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REVIEW

Translational nanoparticle engineering for cancer vaccines

Adam J. Grippin[a,b], Elias J. Sayour[a], and Duane A. Mitchell[a]

[a]Preston A. Wells, Jr. Center for Brain Tumor Therapy, University of Florida Brain Tumor Immunotherapy Program, McKnight Brain Institute, Department of Neurosurgery, University of Florida, Gainesville, FL, USA; [b]J. Crayton Pruitt Family Department of Biomedical Engineering, Biomedical Sciences Building, University of Florida, Gainesville, FL, USA

ABSTRACT

Conventional cancer treatments remain insufficient to treat many therapy-resistant tumors.[1] Cancer vaccines attempt to overcome this resistance by activating the patient's immune system to eliminate tumor cells without the toxicity of systemic chemotherapy and radiation. Nanoparticles (NPs) are promising as customizable, immunostimulatory carriers to protect and deliver antigen. Although many NP vaccines have been investigated in preclinical settings, a few have advanced into clinical application, and still fewer have demonstrated clinical benefit. This review incorporates observations from NP vaccines that have been evaluated in early phase clinical trials to make recommendations for the next generation of NP-based cancer vaccines.

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Introduction

Despite advances in chemotherapy, radiation, and surgical resection, cancer is the second leading cause of death in the United States.[1] Cancer immunotherapy has recently produced significant advancements in treatment and is widely recognized as one of the major recent breakthroughs in clinical oncology.[2] Adoptive cellular therapies, involving the transfer of *ex vivo* expanded tumor-reactive lymphocytes, have produced substantial clinical responses in humans, but are complex modalities that require extensive cell preparation *ex vivo*, resulting in high cost, limited availability, and significant regulatory hurdles to clinical approval.[3] Vaccine therapies hold the promise of engendering long-lasting immunity by inducing innate cells including dendritic cells (DCs) to prime and activate T cells *in situ*. However, to date, cancer vaccines have failed to achieve meaningful and durable responses in the majority of treated patients. Early vaccine development focused on the use of peptides as antigens. However, peptide-based vaccines require immunogenic carriers to activate DCs and are often limited to presentation by specific HLA alleles.[4] Nucleic acid vaccines have been proposed as "universal" vaccines that bypass HLA restriction, but these require protection from proteases and entry into cytoplasm and/or nuclear membrane for translation into immunogenic peptides. NPs have been used to protect cargo from degradation, permit entry into cells, and stimulate DC maturation.[5] As a result, immunostimulatory NP vaccines have been proposed as "off the shelf" vehicles to deliver antigen directly to antigen presenting cells (APCs) *in vivo*.[6,7] Although preclinical work has produced a multitude of NPs capable of delivering antigen to APCs *in vitro* and in mouse models, only a few have been approved for investigational use in humans. Those that have been investigated have largely failed to produce significant clinical benefit in late phase clinical trials.[8-10]

This trend is consistent across NP disciplines. Despite massive increases in the number of articles published each year with the search term "nanoparticle*" in the biomedical literature, a few applications have progressed into clinical evaluation.[11] Analysis of the traits that facilitate rapid translation could inform development of nanotherapeutics likely to reach clinical application and ultimately improve patient outcomes.

This review provides a critical analysis of NPs that have been used as tumor vaccines in humans. Clinical and preclinical literature are synthesized to make recommendations on NP engineering and trial design criteria for optimal antitumor efficacy and translation. Application of the insights gained in this review to early NP development may lower regulatory barriers and hasten the development of effective NP vaccines for cancer treatment.

Overview of nanoparticle vaccines investigated in humans

Of 1,564 clinical trials listed on ClinicalTrials.gov with "(nanoparticle OR liposome) AND cancer" as search terms, only 76 utilize delivery of antigen for cancer treatment. Within this group, clinical trial results are reported in PubMed for only nine nanoparticle products (Table 1). Although this sample size is small, the similarities of these NPs may be useful to develop treatments of rapid clinical use. This review begins with a description of each vaccine and its use in clinical trials. Critical analysis of all of these NPs is then used to develop design criteria for future NP vaccines for cancer therapy.

CONTACT Duane A. Mitchell Duane.Mitchell@neurosurgery.ufl.edu Preston A. Wells, Jr. Center for Brain Tumor Therapy, University of Florida Brain Tumor Immunotherapy Program, Department of Neurosurgery, 1149 South Newell Drive, McKnight Brain Institute, University of Florida, Gainesville, FL 32611, USA.

Table 1. NP-based cancer vaccines for antigen delivery currently being used in humans.

