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Melanoma Vaccines: Mixed Past, Promising Future

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Synopsis

Cancer vaccines were one of the earliest forms of immunotherapy to be investigated. Past attempts to vaccinate against cancer, including melanoma, have mixed results, revealing the complexity of what was thought to be a simple concept. However, several recent successes and the combination of improved knowledge of tumor immunology and the advent of new immunomodulators make vaccination a promising strategy for the future.

Keywords

Melanoma; Vaccine; Immunomodulator; Adjuvant; Immunotherapy

Introduction

Vaccination is the earliest form of immunotherapy, corresponding to the discovery of the immune system itself, and infectious disease vaccinations are perhaps the greatest advance in the history of medicine. Vaccination for cancer has been much more difficult, although it had very auspicious and early beginnings. The first attempt predates our knowledge of the specific mechanisms involved in vaccination. In the late 19th century William B. Coley, a surgeon in New York at the time, was deeply saddened by the death of a 17 year old patient with metastatic Ewing's sarcoma, spurring him to begin to look for novel therapies to treat cancer. [12] He was struck by the case of a patient who had tumor regression after developing erysipelas.[3] He wondered if this phenomenon was due to the infection and, then and took it upon himself to begin inoculating patients with streptococcal organisms in 1891. He reported tumor regression in numerous patients. Coley continued to refine his therapy by using a heat-killed Streptococcus and Serratia combination which became known as Coley's toxin.[1] This administration of an immune adjuvant to the site of a superficial tumor is perhaps the first example of cancer vaccination, albeit using the existing tumor as antigen source. This strategy has interesting echoes in melanoma immunotherapy today, as is discussed below.

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More than a century later, Coley's vision of therapeutic immunology is a reality with the approval of several immune agents in melanoma, and additional promising therapies moving through the development pipeline. Vaccines, however, continue to have a difficult time demonstrating consistent benefit. While several negative vaccine trials have led many to discount the possibility of effective cancer vaccination, there are hopeful signs that continued research efforts are not only justified, but important components of the overall effort to develop effective therapies for melanoma and other cancers. After several decades of failed attempts at developing potent therapeutic vaccines, the first proof-of-concept cancer vaccine Sipeucel-T was approved for use in prostate cancer patients by the FDA in 2010, [4, 5] and as discussed below a trial of peptide vaccination in melanoma showed a significant survival advantage in the vaccine group.[6]

Increased knowledge of the immune system and its interaction with tumors along with a widening array of clinically available immunomodulators make the prospect of effective vaccines increasingly likely. Over several decades, breakthroughs in basic science and an increased knowledge about the role of antibodies in infection made many surmise that vaccination and the establishment of antitumor antibodies may be a possible strategy to cure cancer. [7] Many cancers have been studied extensively with respect to vaccine treatment but, perhaps, no cancer as extensively as melanoma.

Vaccine strategies are highly varied and may be characterized by the antigen source and the adjuvants and/or immune modulators given with the antigen. Much of the early period of vaccine development was characterized by substantial debate regarding the ideal antigen. Options vary from the simplest peptide vaccines to the most complex autologous whole-tumor cells. Each approach has advantages and disadvantages. (Table 1, Figure 1) Generally, simple peptide vaccines are easier to prepare, store, administer and monitor, but they offer the narrowest spectrum of tumor targets and are potentially relevant to fewer patients. More complex vaccines are the most likely to offer antigens that are relevant to any given patient, but are much more difficult to produce and administer. They also present substantial difficulties in monitoring immune responses since those responses may be extremely varied among different individuals. It is now becoming increasingly apparent that the nature of the antigen is only a part of the story, perhaps a small part. What may be more significant is the context of the immune stimulation in terms of both patient characteristics and immunologic adjuvants or other immunomodulators. Modification of these factors could prove much more important than the specific source of antigen for a vaccine.

Autologous Melanoma Vaccines

In autologous vaccines, the patient's own tumor is used as the antigen source. There are several significant advantages to autologous vaccines. First, since the source of antigen is the patient's own tumor, there is, by definition, a HLA-type match ensuring that antigen presentation is adequate. Second, they are likely to contain antigens that are unique to that particular patient and, thus, are personalized. However, the derivation of vaccine from an autologous source is daunting from a practical standpoint, and there is little consensus about the optimal method. In addition, sufficient tumor must be available in order to provide raw material for the vaccine. Thus, patients who have low tumor burden or those who have

undergone complete resection of their disease are not candidates for this type of therapy. Finally melanoma metastases may be genetically and antigenically heterogeneous within any given patient.[8] This could create a situation in which a single metastases, used for vaccine preparation, would not contain enough antigenic diversity to lead to a protective response against every metastatic focus.

