



*Immunotherapy holds  
promise as an innovative  
and more effective approach  
for treatment of melanoma.*

Jennifer Eisenpresser. *Cityscape at Night*. Oil on canvas, 52" × 58".

## Immunotherapy for Melanoma

*Christina J. Kim, MD, Sophie Dessureault, MD, PhD, Dimitry Gabrilovich, MD, PhD,  
Douglas S. Reintgen, MD, and Craig L. Slingluff, Jr, MD*

**Background:** Immunotherapy for cancers is based on the principle that the host's immune system is capable of generating immune responses against tumor cells. Currently available treatments for melanoma patients are limited by poor response rates. Interferon- $\alpha$  has been approved for adjuvant treatment of stage III melanoma with improved survival. New and more innovative approaches with improved efficacy are needed.

**Methods:** We reviewed the various new approaches and strategies for immunotherapy for the treatment of melanoma.

**Results:** Immunotherapy for melanoma includes a number of different strategies with vaccines utilizing whole cell tumors, peptides, cytokine-mediated dendritic cells, DNA and RNA, and antibodies.

**Conclusions:** A variety of approaches can be used to enhance immune reactivity in patients with melanoma. Preclinical studies and initial clinical trials have shown promising results. Additional clinical trials are currently ongoing to evaluate the clinical efficacy and the associated toxicities of these novel treatment strategies.

### Introduction

From the Department of Surgery and Cutaneous Oncology Program at the H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida (CJK, SD, DG, DSR), and the Department of Surgery at the University of Virginia Health Science Center, Charlottesville, Virginia (CLS).

Submitted July 31, 2001; accepted September 29, 2001.

Address reprint requests to Douglas S. Reintgen, MD, Cutaneous Oncology Program, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33612-9497.

Dr Reintgen is on the Speaker's Bureau for Schering Plough Oncology. Dr Slingluff receives grant/research support from Chiron Corp, Immunex Corp, and Argonex Pharmaceuticals. No significant relationship exists between the other authors and the companies/organizations whose products or services may be referenced in this article.

The incidence of melanoma is increasing at a dramatic rate worldwide. One in 75 Americans will have developed melanoma in the year 2000.<sup>1</sup> Most patients present with early-stage disease that is potentially curable with surgery alone in up to 90% of patients. However, the prognosis for patients with more advanced disease with involvement of regional lymph nodes or distant metastasis is poor, with median survival rates of 24 and 6 months, respectively. In the last few decades, there have been significant advancements in the identification of patients at high risk of developing recur-

rences. A number of clinical and histopathologic variables, including thickness and ulceration of primary tumors, the number of lymph node metastasis, and sites of distant metastatic disease, are predictors of survival.<sup>2</sup> Furthermore, lymphatic mapping and sentinel lymph node biopsy techniques have allowed for the identification of patients with regional lymph node metastasis at an even earlier stage of micrometastatic disease. This new surgical procedure combined with a more detailed pathologic examination of the sentinel lymph node provides more accurate staging.<sup>3</sup> Therapeutic intervention in high-risk patients as well as in those with progressive disease could potentially confer long-term benefits.

Current available treatments for patients with melanoma are limited. In patients with metastatic disease, chemotherapy, biologic therapy (eg, interleukin 2 [IL-2], tumor-infiltrating lymphocytes, lymphokine-activated cells) and combination biochemotherapy have yielded low response rates of approximately 20% to 30%.<sup>4,5</sup> For patients who are at a high risk of developing recurrent disease, treatment with interferon  $\alpha$ 2b (IFN- $\alpha$ ) in the adjuvant setting has been shown to improve disease-free survival (3.8 vs 2.8 years) compared to observation alone in patients with stage IIb or III disease.<sup>6,7</sup> IFN- $\alpha$  is now an FDA-approved therapy for adjuvant treatment of patients with resected stage III melanoma. However, toxicity to IFN- $\alpha$  is significant, with moderate to severe flu-like symptoms limiting the completion of therapy in nearly 25% of patients. New and innovative approaches and more effective therapies for melanoma are needed. One approach that holds promise is immune therapy.

## Historical Perspective

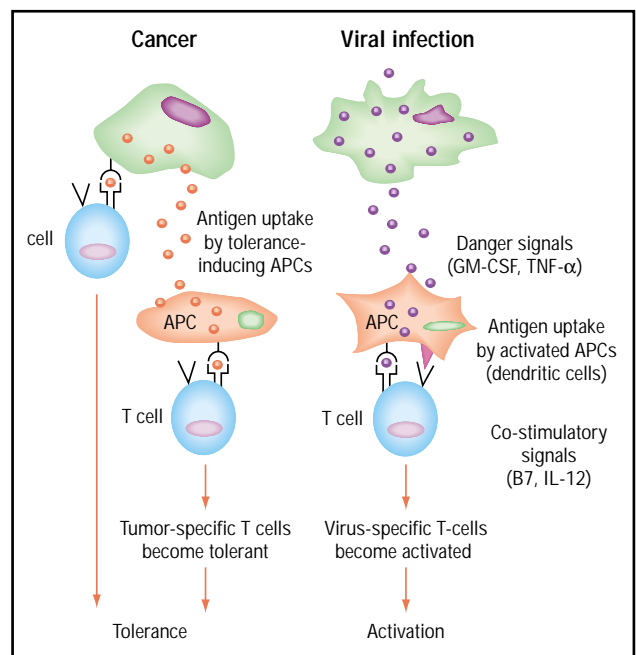
In the 1890s, William Coley first administered a vaccine to treat cancer patients by injecting live bacteria. A review of 30 patients treated with Coley's toxins was published in 1953.<sup>8</sup> The treatment was based on the observation that neoplasms regressed following acute bacterial infection, namely streptococcal infections that produced erysipelas. Examples of complete disappearance without recurrence were documented in patients with extensive, inoperable cancers. However, with the advent of radiation and chemotherapeutic agents, the focus of cancer treatment shifted away from immune activation therapies.