	NP composition	Adjuvant	Antigen	Size (nm)	Disease	Trials	Ref.
Tecemotide (L-BLP25, Stimuvax)	Cholesterol, DMPG, DPPC	BLP25, Monophosphoryl Lipid A	MUC1	150–580	Breast cancer, NSCLC, prostate cancer, CRC	14	8,12,17,19,82
AS15	Monophosphoryl Lipid A, QS-21	CpG7909	MAGE-A3, dHER2	ND	Metastatic melanoma, NSCLC, breast cancer	24	9,10,20,28,77
Lipovaxin-MM	POPC, 3NTA-DTDA, NiSO4	IFNγ	Recombinant proteins from MM200 cells	240	Stage IV melanoma	1	51
DepoVax	Phosphatidyl choline: Cholesterol 10:1	Montanide ISA 51, tetanus toxoid	7 HLA-A2 restricted TAAs or survivin peptides	120	Breast, ovarian, and prostate cancer	6	7,29
RNA-LPX	DOTMA, DOPE	None	MAGEA3, tyrosinase, NY-ESO1, TPTE mRNA	200–400	Stage IIIB–IV melanoma	1	6
OncoVax - Id/ IL-2	DMPC	IL-2	Autologous idiotype protein	NF	Follicular lymphoma	1	50
CHP	Pullalan, cholesterol isocyanate	+/- GMCSF or OK-432	Recombinant MAGE-A4, truncated 146HER2, or NY-ESO-1 protein	20–50	Breast, esophageal, stomach, lung cancer	4	31-34,36
ISCOMATRIX	Cholesterol, phospholipid	Quillaia saponin	Recombinant NY-ESO-1 protein, E6/E7 peptide	40–50	NY-ESO-1 expressing tumors	2	41,43,44
VLP	Qβ bacteriophage	A-type CpG	Melan-A/MART-1 peptides	30	Stage I–IV melanoma	5	47,48

Composition, vaccine design, size, disease targeted, and number of trials registered on ClinicalTrials.gov evaluating use in cancer patients for each of the nine NP vaccines. DMPG, dimyristoyl-phosphatidylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DOPE, 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOTMA,1, 2-di-O-octadecenyl-3-trimethylammonium propane; ND, not determined; POPC, α-palmitoyl-β-oleoyl-phosphatidylcholine; 3NTA-DTDA, 3(nitriilotriacetic acid)-ditetradecylamine.

Tecemotide

Vaccine design

Tecemotide (L-BLP25, StimuVax) is a 150–580 nm liposome composed of cholesterol, dimyristoyl phosphatidylglycerol (DMPG), and dipalmitoyl phosphatidylcholine (DPPC).[12,13] Tecemotide delivers MUC1 glycoprotein, which is overexpressed on the apical surfaces of epithelia in many mucosal cancers, in the presence of the immunostimulatory lipid BLP25 and TLR4 agonist Monophosphoryl Lipid A, which is known to induce a shift toward Th1 polarization and $CD8^+$ T-cell response.[12,14-16]

Clinical studies

Tecemotide is safe, immunogenic, and may provide clinical benefit for subsets of patients with non-small cell lung cancer (NSCLC) (Table 2). Although phase II and III trials failed to demonstrate significant survival benefit for patients with NSCLC, subgroup analyses found significant survival benefit in patients with stage IIIB locoregional disease and patients treated concurrently with cyclophosphamide.[8,12] However, a subsequent study to evaluate Tecemotide with concurrent cyclophosphamide treatment was stopped prematurely after a fatal encephalitis that may have been caused by multiple repeated doses of cyclophosphamide and Tecemotide.[17] Nevertheless, Tecemotide is currently being investigated in an ongoing multinational phase III trial of colorectal cancer following curative resection of hepatic metastases and as maintenance therapy for patients with phase III NSCLC.[18,19]

AS15

Vaccine design

AS15 is an "immunostimulatory lipid" that is co-delivered with Melanoma Associated Antigen 3 (MAGE-A3) protein as a treatment of metastatic melanoma and NSCLC or recombinant HER2 protein (dHER2) as treatment of breast cancer.[10,20,21] MAGE-A3 is a tumor-associated cancer/testes antigen that is expressed in 24% of patients with NSCLC and is associated with poor prognosis.[22,23] Human Epidermal Growth Factor Receptor 2 (HER2) is overexpressed on surfaces of some malignant breast cancer cells.[24] AS15 contains the saponin QS21 that activates the inflammasome in APCs when co-administered with Monophosphoryl Lipid A.[25,26] AS15 has been combined with CpG analogs to further potentiate response.[10] CpG7909 is of particular interest as a TLR9 agonist used to stimulate plasmacytoid DCs to produce robust $CD8^+$ immunity.[10,27]

Clinical trials

AS15 trended toward benefit to overall survival in Phase II and III studies and produced complete responses in 3/36 patients with stage III or IV metastatic melanoma.[9,10] Treated patients demonstrated increased antibody production and $CD4^+$ T-cell activation.[10,21] AS15 induced significantly increased $CD4^+$ and $CD8^+$ T-cell responses in patients with unresected stage IB-III MAGE-A3 positive NSCLC, suggesting that tumor tissue may serve to augment immunologic response.[28] AS15 also demonstrated safety, antibody responses, and a trend toward increased disease free survival using HER2 peptide as antigen in a Phase I trial.[20] However, a large, randomized, double blind Phase III trial to evaluate use of AS15 in NSLC patients failed to improve disease-free survival.[9,21]