An early autologous vaccine to undergo phase 3 trial was of a heat shock protein gp96 peptide complex (HSPPC-96) vaccine derived from autologous tumor.[910] Heat shock proteins are soluble, intracellular “sticky” proteins and bind peptides including antigenic peptides generated within cells. They are thought to play an important role as chaperones for antigen presentation, required for instructing the antigen-specific antitumor immune responses. When heat shock proteins are purified from tumors, non-covalent complexes of these proteins along with peptides expressed by the tumor cell are obtained. When injected into the skin, heat shock proteins may interact with antigen presenting cells through CD91, a heat shock protein receptor. This leads to re-presentation of heat shock protein-chaperoned peptides by Major Histocompatibility (MHC) proteins as well as stimulation and maturation of antigen presenting cells. Despite their extracellular location upon administration, the tumor-associated peptides bound to gp96 may gain access to presentation on MHC class I (cross-priming), important for activation of antitumor killer T cell responses.

The phase 3 trial studied in 322 patients at 71 centers, and randomly assigned subjects 2:1 to receive either the vaccine or physician choice of dacarbazine, temozolomide, interleukin-2 (IL-2), or complete tumor resection.[10] There was no difference in overall survival, although patients with M1a or M1b disease who received more than 10 doeses vaccine survived longer than those receiving fewer treatments. While this type of analysis may be biased, the authors here attempted to control for such bias using “landmark” analysis. In addition, pre-clinical models suggested that at least 4 doses of the vaccine would be required to stimulate a protective immune response. The authors concluded that this M1a and M1b subset of patients may be candidates for further study, and this vaccination strategy remains an area of interest.[11] However, there is also a theoretical concern that chronic stimulation with antigen may lead to tolerance, rather than effective immunity and the ideal duration of cancer vaccination in patients remains unclear. Othy Elsevier er whole-cell autologous vaccines include those developed by Berd and colleagues, subsequently evaluated in clinical trials as M-Vax. [12] This vaccine uses the patients’ irradiated melanoma cells, which are modified with dinitrophenyl, a hapten, which previous research suggests helps improve antigen visibility.[13] The treatment program consists of multiple intradermal injections of DNP-modified autologous tumor cells mixed with bacille Calmette-Guerin as an immunological adjuvant. Administration of DNP vaccine to patients with metastatic melanoma induces the development of inflammation in metastases. [14] In a phase 3 trial, which has completed accrual, patients were assigned to M-Vax or placebo followed by low-dose IL-2. Primary endpoints are best overall tumor response and survival at 2 years. The trial was suspended in 2009, and results have yet to be published. Another autologous, whole-cell strategy was developed by Dillman and colleagues and consists of resected tumor cells which are cultured in vitro and irradiated prior to administration.[15] This vaccine has not been evaluated in phase 3 studies. (Table 2)

An alternative to whole-cell autologous vaccines is the use of tumor lysates pulsed onto dendritic cells. Dendritic cells (DCs) are specialized leukocytes that are the most potent generators of de novo antigen-specific immune responses. While several immune cells are capable of presenting antigens to activate effector cells, the use of dendritic cells as an immune adjuvant for tumor vaccination may provide a more potent source of immune activation. Indeed, a randomized phase II comparison of autologous tumor antigen-pulsed dendritic cells versus autologous whole cell vaccination showed a survival advantage in the dendritic cell arm (HR 0.27, 95% CI 0.098–0.729).[16] Additional considerations of dendritic cell vaccines are considered below.

Allogeneic Vaccines

The use of vaccines derived from stock melanoma cell lines has several theoretical advantages over autologous vaccines. First, the vaccine may be prepared in advance of a patient's need for treatment, eliminating the delay in therapy required when deriving cells from resected tumors. Second, the cells used in the vaccine can be pre-selected for high antigen expression. Third, the presence of foreign, allo-antigens could stimulate a more potent immune response than that engendered by autologous tumor. Several allogeneic whole cell or whole cell lysate vaccines have been evaluated in Phase 3 clinical trials including those using vaccinia melanoma cell lysate (VMCL), vaccinia melanoma oncolysate (VMO), Melacine, and Canvaxin.