In the 1960s, interest in immunizing against tumors had a rebirth with the observation that irradiated tumor cells in adjuvants such as bacille Calmette-Guérin (BCG), *Corynebacterium parvum*, and viruses were efficacious in eradicating some tumors.<sup>9,10</sup> With advances in molecular biology and cellular immunolo-

gy, much progress has been made in the development of cancer vaccines and immunotherapies. In an effort to enhance immune reactivity, tumor cells have been gene-modified to express various cytokines (interleukins, tumor necrosis factor [TNF], granulocyte-macrophage colony-stimulating factor [GM-CSF]), and co-stimulatory molecules (B7.1 and B7.2). The identification of a number of shared melanoma-specific antigens and the peptide fragment epitopes, using molecular cloning techniques, have enabled new approaches targeting tumor cells with greater potency and specificity. These strategies have been tested in clinical trials to treat patients with melanoma. These novel immune therapies in patients have further advanced our knowledge in tumor biology and the interactions with the host immune system.

## Vaccines as Cancer Therapy

Cancer vaccines are based on the principle that the immune system of the host is capable of generating immune responses and stimulating defense mechanisms against tumor cells. Although vaccine therapies have been used for centuries to prevent infectious diseases, there are particular challenges in cancer therapy in that the tumor cells have already been exposed to the immune system and have induced a certain degree of tolerance within the host (Figure). Tumor cells are generally considered to be "poor immunogens." The approaches to vaccine therapy have been either to heighten the immune recognition of tumor cells by



Immune responses to tumor antigens and viral infection. From Pardoll DM. Cancer vaccines. *Nat Med.* 1998;4(5 suppl):525-531. Reprinted with permission. ([www.nature.com/nm](http://www.nature.com/nm)) APCs = antigen-presenting cells.

Table 1. — Types of Melanoma Vaccines

Whole cell tumor vaccines	DNA vaccines
Peptide vaccines	RNA vaccines
Cytokine-modulated vaccines	Antibody
Dendritic cell vaccines	

making the tumor cells appear more “foreign” to the host immune system or to enhance the host response against tumor cells by increased lymphocytic activation.

In general, the goal of vaccine therapy is to introduce the immunizing “foreign” antigen to antigen-presenting cells (APCs) and to elicit long-lasting immunologic memory from T cells. Antigens may be introduced to APCs by various methods, such as exogenous delivery of antigens (whole cell and protein vaccines), direct transduction (recombinant viral and bacterial vaccines), and direct loading of peptide fragments onto APCs. *Active* immunization elicits specific or nonspecific reactivity against a tumor antigen by stimulating the patient’s own immune system, while *passive* immunization is the administration of antitumor antibodies or cells against a tumor antigen.

Melanomas are some of the most immunogenic tumors known and are therefore a good model system for immune-based vaccine therapies. Important developments in melanoma vaccines over the years include (1) the understanding of antigen presentation and the role of T-cell activation in generating an antitumor response, (2) the identification of a number of melanoma-specific tumor antigens that are shared by tumors from different patients, and (3) the elucidation of T-cell recognition of specific peptide fragments that are expressed on the surface of tumor cells. Currently, a variety of strategies of tumor immunizations are actively being studied with ongoing clinical trials and are the focus of this article.

## Strategies for Targeting Melanoma

Generating an effective immune response requires a complex set of events involving both humoral (antibody) and cell-mediated interactions. Several strategies have been used to enhance the immune response to target melanoma tumor cells. Early on, it was found that nonspecific immune reactivity could be generated by injecting tumor cells with bacteria or viruses. More recent strategies for melanoma vaccine therapies include the use of cytokines and growth factors, whole tumor cells, whole tumor cell vaccines, peptides, antibodies, APCs such as dendritic cells (DCs), DNA, and RNA (Table 1). The identification of melanoma-associ-

ated antigens has allowed the strategy of inducing antigen-specific recognition of tumor cells. A number of genetically modified tumor vaccines are aimed at enhancing immunostimulatory mechanisms within the host, including tumor antigen(s) recognition, increased cytokine release, activation of APCs, and the use of co-stimulation to recruit activated immune reactive cells.

