

# Vaccine Therapy for Malignant Melanoma

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## Introduction

The incidence of malignant melanoma of the skin continues to rise worldwide at a rate of about five percent per year.<sup>1</sup> In the United States and Canada, melanoma has increased at a rate exceeding that of any other tumor except lung cancer in women. According to the American Cancer Society, about 38,300 patients will be diagnosed with melanoma this year and 7,300 will die from recurrent advanced disease.<sup>2</sup>

With early diagnosis and adequate surgical treatment, the 10-year cure rate is 90 percent for primary melanomas less than 1.5 mm in thickness (American Joint Committee on Cancer [AJCC] stage I). However, once melanoma metastasizes to distant sites (AJCC stage IV), prognosis is discouraging. At present, five-year survival is rare, and median survival remains a dismal four to eight months, depending on the site of metastasis.

Although in rare instances complete responses do occur, on the whole chemotherapy has failed to improve overall survival. Among single agents, dacarbazine has produced the highest response rates (15 to 25 percent) in patients with soft tissue metastasis, but responses to dacarbazine are less than 10 percent in patients with visceral metastasis. In most cases, responses last only three to six months, with only four percent being complete and only about one percent lasting more than 30 months.

In a large study from the Mayo Clinic, only 10 of 503 patients undergoing various chemotherapeutic regimens achieved complete remission and survived for more than five years.<sup>3</sup> Although biochemotherapeutic regimens combining cytotoxic agents with interleukin-2 and/or interferon alfa appear to increase response rates, significantly prolonged survival is still limited to the small group of complete responders. In short, no regimen of chemotherapy has significantly improved the overall median survival of patients with advanced melanoma, and no single agent or combination of agents has proved superior to dacarbazine, the only drug approved by the Food and Drug Administration for the treatment of metastatic melanoma.<sup>4,5</sup>

Unfortunately, almost all patients with melanoma metastatic to distant sites experience a progressive disease that follows its inextricable course in defiance of

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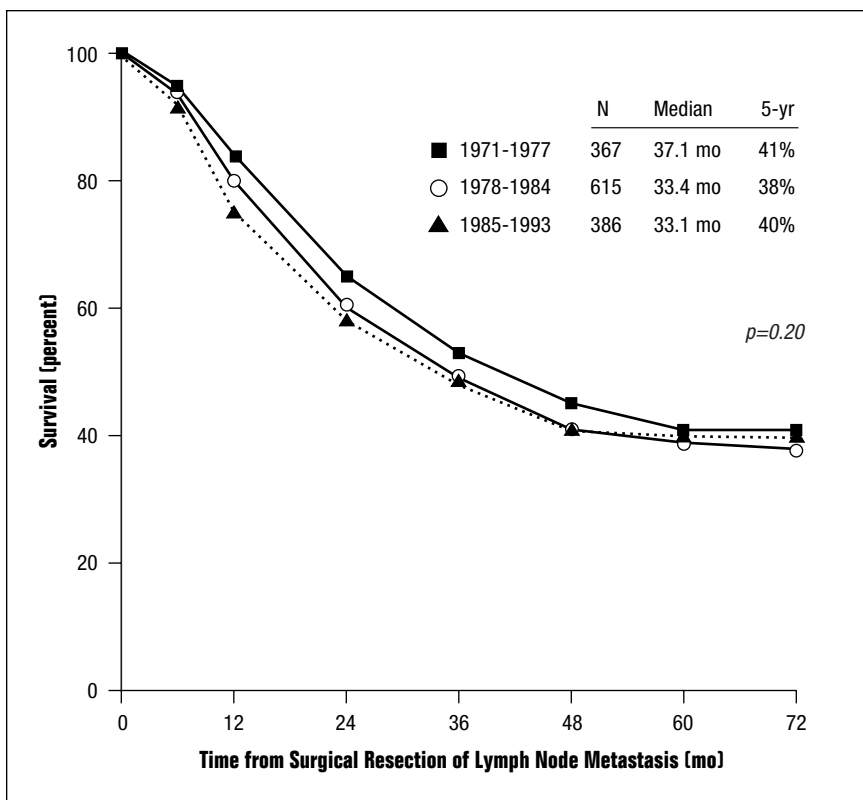


Fig. 1A. Overall survival of patients with stage III melanoma (regional lymph node metastasis) treated with standard therapy. Data based on experience at the John Wayne Cancer Institute.

all available therapies. Moreover, a depressingly high 60 to 80 percent of patients who have metastasis limited to the regional lymph nodes (AJCC stage III) or primary melanomas thicker than 4 mm die from disseminated disease within five years, depending on the number of tumor-involved lymph nodes. Review of our experience at the John Wayne Cancer Institute indicates that there has been no improvement in the overall survival of AJCC stage III or stage IV melanoma patients receiving standard therapy without melanoma vaccines for almost a quarter of a century (Fig. 1).<sup>6</sup> Thus, there is an ur-

gent need for new approaches to the management of melanoma that has metastasized to the regional nodes or distant sites or is at high risk of metastasizing.