The VMCL vaccine employs a single melanoma cell line, which is lysed in vitro using vaccinia virus and injected intradermally. Infection of melanoma cells with vaccinia virus could provide additional stimulation of antitumor immunity by introducing viral pattern recognition ligands in the vaccine. The Phase 3 trial included 700 patients in Australia and was randomized 1:1 against observation as control.[17] Data were analyzed with a median of 8 years of follow up and demonstrated a non-significantly increased overall survival in the vaccine group (5-year OS 60.6% vaccine vs. 54.8% control, HR 0.81, 95% CI 0.64–1.02, p=0.068). Notably, the patients in the control arm also showed longer survival compared to that expected for the time, a seemingly common phenomenon in melanoma vaccine trials.

The next large trial of a whole-cell lysate was the VMO vaccine. This consisted of a lysate of four melanoma cell lines and a trial of 250 patients from 11 North American institutions who were randomized to vaccine or vaccinia only. A study of the long-term results of the trial was published in 2013 and demonstrated no indication of benefit (or harm) for the vaccine group.[18]

A third Phase 3 trial was conducted the Southwest Oncology Group and evaluated Melacine, a lysate of two melanoma cell lines combined with a detoxified Freund's adjuvant (DETOX).[19] Freund's adjuvant is comprised of mycobacterial components which are potent immune stimulators. The study enrolled 689 subjects who were randomized to vaccine or to observation. The study showed a hazard ratio of 0.84 in favor of the vaccine arm, but the difference was not statistically significant. However, examination of subgroups with cross reactivity with the HLA types presented in the vaccine, particularly HLA-A2 and HLA-C3 positive subjects, showed a significant advantage.[20] It is interesting to speculate that if

allogeneic tumor cells were to be employed for further tumor vaccine development, perhaps some level of matching to the recipient's MHC could improve the efficacy of the vaccine. Melacine was approved for use in Canada for Stage IV melanoma, based upon improved quality of life compared to combination chemotherapy. It was not approved in the United States or elsewhere.

The largest Phase 3 clinical experience for an allogeneic whole cell vaccine is with Canvaxin.[21] Canvaxin consists of 3 melanoma cell lines, selected for their spectrum of antigen expression and irradiated at doses so that the cells would be live but replication incompetent upon administration. The vaccine showed excellent results in Phase 2 trials and was evaluated in two large, randomized trials in resected Stage III and Stage IV melanoma. Subjects were randomized to vaccine or placebo with both arms receiving bacillus Calmette-Guerin (BCG) as an immune adjuvant with the first two doses. The trial in Stage III patients enrolled 1,160 subjects, and the Stage IV study enrolled 496.[22] Both trials were halted after interim analyses for futility as there was no significant difference from placebo.

Interestingly again, the Canvaxin studies demonstrated outcomes in the vaccine arms that were very similar to those predicted by Phase 2 results.[23] These vaccine survival times, which were superior to historical controls and appeared better than those of other contemporary adjuvant therapy trials, were no better than those of the control group who only received BCG. While BCG had been evaluated as an immune adjuvant in melanoma before, prior trials used relatively ineffective administration schemes and were inadequately powered to evaluate the therapy. It has also been noted that survival times of the control arms of the study were longer than the vaccine arm, though these values were not technically statistically different at the threshold of an interim analysis (Stage III $p=0.040$, Stage IV $p=0.086$). It is possible that this vaccine strategy and others could be improved with optimization of dosing and schedule since chronic, repeated inoculation with tumor antigens may lead to less robust antitumor responses and instead induce tolerance to tumor antigens. However, the overall survival of all subjects both in the treated and control arms was longer than expected indicating participation in the protocol was not harmful.

Ganglioside Vaccines

Gangliosides are glycolipids that are differentially expressed in several cancer types. Thus, they are also potential targets as tumor-specific antigens for immune therapy and/or vaccination.[24] Two large Phase 3 trials have been performed using the GM2 ganglioside. The first of these was conducted by the Easter Cooperative Oncology Group (ECOG) in collaboration with the North American cooperative groups.[25] This study compared GM2 vaccine (consisting of the ganglioside coupled to keyhole limpet hemocyanin (KLH) and the QS-21 adjuvant) to high-dose interferon-α2b. The trial enrolled 880 patients and demonstrated superior relapse-free and overall survival in the interferon arm.

The second trial was performed by the European Organization for Research and Treatment of Cancer (EORTC) and randomized subjects to either GM2-KLH-QS21 vaccine or observation.[26] The trial included 1314 subjects and was halted at the second interim analysis for futility as early follow up showed no suggestion of a relapse-free survival advantage to

the vaccine group and a possible overall survival disadvantage. With additional follow up, the survival curves are almost overlapping; indicating that while there was no harm by vaccination, there was clearly no benefit.