## Whole Tumor Cell Vaccines

Whole tumor vaccines have the advantage of immunizing the patient with diverse antigens that are present on the tumor surface without knowing the exact antigen(s) that may be responsible for tumor rejection, ie, whole tumor cells are used as the source of antigen(s). Autologous tumor cells are derived from tumors surgically resected from patients who will be vaccinated with their own manipulated tumor cells. These tumor cells are irradiated or attenuated in some fashion to prevent proliferation in vivo and reinjected into patients with or without immunomodulators such as BCG or *C. parvum*.<sup>9,10</sup> However, a limitation to autologous tumor vaccines is that a tumor specimen must be obtained from each patient. To circumvent this problem, allogeneic tumor cell vaccines can be generated using established stable cultured cell lines derived from tumors previously obtained from patients. The rationale for the efficacy of allogeneic tumor cell vaccines is that there are melanoma-specific antigens that are shared by different patients.<sup>11</sup> These shared antigens are immunogenic and can enhance the host’s immune system to generate an effective antitumor response. Allogeneic vaccines also have the advantage of being more readily applicable to a greater number of patients regardless of the availability of bulky tumor.

A major disadvantage of whole-cell tumor vaccines is that tumor cells, as such, are generally not immunogenic. These tumor cells have already demonstrated their tumorigenic and metastatic potential by escaping the host immune system. Other approaches such as the use of purified fractions of allogeneic tumor cell lines as antigens that are shed by the tumor cells into the culture media have been used to improve immunogenicity.<sup>12</sup> Heat shock protein extracts purified from autologous tumor cells have also been shown to have antitumor reactivity against the tumor cells from which they were derived.<sup>13</sup>

Mitchell et al<sup>14</sup> reported their experience using allogeneic tumor vaccines. In a series of studies, 106 patients were treated with Detox vaccine for metastatic disease. Twenty patients had an objective clinical response, 5 with a complete response. This led to the current ongoing phase III randomized trials of vaccine

(Melacine) vs chemotherapy for stage III and IV melanoma. Berd and colleagues<sup>15</sup> treated patients with irradiated autologous tumor vaccine mixed with BCG and low-dose chemotherapy and found response rates of 11% to 13% for stage IV disease and a disease-free survival of 60% at 30 months for stage III disease. Morton et al<sup>16</sup> demonstrated a 34% and 23% 5-year survival rate in patients with stage III and IV disease, respectively, who had been rendered disease-free before treatment. The CancerVax vaccine is currently being tested in an NCI-funded multicenter national trial. However, a large phase III randomized trial comparing vaccinia melanoma oncolysate (derived from allogeneic melanoma cells) vs placebo in patients with resected nodal metastasis showed no survival benefit in the vaccinated patient.<sup>17</sup> Thus, preliminary data from single-arm studies suggest a possible benefit of whole-cell vaccines, but randomized data have not yet confirmed a therapeutic benefit for this approach. There are numerous potential approaches to optimizing immunogenicity of whole-cell vaccines. Thus, the failure of one approach in a randomized, prospective trial cannot be interpreted as failure of this approach generally. However, there is enthusiasm for evaluating other approaches, in which immune responses to the vaccines may be more readily evaluated.

## Synthetic Vaccines Targeting Antibody Responses

An alternative approach is to vaccinate with defined tumor antigens that are created synthetically. Several cell-surface molecules on melanoma cells may be targeted by antibodies, and antibodies to some of these molecules are induced in patients with melanoma.<sup>18</sup> A substantial series of clinical studies has been performed with vaccines intended to induce protective antibody responses to the melanoma antigen, GM2, a cell-surface ganglioside. In a pilot study, Livingston and colleagues<sup>19</sup> showed a trend toward improved survival for patients with stage III disease who received GM2 ganglioside/BCG and low-dose chemotherapy, and they demonstrated that patients developed antibodies to the GM2 ganglioside. This has led to a larger randomized phase III ECOG study comparing GM2/KLH in adjuvant QS-21 vaccine to high-dose IFN- $\alpha$ . This study was recently closed due to an early significant survival benefit associated with adjuvant IFN  $\alpha$ -2b.<sup>20</sup>

## Peptide Vaccines

Evidence demonstrating the presence of shared antigens on melanoma tumor cells of different patients

led to the cloning and identification of several melanoma-associated antigens.<sup>11</sup> Furthermore, it is now known that the antigenic epitopes responsible for eliciting an antitumor response consist of small peptide fragments. These peptides are endogenously processed by the tumor cell or an APC and presented to the T cell in association with a major histocompatibility complex (MHC) molecule. The mechanism of peptide vaccination requires the loading of peptide fragments onto empty MHC class I or II molecules. The peptide is generally administered in conjunction with a delivery system such as incomplete Freund's adjuvant. A number of shared melanoma-associated antigens and their DNA sequences are now known. The majority of peptides have been found to be presented on MHC class I molecules (Table 2).

The antigenic peptides can be produced synthetically from purified amino acids, therefore obviating the need to obtain tissue from patients, as in the case for whole tumor cell vaccines. In addition, the patients can be monitored to detect specific immune reactivity after immunizations. The disadvantage is that because the vaccine consists of one specific antigen or a peptide fragment, it is possible that the tumors will undergo mutations and escape the immune system or that more than one particular tumor antigen may be required to generate a clinically effective antitumor response. It is possible that an effective tumor vaccine may require immunization with multiple antigens. Another disadvantage of the peptide-based vaccine is the need for patient selection based on HLA type, since peptides may bind only to a certain MHC class I motif. Defined peptide epitopes are not necessarily expressed on the surface of tumor cells, and posttranslational modifications may affect the recognition of tumor associated peptides. There is increasing consensus that effective peptide vaccines will incorporate multiple different peptides in order to increase the breadth of the immune response generated. Also, MHC class I-restricted peptides do not engage CD4+ lymphocytes, which have a critical role in the induction of an effective immune response. Thus, there is a rationale for vaccinating with combinations of peptides restricted by MHC class I and MHC class II to permit induction of both CD8+ and CD4+ responses to tumor antigens.

