serotype 6 (boost)	i.m. + EP	93
	II	Recruiting	CEA	-	-	*Saccharomyces cerevisiae*	s.c.	96

Abbreviations: BCG, bacillus Calmette–Guérin; CEA, carcinoembryonic antigen; EBNA1, Epstein-Barr nuclear antigen 1; EP, electroporation; GUCY2C, guanylyl cyclase 2C; HPV-16, human papillomavirus type 16; i.d., intra dermam; i.m., intra musculum; i.v., intra venam; LMP2, latent membrane protein 2; LTB, *E. coli* heat labile toxin, B subunit; MAMA, mammaglobin A; MUC1, mucin 1; MVA, modified vaccinia virus Ankara; n.a., not available; PADRE, pan HLA DR-binding epitope; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; TAA, tumor-associated antigen; s.c., sub cutem; TERT, telomerase reverse transcriptase; VRP, virus-like replicon particle. *Published or initiated between 2013, January 1st and the day of submission.

every 4 wk over 5 consecutive mo. No systemic toxicities were recorded upon vaccination, which was only associated with some discomfort (not requiring analgesia or topical anesthetics) and/or minor skin reactions at the electroporation site. T cells and antibodies specific for xenogenous PSA could be detected in some patients upon vaccination, yet were not associated with clinical efficacy, as all subjects eventually required ADT owing to raising PSA levels. Of note, all but 1 patient exhibited pre-vaccination reactivity against endogenous PSA.[94] This suggests that relapsing tumors had already been immunoedited by a PSA-targeting immunological pressure, possibly explaining the lack of efficacy of this approach.[94]

Tiriveedhi and collaborators characterized the immunological effects of a plasmid coding for secretoglobin, family 2A, member 2 (SCGB2A2, best known as mammaglobin A, MAMA), which is overexpressed by a large fraction (up to 80%) of breast neoplasms,[104-106] in 7 patients with Stage IV metastatic breast carcinoma (NCT00807781).[95] In this context, MAMA-coding plasmids were administered via the intramuscular route (by means of a jet injector) in 3 vaccination sessions at 4-wk intervals from each other. As this Phase I clinical trial is still ongoing, data on safety and efficacy are not available. Nonetheless, Tiriveedhi et al. reported an increase in circulating $CD4^+$ T cells expressing high levels inducible T-cell co-stimulator (ICOS)[107] 6 mo after vaccination, a phenomenon that was paralleled by a decline in the levels of blood-borne $CD4^+FOXP3^+$ regulatory T cells (Tregs).[108-110] Interestingly, MAMA-specific $CD4^+ICOS^{high}$ T cells were found to predominantly express immunosuppressive cytokines such as interleukin-10 (IL-10) before vaccination, but immunostimulatory ones like interferon γ (IFNγ) thereafter. Such a shift in the secretory profile of $CD4^+ICOS^{high}$ T cells was associated with an improved ability to directly lyse MAMA-expressing malignant cells.[95] Thus, MAMA-targeting naked DNA-based vaccines may elicit therapeutically relevant immune responses in breast carcinoma patients. The conclusion of this clinical trial is eagerly awaited to shed light on this possibility.

Diaz and colleagues reported the results of 2 multicenter, Phase I, open-label dose-escalation trials testing a bivalent naked DNA-based vaccine targeting carcinoembryonic antigen (CEA)[111-113] and *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2, best known as HER2),[114-116] alone or combined with a dicistronic adenovirus type 6-based

vaccine with identical specificity.[93] In particular, both the naked DNA-based (V930) and the adenoviral vaccine (V932) encode a truncated variant of HER2 (lacking the cytoplasmic domain) and CEA fused to the B subunit of *Escherichia coli* heat labile toxin (LTB), as an adjuvant.[117,118] In the first study (NCT00250419), 28 patients bearing various HER2- and/or CEA-expressing Stage II-IV malignancies (mainly colorectal, breast, and non-small cell lung carcinomas) received 5 courses of V930 (at 2-wk intervals) by electroporation. With the exception of 2 subjects suffering from Grade 3 abdominal pain and a few individuals reporting minor adverse effects (Grade 1/2 injection site reactions, fatigue, and diarrhea), vaccination was globally well tolerated. However, 3 mo after vaccination, none of the subjects included in this study had developed detectable CEA- or HER2-specific T-cell responses.[93] In the second study (NCT00647114), 11 patients with HER2- and/or CEA-expressing Stage II-IV tumors were treated with V930 and V932 in a heterologous prime-boost setting. In this context, the administration of V930 (priming, performed exactly as for the first study) was followed by the intramuscular delivery of V932 in 2 distinct sessions (boosting, 4 and 6 wk after the end of the priming cycle). Similar to V930, V932 was well tolerated. However, V932 failed to improve the ability of V930 to elicit CEA-or HER2-specific adaptive immune responses.[93] The precise reasons for such a complete lack of efficacy have not yet been identified.