Peptide Vaccines

Peptides are perhaps the most commonly used tumor-associated antigen (TAA) source for melanoma vaccines. These protein fragments are normally presented in the context of Major Histocompatibility (MHC) proteins to be recognized by T-lymphocytes. Cancer-specific peptides were identified in melanoma over two decades ago. [27, 28] The peptides are easily produced, stored and administered. Because of the narrow spectrum of immune responses possible to the peptides, immunological monitoring of peptide vaccination is relatively straightforward. In part as a result of these advantages, several dozen peptide vaccine trials have been performed in melanoma including three randomized Phase 3 trials.[6, 29, 30]

However, the simplicity of these antigens is also a potential weakness. Each peptide generally contains only one epitope for the immune system to target, and peptides are limited in compatibility to HLA-matched patients. Since HLA-A2 is a relatively common allele in melanoma patients, most peptide vaccine studies conducted to date have been limited to peptides which bind HLA-A2 and therefore have only been available to HLA-$A2^+$ patients. In addition, with a narrow spectrum of target epitopes, the potential for antigen loss through immune selection and survival of antigen-negative clones is a concern. With identification of numerous potential peptide antigens with histocompatibility for several HLA types, some of this limitation has been at least partially overcome.[31] It is still not clear whether those improvements will translate into increased effectiveness in the clinical setting. Despite the relative ease of peripheral blood monitoring of immunization, correlations of successful immunization by such monitoring and clinical outcomes have been limited or even inverse (i.e. lower peripheral blood responses in clinical responders).[32, 33] It is also quite possible that the in vitro assays used thus far to monitor peripheral blood responses lack the relevant immune readouts that correlate to clinical benefit.

The results of three Phase 3 trials of peptide vaccination have been mixed. All three trials included a systemic immunomodulating drug and examined the effect of adding peptide vaccines. One examined high-dose interleukin-2, an approved therapy for metastatic melanoma, with or without gp100 peptide and incomplete Freund's adjuvant in patients with measurable metastatic disease. [6] The multicenter trial enrolled 185 subjects and demonstrated a significant improvement in the overall response rate (16% vs 6%, p=0.03) and progression free survival (2.2 vs 1.6 months, p=0.008) in the vaccine group. The trend in median overall survival was improved in the gp100 group compared to the IL-2 alone group, but was not quite statistically significant (17.8 vs 11.1 months, p=0.06). Thus, this is the first peptide vaccine to show a clinical benefit in a phase 3 trial.

Another trial examined the role of both a peptide vaccine and GM-CSF as adjuvant therapies in patients with resected Stage III and IV melanoma. This trial enrolled 815 patients who were assigned to one of the multiple arms of the trial depending on their HLA-A2 status. Only HLA-$A2^+$ patients (n=398) were randomized to receive vaccine or peptide placebo. [30]

The study has only been reported in abstract form, and mature results are expected sometime this year. Preliminary results reported a relapse free survival advantage with GM-CSF, but this finding has lost statistical significance over time and was not accompanied by an overall survival benefit. The addition of peptide vaccination did not appear to improve overall or relapse-free survival.

The third Phase 3 trial involving peptide vaccination was conducted within a larger trial evaluating the efficacy of CTLA-4 blockade with ipilimumab.[29] The trial had three arms: gp100 peptide alone (n=136), ipilimumab alone (n-137) and the combination (n=403). The trial showed a significant survival advantage to ipilimumab but no advantage to peptide vaccination. This study provides an interesting contrast to the trial of peptide vaccination in the context of interleukin-2, which did show a benefit, and highlights the paramount importance of context to determine the clinical impact of an immune therapy. The mixed results of these three peptide vaccine trials indicate much additional work is required to optimize vaccine strategies employing peptides.

Immune adjuvants and immunomodulators

Although our review to this point has been focused on the types of antigen sources that have been used in melanoma vaccines; an equally important, if not more important, consideration may be the context of immunization with regard to the patient population, the frequency of dosing, and the immune adjuvant and immunomodulators that are given with the vaccine. As our knowledge of the immune system and its interaction with melanoma has improved, new opportunities for rational immunization improvement have arisen. (Figure 2)

Vaccines, including many infectious disease vaccines, are given with non-specific immune adjuvants to boost immunologic responses. Vaccine adjuvants serve to increase recognition of antigens, amplify immune responses, and modulate those responses. Many early trials used traditional vaccine adjuvants, such as incomplete Freund's, while other used live or killed microorganism components such as BCG [21] or detoxified mycobacterial cell walls (e.g. DETOX). [19] The discovery of toll-like receptors and elucidation of their importance in the development of immune responses has led to their ligands being incorporated into some vaccination strategies.