Clinical trials using peptide vaccination approaches with epitopes MAGE-1, MAGE-3, MART-1, gp100, tyrosinase, and gp75 have been used for immunization. A phase I trial with MAGE-3 peptide vaccination in incomplete Freund's adjuvant showed promising results in patients with advanced melanoma.<sup>69</sup> Investigators at the NCI demonstrated that a gp100 peptide modified at a single amino acid residue administered with high-dose IL-2 significantly enhanced immuno-

Table 2. — List of Known Peptides Present on Melanoma Cells That May Be Used as Potential Targets in Peptide Vaccinations

Protein	MCH Restriction	Peptide Sequence	Study
Tyrosinase	A1	KCDICTDEY	Kittlesen <sup>21</sup>
Tyrosinase	A2	YMDGTMSQV	Skipper, <sup>22</sup> Wolfel <sup>23</sup>
Tyrosinase	A2	MLLAYLYQL	Wolfel <sup>23</sup>
Tyrosinase	A24	AFLPWHRLF, AFLPWHRLFL	Kang <sup>24</sup>
Tyrosinase	B44	SEIWRDIDF	Brichard <sup>25</sup>
gp100/pMEL17	A2	YLEPGPVTA	Cox <sup>26</sup>
gp100/pMEL17	A2	KTWGQUWQV	Bakker, <sup>27</sup> Kawakami <sup>28</sup>
gp100/pMEL17	A2	ITDQVPFSV	Kawakami <sup>28</sup>
gp100/pMEL17	A2	VLYRYGSFSV	Kawakami <sup>28</sup>
gp100/pMEL17	A2	LLDGTATLRL	Bakker, <sup>29</sup> Kawakami <sup>30</sup>
gp100/pMEL17	A3	ALLAVGATK	Skipper <sup>31</sup>
gp100/pMEL17	A3	MLGTHTMEV	Kawakami <sup>32</sup>
gp100/pMEL17	A3	LIYRRRLMK	Kawakami <sup>33</sup>
gp100/pMEL17	A3	ALNFPGSQK	Kawakami <sup>32</sup>
MART-1/MelanA	A2	AAGIGILTV <sup>†</sup>	Coulie, <sup>34</sup> Kawakami <sup>35</sup>
MART-1/MelanA	A2	ILTIVLGVL	Castelli <sup>36</sup>
gp75/TRP-1	A31	MSLQRQFLR	Wang <sup>37</sup>
TRP-2	A2	SVYDFFVWL	Parkhurst <sup>38</sup>
TRP-2	A31, A33	LLGPGRPYR	Wang <sup>39,40</sup>
CEA	A2	YLSGANLNL	Tsang <sup>41</sup>
HER-2/neu	A2	KIFGSLAFL	Fisk, <sup>42</sup> Lustgarten <sup>43</sup>
HER-2/neu	A2	VMAGVGSPYV	Fisk, <sup>42</sup> Lustgarten <sup>43</sup>
HER-2/neu	A2	IISAVGIL	Yoshino, <sup>44</sup> Linehan, <sup>45</sup> Peoples, <sup>46</sup> Yoshino <sup>47</sup>
PSMA	A2	LLHETDSAV	Tipa <sup>48</sup>
PSMA	A2	ALFDIESKV	Tipa <sup>48</sup>
MAGE-1	A1	EADPTGHSY	Traversari <sup>49</sup>
MAGE-1	A3	SLFRAVITK	Chaux <sup>50</sup>
MAGE-1	Cw*1601	SAYGEPRKL	van der Bruggen <sup>51</sup>
MAGE-2	A2	KMVELVHFL	Visseren <sup>52</sup>
MAGE-2	A2	YLOLVFGIEV	Visseren <sup>52</sup>
MAGE-3	A1	EVDPIGHLY	Gaugler <sup>53</sup>
MAGE-3	A2	FLWGPRLV	van der Bruggen <sup>54</sup>
MAGE-3	B44	MEVDPIGHLY	Fleischhauer, <sup>55</sup> Herman <sup>56</sup>
BAGE	Cw*1601	AARAVFLAL	Boel <sup>57</sup>
GAGE-1,2	Cw6	YRPRRRY	Van den Eynde <sup>58</sup>
GnT-V	A2	VLPDVFIRC	Guilloux <sup>59</sup>
NY-ESO-1	A2	QLSLLMWIT	Jager <sup>60</sup>
NY-ESO-1	A2	SLLMWITQC	Jager <sup>60</sup>
NY-ESO-1	A31	ASGPGGGAPR	Wang <sup>61</sup>
43kD protein	A2	QDLTMKYQIF	Morioka <sup>62</sup>
p15	A24	(E)AYGLDFYIL	Robbins <sup>63</sup>
Mutated beta-catenin	A24	SYLDSGIHF <sup>‡</sup>	Robbins <sup>64</sup>
Mutated elongation factor 2	A*6802	ETVSEQSNV <sup>§</sup>	Hogan <sup>65</sup>
Mutated CASP-8 (FLICE/MACH)	B35.3	FPSDSWCYF	Mandruzato <sup>66</sup>
MUM-1 gene product mutated across intron/exon junction	B*4402	EEKLIVVLF <sup>¶</sup>	Coulie <sup>67</sup>

<sup>†</sup> This peptide AAGIGILTV is also recognized by HLA B45-1-restricted cytotoxic T lymphocyte.<sup>68</sup>

<sup>‡</sup> Phenylalanine (F) at position 9 is the result of mutation. The wild-type sequence is SYLDSGIHS.

