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REVIEW

Melanoma vaccines: trials and tribulations

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Robert O Dillman[1,2]

[1]Hoag Cancer Institute and Hoag Institute for Research and Education, Newport Beach, CA, USA; [2]University of California Irvine, Irvine, CA, USA

Abstract: Metastatic melanoma has been a target of immunotherapy for more than 4 decades. Three immunotherapeutics have received regulatory approval for treating melanoma: interferon-alpha, interleukin-2, and ipilimumab. The antitumor mechanisms of these products depend on enhancing existing immune responses, including autoimmune effects. The combination of autologous, cytotoxic T-lymphocytes plus high-dose interleukin-2 is a promising patient-specific therapy, but has limited clinical application. Other approaches include vaccines targeting melanoma-associated antigens, and patient-specific vaccines that utilize autologous tumor. Non-patient-specific vaccine approaches target melanocyte differentiation antigens (eg, tyrosinase, Melan-A, gp100), antigens identified by cytotoxic T-lymphocytes (eg, NY-Eso-1, Melan-A/Mart-1, Mage-3), and antigens originally identified by murine monoclonal antibodies (gangliosides, gp97, gp225). Self-renewing cells in tumor cell lines may represent tumor stem cells, but vaccines derived from allogeneic tumor cell lines have yielded disappointing results in randomized trials. Patient-specific vaccines can be derived from bulk autologous tumor or autologous tumor cell lines, and intratumoral injections of immunostimulatory fusion products have shown promise. While technically more complex to manufacture, patient-specific vaccines derived from autologous tumor cell lines have the potential to target tumor stem cells and overcome interpatient tumor cell heterogeneity. This article reviews sources of melanoma-associated antigens, costimulatory agents, and clinical trial results for various melanoma vaccines. Comparing Phase II trials is difficult because of the wide range of vaccine strategies and the differences in study patient populations; therefore, randomized trials are necessary to prove the efficacy of such products. Therapeutic vaccines are more likely to enhance, rather than replace, other anti-melanoma immune therapies. In particular, effective vaccines may be synergistic with products that block T-cell immune checkpoint molecules such as ipilimumab and monoclonal antibodies that interfere with programmed death ligand-receptor interactions.

Keywords: melanoma, vaccines, melanoma-associated antigens, melanoma stem cells, dendritic cells, GM-CSF, checkpoint molecules

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Correspondence: Robert O Dillman
Hoag Institute for Research and Education, 3900 Pacific Coast Highway, Suite 210A, Newport Beach, CA 92663, USA
Email robert.dillman@hoag.org

Immunotherapy of melanoma

The adaptive immune system is an iterative process involving processing and presentation of antigen particles to T-lymphocytes by dendritic cells (DC) in the context of human lymphocyte antigens (HLA), also known as histocompatibility antigens. This interaction is regulated by membrane receptors, coexpression molecules, and checkpoint molecules that limit autoimmunity.[1,2] Once induced, T-lymphocytes provide cellular immunity and B-lymphocytes humoral immunity, with the participation of helper and suppressor T-cells, memory T-cells, cytotoxic T-cells, natural killer cells, regulatory

T-cells that help limit autoimmunity, and a process of B-cell differentiation that results in continuous improvement in the affinity and avidity of antibodies to antigen. Cancer immunology is a complex balance between immune recognition of self and non-self, and adaptive processes by cancer cells that help them evade immune recognition.[3] The iterative process of immunoediting results in continuous improvement in specific immune recognition of foreign molecules that affect cancer suppression and promotion.[4] Especially challenging is that cancer cells in each patient contain the same basic genetic material as all other cells in that individual. Consequently, cell mechanisms that protect against autoimmunity can also limit immune recognition of autologous cancer cells. Cancer immunotherapy involves stimulation, augmentation, or suppression of various aspects of the immune system. Cancer immunotherapeutics include foreign agents that enhance inflammatory responses, monoclonal antibodies that target tumor-associated antigens, immune signaling cytokines that have broad nonspecific immune-stimulating effects, agents designed to block specific cell interactions that inhibit autoimmunity, and immune cells.[5]

Anti-melanoma effects mediated by the host immune system have been recognized for many years and include rare cases of spontaneous regression of distant metastases[6,7] and the favorable prognostic implications of tumor-infiltrating lymphocytes in both primary tumors[8] and lymph node metastases.[9] Melanoma has been a focus of anticancer immunotherapy for more than 4 decades. The nonspecific immune stimulating agent bacille Calmette–Guérin (BCG) was tested extensively during the 1970s before it was concluded to be of limited therapeutic benefit for preventing melanoma recurrence or treating metastatic melanoma. However, objective tumor regressions following intratumoral (IT) BCG injections were reported,[10] and enthusiasm persists for BCG as an adjuvant in melanoma vaccines.[11]

Table 1 lists many immunotherapies that have been tested in melanoma patients. Two cytokines with broad immune-stimulating effects have received regulatory approval for treating melanoma from the United States Food and Drug Administration (FDA). In 1995, interferon (IFN)-α was approved for the adjuvant treatment of surgically resected, high-risk melanoma.[12] A pegylated formulation of IFN-α received approval in 2011.[13] Interleukin (IL)-2 has been used to treat metastatic melanoma since its regulatory approval for treating metastatic renal cell carcinoma in 1992 and received marketing approval for treating metastatic melanoma in 1998.[14] In 2010, the anti-cytotoxic T-cell antigen-4 (CTLA-4) monoclonal antibody ipilimumab was approved based on improved survival in patients with metastatic melanoma.[15] Other immune-modulating products, such as the monoclonal antibodies nivolumab and lambrolizumab that target programmed death receptor-1 (PD-1),[16,17] and antibodies that target its ligand (PDL-1),[18] have produced response rates in the range of 25% to 40% in melanoma patients. One or more of these anti-PD-1 agents will almost certainly receive regulatory approval in the near future. All of these immunotherapies are nonspecific, and generalized autoimmune effects are responsible for some or all of their antitumor activity and toxicity. For instance, in the adjuvant treatment of high-risk melanoma, serologic detection of autoimmune antibodies induced by IFN-α is the best predictor of progression-free survival.[19] In patients with metastatic melanoma, autoimmune phenomena such as vitiligo and thyroiditis are associated with clinical benefit of IL-2.[20] Autoimmune inflammation and associated adverse events are associated with clinical benefit in patients receiving ipilimumab.[21]

Unfortunately, clinical use of available immunotherapy agents is limited because of these toxic side effects. IFN-α commonly induces flu-like symptoms, severe fatigue, and debilitating depression that necessitate cessation of treatment.[22] Intravenous infusion of high-dose IL-2 induces the release of numerous cytokines that cause severe flu-like symptoms, dermatitis, and potentially lethal capillary leak syndrome associated with hypotension and decreased renal perfusion.[23,24] For this reason, only a minority of medically fit patients are offered this treatment, which has to be administered in a setting that includes specialized nursing and inpatient monitoring. Ipilimumab is often associated with severe autoimmune toxicities including enterocolitis, dermatitis, and hepatitis, and endocrinopathies such as hypophysitis, thyroiditis, and adrenal insufficiency.[21] These toxicities

Table 1 Immunotherapies for melanoma other than vaccines

Nonspecific microbial immune stimulants
Bacille Calmette–Guérin[10,11,38,44,45]
Cytokines
Interferon-alpha (approved 1995 for adjuvant treatment of high-risk melanoma)[12,13,19,22,123]
Interleukin-2 (approved 1998 for treatment of metastatic melanoma)[14,20,23,24,29]
Granulocyte-macrophage colony-stimulating factor[55–57,60,61]
Monoclonal antibody immune modulators
Ipilimumab (approved 2010 for treatment of metastatic melanoma)[15,21]
Nivolumab and lambrolizumab: anti-programmed-death 1 receptor[16–18]
Adoptive cell therapy
Lymphokine-activated killer cells[25–29]
Tumor infiltrating lymphocytes[30,31]
Cytotoxic T-lymphocytes[32–34]

have resulted in death in some patients. On the other hand, so far the anti-PD-1 antibodies appear to be associated with minimal toxicity.[16,17]

There have been several reports of clinical benefit following adoptive cell therapy with autologous lymphocytes. Early efforts focused on lymphokine-activated killer (LAK) cells that consist of natural killer and T-cells isolated from the peripheral blood and infused with IL-2.[25,26] Unfortunately, subsequent trials failed to confirm such high response rates,[27,28] and a randomized trial failed to clearly establish a therapeutic benefit for the addition of LAK cells.[29] More promising has been the use of tumor-infiltrating lymphocytes (TIL), especially cytotoxic T-lymphocytes (CTL).[30,31] Immunosuppression by lymphodepletion, followed by IL-2 and infusion of billions of tumor antigen-specific, autologous CTL derived from patient tumor, has resulted in some remarkable therapeutic effects,[32,33] but may provide no greater clinical benefit than TIL.[34] Adoption of this approach has been limited by the technical demands of T-cell production, the need for lymphocyte depletion, and infusion of high-dose IL-2.