One of the most promising areas of enhancing vaccine responsiveness is the use of dendritic cells (DC) as immune adjuvants.[34] These cells are primarily responsible for generation of new responses in vivo. Dendritic cells may be obtained from bone marrow, but now for clinical trials are most commonly derived from peripheral blood mononuclear cells. Early studies of DC vaccines showed very encouraging response rates among relatively advanced melanoma patients. Nestle et al. utilized autologous dendritic cells cultured in GM-CSF and interleukin-4 and then pulsed with tumor lysates or peptides before infusion into 16 patients with advanced melanoma. [35] Two out of 16 patients had a complete response (CR) while 3 out of 16 patients had a partial response (PR). More recently a DC vaccine was evaluated in a Phase 3 trial of 108 subjects.[36] The DC in this trial were pulsed with peptides and the control arm was treated with dacarbazine chemotherapy. There was no indication of benefit

to vaccination, and the trial was closed at the recommendation of the Data Safety Monitoring Board.

It has now become clear, however, that not all DC are the same. In fact, depending on the state of maturation of the DC, and the cytokines and chemokines they produce, the cells may not traffic well to present antigen, and may actually skew immune responses toward tolerance. This new knowledge is being incorporated into the design of current DC vaccine trials in melanoma and other cancers. [37]

In a recent pilot trial, dendritic cells were grown with GM-CSF, interleukin-4, tumor necrosis factor (TNF) and CD40 ligand (CD40L). [38] Both TNF and CD40L are important for maturation of dendritic cells. The DC were then pulsed with allogeneic, killed melanoma cells. Of 20 advanced melanoma patients vaccinated, 1 showed a CR while another patient had a PR. This study was proof of concept that HLA restriction could be overcome by loading mature dendritic cells with allogeneic killed melanoma cells.

Additional DC vaccine trials are planned, including two upcoming phase 3 trials. There is hope that improved understanding of DC biology will increase the benefits of vaccination for these studies.

Intralesional Immunotherapy: Tumor (in situ) as vaccine

We have characterized Coley's intralesional injection of bacterial toxins as "vaccination," although the injection itself did not contain tumor antigens. Rather, the antigens were already present, and he simply added the adjuvant. A very similar strategy was pursued by Morton and colleagues starting in the 1960s using BCG.[39] In the first such case, a woman presented with numerous in-transit metastases on her upper extremity. Her other arm had been paralyzed by polio, and so she declined an amputation. Morton, who was in the early stages of developing an allogeneic whole cell vaccine at the time, elected to inject BCG into her melanoma lesions, using the lesion as vaccine. Subsequently, all of her lesions, both injected and non-injected, regressed completely. She remained free of disease for many years thereafter.

BCG was explored with great enthusiasm after early publications of melanoma regression. [40] Subsequent reports documented regression of non-injected metastases, even at visceral sites.[41] However, severe toxicities, including disseminated intravascular coagulation, anaphylaxis and death were reported and enthusiasm for the technique waned.[4243] A few centers continue to use BCG in this way, though at greatly reduced doses, and it is included in the National Comprehensive Cancer Network guidelines as an option for in-transit disease.[44] A recent report used the combination of BCG and imiquimod, the topical toll-like receptor agonist, and demonstrated regression of extensive areas of in-transit metastases with minimal toxicity.[45]

Although radiation is generally understood to be immunosuppressive, it may also have local immunostimulatory effects such as increased antigen expression and/or release. Local radiation therapy may facilitate development of systemic immunity, something known as the abscopal effect. An example of this was recently reported in the context of a patient

previously treated with ipilimumab.[46] Although it is difficult to rule out the possibility of a delayed clinical response to checkpoint blockade, numerous similar examples, and biologically promising mechanisms suggest radiation may be a fruitful avenue for further study to stimulate antitumor immune responses. Several clinical trials are currently evaluating local therapies such as radiation or regional chemotherapy administration as adjuncts to systemic immunotherapies.