Stage I/II colorectal cancer patients are currently being recruited in the context of a Phase I clinical trial aimed at testing an adenovirus type 5-based vaccine that encodes guanylyl cyclase 2C (GUCY2C, best known as GCC), which is universally expressed by colorectal cancer cells,[119,120] and the so-called pan HLA DR-binding epitope (PADRE), a short peptide that operates as adjuvant[121,122] (NCT01972737). Besides evaluating the safety and immunogenicity of this approach, this study aims at investigating whether the development of anti-GCC immunity is related to (1) occult metastases in regional lymph nodes; (2) race (African American vs. Caucasian); and (3) time to recurrence and/or disease-free survival.

Hui and collaborators tested a modified vaccinia virus Ankara (MVA)-based vector[123,124] expressing 2 Epstein–Barr virus (EBV) antigens in patients with EBV-related nasopharyngeal carcinoma.[97] All the patients enrolled in this Phase I clinical trial (18 individuals who were in remission for more than 12 wk after the completion of first-line therapies) received 3 intradermal vaccinations at 3-wk intervals. No dose-limiting toxicity was observed, and T-cell responses to either or both EBV antigens, namely, Epstein–Barr nuclear antigen 1 (EBNA1) and latent membrane protein 2 (LMP2),[125,126] could be documented in 15 out of 18 (83.3%) patients.[97] This vaccination strategy is currently being tested in a larger cohort of patients affected by the same malignancy (NCT01800071).

Three Phase II clinical trials testing recombinant vaccinia and fowlpox viruses as vectors for DNA-based anticancer vaccines have recently been initiated and are currently recruiting patients. Two of the studies investigate the safety and efficacy of an heterologous prime-boost vaccination combined with enzalutamide (an androgen receptor antagonist also known as MDV3100)[127-130] in advanced prostate cancer patients (NCT01867333; NCT01875250). In both these settings, priming is achieved by the subcutaneous injection of a recombinant vaccinia virus expressing PSA plus 3 immunostimulatory molecules, namely, CD80 (also known as B7-1), CD58 (also known as LFA-3), and intercellular adhesion molecule 1 (ICAM1).[131-135] Boosting is performed by the repeated subcutaneous delivery of a recombinant fowlpox virus encoding the same molecules. The third study aims at comparing the efficacy of a similar prime-boost approach combined with bacillus Calmette–Guérin (BCG)-based immunotherapy[63,64] in subjects with high grade non-muscle invasive bladder carcinoma (NCT02015104). In this case, however, the viral vectors employed for vaccination do not encode PSA but CEA and mucin 1 (MUC1), a glycoprotein that is overexpressed or aberrantly glycosylated in multiple tumors.[136-138]

Slovin and colleagues investigated the safety and therapeutic potential of alphavirus-derived virus-like replicon particles (VRPs)[139-141] expressing high levels of folate hydrolase 1 (FOLH1, best known as prostate-specific membrane antigen, PMSA)[142,143] in subjects affected by castration-resistant metastatic prostate cancer.[98,144] In this Phase I dose-escalation study, 12 patients received up to 4 vaccine doses (at 3-wk intervals), followed by a 5th dose 2 mo later. No systemic or local adverse effects were recorded, indicating that this regimen is well tolerated. However, although all patients were immunologically competent (as demonstrated by appropriate T-cell responses to standard mitogenic assays), none of them developed PSMA-specific cellular immunity. Accordingly, none of these individuals obtained clinical benefits from vaccination. As VRP-neutralizing antibodies were detected in some patients, dosing may have been suboptimal.[98] A Phase I clinical trial study has recently been launched to assess the safety and immunogenicity of AVX701, an alphavirus-derived VRP encoding CEA in Stage III colorectal cancer patients (NCT01890213).

Bilusic et al. assessed the clinical profile of a heat-inactivated *Saccharomyces cerevisiae* strain that had been genetically manipulated to express human CEA (GI-6207).[96,145,146] In this Phase I clinical trial, 25 subjects affected by CEA-expressing metastatic carcinomas underwent subcutaneous vaccination every 2 wk for 3 mo, and then monthly. Subcutaneous GI-6207 was well tolerated and was able to induce systemic CEA-specific T-cell responses as well as to decrease the percentage of circulating Tregs, at least in some individuals. Notably, 5 patients experienced stable disease upon vaccination, lasting for more > 3 mo (range: 3.5 to 18 mo).[96] A Phase II study has recently been launched to test the same experimental paradigm in patients with medullary thyroid cancer, which is often associated with increased CEA levels[147,148] (NCT01856920). The primary endpoint of this trial will be the effect of GI-6207 on the levels of calcitonin (a circulating marker that correlates with tumor size)[149-151] 6 mo post-vaccination.