Immunotherapy may be the most important modality for treating melanoma. However, our current armamentarium has resulted in only modest improvement in the survival of melanoma patients, so more effective and less toxic approaches are needed. It is hoped that vaccine approaches will induce new immune responses or enhance existing weak immune responses, including those partially suppressed by checkpoint molecules and regulatory T-cells. Such vaccine approaches are expected to be additive or synergistic with existing and emerging immunotherapies. Administration of vaccines with ipilimumab may facilitate induction of new anti-melanoma immune responses, while administration of vaccines with anti-PD-1 antibodies may facilitate enhancement of existing anti-melanoma immune responses.

Antigen presentation and complementary agents

Table 2 lists a number of agents that attract immune cells and/or enhance immune responses to tumor-associated antigens.

Table 2 Antigen presentation and complementary agents

- Antigen presentation
  - Dendritic cells[81–98]
  - Purified or recombinant-manufactured antigen[100–115]
  - Recombinant presentation products (viral vectors, fusion proteins)[48–50,70,207–212]
  - Histocompatibility antigens (heat shock proteins, B7-beta-2 microglobulin)[177–179,207–209]
- Local chemical immune enhancers
  - Alum (potassium aluminum sulfate)[35]
  - Montanide emulsification of oil and water (incomplete Freund's adjuvant)[36]
  - Dinitrophenol[164,165]
- Foreign antigens
  - Bacterial antigens[11,37–45,155]
    - Mycobacteria including bacille Calmette–Guérin
    - Other bacterial antigens: *Streptococcus, Salmonella, Corynebacterium, Nocardia,* tetanus
  - DETOX[43]
  - Monophosphoryl lipid A from *Salmonella minnesota*[35,36]
  - Viral antigens (especially pox, adeno and herpes viruses)[47–50]
  - Keyhole limpet hemocyanin[46]
  - Saponins[51,52]
- Cytokines
  - Interleukin-2[140,144,169]
  - Interleukin-12[126]
  - Interferons (alpha, beta, and gamma)[66,68,154]
  - Granulocyte-macrophage colony-stimulating factor[53–56,63–68]
- Anti-T-cell products or T-cell modifiers
  - Chemotherapy agents: cyclophosphamide[71–73]
  - Products that target interleukin-2 receptor (CD25)
    - Denileukin diftitox[74,75]
    - Daclizumab[76,77]
  - Monoclonal antibodies to T-lymphocyte checkpoint molecules
    - Cytotoxic T-lymphocyte antigen-4 (CTLA-4): ipilimumab[15,21,78–80]
    - Programmed death 1 (PD-1) receptor and ligand: nivolumab and lambrolizumab)[16–18,78–80]

Adjuvants

Adjuvants that are components of existing FDA-approved vaccines include alum (potassium aluminum sulfate), other aluminum salts (aluminum hydroxide and aluminum phosphate), and ASO4, a mixture of aluminum salts and monophosphoryl lipid A, which is a detoxified component of *Salmonella minnesota* lipopolysaccharide.[35] A popular adjuvant in animal models is complete Freund's adjuvant, which consists of an emulsion of the lipid squalene and water in mineral oil (montanide) and inactivated *Mycobacteria tuberculosis,* but it is considered too toxic for human use. Incomplete Freund's adjuvant, also known as montanide, lacks the mycobacterial component, and has been used as a component of several investigational melanoma vaccines.[36] The immune-stimulating effects of bacterial antigens, and their potential to act as adjuvants in vaccines, has been recognized since the description of Coley's toxins.[37] BCG has been widely used as a bacterial adjuvant and also as a monotherapy.[10,11,38–40] Proteins from *Nocardia, Corynbacteria, Streptococci, Salmonella*, and others have also been used.[40–44] DETOX consists of detoxified endotoxin, MPL, and *Mycobacteria minnesota* cell wall skeletons.[45] Keyhole limpet hemocyanin (KLH) is a highly immunogenic copper-containing metalloprotein isolated from the giant keyhole limpet *Megathura crenulata* found along the California seacoast.[46] Because of immunogenicity of various pox viruses, there has been long-standing interest

in vaccinia (smallpox) as an adjuvant in viral oncolysates[47] and, more recently, in recombinant delivery systems that combine viral and tumor antigens.[48–50] Saponin adjuvants, such as QS-21, are mixtures of soluble triterpene glycosides purified from the South American tree *Quillaja saponaria* Molina, also known as the soap bark tree.[51] $ASO2_B$ combines MPA and QS21, while AS15 adds synthetic oligonucleotides containing CpG motifs that target Toll-like receptor 9 to the MPL and QS-21.[52]

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

Immune-enhancing cytokines may function as adjuvants.[53] IFN-α, IFN-γ, IL-2, and GM-CSF have been commercially available for more than 20 years, which has facilitated their testing in conjunction with vaccines;[54] GM-CSF has been especially popular.[55,56] In standard doses, GM-CSF is associated with mild side effects, and it has stimulating effects on DC and B- and T-lymphocytes. It has been given by either injection, cell transfection, or as a component of engineered fusion products. Adjuvant GM-CSF has been associated with antigen-specific immune responses to various melanoma peptide antigens.[57–59] Phase II trials suggest that maintenance GM-CSF monotherapy may enhance survival in melanoma patients with deep melanomas or positive microscopic regional lymph nodes.[60,61] In trials in which melanoma patients were treated with patient-specific vaccines, the 53-patient subset that received GM-CSF and/or IFN-γ had better survival than 21 patients who received vaccine with no adjuvant, BCG, or IFN-α (3-year survival 29% versus [vs] 0%, $P < 0.001$).[62]

The benefits of GM-CSF have been questioned by some.[63] As summarized in Table 3, some randomized trials have been

Table 3 Randomized trials in melanoma testing the cytokine GM-CSF as an immune stimulant

Author	Stage[213]	Number of patients	Antigen	Adjuvant	Route	Clinical efficacy	Other metrics
Weber et al[64]	II	20	gp100 Tyrosinase	Montanide + GM-CSF	SC	Unclear <2-year F/U	Slightly better immune responses to antigens (NSD)
Weber et al[64]	II	21	gp100 Tyrosinase	Montanide	SC	Unclear <2-year F/U	
Slingluff et al[65]	U-III U-IV	13	gp100 Tyrosinase	GM-CSF Montanide THP LD IL-2	SC and ID	2/13 RR 2/13 vitiligo	More antigen specific T-cell responses ($P = 0.02$)
Slingluff et al[65]	U-III U-IV	13	gp100 Tyrosinase	DC THP LD IL-2	SC and ID	1/13 RR 0/13 vitiligo	
Dillman et al[66]	U-III and IV	25	Autologous tumor cell lines	GM-CSF	SC	1/10 RR 15-month med OS 31% 5-year OS	22% DTH-T conversion NSD
Dillman et al[66]	U-III and IV	26	Autologous tumor cell lines	IFN-γ	SC	0/10 RR 21-month med OS 31% 5-year OS	27% DTH-T conversion NSD
Faries et al[67]	II R-III R-IV	46	Allogeneic tumor cell lines	GM-CSF BCG	ID	60% 4-year OS $P = 0.10$	↓ DTH-V, $P = 0.006$
Faries et al[67]	II R-III R-IV	48	Allogeneic tumor cell lines	BCG	ID	80% 4-year OS	
Kirkwood et al[68]	IV	56	gp100 Mart-1 Tyrosinase	GM-CSF ± IFN-α	SC	14.3-month med OS ≈28% 2-year OS	NSD
Kirkwood et al[68]	IV	61	gp100 Mart-1 Tyrosinase	None or IFN-α	SC	10.4-month med OS ≈26% 2-year OS	
Lawson et al[69]	R-III R-IV	≈400	None	GM-CSF	SC	12-month med DFS 72-month med OS	$P = 0.14$ $P = 0.55$
Lawson et al[69]	R-III R-IV	≈400	None	Placebo	SC	9-month med DFS 60-month med OS	