Another developing local immunotherapy is talimogene laherparepvec (T-VEC), an oncolytic herpes simplex virus, which was engineered to express granulocyte colony-stimulating factor. [47] Tumor destruction is thought to stem from both the oncolytic effect of the herpes simplex virus which causes the melanoma cell to lyse and GM-CSF which is expressed upon infection and may attract and activate dendritic cells to present antigens from the tumor lysate inducing an antitumor immune response more systemic immune response. [47–49] Recently, the results of the OPTiM trial, a phase3 randomized control trial comparing T-VEC to GM-CSF alone, was presented at the American Society of Clinical Oncology. [50] Unresectable Stage IIIB/C or Stage IV patients with injectable cutaneous, subcutaneous or nodal lesions were randomized to intralesional T-VEC or subcutaneous GM-CSF. The objective response rate (ORR) with T-VEC was 26% with 11% complete response (CR) compared to GM-CSF alone which had a 6% ORR and 1% CR. Durable response rate for T-VEC was 16% compared to 2% for GM-SCF. Interim overall survival analysis showed a trend in benefit toward T-VEC. Thus, T-VEC is the first such melanoma local immune therapy to show benefit in overall survival in a phase 3 randomized clinical trial in melanoma.

Future

Although many of the clinical trials evaluating melanoma vaccines have not demonstrated a benefit, and some have even raised concerns that vaccination can be harmful, there are many reasons to support that vaccines will be an important component of optimal therapy for melanoma in the future. Successful deployment of melanoma vaccines was probably hampered by the fact that they were the first immune therapies to be developed. Introduction in an era of less sophisticated knowledge of tumor immunology and clinical trial design led to studies conducted with insufficient sample sizes or in populations that could not be accurately stratified due to inadequate staging. Despite these challenges some successes have kept cancer vaccination research alive. Hopeful signs include two positive randomized trials in the peptide/interleukin-2 study [6] and the T-VEC trial [49] as well as the availability of new and increasingly diverse immunomodulators.

Perhaps the most important challenge that faces vaccine development is the identification of reliable surrogates for clinical outcomes. Traditionally clinical development begins with pre-clinical investigations and many early phase trials use surrogates, such as immune endpoints to select therapies to take forward. This model appears to be unreliable in melanoma vaccines. For example, pre-clinical models had suggested a strong synergy between peptide vaccination and checkpoint blockade with anti-CTLA-4 antibody but was not borne out by the completed Phase 3 trial. Numerous immune surrogates have been used to guide modification and combination of immune therapies. One example is that of GM-CSF, which

leads to improved antibody responses, but not to improved clinical outcomes in multiple randomized trials. [51] Another is the measurement of number of circulating antigen-specific lymphocytes. In a study performed at the National Cancer Institute, several cytokines were added to peptide vaccines and peripheral blood responses were monitored. The only group with significant clinical responses, that in which peptides were combined with interleukin-2, had decreased numbers of circulating antigen-specific cells.[32] Development of other measures of productive anti-tumor responses is needed and may include evaluation of tumor material to assess immune infiltrates or down regulation of immunosuppressive factors. The current wealth of agents that are potentially useful as adjuncts to vaccination are most welcome, but will require improved means of assessment to sort through. Reliance on large randomized trials with survival endpoints will be too slow to provide answers in the time frame many of our patients need.

Coley's vision of curing cancer through vaccination has become a reality for some patients.[4] The advent of new immune agents and new means of applying immunotherapies make the prospect of extending benefits to more patients increasingly likely. We should remember Coley's pioneering spirit, including the courage and tenacity he needed to inject patients with his toxin as we enter a critical second phase of melanoma vaccine development.

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Key Points

1. Numerous vaccine antigen sources have been evaluated, and each has advantages and disadvantages.
2. Most phase 3 vaccine trials to date have not shown clinical benefit, although there have been a few successes and suggestions of activity.
3. Novel vaccine strategies using the tumor in vivo as an antigen source bypass the need to define tumor antigens; allow simple, yet personalized therapy; and are perhaps the most interesting current method of vaccination.
4. Numerous immunomodulators are now available or in development that could enhance vaccination.
5. Adequate immune monitoring with clinically meaningful surrogate endpoints will be critical for additional vaccine development.