DepoVax

Vaccine design

DepoVax (DPX) is a 120-nm liposomal vaccine composed of a 10:1 mix of Phosphatidyl Choline:Cholesterol that is used in multiple formulations.[7,29] DPX-0907 delivers seven tumor-associated antigens (TAAs) that are expressed by MHC I on an ovarian cancer cell line.[7] DPX-Survivac delivers survivin, an inhibitor of apoptosis protein that is overexpressed in a variety of cancers and expressed on normal tissues at only low levels.[30] Both DPX vaccines are co-delivered with tetanus toxoid, a

Table 2. Overview of published clinical trials using NP vaccines.

Vaccine	Phase	Design	Patients	Control treatment	Endpoint	Result	Immune response (T cell//Ab)	Disease	Combinatorial treatments	Ref.
Tecemotide	Phase I	R*, 2 arm	17	NUC	Safety/Immunogenicity	pos/pos	neg//neg	Stage IIIB/IV NSCLC	Cyclophosphamide: single IV dose	13
	Phase IIB	R, NB, 2 arm, ITT	171	BSC	Median survival/safety	neg/pos	neg//NR	Stage IIIB/IV NSCLC	Cyclophosphamide: single IV dose	12
	Phase III	R, DB, 2 arm, MITT	1513	Placebo	Overall survival	neg	pos//NR	Stage III NSCLC	Cyclophosphamide: single IV dose	8
	Phase II	R, NB, 2 arm	34	NUC	Antigen-specific T-cell response	pos	pos//NR	Multiple myeloma, stage I/II untreated or stable II/III	Cyclophosphamide: single IV dose or metronomic low doses	17
	Phase II	NR, NB	22	NUC	Safety	pos	NR//NR	Unresectable stage III NSCLC	Cyclophosphamide: single IV dose 3 d before treatment	83
	Phase I/II	NR, NB	6	NUC	Safety	pos	NR//NR	Unresectable stage III NSCLC	Cyclophosphamide: single IV dose 3 d before treatment	19
	Pilot study	NR, NB, single arm	16	NUC	Prolonged PSA doubling time, safety	pos/pos	NR//NR	Prostate cancer	Cyclophosphamide: single IV dose 3 d before treatment	84
AS15	Phase IIB	R, NB, 2 arms (AS15 vs AS02)	75	AS02	Survival/Safety	neg/pos	pos//pos	Stage IIIB/IV melanoma		10
	Phase III	R, DB, 2 arms, T	2,312	Placebo	Disease-free survival	neg	//pos	Stage IB, II, IIIA NSCLC	With or without cisplatin	9,21
	Phase 0	R, 2 arms	25	NUC	Safety/ T-cell response	pos/pos	pos//pos	Stage IIB–IV		77
	Phase I/II	4 arms, NR, NB	67	NUC	Safety/T-cell response	pos/pos	pos//pos	Stage IB–III NSCLC	Concurrent with, after, or without cisplatin and vinorelbine	28
	Phase I	4 arms, NR, DE	61	NUC	Safety/Humoral response	pos/pos	NR//pos	Stage II–III HER2-+ breast cancer		20
	Phase I	NR, NB	12	NUC	Safety/Humoral immunity	pos/pos	neg//pos	HER2+ breast cancer	Lapatinib	85
CHP-MAGE-A4	Phase I	NR, NB	20	NUC	Safety/Immunogenicity	pos/pos	neg//neg	Advanced esophageal, stomach, and lung cancer refractory to surgery		33
CHP-NY-ESO-1	Phase I	NR, NB, 2 doses	13	NUC	Safety/humoral immunity	pos/pos	pos//pos	Stage IV esophageal cancer		34
	Phase I	NR, NB	9	NUC	Humoral immunity	pos	ND//pos	Esophageal cancer, prostate cancer, and melanoma		39
	Phase I	2 Arm NR, DE, T	25	NUC	Safety/Humoral immunity/ overall survival	pos/pos/ pos	ND//pos	Esophageal cancer		40
CHP-NY-ESO-1 and CHP-HER2	Phase I	NR, NB, single arm	8	NUC	Safety/Immunogenicity	pos/pos	ND//pos	NY-ESO-1^{+} HER-2^{-} esophageal cancer	OK-432	36
CHP-HER2	Phase I	NR, NB, 2 arm	9	NUC	Safety/TC response	pos/pos	pos//ND	HER2+ cancers	GMCSF or OK-432	31
CHP-HER2	Phase I	NR, NB, 2 arms	15	NUC	Safety/Humoral immunity	pos/pos	pos//pos	HER2+ cancers	GMCSF (75 μg $\times$ 5 d)	32
ISCOMATRIX	Phase I	NR, DB, DE,2 arms	46	Placebo	Safety/Immunogenicity	pos/pos	pos//pos	NY-ESO-1+ tumors (melanoma, bladder, rectal, breast)		42
	Phase I	R, B, DE	31	Placebo	Safety/Immunogenicity	Pos/pos	pos//pos	Cervical intraepithelial neoplasia		43
	Phase II	R, NB, 2 arms	46	NUC	Safety/Immunogenicity	pos/pos	pos//pos	Stage IV or unresectable stage III NY-ESO-1 or LAGE-1+ melanoma	Cyclophosphamide: single IV dose	46,57
	Phase II	R, B, 2 arms	39	NUC	T-cell response	pos	pos//pos	Resected melanoma patients vaccinated with NY-ESO-1 ISCOMATRIX	Fowlpox virus containing recombinant full length NY-ESO-1	44
DPX-0907	Phase I	NR, NB, 2 doses	23	NUC	Safety	pos	pos//ND	Breast, ovarian, and prostate cancer		7
DPX-Survivin	Phase I	NR, NB, 3 arms	19	NUC	Safety/Immunogenicity	pos/pos	pos//ND	Ovarian cancer in 1st or 2nd remission	Concurrent metronomic cyclophosphamide or single dose	29
OncoVAX-Id/IL-2	Phase I	NR, NB, 1 arm	10	NUC	Safety/Immunogenicity	pos/pos	pos//neg	Follicular lymphoma	>6 cycles of PACE regimen completed before treatment	50
Lipo-MERIT	Phase I	NR, NB, DE	3	NUC	Safety	ND	pos//ND	Stage IIIB–IV melanoma		6
VLP	Phase I/II	R/NR, NB, 4 arms	22	NUC	Safety/Immunogenicity	pos	pos//ND	Stage II–IV melanoma		47
	Phase IIa	4 arms	21	NUC	Immunogenicity/Safety	pos/pos	pos//ND	Stage III–IV melanoma	Imiquimod cream or IFA	48