Abbreviations: BCG, bacille Calmette–Guérin; DFS, disease-free survival; DTH-T, delayed-type hypersensitivity reaction to tumor cells; DTH-V, delayed-type hypersensitivity reaction to vaccine; F/U, follow-up; GM-CSF, granulocyte-macrophage colony-stimulating factor; ID, intradermal; IFN, interferon; IL, interleukin; LD, low-dose; med, median; NSD, no significant difference; OS, overall survival; R, resected; RR, response rate; SC, subcutaneous; THP, tetanus helper peptide; U, unresectable; DC, dendritic cells.

conducted to better define the role of GM-CSF in vaccines, but none were definitive.[64–69] The largest (ECOG 4697) is an intergroup trial for which interim results have been presented in abstract form.[69] Patients who were HLA-A2 positive were randomized to gp100 vaccine or placebo with a secondary randomization to GM-CSF or placebo, while HLA-A2-negative patients were randomized to GM-CSF or placebo. The interim analysis found no advantage for GM-CSF, but among 258 patients with stage IV disease, there was a trend toward improved disease-free survival and overall survival.

In addition to injection of various adjuvants, molecular and transfection techniques make it possible to create a variety of combinations and permutations of antigens and immune-stimulating proteins. For instance, a fusion protein of GM-CSF and prostatic acid phosphatase was the basis for the immune therapy sipuleucel-T that was recently approved for the treatment of prostate cancer.[70]

T-cell-mediated immune modifiers

T-lymphocytes provide oversight of the immune system to protect against debilitating autoimmune responses. In addition to helper, memory, and suppressor T-cells, there are also regulatory T (CD4+ CD45+ FoxP3+)-cells that inhibit interactions between DC and T-lymphocytes. A few small trials have explored the immune-modulating effects of anti-T-lymphocyte agents such as cyclophosphamide chemotherapy,[71–73] the anti-CD25 immunotoxin denileukin diftitox,[74,75] and the anti-CD25 monoclonal antibody daclizumab.[76,77] Whether addition of these agents can enhance vaccine therapy remains to be seen. There is great optimism for the role of antibodies that target T-cell checkpoint molecules, including CTLA-4, PD-1, and its ligand PDL-1,[78–80] not only as monotherapies but as adjuncts to vaccine therapies. CTLA-4 inhibits induction of T-cell-mediated immunity by competing with the CD28 receptor on T-cells that binds to dendritic cells via CD80 and CD86. PDL-1, which is present at sites of inflammation and is produced by many tumors, induces apoptosis of antigen-activated T-cells by binding to their PD-1 receptors. As single agents in patients with relapsed melanoma, the anti-PD-1 monoclonal antibodies nivolumab and lambrolizumab have produced objective response rates of 28% to 38%.[16,17] Ipilimumab was associated with a response rate of less than 15%, but provided a survival benefit in a randomized, placebo-controlled trial.[15] However, immunization with gp100 provided no additional benefit beyond ipilimumab alone. Trials that combine other vaccines with these monoclonal antibodies will be forthcoming.

DC

DC are now recognized as the most important of the antigen-presenting cells.[81–83] They can be derived from bone marrow or peripheral blood mononuclear cells. Immature DC are preferred for antigen-loading, but the subsequent maturation is crucial for antigen presentation.[84–86] Although apoptotic tumor cells may be associated with tolerance in the tumor microenvironment,[87,88] apoptotic rather than necrotic cells are preferred for ex vivo DC loading.[89] Of practical importance for manufacturing, studies have shown that DC are phenotypically and functionally similar before freezing and after thawing.[90,91] Even though few DC actually reach regional lymph nodes after injections, it only takes a small number migrating there to induce a robust immune response against new antigens.[92] DC produced using different manufacturing conditions may differ biologically and functionally.[93] Since the earliest reports of tumor response after injections of DC pulsed with autologous tumor lysate or melanoma-associated antigen (MAA) peptides,[94] DC have been used to present a variety of MAA as part of vaccine investigation.[95–98]

Other variables in vaccine delivery

Another variable for melanoma vaccines is route of administration. Possibilities include intranodal (IN), intralymphatic (ILY), IT, intravenous (IV), subcutaneous (SC), intradermal (ID), intramuscular (IM), and combinations of these. There is no convincing evidence that there is a preferred route,[99] but SC and IM are most practical in terms of ease of administration and the volume of vaccine that can be administered while avoiding the risks of toxicity associated with IV administration. For a variety of different reasons, including production limitations and study power, vaccine trials have failed to define minimally effective doses, maximum tolerated doses, or optimum doses or dose ranges of antigen exposure. There is also no convincing evidence that there is a preferred schedule of administration. Daily, weekly, and monthly approaches have been used as well as combinations of the above with different induction and booster or maintenance schedules. The optimal duration of vaccine therapy is also unclear and can only be tested when a vaccine has been validated to be therapeutically beneficial.

Melanoma antigens and clinical trials

Table 4 summarizes various antigen sources that have been or are being tested in clinical trials. The rest of this review focuses on these antigens and results of clinical trials, especially randomized trials.

Table 4 Sources of melanoma-associated antigens for therapeutic vaccines

Melanoma-associated antigens identified by monoclonal antibodies[100–110]
Melanoma-associated antigens identified by cytotoxic T-cells[111–115]
Anti-idiotype antibodies mimicking melanoma antigen[106,109,118,119]
Allogeneic tumor cell lines[149–155]
Autologous tumor ex vivo[162–169,177–179,201–212]
Autologous tumor cell lines[62,180,181,191–200]

Characterized MAA

Murine monoclonal antibodies, human antitumor antibodies, and human CTL from patients responding to TIL therapy have identified numerous MAA that could be targeted for therapy. The first MAA were identified by murine monoclonal antibodies and included high-molecular-weight chondroitin sulfate proteoglycan (gp225), also known as human high-molecular-weight-melanoma-associated antigen (HMW-MAA);[100,101] gp97 melanotransferrin;[100,101] gangliosides;[102] including GM2,[103,104] GM3,[105] GD2,[106,107] and GD3;[108,109] and other antigens of various molecular weights.[110] HMW-MAA is expressed on 80% of melanomas and is believed to activate several signaling cascades that affect cell adhesion, migration, and invasion. An increasing number of different melanoma peptide antigens have been recognized by CTL; examples are Mage-1, Mage-3, Mart-1 (Melan-A), tyrosinase, gp100, and NY-Eso-1.[111–115] These may be classified as oncofetal or cancer testis antigens, or as melanocyte differentiation antigens. Oncofetal antigens include the melanoma gene families referred to as A-MAAs (Mage, including Mage-A1, Mage-A3, and Mage-A4), B-MAAs (Bage) and G-MAAs (Gage), and NY-Eso-1. Many of these are HLA-phenotype restricted, none are expressed on all melanoma tumor cells, and many are expressed on less than 50% of tumor samples. Melanocyte differentiation antigens involved in melanin production include tyrosinase, gp100 (recognized by HMB-45) and Melan-A (Mart-1), and tyrosine-related protein-2 (TRP-2). Many unique antigens have been detected that result from mutations or aberrant expression on malignant cells. In addition to being tumor-specific MAA, some may function as oncogenes, inhibit suppressor genes, induce angiogenesis, cause cell cycle dysregulation, alter epigenetic regulation, and increase resistance to apoptosis. Examples include multiple myeloma oncogene-1 (MUM-1), which is normally involved in immunoglobulin gene expression in B-cells; cyclin-dependent kinase 4 (CDK4) that leads to dysregulated cell cycle activity; p15, which normally inhibits CDK4, the cell adhesion molecule beta-catenin; N-acetylglucosaminyltransferase V, which can be upregulated by transforming growth factor-beta; variants of gp100; fatty acid-binding protein-7; and homeobox (HOX) transcription factors involved in protein regulation. In one study, Mage-1 and Mage-4 were more likely to be expressed on sites of metastatic disease than primary melanoma, while NY-Eso-1 was expressed on about 45% of both sites.[116] Patients with pre-existing T-lymphocytes that recognize Melan-A and NY-Eso-1 have a better prognosis than patients who lack such T-cells.[117] Mage-1 and Mage-3 are HLA-A1-restricted, while Mart-1 (Melan-A), tyrosinase, and gp100 are HLA-A2-restricted, which limits their testing to specific patient populations.