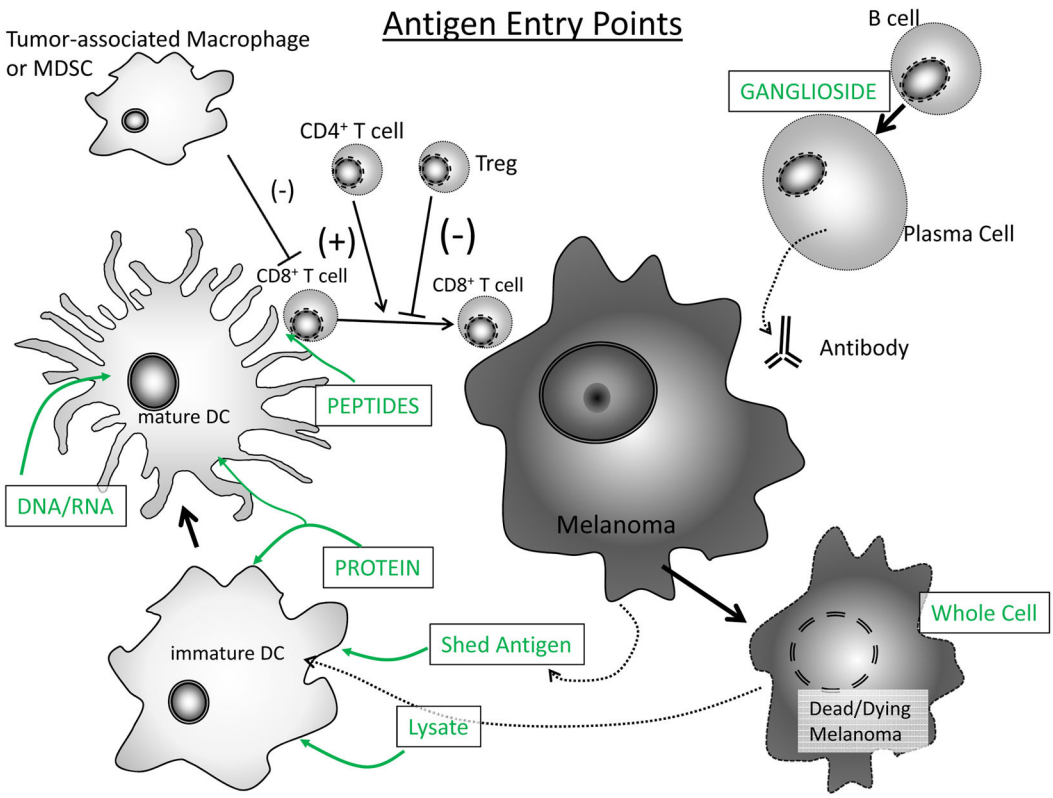

Figure 1.

Antigen Entry Points: Numerous options exist for vaccine antigen and each may enter at a different point in immune response development. Peptides, the simplest antigen, enter at the immunologic synapse between T-cell and antigen presenting cell and require not processing. Others require uptake, processing and presentation to be recognized. Antigen may also be released from dead or dying cancer cells, either injected as a prepared vaccine or produced from existing metastases.