### Clinical Evidence of an Immune Response to Melanoma

The development of novel approaches using active specific immunotherapy with therapeutic melanoma vaccines has been prompted by clinical observations suggesting an important role of the immune system in melanoma.<sup>7-12</sup> These observations include waxing and waning of

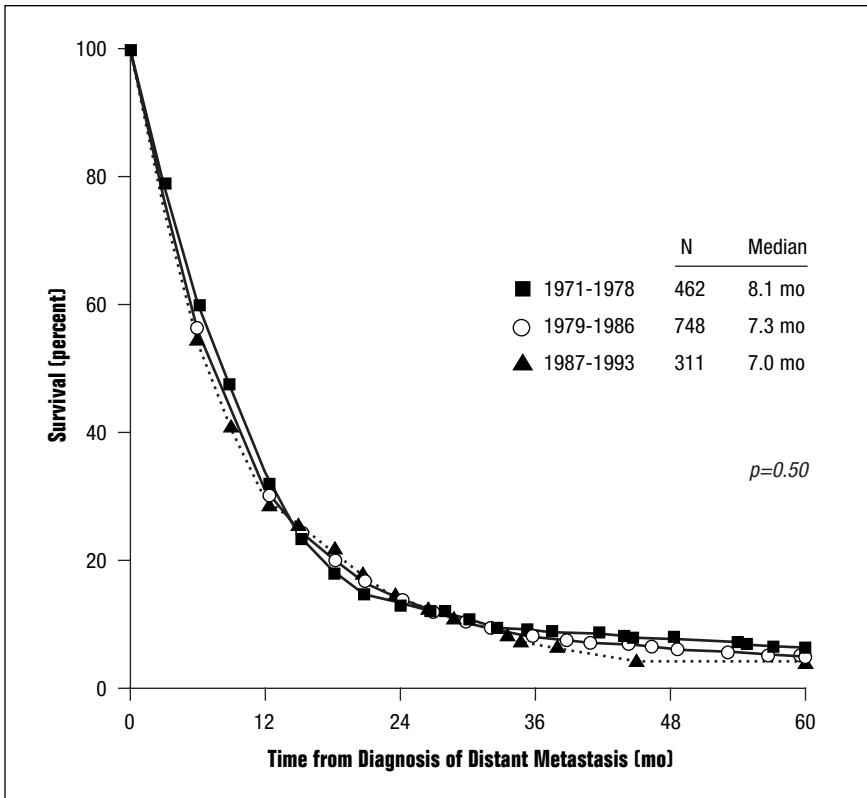


Fig. 1B. Overall survival of patients with stage IV melanoma (distant metastasis) treated with standard therapy. Reprinted with permission from Barth et al.<sup>6</sup>

melanoma lesions; the highly variable rate of disease progression; the possibility of late relapse; the increased incidence of malignant melanoma in immunosuppressed patients; and the therapeutic efficacy of interleukin-2, which mediates its antitumor effect immunologically. A striking observation is spontaneous regression of malignant melanoma. In the experience at the John Wayne Cancer Institute, almost 30 percent of primary melanomas show some evidence of regression, and about 14 percent of nodal metastases occur without an identifiable primary (presumably due to spontaneous

regression of the cutaneous melanoma). Regression of melanoma metastatic to distant sites is a frequently discussed but rarely observed phenomenon usually associated with bacterial infections. Nevertheless, multiple cases have been documented since Bennett's original report in 1899.<sup>13</sup>

The success of prophylactic vaccines against infectious disease prompted investigation of vaccines against cancer. At present, most cancer vaccines are therapeutic rather than prophylactic; they are administered after the advent of disease. Like vaccines against infectious disease,

cancer vaccines are made from attenuated whole cells, cell walls, specific antigens, or nonpathogenic strains of living organisms.<sup>14</sup> Active immunotherapy with cancer vaccines is administered to stimulate the patient's immune system to destroy tumor cells and thereby overcome the immunosuppression produced by tumor-derived factors. Active immunotherapy also attempts to enhance the ability of tumor-associated antigens to stimulate a clinically beneficial immune response.

Our early attempts to develop vaccines against melanoma were based on the observation that serum from melanoma patients contains antibodies that react with antigens on autologous melanoma cells.<sup>10</sup> These early vaccines were crude mixtures of whole or fractionated, autologous or allogeneic melanoma cells. Most were unsuccessful. However, the application of modern techniques of mol-

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ecular biology and immunology has allowed definition of a large number of melanoma-associated antigens (MAA) that can induce an immune response in patients with melanoma. Although early investigators doubted the existence of such antigens, their clear demonstration has awakened new interest in development of vaccines that can upregulate a patient's immune response to melanoma.

Common MAA shared by melanomas from different patients (i.e., allogeneic melanomas) are the basis for "generic" melanoma vaccines. These common MAA were defined initially by their reaction with polyclonal antibodies from melanoma patients and later by

their reaction with highly specific human monoclonal antibodies and cytotoxic T cells (Table 1).<sup>15-30</sup> The ability to quantify the humoral and cell-mediated immune responses to these MAA has transformed melanoma vaccine research from a series of interesting clinical observations to an exciting scientific discipline.

### **Tumor Regression Induced by Chemotherapy Versus Cancer Vaccines**

The timing of the response to therapeutic cancer vaccines is quite different from the response to chemotherapy (Table 2).

Chemotherapeutic agents are cytotoxic: they kill tumor cells immediately and therefore can cause clinical regression within two to four weeks. By contrast, cancer vaccines have no direct cytotoxic effect on existing metastatic disease. Instead, they elicit humoral and cell-mediated immune responses, which evolve over a period of 12 to 14 weeks and may require six to eight months to induce complete regression.<sup>31</sup> After the initial immunization, disease may show progression for four to eight weeks before the growth rate slows and is followed by a period of tumor stability and subsequently by signs of regression. The delayed response to cancer vaccines is somewhat analogous to the delay of six to eight weeks commonly observed with the responses to hormonal therapy of breast cancer. Although a therapeutic cancer vaccine usually requires three to four months to induce clinical regression or slowing of tumor growth, the response is usually durable, lasting from months to years. This is in marked contrast to the short-term response to most chemotherapy regimens.