<sup>§</sup> Glutamine (Q) at position 6 is the result of somatic mutation. The wild-type sequence is ETVSEESNV.

<sup>¶</sup> Isoleucine (I) at position 5 is the result of mutation. The wild-type sequence is EEKLSVVLV.

From Brinckerhoff LH, Thompson LW, Slingluff CL Jr. Melanoma vaccines. *Curr Opin Oncol.* 2000;12:163-173. Reprinted with permission.

genicity compared to wild-type gp100. In phase I clinical trials, patients treated with the modified gp100 vaccine had responses with clinical tumor regression. A multi-institutional clinical trial randomizing patients with melanoma to modified gp100 vaccine plus high-dose IL-2 vs IL-2 alone was recently opened for patient accrual.<sup>70</sup>

## Cytokine and Growth Factor Modulation

Cytokines are soluble proteins that are important in the regulatory function of the cells of the immune system. The number of cytokines identified continues to expand, but the cytokines that have been used clinically to treat patients with cancer include IL-2, interferons (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ ), GM-CSF, and TNE. Intravenous administration of recombinant IL-2 has been used in clinical trials for patients with metastatic melanoma with overall response rates of approximately 20%.<sup>4,71</sup> In a study by Leong et al,<sup>72</sup> rhGM-CSF in addition to autologous tumor vaccine was used to treat patients with metastatic melanoma with response rates of 20% and a 10% CR rate.

Genetically modified cell-based vaccines encoding for cytokines and co-stimulatory molecules allow sustained local release of cytokines to enhance a potent local inflammatory response without generating systemic side effects. GM-CSF appears to be a strong promoter of local DCs at the vaccination site.<sup>73</sup>

## Antigen-Presenting Cells/Dendritic Cells

DCs are professional APCs that are critical in the initiation of cellular responses in naive T lymphocytes. They take up antigen, circulate and migrate to secondary lymphoid organs, and stimulate resting T cells in an MHC class I and class II restricted manner. The majority of the studies showing efficacy of DCs in cancer therapy have been in preclinical mouse models. Antigenic peptides have been directly loaded onto DCs to induce specific antitumor response. In these animal models, peptide-pulsed DC vaccines appear to be a more efficient method of inducing an antitumor response than peptide alone.<sup>74,75</sup>

New methods in isolating DCs in humans from peripheral blood in larger quantities have allowed the use of DCs as a therapeutic modality. Clinical vaccination trials using DCs have shown promise as a potent antitumor therapy. A study reported by Nestle et al<sup>76</sup> using autologous DCs were pulsed with autologous tumor lysates or peptides showed efficacy. Sixteen patients with metastatic melanoma were immunized,

and objective responses were seen in 5 of the 16 patients (2 CRs and 3 PRs). Delayed hypersensitivity reaction to peptide-pulsed DCs and peptide-specific CTLs were demonstrated. In another trial,<sup>77</sup> 16 patients with metastatic melanoma were treated with DCs pulsed with tyrosinase<sub>368-376</sub>(370D) and gp100<sub>209-217</sub>(210M) peptides restricted by HLA class IA\*0201. One patient had a CR of lung and pleural disease after 2 cycles of DC therapy. Two additional patients had stable disease, and 2 patients had mixed responses. Five of 16 patients had an immune response to gp100 or tyrosinase as demonstrated by INF- $\gamma$  enzyme-linked immunosorbent assay (ELISA). Four of 5 were clinically stable or had tumor regression.<sup>77</sup> The optimal strategy of using DCs in vaccination is critically important for the success of immunization. It has been reported that nonactivated, relatively immature DCs were not effective in patients with melanoma.<sup>78</sup> However, Schuler-Thurner and colleagues<sup>79</sup> recently reported that 3-5 biweekly vaccinations of DCs pulsed with MAGE-3A2.1 tumor and the recall Ag-tetanus toxoid generated Ag-specific effector CD8+ T cells in all 8 patients treated for metastatic melanoma. Future studies are needed to address the issues such as optimal dosage, route of administration, and antigens presented.