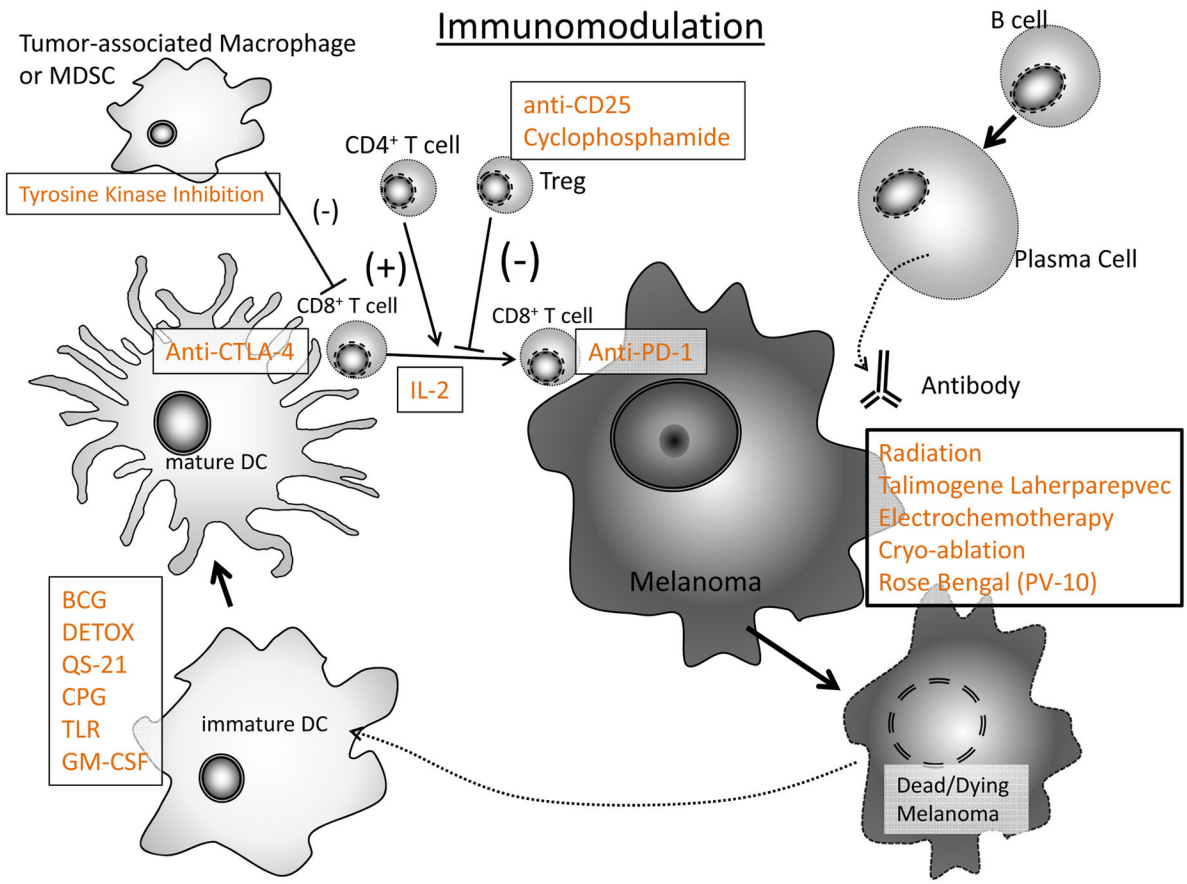

Figure 2.

Immunomodulation: The spectrum of available agents to boost or modify immune responses has increased dramatically in recent years and can interact with the system in numerous places.

Table 1

Antigen Types

Antigen Type	Pros	Cons
peptide	Easy preparation Easy storage Simple monitoring	Narrow antigen spectrum HLA restriction Many limited to Class I presentation
protein	No HLA restriction Relatively simple preparation	Requires antigen cross-presentation to sensitize CD8 T cells Still fairly narrow antigen spectrum
DNA/RNA	Relatively simple preparation No HLA restriction	May be difficult to generate both CD4 and CD8 responses Requires delivery of genetic material
ganglioside	Immune monitoring (antibody response) is relatively simple	Relies largely on humeral response for effect
lysate	Broad antigen spectrum	Preparation/storage more difficult
whole cell	Most diverse antigen spectrum	Preparation/storage most difficult

Table 2

Phase 3 trials of melanoma vaccines

Author	year	n	Arms	HR	CI	p-value	Notes:
Kirkwood	2001	880	HD-IFN vs GM-2KLH/QS-21	RFS: 1.47 OS: 1.52	(1.14–1.90) (1.07–2.15)	0.0015 0.009	
Hersey	2002	700	VMCL vs. Obs	RFS: 0.86 OS: 0.81	(0.7–1.07) (0.64–1.02)	0.17 0.068	
Sondak	2002	698	Melacine vs. Observation	0.84 (rec or death)	(0.66–1.08)	0.17	HLA-A2^{+}, C3^{+} significant
Schadendorf	2006	108	DC+ peptides vs. DTIC	OS		0.48	AWD Stage IV
Morton	2007	1160	Canvaxin vs. Placebo	1.26		0.040	Stage III
Morton	2007	496	Canvaxin vs. Placebo	1.29	(0.97–1.72)	0.086	Stage IV
Testori	2008	322	hsp96 vaccine vs. BAC			0.316	Stage IV
Hodi	2010	676	ipilimumab vs. peptide vs. both	OS: 1.04		0.76	AWD
Lawson	2010	398	GM-CSF +/− peptides	OS: 0.94 DFS: 0.93	(0.70–1.26) (0.73–1.27)	0.670 0.709	
Schwartzentruber	2011	185	IL-2 +/− peptides	RR: PFS: OS:		0.03 0.008 0.06	AWD, all favor vaccine
Eggermont	2013	1314	GM2-KLH/QS-21 vs Observation	RFS: 1.03 OS: 1.66	(0.84–1.25) (0.90–1.51)	0.81 0.25	
Suriano	2013	250	VMO vs. Vaccinia	OS		0.70	
Unpublished	2013	?	MAGE-A3 vs. placebo	OS		NS	subgroups pending