Trials with MAA recognized by murine monoclonal antibodies and/or gangliosides are summarized in Table 5.[118–123] A construct consisting of recombinant vaccinia virus and p97 (melanotransferrin) called v-p97NY appeared promising in mouse models, but apparently was not evaluated further.[124] The mouse anti-idiotype monoclonal antibody MK2-23 mimics HMW-MAA.[118] The product TriGem™ (Titan Pharmaceuticals, South San Francisco, CA, USA) consisted of anti-idiotype antibody-mimicking GD2 mixed with QS-21.[106] One anti-GM3 vaccine consists of N-glycolylneuraminyl-lactosylceramide (NeuGcGM3) in a proteoliposome of *Neisseria meningitides* with Montanide™ ISA (SEPPIC Fairfield, NJ, USA).[120] The anti-GD3 anti-idiotype BEC2 was used to create the GD3-lactone-KLH/QS21 product.[121] In randomized trials, the GM2-BCG vaccine was not clearly superior to BCG alone,[122] and a trial comparing a GM-2 vaccine to high-dose IFN-α was stopped after only 16 months' median follow-up when an interim analysis showed it was inferior to IFN-α.[123] In summary, trials of ganglioside antigens have not been associated with high response rates or survival benefit in patients with metastatic disease,[118,119] have not been associated with encouraging disease-free or overall survival rates in patients with resected disease,[121,122] and were inferior to IFN-α in a large, randomized trial in patients with resected high-risk stage II and III disease.[123]

There was great interest in testing peptide MAA recognized by CTL from patients who had responded to TIL treatment.[111] Numerous trials have been reported that used melanoma peptide antigens with various adjuvants or loaded on DC (Table 6).[59,94,124–139] These trials varied in terms of disease stage, whether or not tumor had been resected prior to treatment, and the use of adjuvants and immune modifiers. They confirmed that immunization with such peptides could induce or enhance antigen-specific T-cell responses to various MAA, but there were no striking differences related to different adjuvants or antigen-delivery by DC. Even though

Table 5 Single-arm and randomized trials involving ganglioside antigens

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Mittelman et al[118]	U-III IV	52	HMW-MAA (anti-id)	None, CTX, KLH, BCG	SC	4.4-month med OS	3/52 made antigen specific antibodies
Foon et al[119]	IV	47	GD2 (anti-id)	QS-21	SC	1/47 RR, CR 16-month med OS	40/47 made anti-GD2 antibodies
Osorio et al[120]	R-IV	35	NeuGcGM3 ganglioside	Neisseria and Montanide in liposome	SC	5/35 RR, 2 CR 20-month med OS	28/30 made anti-GM3 antibodies
Chapman et al[121]	R-III R-IV	12	GD3→GD3 anti-id	KLH + QS-21 BCG	ID	45-month med OS for all patients (22/24 R-III)	5/12 made anti-GD3 antibodies
Chapman et al[121]	R-III R-IV	12	GD3 anti-id→GD3	BCG KLH + QS-21	ID	No difference for sequence	5/12 made anti-GD3 antibodies
Livingston et al[122]	R-IV	58	GM-2	BCG	ID	70% 2-year OS 60% 3-year OS $P = 0.22$	50/58 made anti-GM2 antibodies
Livingston et al[122]	R-III R-IV	64	None	BCG	ID	70% 2-year OS 45% 3-year OS	7/64 made anti-GM2 antibodies
Kirkwood et al[123]	R-II R-III	440	GM-2	KLH, QS-21	ID	51% 2-year DFS 73% 2-year OS	DFS $P = 0.0015$
Kirkwood et al[123]	R-II R-III	440	None	HD-IFN-α	ID	55% 2-year DFS 78% 2-year OS	OS $P = 0.009$

Abbreviations: anti-id, anti-idiotype; BCG, bacille Calmette–Guérin; CR, complete response; CTX, cyclophosphamide; DFS, disease-free survival; HD, high-dose; HMW-MAA, high-molecular-weight melanoma-associated antigen; ID, intradermal; IFN, interferon; KLH, keyhole limpet hemocyanin; med, median; OS, overall survival; R, resected; RR, response rate; SC, subcutaneous; U, unresectable.

antigen-specific responses were detected in many patients, antitumor responses with vaccine monotherapy occurred in less than 10% of patients with measurable disease. The 10% response rate for DC-based approaches[94,130,134,137,138] was not statistically higher (10/97 vs 10/174, $P = 0.17$) than the 6% response rate for non-DC approaches[125,126,131–133,136,139] The 9% response rate for multivalent vaccines[94,130,133,134,137–139] was not statistically higher (15/165 vs 5/106, $P = 0.18$) than the 5% response rate for monovalent vaccines.[125,126,131,132] The 6% response rate for vaccines containing Mage-3[131–133,137–139] was not dissimilar (9/150 vs 11/121, $P = 0.33$) to the 9% response rate for vaccines that did not contain Mage-3.[94,125,126,130,134,136] The objective response rate following IL-2 historically was about 17%,[14] and was still 17% despite the addition of gp100.[129] Disease recurrence was still high in patients with advanced disease who were disease-free at the time of vaccination, and survival was influenced more by patient selection than by vaccine therapy. The importance of patient selection is evidenced by the 45% 5-year survival rate for patients treated by complete surgical resection of metastatic melanoma just prior to treatment with any of several peptide vaccines.[135]

Table 7 summarizes randomized trials that tested peptide vaccines;[15,73,140–144] only two were powered to compare treatment arms in a Phase III trial.[15,144] Again, these trials vary in terms of disease stage, antigens, adjuvants, and other immune modifiers. None of the smaller Phase II trials showed definitive differences between or among study arms, although trends were used to select products for further investigation. It is noteworthy that stage IV patients treated with DC loaded with a combination of peptide antigens did no worse than patients treated with dacarbazine, but the vaccine was less toxic.[141] In the largest trial, gp100 did not add benefit to ipilimumab in patients with measurable stage IV disease,[15] but, in a much smaller trial, gp100 appeared to add benefit to patients who were healthy enough to receive high-dose IL-2,[144] although the response rate to IL-2 alone was much lower than the 17% reported in trials.[14,129]

It is hoped that oncofetal peptide MAA will produce more striking results because of their frequent expression on tumor stem cells. In 25 melanoma patients (72% stage IV and 28% stage III), preexisting anti-NY-Eso-1 CD4+ and CD8+ cells were present in 52% and 40%, respectively, before vaccination, but increased to 78% and 88% after vaccination with recombinant vaccinia and fowlpox vectors expressing NY-Eso-1 antigen.[145] The objective tumor response rate was 3/21 and median survival 48 months for all 25 patients. However, the major clinical benefit was in patients who already had preexisting antibody and T-cell recognition of NY-Eso-1, and the presence of preexisting anti-NY-Eso-1 antibodies was more predictive of survival than induction of anti-NY-Eso-1 antibodies after immunization. Several trials with NY-Eso-1 and other oncofetal antigens are in progress.

A Mage-A3 antigen-specific cancer immunotherapeutic (ASCI) product is being tested in combination with the AS15