Because MAA are weak antigens, melanoma patients receiving therapeutic vaccines are usually immunized repeatedly for prolonged periods. A melanoma vaccine's success (i.e., its ability to prolong survival) can be predicted by quanti-

**Table 1**  
**Some Immunogenic Antigens**  
**Identified in Human Melanoma Cells\***

Common Tumor-Associated Antigens (TAA)	Melanoma-Associated Antigens (MAA)
TAA are found not only in melanoma but also in kidney, lung, breast, and other solid neoplasms	MAA are found primarily in melanocytes/melanoma (and rare neoplasms of neural crest origin)
†Urinary TAA (glycoprotein 90) <sup>18</sup>	†Lipoprotein180 <sup>19</sup>
†Fetal antigen (glycoprotein 70) <sup>17</sup>	Tyrosinase <sup>22</sup>
†810 peptide (43 kd) <sup>23</sup>	MART-1/Melan A <sup>26,27</sup>
†MAGE 1 <sup>20</sup>	Glycoprotein 75 (gp 75.TRP) <sup>28</sup>
†MAGE 3 <sup>21</sup>	Glycoprotein 100 (gp 100/pm17) <sup>24,25</sup>
†GM2 <sup>16</sup>	High molecular weight melanoma antigen <sup>29,30</sup>
†GD2 <sup>16</sup>	
†O-acetyl GD3 <sup>16</sup>	
†GM3 <sup>15</sup>	
*All of these antigens have been identified in the three melanoma cell lines used in the John Wayne Cancer Institute's living allogeneic melanoma cell vaccine (CancerVax). Some have not yet been fully characterized.	
†Antibody responses to these antigens have been demonstrated in the serum of patients receiving active immunotherapy with CancerVax.	

tating the patient's immune response during this time. The induction of humoral antibodies and the development of cell-mediated responses are directly related to the duration of survival.<sup>31</sup>

Attempts have been made to enhance the efficacy of melanoma vaccine therapy by administering a vaccine with nonspecific adjuvants, such as bacille Calmette-Guérin (BCG), or with immunomodulators, such as cyclophosphamide, cimetidine, or indomethacin. These agents may help counteract the tumor-associated immunosuppression induced by T-suppressor cells. Adjuvants can enhance and often modify a patient's immune response to cancer vaccines by providing

carriers, immune stimulants, and antigen targeting to particular cells or organ sites. In addition to BCG, currently used adjuvants in human melanoma include DETOX, QS-21, and MF-59. The increasing interest in subunit vaccines (e.g., peptides) will increase the need for effective adjuvants. Recently, investigators have also begun to use DNA sequences from BCG to augment immune responses.

In this brief review, we will summarize the present status of melanoma vaccines as therapy for distant metastatic disease (AJCC stage IV) and as adjuvant therapy following surgical resection of regional lymph node metastasis (AJCC

**Table 2**  
**Antitumor Responses Induced by Chemotherapy**  
**Versus Active (Vaccine) Immunotherapy**

	<b>Chemotherapy</b>	<b>Active Immunotherapy with Vaccines</b>
Mechanism of Response	<b>Direct:</b> Drug is toxic to cancer cells	<b>Indirect:</b> Cytotoxic T cells and antibodies are activated to kill tumor cells
Intermediate Steps	<b>Rapid:</b> Drug quickly metabolizes to active state	<b>Slow:</b> Maximum activation of immune response requires 8-12 weeks
Clinical Response	<b>Rapid:</b> 2-4 weeks	<b>Slow:</b> 3-6 months
Duration of Response	<b>Short:</b> Weeks to months	<b>Long:</b> Usually months to years
Side Effects	<b>Substantial:</b> Severe toxicity may occur	<b>Few:</b> Usually mild

stage III). Because we are most familiar with our work, much of this review will concentrate on our own experience during 30 years of research to develop an effective therapeutic vaccine against melanoma.

### **John Wayne Cancer Institute Therapeutic Vaccine Trials for AJCC Stage IV Melanoma**

Despite new treatment options, the prognosis of patients with melanoma metastatic to distant sites has not changed significantly over the past two decades (Fig. 1).<sup>6</sup> Median overall survival is 7.5 months, and the five-year survival rate is less than five percent. At the John Wayne Cancer Institute, we have developed a living whole-cell melanoma vaccine called CancerVax. CancerVax is an irradiated mixture of three allogeneic melanoma cell lines selected for their high content of immunogenic MAA, including all antigens listed in Table 1.

Since 1984, we have been investigating the response to CancerVax in patients with melanoma metastatic to distant sites (AJCC stage IV). In 1992, a first analysis

of our phase II experience with 75 patients revealed a response rate of 22 percent.<sup>31,32</sup> Median survival was significantly longer in 75 patients treated with CancerVax than in 1,275 historical controls receiving other types of treatment (23 months versus 7.5 months,  $p=0.0001$ ). This series has recently been updated and now includes 157 CancerVax patients and 1,521 controls.<sup>33</sup> Median survival is still an impressive 23 months, with a five-year survival rate of 25 percent (Fig. 2). In a series of melanoma patients undergoing treatment for pulmonary metastasis, median overall survival following CancerVax therapy was 13 months, compared with eight months following other treatments, such as chemotherapy or BCG immunotherapy.<sup>34</sup>

The survival benefit is highest among patients who have undergone complete resection of metastatic disease (often to multiple sites) prior to CancerVax therapy. For example, patients with visceral metastasis (AJCC stage IV M1b) who were rendered free of disease prior to vaccine therapy experienced a five-year survival rate of 33 percent compared with 10 percent for similar patients un-