Design and outcome information for each nanoparticle vaccine with published clinical trial results. "Results" represents the outcome of the primary endpoint for the general treated population. Therefore, a negative result does not preclude effect in subgroup analysis.

BSC, best supportive care; DB, double blind; DE, dose escalation; ITT, intention to treat analysis; MITT, modified ITT; NB, nonblinded; ND, not determined; neg, no statistically significant improvement or an increase in less than 50% of treated patients if no control cohort is available for comparison; NR, non-randomized; NUC, no untreated control; pos, statistically significant improvement or increase in greater than 50% of patients if no control cohort is available for comparison; R, randomized; T, only treated patients considered in statistical analysis. "T-cell response" includes both flow cytometry to quantify numbers of increased antigen-specific T cells and ELISAs of peripheral blood to evaluate IFNγrelease after restimulation with tumor antigen.

proprietary TLR9 agonist, and Montanide ISA 51, which is a Freund's Adjuvant called Montanide ISA 51, and a proprietary TLR9 agonist.[7,29]

Clinical trials

DPX-0907 induced persistent antigen-specific T-cell responses in 39% of patients with breast, ovarian, or prostate cancer in a Phase I trial.[7] Interestingly, individual patients responded to unique sets of antigens.[7] A trial using DPX-Survivac to treat patients with ovarian cancer included a metronomic cyclophosphamide regimen to selectively eliminate regulatory T cells (T_{Reg}) while allowing the proliferation of effector cells targeted against multiple survivin peptides.[29] Although no patients demonstrated evidence of objective clinical response, all patients in this trial generated antigen-specific $CD8^+$ T-cell responses in peripheral blood after three subcutaneous vaccines.[29]

CHP

Vaccine design

Cholesteryl pullalan (CHP) nanogels deliver peptides of MAGE-A4 and NY-ESO-1 or recombinant proteins of HER2.[31-34] Preclinical models suggest that these "immunologically stealth" particles act mainly via medullary macrophages in lymph nodes after subcutaneous injection.[35] CHP-HER2 NPs are sometimes combined with GMCSF or OK-432, a killed, low virulence strain of *Streptococcus pyogenese* that activates TLR-4.[31,36]

Clinical trials

CHP-NY-ESO-1 demonstrated widespread T-cell and antibody responses that correlated with PSA stabilization in prostate cancer patients and tumor regression in esophageal cancer and melanoma patients whose tumors expressed NY-ESO-1 protein.[37-39] Subsequent studies found enhanced survival benefit in esophageal cancer patients who received 200 μg dose compared with 100 μg.[40] CHP-MAGE-A4 induced substantial antibody responses that correlated with prolonged progression free and overall survival among esophageal cancer patients whose tumors highly expressed MAGE-A4 protein.[33] However, treatment also resulted in increased prevalence of $CD4^+FoxP3^{hi}CD45RA^-$ regulatory T cells in peripheral blood.[33] Likewise, CHP-HER2 induced antibody responses in almost all patients but was associated with loss of tumor antigen, presence of $CD68^+$ macrophages, and reduced $CD4^+$ and $CD8^+$ infiltration in tumor after treatment.[32,34]