Table 6 Single-arm trials testing peptide vaccines and other agents

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Cormier et al[125]	IV	23	Melan-A/ Mart-1	Montanide	SC	0/23 RR	Increased antigen-specific cytotoxicity
Peterson et al[126]	IV	20	Melan-A/ Mart-1	PBMC IL-12	SC	2/20 RR, 2 CR 12-month med OS	Increased antigen-specific T-cell response
Smith et al[127]	I-II R-III	30	gp100	Montanide	SC	No data	28/29 increased antigen-specific T-cell responses
Rosenberg et al[128]	II R-III R-IV	95	gp100	Montanide	SC	≈50% 3-year DFS	High levels of antigen-specific T-cell responses
Sosman et al[129]	IV	131	gp100	HD-IL-2	SC	20/121 RR 15-month med OS ≈35% 2-year OS	No clinical correlation with antigen-specific responses
Lesterhuis et al[130]	IV	27	gp100 Tyrosinase	DC KLH	IV and ID	2/27 RR	3/27 antigen-specific responses
Marchand et al[131]	IV	33	Mage-3	MPL QS-21	IM	2/33 RR	
Kruit et al[132]	IV	30	Mage-3	*Haemophilus influenzae* protein D	SC and ID	1/30 RR	Rare antigen-specific responses
van Baren et al[133]	IV	37	Mage-1 Mage-3	Canary pox	SC and ID	1/30 RR	
Nestle et al[94]	IV	16	gp100 Melan-A Tyrosinase or autologous tumor	DC KLH	IN	5/16 (2 CR)	DTH-peptide pulsed DC 11/16
Hersey et al[134]	IV	14	gp100 Melan-A Tyrosinase	DC immature KLH	IN	0/14 RR	
Atzpodien and Reitz[59]	II R-III R-IV	24	gp100 Melan-A Tyrosinase Mage-1	GM-CSF	SC	<10% still disease-free at 2 years	20/24 DTH-V
Tagawa et al[135]	R-IV	41	Melan A gp100 Tyrosinase	Various 8 different trials	SC	3.8-year med OS 45% 5-year OS	
Tarhini et al[136]	U-III U-IV	22	Melan-A gp100 Tyrosinase	Montanide GM-CSF mRNA	SC	2/21 RR	9/20 antigen-specific response
Banchereau et al[137]	IV	22	gp100 Melan-A Tyrosinase Mage-3	DC ± KLH	SC	0/22 RR 12-month med OS	No increase in antigen-specific T-cell response
Hersey et al[138]	IV	18	gp100 Melan-A Tyrosinase Mage-3	DC mature ± LD IL-2	IN	2/9 RR + IL-2 1/9 RR no IL-2 18-month med OS	
Slingluff et al[139]	III-B IV	37	6 peptide Melan-A gp100 Tyrosinase Mage-3	GM-CSF Montanide	SC and ID	2/17 RR 72% 1.5-year OS	30/37 had antigen-specific T-cell responses

Abbreviations: CR, complete response; DC, dendritic cells; DFS, disease-free survival; DTH, delayed-type hypersensitivity; DTH-V, DTH reaction to vaccine; GM-CSF, granulocyte-macrophage colony-stimulating factor; HD, high-dose; ID, intradermal; IL, interleukin; IN, intranodal; KLH, keyhole limpet hemocyanin; LD, low-dose; med, median; MPL, monophosphoryl lipid A; mRNA, messenger ribonucleic acid; OS, overall survival; PBMC, peripheral blood mononuclear cells; R, resected; RR, response rate; SC, subcutaneous; U, unresectable.

Table 7 Randomized trials testing peptides and other agents

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Slingluff et al[140]	R-IIB R-III R-IV	20	gp100 Tyrosinase	THP Montanide GM-CSF IL-2 day 7	SC and ID	39% 2-year DFS $P = 0.32$	T-cell responses 37% PBL 38% SLN
Slingluff et al[140]	R-IIB R-III R-IV	20	gp100 Tyrosinase	THP Montanide GM-CSF IL-2 day 28	SC and ID	50% 2-year DFS $P = 0.32$	T-cell responses 53% PBL 83% SLN
Schadendorf et al[141]	IV	53	gp100 Tyrosinase Mage-1 Mage-3	DC	SC	2/41 RR 9.3-month med OS 20% 2-year OS	NSD $P = 0.48$
Schadendorf et al[141]	IV	55	None	DTIC	IV	3/52 RR 1.6-month med OS 20% 2-year OS	NSD $P = 0.48$
Slingluff et al[142]	R-IIB R-III R-IV	26	4 peptide gp100 Tyrosinase	THP Montanide GM-CSF	SC and ID	Med DFS and OS not provided	
Slingluff et al[142]	R-IIB R-III R-IV	25	12 peptide gp100 Tyrosinase Mage-A1 Mage-A3 Mage-A10 NY-ESO-1	THP Montanide GM-CSF	SC and ID	Med DFS and OS not provided	Greater immune responses
Slingluff et al[73]	R-IIB R-III R-IV	41	12 peptide	THP Montanide GM-CSF	SC and ID	≈75%–80% 3-year OS	78% CD8 response 93% CD4 response
Slingluff et al[73]	R-IIB R-III R-IV	41	12 peptide	THP Montanide GM-CSF CTX	SC and ID	≈75%–80% 3-year OS	CTX had no effect
Slingluff et al[73]	R-IIB R-III R-IV	42	12 peptide + 6 peptide	THP Montanide GM-CSF	SC and ID	≈75%–80% 3-year OS	19% CD8 response 48% CD4
Slingluff et al[73]	R-IIB R-III R-IV	43	12 peptide + 6 peptide	THP Montanide GM-CSF CTX	SC and ID	≈75%–80% 3-year OS	CTX had no effect
Kruit et al[143]	U-III M1a	36	Mage-A3	AS15	IM	4/36 RR 3 CR 3-month med PFS 33-month med OS 59% 2-year OS	*P*-values not reported
Kruit et al[143]	U-III M1a	36	Mage-A3	$ASO2_B$	IM	1/36 RR 3-month med PFS 20-month med OS 37% 2-year OS	
Hodi et al[15]	IV	403	gp100	Ipilimumab	SC	10.0-month OS 5.7% RR	$P < 0.001$ vs gp100 alone
Hodi et al[15]	IV	136	gp100	Placebo	SC	6.4-month OS 1.4% RR	
Hodi et al[15]	IV	137	Placebo	Ipilimumab	SC	10.1-month OS 10.9% RR	$P = 0.003$ vs gp100 alone

(*Continued*)

Table 7 (*Continued*)

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Schwartzentruber et al[144]	IV	85	gp100	Montanide HD-IL-2	SC	16% RR 3.9-month PFS 25-month OS	$P = 0.03$ $P = 0.008$ $P = 0.06$
Schwartzentruber et al[144]	IV	93	None	HD-IL-2		6% RR 1.8-month PFS 11-month OS	

Abbreviations: CR, complete response; CTX, cyclophosphamide; DC, dendritic cells; DFS, disease-free survival; DTIC, dacarbazine; GM-CSF, granulocyte-macrophage colony-stimulating factor; HD, high-dose; ID, intradermal; IL, interleukin; IM, intramuscular; IV, intravenous; med, median; NSD, no significant difference; OS, overall survival; PBL, peripheral blood lymphocytes; PFS, progression-free survival; R, resected; RR, response rate; SC, subcutaneous; SLN, sentinel lymph node lymphocytes; THP, tetanus helper peptide; U, unresectable.

adjuvant (CpG 7909, MPL, and QS-21) in a Phase III trial (DERMA) in patients with stage IIIB or IIIC melanoma whose tumors express Mage-A3. It is estimated that 60% of melanomas in Europe express the antigen.[143] Previous trials suggested some immune response to vaccination with Mage-3 but limited clinical activity (Table 6).[131–133] A randomized Phase II trial suggested that Mage-3 might have greater activity when administered with AS15 rather than $ASO2_B$ (Table 7).[143]

Tumor-derived products

Rather than focusing on specific antigens, many investigators have pursued tumor-derived products because of cancer heterogeneity, the extent of mutations in metastatic melanoma, and the inability to know every possible antigen for targeting.

Preparations from whole allogeneic tumor

One investigator prepared a lysate from a large amount of tumor harvested from a single patient. This lysate was injected into 129 patients with stage I melanoma and 61 with stage II disease and was associated with 5-year survival rates of 88% and 64%, respectively.[146] The subset of stage II patients appeared to have improved survival compared to published data. This approach is no longer desirable given the availability of allogeneic cell lines and the theoretical advantages of autologous tumor. The investigator himself abandoned this approach in favor of a lysate prepared from an allogeneic cell line, II-B-MEL-J,[147] but a Phase II trial with this product was not completed.