## DNA and RNA Vaccines

Injection of nucleic acids has been shown to induce activation of APCs which then are responsible for presenting the antigens to T cells.<sup>80</sup> One advantage of DNA and RNA vaccines is that they deliver genetic material without the difficulties associated with the development of immune reactivity against viral vectors. Approaches to DNA vaccines to date have used DNA encoding for cytokines, co-stimulatory molecules (eg, B7-1), or melanoma-associated antigens. In animal models, DNA expressing melanoma-specific antigen gp100/pm117 was used to effectively immunize mice and reduce tumor formation by 50%.<sup>81</sup> Investigators from Duke University treated patients with central nervous system tumors and metastatic malignancies using tumor RNA pulsed or transfected into DCs to induce antitumor activity.<sup>82,83</sup>

## Conclusions

There is a major collaborative effort in the United States and Europe involving the NCI, pharmaceutical companies, hospitals, and institutes for research and development of cancer vaccines. More than 95 tumor vaccines are in development, many of which are for the treatment of melanoma patients. A variety of approaches can be used to enhance immune reactivity against

tumor cells. In November 2000, the first therapeutic melanoma vaccine, Melacine, became available for commercial sale in Canada. Melacine vaccine consists of two lysed melanoma tumor cell lines in combination with an adjuvant, lipid A, and mycobacterial cell wall skeleton. In the United States, the vaccine is still in phase III clinical trials.<sup>84</sup> The future of cancer vaccines is exciting and promising. However, many unanswered questions and challenges lie ahead. Optimization of strategies, route, timing, and dose of administration will be critical in the development of melanoma vaccines. Continued efforts, clinical trials, and scientific progress will allow the development of more potent targeted therapies for melanoma patients.

## References

- Balch CM, Reintgen DS, Kirkwood JM, et al. Cutaneous melanoma. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. 5th ed. Philadelphia, Pa: Lippincott; 1997:1935-1993.
- Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol*. 2001;19:3635-3648.
- Gershenwald JE, Thompson W, Mansfield PF, et al. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol*. 1999;17:976-983.
- Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. *JAMA*. 1994;271:907-913.
- Rosenberg S. Principles of cancer management: biologic therapy. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. 5th ed. Philadelphia, Pa: Lippincott; 1997:349.
- Kirkwood JM, Strawderman MH, Ernstoff MS, et al. Interferon alpha-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol*. 1996;14:7-17.
- Kirkwood JM, Ibrahim JG, Sondak VK, et al. High- and low-dose interferon alpha-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J Clin Oncol*. 2000;18:2444-2458.
- Nauts H. A review of the influence of bacterial infection and of bacterial products (Coley's toxin) on malignant tumors in man. *Acta Med Scand Suppl*. 1953;276:1.
- Key ME, Hanna MG Jr. Mechanism of action of BCG-tumor cell vaccines in the generation of systemic tumor immunity. II. Influence of the local inflammatory response on immune reactivity. *J Natl Cancer Inst*. 1981;67:863-869.
- Halpern BN, Biozzi G, Stiffel C, et al. Inhibition of tumour growth by administration of killed *Corynebacterium parvum*. *Nature*. 1966;212:853-854.
- Darrow TL, Slingluff CL Jr, Seigler HF. The role of HLA class I antigens in recognition of melanoma cells by tumor-specific cytotoxic T lymphocytes: evidence for shared tumor antigens. *J Immunol*. 1989;142:3329-3335.
- Bystryn JC. Immunogenicity and clinical activity of a polyvalent melanoma antigen vaccine prepared from shed antigens. *Ann NY Acad Sci*. 1993;690:190-203.
- Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science*. 1995;269:1585-1588.
- Mitchell MS, Harel W, Kan-Mitchell J, et al. Active specific immunotherapy of melanoma with allogeneic cell lysates. Rationale, results and possible mechanisms of action. *Ann NY Acad Sci*. 1993;690:153-166.
- Berd D, Maguire HC Jr, McCue P, et al. Treatment of metastatic melanoma with an autologous tumor-cell vaccine: clinical and immunologic results in 64 patients. *J Clin Oncol*. 1990;8:1858-1867.
- Morton DL, Hoon DS, Nizze JA, et al. Polyvalent melanoma vaccine improves survival of patients with metastatic melanoma. *Ann NY Acad Sci*. 1993;690:120-134.
- Wallack MK, Sivanandham M, Balch CM, et al. A phase III randomized, double-blind multiinstitutional trial of vaccinia melanoma oncolysate-active specific immunotherapy for patients with stage II melanoma. *Cancer*. 1995;75:34-42.
- Jager E, Gnjatich S, Nagata Y, et al. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc Natl Acad Sci U S A*. 2000;97:12198-12203.
- Livingston PO, Natoli EJ, Calves MJ, et al. Vaccines containing purified GM2 ganglioside elicit GM2 antibodies in melanoma patients. *Proc Natl Acad Sci U S A*. 1987;84:2911-2915.
- Kirkwood JM, Ibrahim JG, Sosman JA, et al. High-dose interferon alpha-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. *J Clin Oncol*. 2001;19:2370-2380.
- Kittleson DJ, Thompson LW, Gulden PH, et al. Human melanoma patients recognize an HLA-A1-restricted CTL epitope from tyrosinase containing two cysteine residues: implications for tumor vaccine development. *J Immunol*. 1998;160:2099-2106.
- Skipper JC, Hendrickson RC, Gulden PH, et al. An HLA-A2-restricted tyrosinase antigen on melanoma cells results from post-translational modification and suggests a novel pathway for processing of membrane proteins. *J Exp Med*. 1996;183:527-534.
- Wolfel T, Van Pel A, Brichard V, et al. Two tyrosinase nonpeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. *Eur J Immunol*. 1994;24:759-764.
- Kang X, Kawakami Y, el-Gamil M, et al. Identification of a tyrosinase epitope recognized by HLA-A24-restricted, tumor-infiltrating lymphocytes. *J Immunol*. 1995;155:1343-1348.
- Brichard VG, Herman J, Van Pel A, et al. A tyrosinase nonapeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. *Eur J Immunol*. 1996;26:224-230.
- Cox AL, Skipper J, Chen Y, et al. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science*. 1994;264:716-719.
- Bakker AB, Schreurs MW, Tafazzul G, et al. Identification of a novel peptide derived from the melanocyte-specific gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line. *Int J Cancer*. 1995;62:97-102.
- Kawakami Y, Eliyahu S, Jennings C, et al. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. *J Immunol*. 1995;154:3961-3968.
- Bakker AB, Schreurs MW, de Boer AJ, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J Exp Med*. 1994;179:1005-1009.
- Kawakami Y, Eliyahu S, Delgado CH, et al. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. *Proc Natl Acad Sci U S A*. 1994;91:6458-6462.
- Skipper JC, Kittleson DJ, Hendrickson RC, et al. Shared epitopes for HLA-A3-restricted melanoma-reactive human CTL include a naturally processed epitope from Pmel-17/gp100. *J Immunol*. 1996;157:5027-5033.
- Kawashima I, Tsai V, Southwood S, et al. Identification of gp100-derived, melanoma-specific cytotoxic T-lymphocyte epitopes restricted by HLA-A3 supertype molecules by primary in vitro immunization with peptide-pulsed dendritic cells. *Int J Cancer*. 1998;78:518-524.
- Kawakami Y, Robbins PF, Wang X, et al. Identification of new melanoma epitopes on melanosomal proteins recognized by tumor infiltrating T lymphocytes restricted by HLA-A1, -A2, and -A3 alleles. *J Immunol*. 1998;161:6985-6992.
- Coulie PG, Brichard V, Van Pel A, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med*. 1994;180:35-42.
- Kawakami Y, Eliyahu S, Sakaguchi K, et al. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J Exp Med*. 1994;180:347-352.