A Phase I clinical trial has been initiated a few months ago to test the therapeutic profile of a prokaryotic vector for DNA-based anticancer vaccination (NCT02002182). This study involves the intravenous administration of a live attenuated strain of *Listeria*

monocytogenes engineered to express the E6/E7 antigens of human papillomavirus (HPV),[152-155] named ADX11-001, to patients with Stage II-IV HPV+ oropharyngeal cancer scheduled to undergo ablative surgery. In particular, ADX11-001 will be evaluated for its safety and ability to elicit HPV-specific circulating and intratumoral cytotoxic T-lymphocyte responses.

As for the clinical trials listed in our previous Trial Watch dealing with this topic,[56] the following studies have changed status: NCT00669136, now listed as "Terminated"; NCT00629057, NCT01147965, NCT01152398, and NCT01191684, now listed as "Completed"; NCT00669734 and NCT01304524, now listed as "Active, not recruiting"; and NCT01116245 and NCT01064375, whose status is "Unknown" (source http://www.clinicaltrials.gov). NCT00669136, aimed at testing an heterologous prime-boost vaccine targeting the α-fetoprotein (AFP)[156] in subjects with hepatocellular carcinoma, was terminated owing to poor accrual and insufficient target population for future accrual. Among "Completed" studies, preliminary results appear to be available only for NCT01147965, a Phase I/II clinical trial aimed at assessing the safety and therapeutic potential of a CEA-encoding adenoviral vector in breast, colorectal, and lung carcinoma patients. In particular, the authors of this study reported that their adenoviral vector is able to elicit TAA- (CEA-)specific cellular immune responses in colorectal cancer patients, in spite of the presence of neutralizing antibodies.[157] These findings indicate that some vectors for DNA-based vaccination may be less sensitive than others to natural or immunization-induced humoral responses.

Interestingly, Gavazza and colleagues have recently evaluated the therapeutic profile of a heterologous prime-boost approach in a veterinarian trial.[158] In this context, 42 client-owned dogs with Stage III/IV B-cell lymphosarcoma received a conventional chemotherapeutic regimen consisting of cyclophosphamide (an immunogenic alkylating agent),[159-162] vincristine (a microtubular poison),[163] and prednisolone (a glucocorticoid),[164] either alone or combined with a prime-boost vaccine targeting endogenous telomerase reverse transcriptase (TERT), which is hyperactivated in a significant fraction of canine neoplasms.[165] Priming relied on 2 intramuscular injections (at a 2-wk interval) of an adenoviral vector expressing a catalytically inactive variant of canine TERT. Boosting was performed 4–6 wk later by administering a naked plasmid coding for the same protein fused to the leader sequence of the human plasminogen activator (at the N-terminus) and LTB (at the C-terminus). Boosting injections were repeated up to 3 times, at 2-wk intervals from each other. Both treatments were extremely well tolerated by the cohort (no signs of toxicities), and 19 out of 21 (90.5%) vaccinated dogs developed a robust T-cell response against canine TERT. Moreover, vaccinated dogs exhibited a significant survival advantage as compared with dogs receiving chemotherapy only (> 76.1 vs. 29.3 wk, respectively).[158] These results validate the efficacy of TERT-targeting vaccines in dogs bearing B-cell malignancies,[166,167] and support the evaluation of a similar approach for other veterinary neoplasms. TERT-targeting vaccines (including DNA-based, DC-based, and purified component-based formulations) have been intensively investigated as a therapeutic measure against multiple human neoplasms. However, no TERT-targeting preparations are currently approved by the US FDA or other international regulatory agencies for use in cancer patients.[6,9,56]

Concluding Remarks

As discussed above, several strategies have been devised in the past 2 decades to elicit therapeutically relevant TAA-specific immune responses in cancer patients.[5-11] However, only one of these approaches is currently approved by the US FDA and other international regulatory agencies for use in humans. This is the DC-based preparation known as sipuleucel-T (Provenge®), which is currently licensed for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer.[168] Conversely, in spite of encouraging results, no purified component-based and DNA-based anticancer vaccine has yet entered the clinical practice.[9,10,56]

During the last 13 mo, only a few clinical trials have been initiated to test the safety, immunogenicity and therapeutic potential of DNA-based anticancer vaccines, as if the initial enthusiasm about this (at least hypothetically) powerful immunotherapeutic paradigm were decreasing. Perhaps, this trend reflects a significant number of Phase I/II clinical studies in which DNA-based vaccines were shown to be well tolerated by cancer patients and exerted immunogenic effects, yet failed to elicit therapeutically relevant immune responses.[56] We surmise that additional insights into the molecular and systemic factors that allow for the elicitation of robust TAA-specific immunity are required to conceive not only safe, but also efficient DNA-based anticancer vaccines. As vaccines are normally administered in the presence of potent adjuvants, which generally operate via pattern recognition receptors,[169] we expect agents other than Toll-like receptor agonists to mediate optimal immunostimulatory effects in this context. The future will tell which, if any, of the multiple immunochemotherapeutic combinatorial regimens that are currently being developed[170] endows DNA-based vaccines with the ability to elicit clinically meaningful anticancer immune responses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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