Allogeneic tumor cell lines

Allogeneic tumor cell lines consist of proliferating, self-renewing cancer cells that can be selected for expression of common MAA, although they cannot be expected to express all relevant MAA because of inter-patient tumor heterogeneity and unique patient-specific neoantigens.[148] Allogeneic cell line products can be standardized and reproduced for clinical investigation, although they have to be continually monitored for genetic and phenotypic drift while in continuous culture. Several large randomized trials in melanoma patients have been conducted using whole tumor cells or lysates from allogeneic cell lines as antigen sources (Table 8).[149–155] It should be noted that all of these trials were conducted in patients whose melanoma had been completely resected and had not recurred at the time of treatment. Two large trials used similar allogeneic viral oncolysates in patients with resected metastatic disease.[150,151] The US trial, which enrolled patients with lymph node metastases, indicated stage II disease in one report[150] but stage III subsequently.[151] This trial was negative, but an Australian trial that enrolled patients with stage IIB disease and resected stage III showed trends favoring the vaccine.[152]

Melacine™ (Corixa Corporation, Seattle, WA, USA) is an allogeneic cell lysate mixed with DETOX that was no better than observation in stage IIB patients[153] but was not clearly inferior to IFN-α in patients with resected stage III melanoma.[154] Canvaxin™ (CancerVax Corporation, Carlsbad, CA, USA), which consists of cells from two allogeneic cell lines admixed with BCG, was associated with a doubling of 5-year overall survival in patients with resected stage III melanoma and a tripling of 5-year survival in stage IV melanoma, compared to historical controls.[156] In another retrospective comparison between patients who had undergone complete resection of metastatic melanoma lesions, 150 treated with Canvaxin™ had a 39% 5-year survival compared to only 19% for 113 who did not receive the vaccine.[157] However, results of two large double-blinded, placebo-controlled trials of Canvaxin™ in patients with resected stage III and IV disease were negative. The stage III trial was stopped for futility after a third analysis, and the stage IV trial was halted after a second interim analysis, at which time survival actually was better in the BCG placebo arm ($P = 0.04$).[155] These disappointing results with allogeneic

Table 8 Randomized trials testing allogeneic vaccines and other agents

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Bystryn et al[149]	R-III	24	Allogeneic tumor cell line shed antigen	Alum	ID	19-month med DFS 67% 2-year OS 3.8-year med OS	$P = 0.03$ NSD
Bystryn et al[149]	R-III	14	None	Alum	ID	7-month med DFS 61% 2-year OS 2.7-year med OS	
Wallack et al[151]	R-III	104	Allogeneic tumor cell line lysate	Vaccinia	ID	42% 5-year DFS 49% 5-year OS	$P = 0.61$ $P = 0.79$
Wallack et al[151]	R-III	113	None	Vaccinia		40% 5-year DFS 48% 5-year OS	
Hersey et al[152]	IIB R-III	338	Allogeneic tumor cell line lysate	Vaccinia	ID	6.9-year med PFS 12.6-year med OS 61% 5-year OS	$P = 0.17$ $P = 0.07$
Hersey et al[152]	IIB R-III	335	None	Vaccinia		3.6-year med PFS 7.3-year med OS 55% 5-year OS	
Sondak et al[153]	IIB	300	Allogeneic tumor cell line lysate	DETOX	IM	66% 5-year DFS 80% 5-year OS	$P = 0.51$
Sondak et al[153]	IIB	300	None			62% 5-year DFS 80% 5-year OS	
Mitchell et al[154]	R-III	294	Allogeneic tumor cell line lysate	DETOX LD IFN-α	IM	16% 5-year DFS 62% 5-year OS	$P = 0.91$ $P = 0.91$
Mitchell et al[154]	R-III	277	None	HD IFN-α		13% 5-year DFS 58% 5-year OS	
Morton et al[155]	R-III	≈580	Allogeneic tumor cell line cells	BCG	ID	≈63% 5-year OS	NSD
Morton et al[155]	R-III	≈580	None	BCG	ID	≈63% 5-year OS	
Morton et al[155]	R-IV	≈248	Allogeneic tumor cell line cells	BCG	ID	≈42% 5-year OS	
Morton et al[155]	R-IV	≈248	None	BCG	ID	≈42% 5-year OS	$P = 0.04$

Abbreviations: alum, potassium aluminum sulfate; BCG, bacille Calmette–Guérin; DFS, disease-free survival; HD, high-dose; ID, intradermal; IFN, interferon; IM, intramuscular; LD, low-dose; med, median; NSD, no significant difference; OS, overall survival; PFS, progression-free survival.

cell lines remind us that no matter how encouraging immune response data and survival compared to historical controls may be, randomized trials are necessary to establish the benefit of vaccine therapies.

A polyvalent vaccine prepared from antigens shed into culture by one xenogenic and three allogeneic cell lines appeared safe and induced some immune-enhancing effects in 36 stage II and 19 stage III melanoma patients.[158] In a 38-patient randomized trial, ID injections of antigens shed from an allogenic cell line and suspended in alum (n = 24) was associated with a survival that was no better than that observed in patients injected with alum plus albumin (n = 14), and the median survival was less than 4 years.[149] The shed antigens have been characterized,[110] and, in 2012, a randomized, double-blind, placebo-controlled trial in resected stage IIB, IIC, and III melanoma was initiated with a product called POL-103A™ (Polynoma, Inc., San Diego, CA, USA) polyvalent melanoma vaccine.

In recent years, a few small trials have been conducted using autologous DC pulsed with antigens from allogeneic cell line lysates (Table 9).[58,159–161] Antigen-specific immune responses were reported in all trials, but fewer than 10% of patients experienced objective responses and survival rates were not very encouraging. Given the generally disappointing results with allogeneic cell lines, it is not clear whether such products will be further developed.

Preparations from whole autologous tumor

Fresh or frozen autologous tumors have been used to prepare cell suspensions, lysates, or mechanically enriched tumor cell populations. Autologous tumors offer the theoretical advantage of being patient-specific in terms of tumor and histocompatibility antigens and are easier to prepare and more rapidly available than autologous tumor cell lines. However, this is only an option for patients who have surgically accessible

Table 9 Trials of dendritic cells loaded with allogeneic antigens

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Salcedo et al[159]	U-III IV	15	Allogeneic tumor cell line lysate	Hepatitis B protein and/or tetanus toxoid	SC ID IN	1/15 RR	Antigen-specific immune responses
Palucka et al[58]	IV	20	Allogeneic tumor cell line lysate	DC	SC	2/20 RR 22-month med OS	3/13 antigen-specific immune response
Lopez et al[160]	IV	43	Allogeneic tumor cell line lysate	DC Alum or KLH	ID or SC	15-month med OS 12% 5-year OS	21/39 DTH-T response
Ribas et al[161]	U-III IV-M1b	33	Allogeneic tumor cell line lysate	DC	ID and SC	3/33 RR, 1 CR	26/29 antigen-specific responses

Abbreviations: alum, potassium aluminum sulfate; CR, complete response; DC, dendritic cells; DTH-T, delayed-type hypersensitivity reaction to tumor cells; ID, intradermal; IN, intranodal; KLH, keyhole limpet hemocyanin; med, median; OS, overall survival; RR, response rate; SC, subcutaneous; U, unresected.

tumors large enough to contain the desired number of cells for vaccine manufacturing. Furthermore, such vaccines often include variable numbers of immune cells and stromal cells in addition to malignant cells. Any tumor that is greater than a few millimeters in size mostly consists of more differentiated tumor cells rather than self-renewing tumor cells that may be a more critical target for complete eradication of tumor. Challenges that this approach shares with autologous tumor cell lines include the need to manufacture a specific product for each patient and the inter-patient variation in final treatment products. Trials utilizing cells from autologous tumor masses are summarized in Table 10.[91,134,138,162–172] In patients with measurable disease, the response rate was 15% (23/149 vs 12/82) whether the autologous tumor cell products were loaded onto DC[91,134,138,167–169] or not,[162,163,166] but the response rate was only 5% (2/37) for the dendritoma products.[170–172] Long-term survival data were limited.

Autologous tumor cell suspensions

The process required for this approach is exemplified in a trial in which eligible patients had to have more than 5 g of non-necrotic tumor resected, from which aliquots of cells were prepared and cryopreserved.[166] At the time of each treatment, an aliquot of frozen cells was thawed, washed, exposed to ultraviolet light, placed in cell culture for 24 hours, washed, suspended in phosphate-buffered saline, then mixed with DETOX for ID injection. This process was repeated every 2 weeks for six doses and then every 4 to 6 weeks.