36. Castelli C, Storkus WJ, Mauerer MJ, et al. Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8+ cytotoxic T lymphocytes. *J Exp Med.* 1995;181:363-368.
37. Wang RF, Robbins PF, Kawakami Y, et al. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. *J Exp Med.* 1995;181:799-804.
38. Parkhurst MR, Fitzgerald EB, Southwood S, et al. Identification of a shared HLA-A\*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). *Cancer Res.* 1998;58:4895-4901.
39. Wang RF, Appella E, Kawakami Y, et al. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. *J Exp Med.* 1996;184:2207-2216.
40. Wang RF, Johnston SL, Southwood S, et al. Recognition of an antigenic peptide derived from tyrosinase-related protein-2 by CTL in the context of HLA-A31 and -A33. *J Immunol.* 1998;160:890-897.
41. Tsang KY, Zarella S, Nieroda CA, et al. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst.* 1995;87:982-990.
42. Fisk B, Blevins TL, Wharton JT, et al. Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines. *J Exp Med.* 1995;181:2109-2117.
43. Lustgarten J, Sherman L. Generation of xenogeneic cytotoxic T cells from peptides derived from the her-2/neu protooncogene. *Ninth Int Congress Immunol.* 1995:663. Abstract.
44. Yoshino I, Peoples GE, Goedegebuure PS, et al. Association of HER2/neu expression with sensitivity to tumor-specific CTL in human ovarian cancer. *J Immunol.* 1994;152:2393-2400.
45. Linehan DC, Peoples GE, Parikh AS, et al. Ovarian and breast tumor associated T lymphocytes stimulated with an antigenic peptide derived from her2/neu show enhanced cytotoxicity against autologous tumor. *Surg Forum.* 1994;45:656-570.
46. Peoples GE, Goedegebuure PS, Smith R, et al. Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. *Proc Natl Acad Sci U S A.* 1995;92:432-436.
47. Yoshino I, Goedegebuure PS, Peoples GE, et al. HER2/neu-derived peptides are shared antigens among human non-small cell lung cancer and ovarian cancer. *Cancer Res.* 1994;54:3387-3390.
48. Tjoa BA, Erickson SJ, Bowes VA, et al. Follow-up evaluation of prostate cancer patients infused with autologous dendritic cells pulsed with PSMA peptides. *Prostate.* 1997;32:272-278.
49. Traversari C, van der Bruggen P, Luescher IE, et al. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. *J Exp Med.* 1992;176:1453-1457.
50. Chaux P, Luiten R, Demotte N, et al. Identification of five MAGE-A1 epitopes recognized by cytolytic T lymphocytes obtained by in vitro stimulation with dendritic cells transduced with MAGE-A1. *J Immunol.* 1999;163:2928-2936.
51. van der Bruggen P, Szikora JP, Boel P, et al. Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw\*1601. *Eur J Immunol.* 1994;24:2134-2140.
52. Visseren MJ, van der Burg SH, van der Voort EI, et al. Identification of HLA-A\*0201-restricted CTL epitopes encoded by the tumor-specific MAGE-2 gene product. *Int J Cancer.* 1997;73:125-130.
53. Gaugler B, Van den Eynde B, van der Bruggen P, et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J Exp Med.* 1994;179:921-930.
54. van der Bruggen P, Bastin J, Gajewski T, et al. A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. *Eur J Immunol.* 1994;24:3038-3043.
55. Fleischhauer K, Fruci D, Van Endert P, et al. Characterization of antigenic peptides presented by HLA-B44 molecules on tumor cells expressing the gene MAGE-3. *Int J Cancer.* 1996;68:622-628.
56. Herman J, van der Bruggen P, Luescher IE, et al. A peptide encoded by the human MAGE3 gene and presented by HLA-B44 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE3. *Immunogenetics.* 1996;43:377-383.
57. Boel P, Wildmann C, Sensi ML, et al. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity.* 1995;2:167-175.
58. Van den Eynde B, Peeters O, De Backer O, et al. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med.* 1995;182:689-698.
59. Guilloux Y, Lucas S, Brichard VG, et al. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the N-acetylglucosaminyltransferase V gene. *J Exp Med.* 1996;183:1173-1183.
60. Jager E, Chen YT, Drijfhout JW, et al. Simultaneous humoral and cellular immune response against cancer-testis antigen NYESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J Exp Med.* 1998;187:265-270.
61. Wang RF, Johnston SL, Zeng G, et al. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. *J Immunol.* 1998;161:3598-3606.
62. Morioka N, Kikumoto Y, Hoon DS, et al. A decapeptide (Gln-Asp-Leu-Thr-Met-Lys-Tyr-Gln-Ile-Phe) from human melanoma is recognized by CTL in melanoma patients. *J Immunol.* 1994;153:5650-5658.
63. Robbins PE, el-Gamil M, Li YF, et al. Cloning of a new gene encoding an antigen recognized by melanoma-specific HLA-A24-restricted tumor-infiltrating lymphocytes. *J Immunol.* 1995;154:5944-5950.
64. Robbins PE, El-Gamil M, Li YF, et al. A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J Exp Med.* 1996;183:1185-1192.
65. Hogan KT, Eisinger DP, Cupp SB 3rd, et al. The peptide recognized by HLA-A68.2-restricted, squamous cell carcinoma of the lung-specific cytotoxic T lymphocytes is derived from a mutated elongation factor 2 gene. *Cancer Res.* 1998;58:5144-5150.
66. Mandruzzato S, Brasseur F, Andry G, et al. A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. *J Exp Med.* 1997;186:785-793.
67. Coulie PG, Lehmann E, Lethé B, et al. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proc Natl Acad Sci U S A.* 1995;92:7976-7980.
68. Schneider J, Brichard V, Boon T, et al. Overlapping peptides of melanocyte differentiation antigen Melan-A/MART-1 recognized by autologous cytolytic T lymphocytes in association with HLA-B45.1 and HLA-A2.1. *Int J Cancer.* 1998;75:451-458.
69. Marchand M, Weynants P, Rankin E, et al. Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. *Int J Cancer.* 1995;63:883-885.
70. Schwartzentruber DJ. Melanoma vaccines: any evidence that they actually work? Presented at the Society of Surgical Oncology 54th Annual Cancer Symposium; March 15-18, 2001; Washington, DC.
71. Rosenberg SA. Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. *Cancer J Sci Am.* 2000;6(suppl 1):S2-S7.
72. Leong SP, Enders-Zohr P, Zhou YM, et al. Recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) and autologous melanoma vaccine mediate tumor regression in patients with metastatic melanoma. *J Immunother.* 1999;22:166-174.
73. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A.* 1993;90:3539-3543.
74. Paglia P, Chiodoni C, Rodolfo M, et al. Murine dendritic cells loaded in vitro with soluble protein prime cytotoxic T lymphocytes against tumor antigen in vivo. *J Exp Med.* 1996;183:317-322.
75. Mayordomo JI, Zorina T, Storkus WJ, et al. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nat Med.* 1995;1:1297-1302.
76. Nestle FO, Aljagic S, Gilliet M, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med.* 1998;4:328-332.
77. Lau R, Wang F, Jeffery G, et al. Phase I trial of intravenous peptide-pulsed dendritic cells in patients with metastatic melanoma. *J Immunother.* 2001;24:66-78.