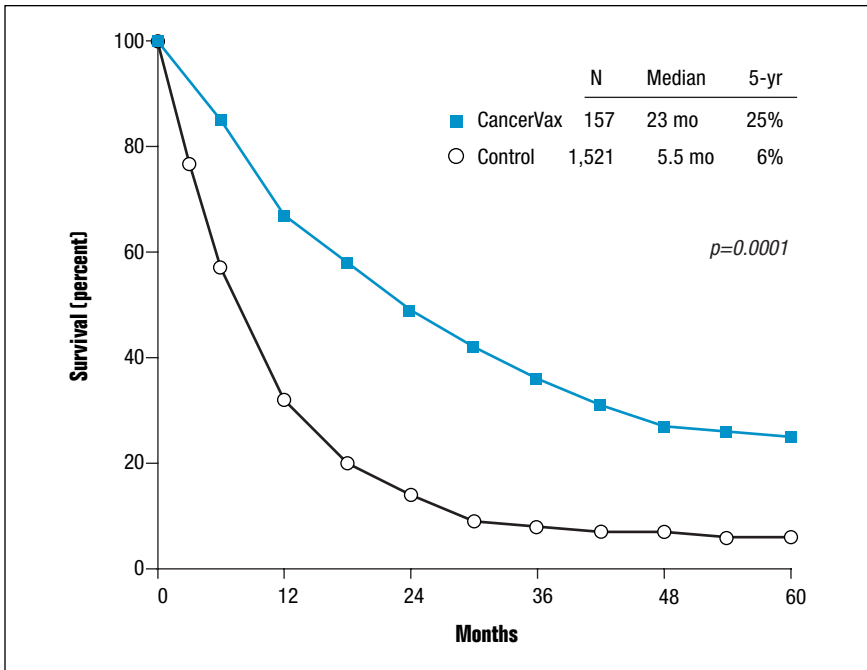


Fig. 2. Survival of 157 patients with AJCC stage IV melanoma treated with CancerVax versus 1,521 historic controls treated with nonvaccine therapies. Five-year survival was 25 percent for the CancerVax patients versus six percent for the historic controls ( $p=0.0001$ ).

dergoing surgical resection followed by non-CancerVax therapy (Fig. 3).

Although 15 to 20 percent of patients with evaluable disease undergo partial or complete regression of metastasis following immunotherapy with CancerVax,<sup>33</sup> vaccine-induced regression occurs primarily in patients with metastases less than 2 cm in diameter (Fig. 4).<sup>31</sup> A much more frequent clinical response to CancerVax is slowing of tumor growth, which can be measured by an increase in tumor doubling time or a period of prolonged stability in tumor size (Fig. 5).<sup>31,34-36</sup>

In addition to its effect on macroscopic disease, CancerVax appears to inhibit blood-borne micrometastasis. Reverse transcriptase-polymerase chain reaction (RT-PCR) techniques indicate

that up to 95 percent of patients with metastasis to distant organ sites have tumor cells circulating in the bloodstream, at least some of which will be able to induce secondary metastasis.<sup>37</sup> Cytotoxic T cells and antibodies can attack these tumor cells at some step in the metastatic process, including intravasation into the microcirculation, circulation in the bloodstream, and extravasation into the interstitial space. The ability to inhibit the metastatic process may be an important benefit of active immunotherapy because our data indicate more than a doubling in the survival rate of patients who respond to vaccine therapy by formation of cytotoxic T cells<sup>38</sup> or IgM antibodies with specificity for melanoma cell-surface antigens.<sup>31</sup>

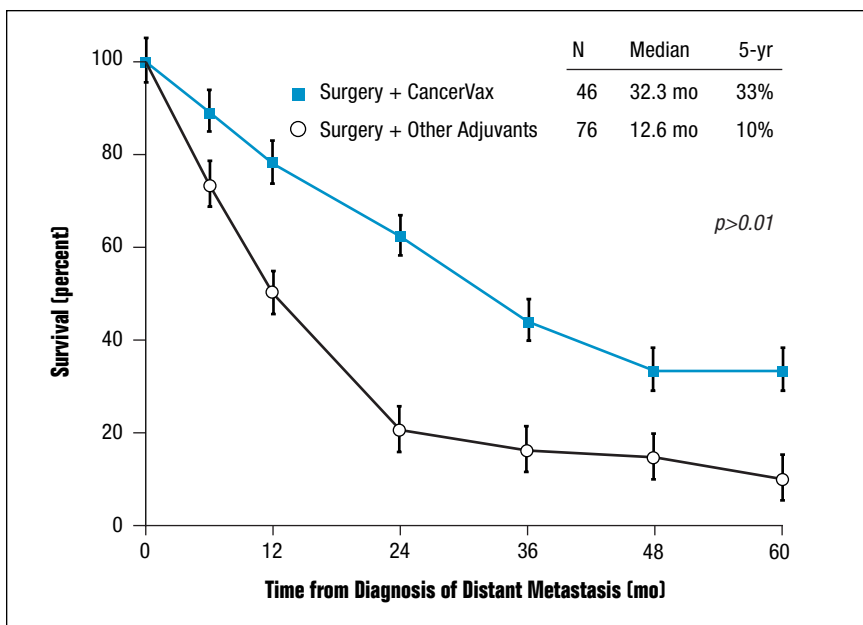


Fig. 3. Survival of melanoma patients receiving CancerVax versus other adjuvant therapies after complete resection of visceral metastasis (AJCC stage IV M1b disease). Other adjuvant therapies were immunotherapy with bacille Calmette-Guérin and/or chemotherapy.

Our studies of brain metastasis in AJCC stage IV melanoma show that as many as 65 to 75 percent of patients have central nervous system involvement at the time of death.<sup>39</sup> The brain is the initial site of distant metastasis in about 20 percent of patients, whereas about 50 percent exhibit initial metastasis to noncerebral sites and later develop secondary cerebral metastasis. However, the incidence of secondary brain metastasis in patients receiving CancerVax is only 22 percent, even though their survival is twofold the survival of control patients from our historical data base who had an incidence of 49 percent for secondary brain metastasis (Table 3). We believe that CancerVax may inhibit the metastatic cascade by activating cytotoxic T cells and antibodies that attack melanoma tumor cells which have entered the blood-

stream during transit to the brain and thereby delay or inhibit the development of blood-borne cerebral metastasis. Thus, even in the absence of a quantifiable lesion response, vaccine treatment may be beneficial by preventing secondary metastasis and improving the response to chemotherapy or biotherapy in patients whose disease progresses on immunotherapy alone.<sup>40,41</sup>

Because of our encouraging results with CancerVax in stage IV disease, we will shortly begin a randomized, multicenter phase III trial of this vaccine.