ISCOMATRIX

Vaccine design

ISCOMATRIX is a 50-nm particle containing saponin, cholesterol, and phospholipid designed to increase cross-presentation of MHC class I restricted epitopes.[41] ISCOMATRIX has been used to deliver recombinant E6/E7 peptide to treat cervical intraepithelial neoplasia (CIN) or NY-ESO-1 protein as treatment of NY-ESO-1 positive melanoma, bladder, rectal, and breast cancer.[42,43]

Clinical trials

ISCOMATRIX originally garnered enthusiasm after a Phase I trial demonstrated dose-dependent antibody responses and reduced risk of relapse for patients with NY-ESO-1 positive tumors.[42] However, Phase II trials failed to demonstrate $CD8^+$ T-cell responses in patients with advanced disease and instead revealed production of an antigen-specific T_{Reg} population in vaccinated patients.[44-46] A current search on ClinicalTrials.gov revealed two terminated studies and one suspended study with this drug for cancer treatment (NCT01341496, NCT02054104, and NCT01258868).

VLPs

Vaccine design

Virus-like nanoparticles (VLPs), also known as CYT004-MelQbG10, are generated from the coat protein of bacteriophage Qβ and loaded with the CpG "G10."[47] This A-type CpG is known to stimulate IFN-α production by plasmacytoid dendritic cells (pDCs) to a greater degree than the B-Type CpG found in Montanide via stimulation of TLR9, but was previously unable to be used in humans due to rapid degradation by DNAse.[47] VLPs deliver peptides of melanoma-associated antigens Melan-A/MART-1.[47,48]

Clinical trials

A randomized Phase I trial found that VLPs safely generate T-cell responses in most patients with Stage II–IV melanoma.[47] Comparisons of multiple vaccination routes demonstrated increased production of antigen-specific T cells and effector memory and central memory subsets of antigen-specific T cells with intradermal or subcutaneous injection compared with intranodal injection.[48]

OncoVAX-Id/IL-2

Vaccine design

OncoVAX-Id/IL-2 was designed to generate more robust responses and a more homogeneous product after a protein conjugate vaccine demonstrated efficacy in follicular lymphoma patients in complete remission.[49] OncoVAX-Id/IL-2 incorporates protein for autologous lymphoma idiotypes and recombinant IL-2 as adjuvant inside a dimyristoylphosphatidylcholine (DMPC) liposome.[50]

Clinical trials

A Phase I trial with 10 patients found that OncoVAX-Id/IL-2 safely induced T-cell responses in 100% of patients and antibody responses in 40% of follicular lymphoma patients in clinical remission.[50]

Lipovaxin

Vaccine design

Lipovaxin is a 240-nm liposomal NP that delivers recombinant protein antigen and IFNγ intravenously (IV) to treat Stage IV melanoma.[51] This vaccine is composed of membrane vesicles from MM200 melanoma cells fused to POPC (α-palmitoyl-β-oleoyl-phosphatidylcholine) liposomes for stability and an anti-DC SIGN (DC-specific intercellular adhesion molecule 3 grabbing nonintegrin) immunoglobulin single variable domain to increase DC uptake.[51]

Clinical trials

A Phase I trial evaluating Lipovaxin was completed in 2012, but no results have been reported (NCT01052142).[54]

Lipo-MERIT

Vaccine design

Lipo-MERIT is a 200-nm liposomal vaccine that delivers mRNA encoding four common melanoma antigens (NY-ESO-1, MAGE-A3, tyrosinase, and TPTE) to APCs.[6] The 200–400 nm particle contains DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane) and DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) and is targeted to lymphoid organs after systemic injection by modifying lipid:RNA ratios, so that the net particle charge is negative.[6]

Clinical trials

Lipo-MERIT is currently in a Phase I trial to assess safety and dosing for patients with stage IV melanoma (NCT02410733). Although final results have not been reported, the first three patients developed flu-like symptoms, released IFN-α and IP-10, and increased levels of antigen-specific $CD4^+$ or $CD8^+$ T cells.[6]

Critical analysis of clinical outcomes

These trials and NPs have many similarities. While all studies report either antibody or T-cell response, no trials demonstrated statistically significant survival benefit in primary analysis.[8-10] The lack of clinical benefit despite immune outputs indicates that responses are either of the wrong character, insufficient magnitude, or too brief in duration. These insufficient outcomes may result from poor biodistribution or immunosuppression via tumor-induced mechanisms such as immune checkpoint activation. Understanding the impact of particle characteristics, combination therapies, and study design may inform development of successful therapeutics.