78. Panelli MC, Wunderlich J, Jeffries J, et al. Phase 1 study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma-associated antigens MART-1 and gp100. *J Immunother.* 2000;23:487-498.

79. Schuler-Thurner B, Dieckmann D, Keikavoussi P, et al. Mage-3 and influenza-matrix peptide-specific cytotoxic T cells are inducible in terminal stage HLA-A2.1+ melanoma patients by mature monocyte-derived dendritic cells. *J Immunol.* 2000;165:3492-3496.

80. Pardoll DM, Beckerleg AM. Exposing the immunology of naked DNA vaccines. *Immunity.* 1995;3:165-169.

81. Nawrath M, Pavlovic J, Dummet R, et al. Reduced melanoma tumor formation in mice immunized with DNA expressing the melanoma-specific antigen gp100/pm17. *Leukemia.* 1999;13(suppl 1):S48-S51.

82. Ashley DM, Faiola B, Nair S, et al. Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce anti-tumor immunity against central nervous system tumors. *J Exp Med.* 1997;186:1177-1182.

83. Nair SK, Boczkowski D, Morse M, et al. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. *Nat Biotechnol.* 1998;16:364-369.

84. Finton L. Melanoma vaccine momentum spurs interest, investment. *J Natl Cancer Inst.* 2000;92:1205-1207.