### Other Vaccine Studies in Patients with Stage IV Melanoma

In several phase I/II studies, Mitchell's group tested preparations of two mechanically disrupted melanoma cell lines

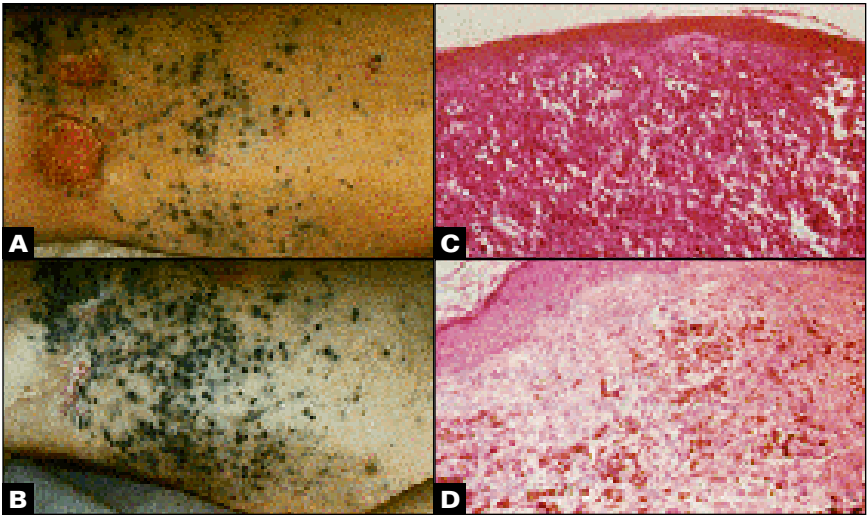


Fig. 4. Cutaneous melanoma metastases on thigh (A) before and (B) four months after initiation of CancerVax immunotherapy. Corresponding photomicrographs show that (C) viable melanoma cells (D) disappear as regression occurs. This patient remains free of disease at 7.5 years of follow-up. Reprinted with permission from Morton et al.<sup>31</sup>

(Melacine) injected subcutaneously in combination with the adjuvant DETOX. Median overall survival of the 106 patients was 12.2 months, but 20 patients (19 percent) had objective clinical regression of tumor masses, five with complete responses.<sup>42</sup> The median duration of response was 46 months. Clinical response correlated with an increase in the level of cytotoxic T-cell precursors in the blood as well as a partial match of the patient's HLA phenotype with vaccine cell lines.<sup>43,44</sup>

A randomized phase III trial of 140 AJCC stage IV melanoma patients was recently completed with patients receiving vaccine lysate (Melacine) plus DETOX or combination chemotherapy (dacarbazine, cisplatin, BCNU, tamoxifen).<sup>45</sup> Interim analysis revealed that median survival was not significantly different among evaluable patients receiving Melacine (329 days) versus chemotherapy (373 days). Although toxic side effects were significantly less frequent with

Melacine, the response rate among evaluable patients was significantly higher with chemotherapy (four percent complete, 24 percent partial, and 42 percent stable) than with the vaccine (four percent, two percent, and nine percent, respectively). No definitive long-term survival analysis is available as yet.

Subsequent administration of interferon alfa-2b (IFN alfa-2b) at a dose of  $5 \times 10^6$  U/m<sup>2</sup> subcutaneously, three times per week, induced responses in eight of 18 patients who failed Melacine treatment regardless of their HLA phenotype.<sup>41</sup> Based on these results, a national confirmatory phase III trial will compare Melacine plus IFN alfa-2b with IFN alfa-2b alone. This trial will include 300 patients and is scheduled to begin this year.

Three monoclonal anti-idiotypic antibodies mimicking high molecular weight (HMW)-MAA have been developed by Ferrone's group. Anti-idiotypic antibodies (anti-ids) theoretically can

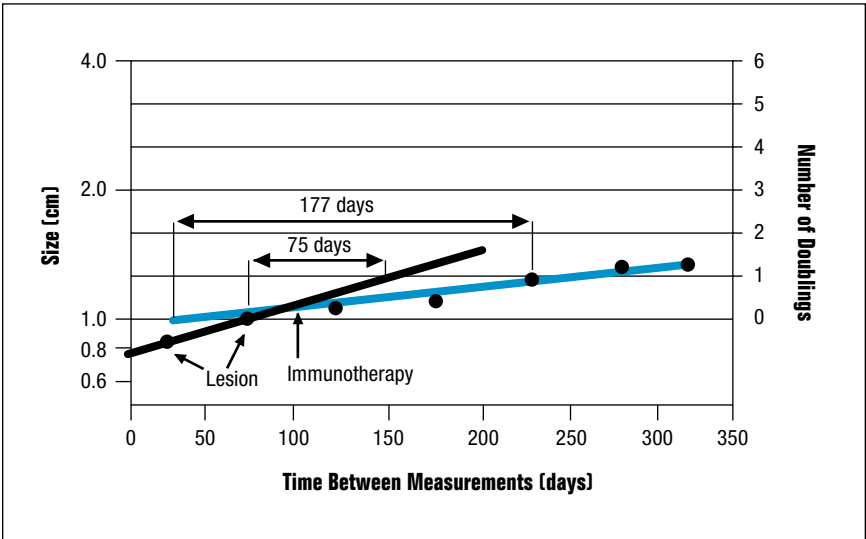


Fig. 5. Tumor doubling time (TDT), a measure of the rate of tumor growth, is exponential during most of the growth of clinically detectable pulmonary metastasis.<sup>36</sup> TDT increases after initiation of CancerVax immunotherapy, indicating a decreased rate of tumor cell growth or increased cell death. Before CancerVax, the tumor required only 75 days to double its volume; after initiation of CancerVax, it required 177 days. The longer the TDT, the less aggressive the tumor. Reprinted with permission from Barth et al.<sup>35</sup>

stimulate “silent” clones that are unresponsive to the nominal antigen and thereby induce humoral and possibly cellular immune response. Two of the anti-ids have been used in phase I/II clinical studies of patients with stage IV melanomas.<sup>29,46,47</sup> Coupled to keyhole limpet hemocyanin and mixed with BCG as an adjuvant, these anti-ids elicited antibodies against HMW-MAA in 60 percent of the patients.<sup>48</sup> The fact that these anti-HMW-MAA antibodies are both IgM and IgG suggests a T-cell-dependent immune response to the anti-id. Development of anti-HMW-MAA immunity was associated with significantly increased survival.<sup>30</sup>