Several other trials using autologous tumor were conducted in the pre-DC era.[162–165] The approach included enzyme-digested cell suspensions of autologous tumor that were cryopreserved and subsequently thawed, washed, irradiated, suspended in a saline solution, and injected with BCG as an adjuvant. Dinitrophenol was later conjugated to tumor cells as a hapten, and patients were pretreated with low doses of cyclophosphamide to suppress T-cells. Each injection consisted of 10 to 25 million viable tumor cells. Over the years, objective tumor responses were reported for 11/83 stage IV patients, stage III patients had a 5-year survival rate of 44%, and patients who had a delayed-type hypersensitivity response had twice the 5-year survival rate as patients who did not (59% vs 29%, $P < 0.001$).[165] Such correlations are typical in vaccine trials, but such immune reactions may just be epiphenomena of immune competence rather than proof of an immune-induced benefit. Efforts were made to commercialize this product as M-Vax™ (AVAX Technologies, Philadelphia, PA, USA),[173] but Phase III trials were never completed because of regulatory and financial hurdles.[174]

DC pulsed with autologous tumor lysates

More recent trials have focused on loading antigens from irradiated autologous tumor lysates onto autologous DC.[91,134,138,167–169] Tumor size is not as critical for this approach because the final product is DC rather than tumor cells. Response rates in stage IV patients have ranged from 0% to 35%, even with what appear to be identical approaches.[168,169] The few complete responses reported have been durable, with responders surviving for several months to over a year.

Autologous DC/tumor cell hybridomas

Another approach with autologous tumor and DC is the creation of hybridomas of autologous tumor cells and autologous DC, which have also been termed dendritomas.[175,176] The hybridomas have been created by electrofusion and polyethylene glycol methods. Small trials of such products have demonstrated some immune-mediated effects, but rarely tumor responses.[170–172]

Table 10 Clinical trials utilizing autologous tumor samples in the vaccines

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Laucius et al[162]	IV	18	Autologous tumor	BCG	ID	4/18 RR	
Berd et al[163]	R-III IV	64	Autologous tumor	BCG CTX	ID	6/40 RR	20/62 DTH-T
Berd et al[164]	R-III	62	Autologous tumor	DNP BCG	ID	58% 5-year OS	21/24 DTH-T
Berd et al[165]	R-III 2.5 cm tumor	214	Autologous tumor	DNP BCG CTX	ID	33% 5-year DFS 44% 5-year OS	47% DTH-T
Eton et al[166]	R-IV U-IV 5 g tumor	42	Autologous tumor	DETOX	ID	2/24 RR 16-month med OS	8/35 DTH-T
O'Rourke et al[167]	IV	17	Autologous tumor	DC	ID	6/17 RR, 3 CR	
O'Rourke et al[91]	IV	46	Autologous tumor	DC	ID	6/46 RR, 3 CR	
Ridolfi et al[168]	IV	27	Autologous tumor	DC KLH LD IL-2	SC or ID	8/27 RR, 2 CR 16-month med OS	DTH-V positive in 15/15 non-PD
Redman et al[169]	IV	24	Autologous tumor	DC KLH LD or HD IL-2	ID	0/24 RR	3/14 converted DTH-T
Hersey et al[134]	IV	19	Autologous tumor	Immature DC KLH	IN	3/19 RR	5/15 converted DTH-T
Hersey et al[138]	IV	16	Autologous tumor	Mature DC KLH ± LD IL-2	IN	0/16 RR 18-month med OS	
Krause et al[170]	IV	17	Autologous tumor	DC dendritoma	SC	1/17	
Haenssle et al[171]	IV	11	Autologous tumor	DC dendritoma	ID or SC	0/11	
Wei et al[172]	IV	9	Autologous tumor	DC dendritoma	SC	1/9	

Abbreviations: BCG, bacille Calmette–Guérin; CR, complete response; CTX, cyclophosphamide; DC, dendritic cells; DFS, disease-free survival; DNP, dinitrophenol; DTH-T, delayed-type hypersensitivity reaction to tumor cells; DTH-V, delayed type hypersensitivity reaction to vaccine; HD, high-dose; ID, intradermal; IL, interleukin; IN, intranodal; KLH, keyhole limpet hemocyanin; LD, low-dose; med, median; OS, overall survival; PD, progressive disease; R, resected; RR, response rate; SC, subcutaneous.

Heat shock proteins

Heat shock proteins carry or "chaperone" tumor antigens in the context of HLA-class I antigens, and can be isolated from other cells present in whole autologous tumor. The product referred to as HSPPC-96 (vitespen) consists of heat shock proteins associated with the gp96 antigen.[177] Although whole tumor cells are not involved in this approach, sufficiently large tumors are needed to obtain the quantity of HSPPC-96 needed for a reproducible product. Reported trials with this approach are summarized in Table 11.[178,179] The Phase III trial was conducted using a 2:1 randomization in stage IV melanoma patients to compare vitespen to a control group in which physicians were to choose among complete tumor resection, dacarbazine, temozolomide, or IL-2 for treatment.[179] Unfortunately, a satisfactory vitespen product could only be prepared for 141/215 patients (66%), and only 133 started therapy, a median of 41 days after randomization. Furthermore, only 86/117 patients randomized to the control arm remained in the study. This illustrates the challenges of an intent-to-treat design for patient-specific products. There

Table 11 Heat shock protein vaccine derived from autologous tumor

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Belli et al[178]	IV	42	Autologous gp96	Heat shock protein	SC or ID	2/28 RR, 2 CR	Increased antigen-specific immune response
Testori et al[179]	IV	215 133	Autologous gp96	Heat shock protein	SC	<20% 3-year OS	$P = 0.31$ by ITT analysis $P = 0.25$ by actual treatment
Testori et al[179]	IV	107 86	None	IL-2 or DTIC or TMZ		<20% 3-year OS	

Abbreviations: CR, complete response; DTIC, dacarbazine; DTH-T, delayed-type hypersensitivity reaction to tumor cells; ID, intradermal; IL, interleukin; ITT, intent-to-treat; OS, overall survival; RR, response rate; SC, subcutaneous; TMZ, temozolomide.

was no difference in progression-free or overall survival by the intent-to-treat analysis from the date of randomization, nor for patients who were actually treated per the protocol.

Autologous tumor cell lines

Theoretically, self-renewing, continuously proliferating autologous tumor cells may be the best antigen source for vaccine therapy.[180,181] Pure autologous tumor cells maximize the potential to present any and all MAA, including unique neoantigens, and assure lack of exposure to irrelevant allogeneic antigens that might actually diminish the immune response to important MAA. Autologous tumor cell lines can be a renewable source of tumor cells and tumor antigen for correlative laboratory experiments. The abilities to self-renew and proliferate are prerequisites for tumor stem cells and early tumor progenitor cells. Melanoma tumor stem cells have been characterized by phenotype and metastatic potential.[182–188] Such cells are characterized by inherent resistance to cytotoxic therapy and protection from the host immune system, and appear to be responsible for tumor recurrence at new and previous sites of disease despite other anti-melanoma therapies. Even though targeting a small subpopulation of tumor stem cells would not be expected to produce a rapid or dramatic effect on large sites of metastatic melanoma, this approach may be needed to prevent recurrence of melanoma. Animal experiments have shown that small tumors can be completely eradicated by targeting a small subset of cells rather than trying to target antigens expressed on the highest percentage of tumor cells.[189] Long-term clinical benefit in patients treated with vaccines targeting such self-renewing cells may be due to an immune response against melanoma stem cells.[190] In patients with extensive metastatic disease, other therapies would be needed to rapidly reduce the tumor burden of more differentiated melanoma cells while the autologous cell line vaccine was being prepared.

All surgically removed tissue is consumed during the diagnostic evaluation of primary melanomas and sentinel lymph nodes; therefore, vaccines derived from autologous tumor cell lines are an option only for patients who have gross metastatic disease. Thus, this approach cannot be used to prevent melanoma or as an adjuvant treatment to prevent recurrence of deep primary melanoma or patients with microscopic stage III nodal metastases. However, regional recurrences and distant melanoma metastases often occur in sites that are readily accessible to biopsy and/or surgical excision, which provides an opportunity to obtain fresh tissue from which to establish short-term cell cultures. Challenges related to this approach include having to establish a cell line for each patient, the inability to establish a cell line for every patient, and the time needed to establish the autologous tumor cell line and expand it to sufficient numbers for therapy. Better media for growing tumor stem cells would make this approach more attractive.