Study design

Outcome variable

Primary endpoints for each trial are included in Table 2. Although T cells are intended mediators of antitumor responses, their activation and proliferation is often not a direct predictor of clinical response. As an example, clinical responders to AS15 did not have increased rates of $CD4^+$ or $CD8^+$ T-cell responses.[10] Similarly, although proliferative response of antigen-specific T cells in the blood correlated with increased median survival among all patients vaccinated with Tecemotide ($n = 88$), the subset of patients that showed a survival benefit compared with controls ($n = 35$) included only two patients with proliferative T-cell responses.[12] Both analyses are limited by sample size and tepid responses to treatment, but this combined evidence may indicate that detectable levels of antigen-specific T cells are neither necessary nor sufficient to produce clinical response to vaccination.

Another possibility is that T-cell characterization as $CD4^+$ and $CD8^+$ is insufficient to predict outcome. Instead, a tipping of the balance between effector, memory, and regulatory T cells may be necessary for rational vaccine design to induce clinical antitumor immunity.[55] This effect was evaluated in Tecemotide, in which immunologic responders generated reduced effector and effector memory $CD4^+$ T cells.[17] In another approach, Berinstein and colleagues differentiated T cells functionally and found that DepoVax induced increased production of polyfunctional central and effector memory T cells.[7,29] Both analyses were applied to evaluate responses to VLPs, which induce production of multifunctional effector memory and central memory antigen-specific T cells.[47]

Furthermore, regulatory responses may counteract effector T cells. CHP-MAGE-A4 nanogels and NY-ESO-1/ISCOMATRIX both induced significant formation of regulatory T cells in many patients.[33,45] In one patient with metastatic melanoma, CHP-NY-ESO-1 induced systemic humoral and cellular responses but also high levels of $CD4^+CD25^+Foxp3^+$ regulatory T cells and macrophages at the tumor sites.[56] This patient ultimately succumbed to metastatic pulmonary infiltrates.[56] Likewise, histologic analysis of tumor from a small number of patients treated with CHP-HER2 revealed increased $CD68^+$ macrophages, reduced $CD4^+$ and $CD8^+$ infiltration, and loss of antigen expression after treatment.[34] Interestingly, clinical responses to CHP-MAGE-A4 correlated with antibody production.[33]

Studies to corroborate the relationship between these immunological classifications and clinical outcomes will likely demonstrate roles for $CD8^+$ T-cell phenotype, regulatory cells, and $CD4^+$ T cells in evaluation of vaccine efficacy.

Combinatorial treatments

Synergy between antigen-specific T-cell activation and chemotherapy has significant implications on future trial design. Combination chemotherapy improved quality or quantity of T cell responses generated by AS15, Tecemotide, ISCOMATRIX, and DepoVax.[7,8,10,29,57,58] One proposed explanation is that cyclophosphamide selectively reduces the regulatory T-cell compartment [59] Additionally, the lymphodepletion induced by chemotherapy may induce homeostatic proliferative responses that benefit vaccine-induced T cells. Although somewhat counterintuitive, we also observed enhancing effects of lymphodepletive chemotherapy in vaccinated patients with glioblastoma.[60-62] Murine experiments suggest that lymphodepletion is followed by a surge of IL-7 that stimulates lymphocyte proliferation..[62,63] In the setting of vaccination, IL-7 can be

co-opted to dramatically enhance expansion of tumor-reactive T cells.[62,64]

Combination therapy with other vaccine approaches may also avoid barriers to efficacy. ISCOMATRIX vaccine demonstrated this principle in showing increased generation of $CD8^+$ T-cell responses when paired with a fowlpox virus vaccine bearing the same antigen.[44] Although unexplored for NP-based vaccines, treatment with immune checkpoints may also potentiate vaccine efficacy by reducing the effect of vaccine-generated T_{Regs} and potentially overcoming tumor-induced immunosuppression.

Vaccine design

Antigen selection

Choice of antigen may also explain low treatment efficacy. None of the described trials targeted tumor-specific neoantigens. Instead, these studies generated responses against overexpressed antigens (Her2, survivin, and telomerase), lineage-restricted antigens (tyrosinase), and cancer-testis antigens (MAGE and NY-ESO). The low immunogenicity of overexpressed peptide antigens may explain the presence of antibody and $CD4^+$ T-cell responses without clinical response.[3,8,9,12,20,28] Selection of appropriate peptides within a protein antigen may also improve vaccinations. The importance of peptide selection was demonstrated with ISCOMATRIX, in which some epitopes stimulated antitumor T-cell responses to cryptic antigens and others generated functional T_{Regs} specific for tumor antigens.[45,65]

Many vaccines contained only a single cancer antigen. However, this strategy allows tumor cells to escape immune detection upon loss of the selected vaccine target.[66] This "antigen loss" was recorded after use of both VLRs and CHP nanogels.[34,48] Efforts to reduce this risk focus on the use of multiple antigens. Individual patients vaccinated with the seven TAA's encoded within DPX-0907 responded to unique subsets of these antigens.[7] The same was true of responses to LipoMERIT, which went further by using a variety of types of cancer antigens.[6] Although sample size is small, each of the three patients responded to a unique set of two of the four antigens in the vaccine.[6] However, studies with CHP showed that vaccination with multiple antigens may reduce the magnitude of humoral responses to specific antigens compared with single antigen vaccination.[36] Therefore, multiple antigens are likely needed to induce clinically significant responses, but further studies are needed to understand how distinct cancer antigens can be combined effectively.