Berd’s group has used a vaccine preparation made from autologous tumor mixed with BCG as an adjuvant. Because melanoma cells are not cultured,

the number of vaccinations is limited. Five of 40 patients responded to treatment with autologous melanoma vaccine; four responses were complete.<sup>49</sup> Eighty percent of the patients with tumor regression exhibited delayed-type hypersensitivity to autologous tumor. Berd’s group has started coupling autologous melanoma cells to dinitrophenol (DNP), to enhance their low antigenicity.<sup>50,51</sup>

### Adjuvant Vaccine Immunotherapy Following Surgical Resection of Melanoma-Involved Lymph Nodes (AJCC Stage III Melanoma)

Adjuvant active immunotherapy refers to administration of a cancer vaccine after surgical resection of all clinically evident tumor. In patients with AJCC stage III melanoma, adjuvant immunotherapy is

**Table 3**  
**Incidence of Secondary Brain Metastasis at Time of Death**  
**in Patients Whose AJCC Stage IV Melanoma Was Initially**  
**Diagnosed in Noncerebral Sites\***

Initial Site of Distant Metastasis	CancerVax			Other Therapies <sup>†</sup>		
	Total No.	Median Survival (months)	No. (percent) with Secondary Cerebral Metastasis	Total No.	Median Survival (months)	No. (percent) with Secondary Cerebral Metastasis
Liver/bone	16	9.5	2 (13)	157	4.5	55 (35)
Skin/gastrointestinal tract	63	21.4	13 (25)	143	10.4	75 (52)
Lung	63	15.4	16 (25)	235	7.9	134 (57)
Total	142	16.8	31 (22)	535	7.5	265 (49)

\*Incidence of secondary brain metastasis significantly lower ( $p < 0.001$ ) in CancerVax patients than in historical controls for each initial distant site.

<sup>†</sup>Other therapies include chemotherapy, surgery, and/or non-CancerVax immunotherapy.

intended to prevent the outgrowth of micrometastases remaining after removal of tumor-involved regional lymph nodes. Our RT-PCR studies indicate the presence of circulating tumor cells in about 72 percent of AJCC stage III patients.<sup>37</sup> Active immunotherapy is far more realistic in these patients than in those with a larger tumor burden, because a growing tumor mass of even 1 cm in diameter contains up to one billion tumor cells that have already successfully evaded the patient's immune system. Moreover, without adjuvant immunotherapy, an estimated 60 to 80 percent of AJCC stage III patients will experience recurrence and death within five years after lymphadenectomy, depending on the initial number of tumor-involved regional lymph nodes.<sup>52</sup> However, the median time to recurrence is one to two years, a period

long enough to accommodate the induction of a clinically beneficial response to vaccine immunotherapy.

Adjuvant immunotherapy for patients with AJCC stage III melanoma has undergone intense investigation in trials of specific melanoma vaccines, nonspecific immune stimulants such as BCG and levamisole, and biologic response modifiers such as the interferons. Until recently, all of these trials have been negative or of borderline significance.<sup>53</sup>

#### Adjuvant Therapy with IFN alfa-2b

The treatment of melanoma was significantly changed in January 1996, when Kirkwood et al<sup>54</sup> from the Eastern Cooperative Oncology Group (ECOG) reported that high-dose IFN alfa-2b significantly prolonged both relapse-free and

overall survival following surgical resection of high-risk primary melanoma (AJCC stage II-B) or regional lymph node metastasis (AJCC stage III). IFN alfa-2b administered intravenously (20 million U/m<sup>2</sup>/day) for four weeks and then subcutaneously (10 million U/m<sup>2</sup> three times a week) for 48 weeks increased median disease-free survival by one to 1.7 years and median overall survival by 2.8 to 3.8 years compared with observation. Interferon treatment also increased the five-year survival rate by 24 percent.

This important study was the first randomized, controlled trial to show a significant benefit of adjuvant therapy in prolonging relapse-free and overall sur-

port of their results showed no difference in either disease-free or overall survival,<sup>55</sup> which indicates that lower-dose interferon, although less toxic, is not as effective as the high-dose regimen used in the ECOG study.

Another approach to adjuvant therapy was pursued by the North Central Cancer Treatment Group and recently reported by Creagan et al.<sup>56</sup> This randomized trial compared high-dose IFN alfa-2a (20 million U/m<sup>2</sup>, intramuscularly, three times per week for 12 weeks) with observation in 102 patients with high-risk primary melanomas (>1.69 mm) and 160 patients with regional nodal metastasis. Although there was no significant difference in either disease-free or overall sur-

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***Adjuvant active immunotherapy refers to administration of a cancer vaccine after surgical resection of all clinically evident tumor.***

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vival of high-risk melanoma patients. Based on its results, the FDA approved IFN alfa-2b for postoperative adjuvant therapy in melanoma patients at high risk for systemic recurrence. However, one year of high-dose interferon therapy costs about \$35,000 and produces significant toxic side effects. In the ECOG study, toxicity required dose modifications in 65 percent of patients, 43 percent during the induction phase and 46 percent during maintenance therapy.