The labor intensiveness and complexity of establishing autologous cell lines has discouraged most investigators and companies from pursing such an approach, although it is technically feasible.[180,191,192] Clinical trials in which short-term autologous tumor cell lines were used as antigen sources for patient-specific vaccines have yielded encouraging long-term survival results (Table 12).[62,193,194] Two products have been tested: irradiated tumor cells and DC loaded with antigens from the irradiated cells. Comparison of successive trials and a small randomized Phase II trial suggest that survival is increased in patients receiving the DC vaccine.[193,194] The survival curves in each arm of the randomized trial were similar to those generated in the two previous single-arm

Table 12 Vaccines using antigens from autologous tumor cell lines

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Dillman et al[62]	23-III 51-IV	74	Autologous tumor cell line	GM-CSF and/or IFN-γ, IFN-α, BCG, none	SC	3/38 RR 20-month med OS 31% 2-year OS 29% 5-year OS	10/43 DTH-T conversion
Dillman et al[193]	14-III 40-IV	54	Autologous tumor cell line	DC GM-CSF	SC	0/15 RR 5-year med OS 73% 2-year OS 54% 5-year OS	13/53 DTH-T conversion
Dillman et al[194]	6-III 18-IV	24	Autologous tumor cell line	GM-CSF	SC	31% 2-year OS	
Dillman et al[194]	3-III 15-IV	18	Autologous tumor cell line	DC GM-CSF	SC	72% 2-year OS 1 CR	$P = 0.007$

Abbreviations: BCG, bacille Calmette–Guérin; CR, complete response; DC, dendritic cells; DTH-T, delayed-type hypersensitivity reaction to tumor cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; med, median; OS, overall survival; R, resected; RR, response rate; SC, subcutaneous.

trials. Long-term progression-free survival was documented for many patients who, despite various therapies, previously had been progression-free for no more than a few weeks to months.[190] One patient treated with the DC vaccine, who was progression-free for no more than a few weeks during her first year after being diagnosed with metastases to the cervical spine, had complete remission of SC metastases, which persisted for at least 5 years.[195] Cell lines were established for more than half of patients who submitted tumor samples, and the median time needed to establish and expand cell lines was about 4 months. In all of these trials, vaccine doses were injected weekly for 3 weeks, then monthly for up to 5 additional months. The major criteria for treatment were the successful establishment of the autologous tumor cell line and the managing oncologist referring the patient for vaccine therapy. A multivariate analysis suggested that the most important laboratory predictor of survival was resistance of the tumor cells to IFN-γ, a feature that is important for survival of tumor stem cells.[196,197] A confirmatory double-blind, randomized Phase III trial of this autologous dendritic cell-tumor cell product, melapuldencel-T, has been submitted to FDA, using stem cell media that decrease the time needed to establish a tumor cell line and increase the probability of establishing a cell line.

Cells from autologous melanoma cell lines have been molecularly engineered to secrete adjuvants. In a Phase I dose escalation trial, 20 patients were safely treated with autologous melanoma cells that had been gene-modified to secrete GM-CSF.[198] At least two Phase I trials have been performed utilizing cells from autologous melanoma cell lines that had been gene-modified to secrete GM-CSF. Toxicity was insignificant in both trials and clinical responses were reported for 1/30 patients[199] and 2/34 patients.[200] No further studies with these products have been reported.

Injections into existing tumors

An approach that obviates the need to administer an antigen product involves the injection of immune-stimulating substances into existing tumor lesions. For many years, investigators have injected various immune-stimulating substances, such as BCG and various cytokines, into tumor masses, not only for a local tumor response and regression of SC and in-transit metastases, but also in an effort to induce or enhance the endogenous antitumor immune response.[201] A variety of agents have been injected for this purpose, including BCG,[202] IFN-α,[203] IFN-γ,[204] IL-2,[205] and GM-CSF.[206] Local antitumor effects have been reported for many of these patients, but most of this experience has been in soft tissue disease. Unfortunately, the desired effects of distant tumor control and increased survival, an indication of induction of systemic anticancer immune effects, has not been demonstrated.

Fusion products for IT injection have been designed, and two have proceeded to Phase III trials (Table 13). The first, Allovectin-7™ (Vical, Inc., San Diego, CA, USA) (velimogene aliplasmid), is a DNA plasmid-liposome product that contains genes for an allogeneic HLA-B7 class I histocompatibility antigen and beta2-microglobulin.[207–209] The duration of disease control was disappointing, even though patients with an elevated lactic dehydrogenase

Table 13 Injections into autologous tumor

Author	Stage[213]	Number of patients	Antigen	Adjuvant or modifier	Route	Clinical efficacy	Other metrics
Stopeck et al[208]	U-III IV	52	Autologous tumor	HLA-B7 β2M liposome	IT	2/51 RR	4/51 regression of injected lesions
Bedikian et al[209]	U-III IV	133	Autologous tumor	HLA-B7 β2M liposome	IT	15/127 RR 1.6-month med PFS	Excluded patients with ↑ LDH
Senzer et al[210]	U-IIIB U-IIIC IV	50	Autologous tumor	Herpes simplex-GM-CSF	IT or IN	26% RR, 8 CR 50% 1-year OS	
Kaufman et al[212]	U-IIIB U-IIIC U-IV	≈290	Autologous tumor	Herpes simplex-GM-CSF	IT	26% RR 16% RR > 6-month 8-month med PFS 23-month med OS	$P < 0.001$ $P < 0.001$ $P < 0.001$ $P = 0.07$
Kaufman et al[212]	U-IIIB U-IIIC U-IV	≈145		GM-CSF	SC	6% RR 2% RR > 6-month 3-month med PFS 19-month med OS	

Abbreviations: β2M, beta-2 microglobulin; CR, complete response; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human lymphocyte antigen; IN, intranodal; IT, intratumoral; LDH, lactic dehydrogenase; med, median; OS, overall survival; PFS, progression-free survival; RR, response rate; SC, subcutaneous; U, unresectable.

were excluded. The second, OncoVEX™ (Amgen, Inc., Thousand Oaks, CA, USA) GM-CSF (T-Vec, talimogene laherparepvec) consists of an attenuated oncolytic herpes simplex virus transfected with a GM-CSF gene, which is believed to replicate preferentially in tumor cells.[210] Six responses were documented after initial disease progression, and some responses were not evident for many months after discontinuation of treatment. Importantly, tumor regressions were documented both for injected lesions and lesions that had not been injected.[211] The results for a randomized Phase III trial (OPTIM) appear to be positive,[212] although the control arm was SC GM-CSF rather than IT GM-CSF, which would have been a more appropriate control, and median overall survival was less than 2 years.

Summary

There are numerous strategies for immunotherapy with MAA, but, to date, no anti-melanoma vaccine has received regulatory approval. In clinical trials, vaccines have been associated with minimal toxicity while inducing or enhancing immune responses against specific MAA, but these effects do not necessarily translate into clinical benefit. It appears that various adjuvants and cytokine biological response modifiers can enhance immune responses to MAA, but there are insufficient comparative data to determine an optimal strategy with regard to incorporating these non-MAA vaccine components. However, the one FDA-approved therapeutic cancer vaccine, sipuleucel-T for prostate cancer, includes GM-CSF and antigen presentation by DC. Randomized trials using MAA from allogeneic melanoma cell lines have failed to provide a survival benefit for patients with resected disease.[149,151–155] In patients with measurable disease, the 15% response rate for autologous tumor cell vaccines[62,91,134,138,162,163,166–169,193] appears higher (38/284 vs 20/271, $P = 0.021$) than the 7% response rate for peptide vaccines.[94,125,126,130–134,136–139] Although there is no apparent difference in response rates for DC-based autologous tumor vaccines[91,134,138,167–169] compared to autologous tumor,[162,163,166] a recent randomized trial showed a survival advantage for patients treated with GM-CSF/DC-tumor cell vaccines compared to GM-CSF/autologous tumor cell vaccines.[194] Thus, it appears that autologous approaches will be required to optimize therapeutic benefit by inducing and/or enhancing patient-specific polyvalent immune responses. Experience with immunotherapeutics has shown that the most important endpoints for clinical trials are not tumor response rates or progression-free survival, but rather long-term overall survival, which can only be established by randomized, controlled, prospective trials.[15,70] Therapeutic vaccines are expected to enhance, rather than replace, other anti-melanoma immune therapies. Despite the failure of gp100 to add benefit to ipilimumab,[15] there is reason to believe that effective vaccines will work synergistically with monoclonal antibodies that interfere with T-cell checkpoint molecules.

Disclosure

The author and/or immediate family have stock in several companies whose products are mentioned in the article (Bristol-Myers Squibb, GlaxoSmithKline, Merck) and stock options in the company California Stem Cell, Inc., which is pursuing a patient-specific melanoma tumor cell vaccine product, having acquired the rights to the clinical data and intellectual property through an agreement with Hoag Hospital. The author also has received honoraria through speakers bureaus sponsored by Prometheus and Genentech. The author reports no other conflicts of interest in this work.

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