Antigen form

Peptides bind avidly to MHC molecules but are limited to patients who express certain HLA molecules. Recombinant protein allows presentation of additional MHC I and MHC II epitopes, but requires a carrier that facilitates antigen presentation on both MHC molecules.[42] While protein and peptide vaccines have consistently achieved measurable immunologic responses, low clinical efficacy and HLA restriction encouraged the development of nucleic acid based vaccination strategies.[6,7,10] Although the bulk of work with nucleic acid vaccines uses DNA, mRNA is attractive for NP delivery because it is innately immunogenic and does not require entry into the nuclear membrane. LipoMERIT was the first human trial to evaluate the systemic administration of mRNA liposomes as vaccines in humans.[6] While preliminary data are promising, survival benefit will be necessary to determine the utility of this development.

Adjuvant

Multiple adjuvants have been used to bolster clinical responses. These include IFA, which increased numbers of antigen-specific and effector memory $CCR7^-CD45RA^-$ T cells in response to VLPs, GMCSF, which accelerated antibody production after vaccination with CHP-HER2, and CPG7909, which trended toward providing survival benefit when combined with AS15.[10,32,48] Other adjuvants produced mixed results, such as the TLR7 agonist Imiquimod, which reduced percentages of antigen-specific T cells but increased $CCR7^+/CD45RA^-$ central memory phenotype and $CD127^+$ T cells.[48]

Effects of NP composition on DC activation

While insufficient information on characteristics of the available NP constructs makes clinical comparisons difficult, preclinical evidence suggests that NP composition can significantly alter DC response. Many NPs, including LipoMERIT, are known to be taken up by DCs via multiple endocytosis pathways including macropinocytosis, but definitive analysis of the best pathway for subsequent antigen presentation remains elusive.[6,67] Studies in other cell types have suggested that addition of cationic lipids to otherwise neutral NPs changed the intracellular destination of NPs.[68] Notwithstanding the lack of mechanistic explanation, cationic NPs have generally emerged as more effective than neutral or anionic NPs for stimulating DC activation *in vitro*.[69,70] Soema et al. recently explored multiple variables with a Design of Experiment model to determine that optimal DC activation occurs at a NP charge of +30 mV.[71] Other groups found similar benefits of cationic NPs with diverse starting materials using elegant screening methods *in vitro*.[72,73] However, *in vitro* studies are likely not sufficient to draw conclusions on translational potential since the characteristics that determine particle uptake are also thought to govern NP localization after injection.

Delivery method

Effect of composition on localization

Clinical trials included subcutaneous (SC), intramuscular (IM), intradermal (ID), and intravenous (IV) vaccine administration (Table 3). Differences in trial outcomes may be reflected by the different cell populations targeted with each method. ID and SC administration are thought to lead to NP uptake by immature DC subsets including Langerhans cells and $CD14^+$ dermal DCs that migrate to lymph nodes and stimulate $CD8^+$ T-cell responses.[74] However, $CD8^+$ T-cell response may be more readily generated with transfection of multiple DC subtypes including those deep within lymph nodes.[75,76] IV vaccines, on the other hand, are thought to circulate before transfecting APCs in lymphoid organs.[6] Consideration of the DC subsets that will be activated by each injection route should influence selection of injection method. Although $CD8^+$ T-cell responses were seen with IM administration of AS15, a recently reported human study of AS15 found that while no routes induced

Table 3. Injection methods and vaccination schedule for NP vaccines in clinical trials.

NP	Vaccination schedule	Vaccination method	References
Tecemotide	8 doses weekly, then continued doses every 6 weeks until progression	4 SC sites	8,12,17,83,84
	Weeks 0, 2, 5, and 9	Upper arm, anterolateral thigh	13
	> 4 weekly doses	4 SC sites	19
AS15	Biweekly for 12 weeks, triweekly for 18 weeks, every 6 weeks for 4 mo, quarterly for 1 y, and biannually for 3 y	IM	10
	13 injections in 27 mo	IM	9
	8 doses triweekly	IM	28
	6 doses over 14 weeks	IM or ID/SC	20,77
	Biweekly for 12 weeks	IM	85
DepoVax	3 doses triweekly	SC	7, 29
LipoVaxin	ND	IV	54
Lipo-MERIT	Weekly vaccinations with increasing dose	IV	6
OncoVAX - Id/IL-2	Months 0, 1, 2, 3, and 5	4 SC sites	50
CHP-NY-ESO-1 or CHP-HER2	6 doses biweekly	SC	31,33,36,40
	14 biweekly doses	SC	34
	3 doses biweekly	SC	31
	4 doses biweekly, then regularly up to 12 vaccinations	SC	32,39
ISCOMATRIX	3–6 doses monthly	IM	42,44,51
	Weeks 1, 3, 5, 9, then 3 monthly, then 1 every 12 weeks until progression	IM	46,57
	1–3 doses weekly	IM	43,57
VLP	Weekly or daily for 14 weeks	SC or IM	47
	3 weekly, then 3 monthly	SC, ID, or intranodal	48