Other investigators have been seeking less toxic and less costly doses and schedules for administration of adjuvant interferon. Cascinelli and colleagues at the World Health Organization have conducted the largest study thus far. Four hundred forty-four melanoma patients with regional lymph node metastases were randomized to an observation arm or to treatment with low-dose IFN alfa-2a (3 million U 3 times/week subcutaneously) for three years. The most recent re-

vival, subset analysis showed borderline improvement in the disease-free survival of patients with nodal metastasis.

In summary, low doses of interferon for a long period or extremely high doses for a very short period seem to be less effective than high/intermediate doses for about one year. However, the cost and toxicity of high-dose interferon make this regimen less than optimal.

### **Melanoma Vaccines as Postsurgical Adjuvants**

In the early 1970s, we conducted a randomized study of a living allogeneic melanoma vaccine (a CancerVax predecessor) in AJCC stage III melanoma patients undergoing resection of lymph node metastasis.<sup>57,58</sup> Although vaccine recipients appeared to have a higher five-year survival rate (53 percent) than patients in the observation group (39 percent), this difference was not significant due to the small

**Table 4**  
**Clinical Trials of Adjuvant Vaccine Therapy for AJCC Stage III Melanoma Patients Undergoing Resection of Regional Lymph Node Metastasis**

Reference	Type of Trial	Number of Immunized Patients	Year of Study	Type of Vaccine	No. of Cell Lines	Components	Adjuvant	Survival Correlated with Immune Response
Morton et al <sup>58</sup>	Phase III	44	1978	Allogeneic	3	Living irradiated cells	BCG	IgM Abs to cell-surface Ags
Livingston et al <sup>67</sup>	Phase III	58	1994	NA	NA	GM2	BCG	Anti-GM2 Ab
Wallack et al <sup>69</sup>	Phase III	104	1995	Allogeneic	4	Lysate	Vaccinia	NR
Morton et al <sup>31</sup>	Phase II	283	1992	Allogeneic	3	Living irradiated cells	BCG	Anti-GM2, anti-GD2, and anti-GD3 Ab; antibody to protein Ags; DTH; CTL
Hersey <sup>61</sup>	Phase II Phase III ongoing	182	1993	Allogeneic	1	Lysate	Vaccinia	NR
Bystryn <sup>64</sup>	Phase II	90	1993	Allogeneic and xenogeneic (hamster)	4	Shed antigens	Alum	DTH
Berd et al <sup>65</sup>	Phase II	37	1993	Autologous	1	Living irradiated cells	DNP/BCG	DTH
Mitchell et al <sup>66</sup>	Phase II	29	1995	Allogeneic	2	Lysate	DETOX	Possibly CTL precursors

Ab = antibody; Ag = antigen; BCG = bacille Calmette-Guérin; CTL = cytotoxic T lymphocyte; DTH = delayed cutaneous hypersensitivity; DNP = dinitrophenol; IgM = immunoglobulin M; NA = not applicable; NR = not reported.

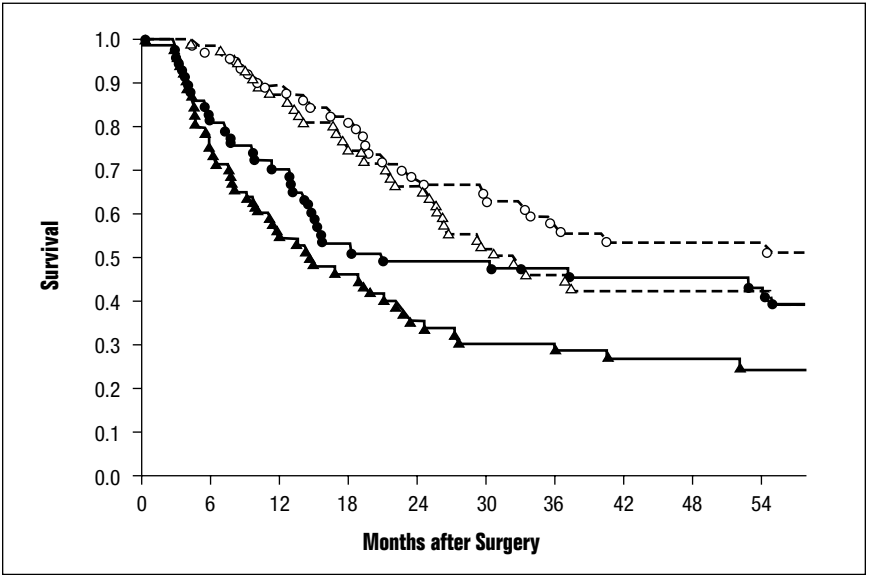


Fig. 6. Disease-free (solid lines) and overall (dashed lines) survival of melanoma patients randomized to receive bacille Calmette-Guérin (BCG) (▲, n=64) or GM2/BCG (●, n=58) as adjuvant therapy following resection of regional nodal metastasis (AJCC stage III disease). Reprinted with permission from Livingston et al.<sup>67</sup>