ID, intradermal; IM, intramuscular; SC, subcutaneous; triweekly, every third week.

robust $CD8^+$ T-cell responses, ID and SC administration tended toward increased $CD4^+$ T-cell responses compared with IM vaccination.[77] Studies with VLP corroborate a lack of difference between ID and SC injection and demonstrate reduced antigen-specific, effector, and memory T cells after direct intranodal injection.[48] Other injection methods have been explored preclinically, including intranasal vaccination, which has been shown to generate robust DC accumulation in pulmonary LNs and produce $CD8^+$ T-cell and antibody responses in peripheral blood in mice.[78] Future human studies should consider all available forms of vaccination and select the most optimal based on NP design and desired response.

Once a delivery method is decided, simple modifications can be made to enhance delivery to target organs. None of the clinical studies track NP fate, but lessons from preclinical work may be extrapolated to understand results of clinical trials. Although cationic liposomes increase DC function and activation in culture, positively charged particles accumulate in lungs in murine models after IV injection, precluding immune responses in lymphoid organs.[6] LipoMERIT avoids this problem by increasing the concentration of negatively charged RNA molecules, effectively reducing net particle charge to negative values.[6] The same principle applies to subcutaneous administration in murine models, where reducing surface charge by addition of PEG to cationic liposomes dramatically increases their uptake in lymph nodes.[79] However, changing route of administration may avoid this dilemma. After intranasal administration, cationic NPs are more readily taken up by nasopharyngeal-associated lymphoreticular tissue (NALT) DCs than anionic NPs.[80] Therefore, NP charge should be selected based on the desired injection method, with neutral NPs for ID/SC administration, cationic NPs for nasal administration, and cationic NPs with net negative charge for systemic administration.

NP size also dictates localization within lymph nodes.[75] However, pleiotropic effects make guidelines difficult. As an example, in a study using poly(lactic-co-glycolic acid) (PLGA) NPs to deliver antigen to DCs, 40 nm NPs migrated to lymph nodes more effectively after SC administration, but 100 and 200 nm NPs each delivered more DNA per NP.[81] However, many treatments used in humans appear to be in the range of 100 nm to 1 μm, which is larger than recommended for optimal migration to LN after SC injection in murine models.[81] Analysis of the effects of size and charge in humans will require consistent reporting of size and charge in ongoing trials.

Despite the clear importance of particle localization, none of the evaluated trials track particle fate in humans. Future particle development will require adoption of clinically relevant methods to determine effects of particle characteristics on particle localization in humans.

Future trial design

Although promising, nanoparticle vaccines face many challenges in practice. Tolerance induced by long-term exposure to tumor antigens and the immunoregulatory tumor microenvironment may contribute significantly to this underwhelming clinical benefit. However, combination with immune checkpoint inhibitors may overcome these barriers. By "turning off the brakes" on the immune system, anti-CTLA-4, anti-PDL-1, and anti-PD-1 antibodies prevent tolerance induction and allow unfettered T-cell activation. Although combination therapies are more challenging to evaluate in clinical trials, the clinical approval and acceptance of ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1), and nivolumab (anti-PD-1) for a variety of advanced malignancies has paved the way for the initiation of a myriad of combinatorial therapeutic trials. Therefore, to unleash the full therapeutic potential of nanoparticle vaccines, future trials will likely be designed in combination with agents that remodel the intratumoral microenvironment.

Conclusions

NP vaccines that have been investigated in humans have many similarities. Most contain components that were approved for use in other products and are co-delivered with previously approved immunostimulatory adjuvants. Analysis of these early studies indicates the need for multiple, tumor-specific antigens, rational selection of combinatorial treatments, and NP design specific to route of administration. Future studies should consider the impact of NP characteristics on particle localization, determine immune correlates that accurately predict clinical outcomes, and consider combination therapy with immune checkpoint inhibitors. Consistent reporting of NP characteristics and immunological outcome variables in human and preclinical trials would facilitate this work and inform a generation of NP vaccines that provide significant survival advantage to patients with malignant tumors.

Disclosure of potential conflicts of interest

DAM receives research funding from Immunomic Technologies, Inc., a cancer therapeutics company. AJG and EJS declare no conflicts of interest.

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