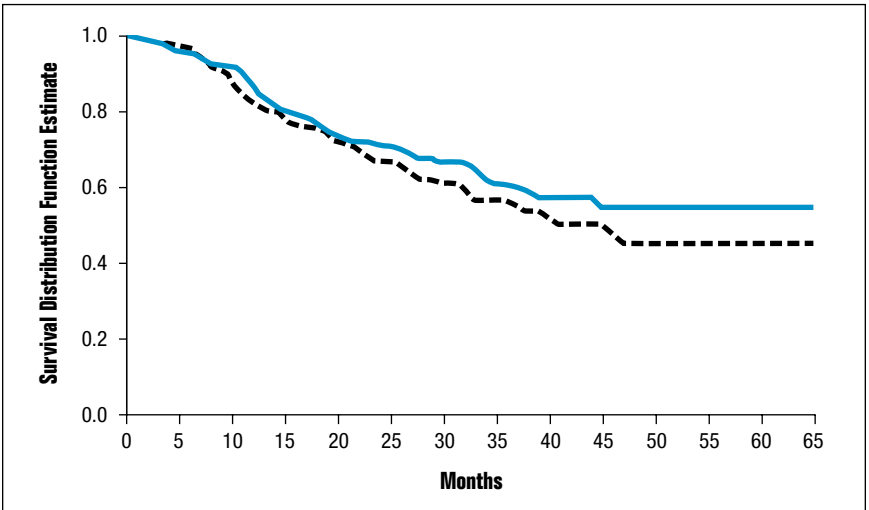


Fig. 7. Overall survival of melanoma patients randomized to receive either vaccinia melanoma oncolysate (VMO) (n=104, solid blue line) or vaccinia virus alone (n=113, broken black line) as adjuvant therapy after resection of regional nodal metastasis (AJCC stage III disease). VMO therapy was associated with a nonsignificant increase in overall survival ( $p=0.88$ ). Reprinted with permission from Wallack et al.<sup>69</sup>

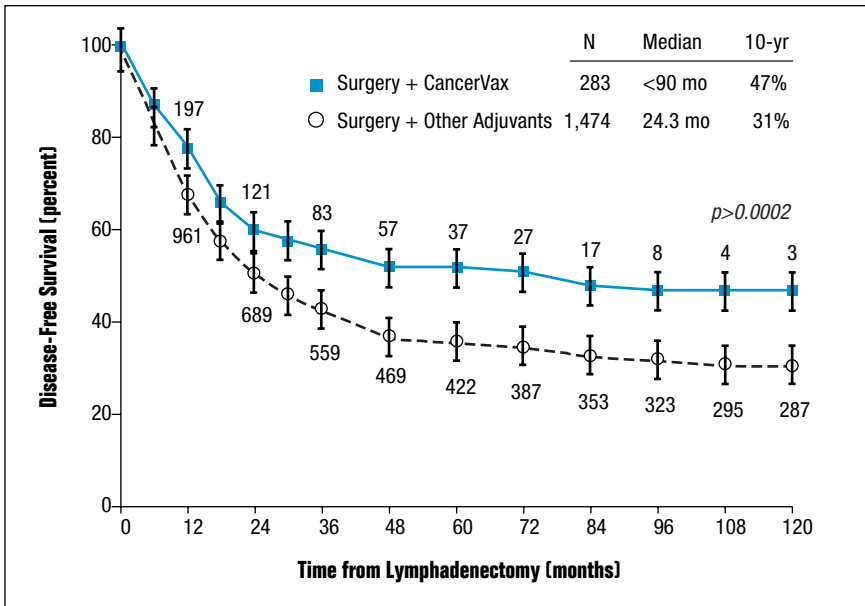


Fig. 8. Disease-free survival of melanoma patients receiving adjuvant CancerVax versus other adjuvant therapies or no adjuvant therapy following resection of regional nodal metastasis (AJCC stage III disease). Other adjuvant therapies were immunotherapy with bacille Calmette-Guérin and/or chemotherapy. Numbers indicate the number of patients observed and at risk for each observation period.

sample size. Patients who developed a high titer of IgM antibodies against MAA experienced prolonged survival,<sup>59</sup> but because of the vaccine's low potency, only one third of patients were responders.

Since that time, we and others have conducted phase II investigations of vaccines as postoperative adjuvants in melanoma (Table 4). These trials have examined irradiated whole cell vaccines,<sup>31,38</sup> vaccinia melanoma cell lysates (n=182),<sup>60,61</sup> shed polyvalent antigen vaccine (n=90),<sup>62-64</sup> DNP-conjugated autologous vaccine (n=37),<sup>65</sup> and melanoma lysate vaccine (n=29).<sup>66</sup> Because the phase II data suggested a survival benefit, results of prospective, randomized (phase III) trials have been awaited with great interest.

In the phase III trial conducted by Livingston et al,<sup>67,68</sup> 122 patients with resected AJCC stage III melanoma received either purified GM2 ganglioside with BCG or BCG alone after pretreatment with low-dose cyclophosphamide. After a median follow-up of 63 months, there was no difference in disease-free and overall survival between the two treatment arms (Fig. 6). However, when patients with an elevated prestudy anti-GM2 antibody level were excluded from the analysis, there was a significant improvement in disease-free survival.

In the phase III, multicenter trial conducted by Wallack et al,<sup>69</sup> 217 eligible patients with resected AJCC stage III melanoma received either vaccinia melanoma oncolysate (VMO) (n=104) or vac-

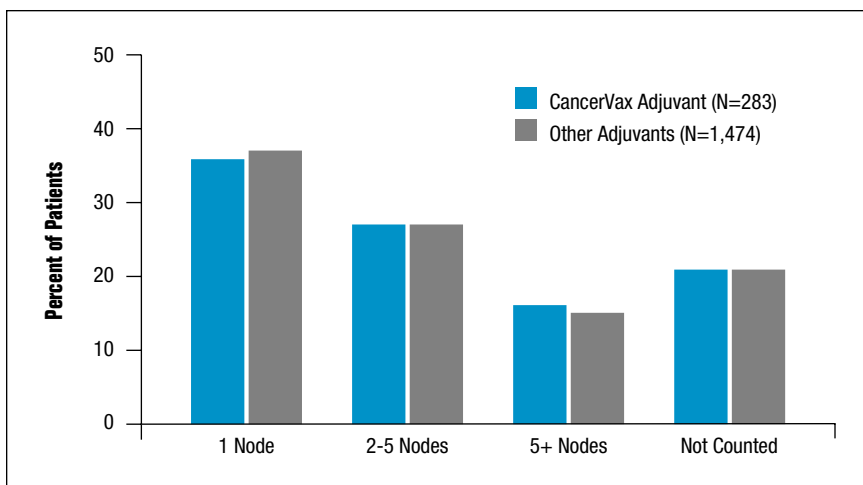


Fig. 9. Number of tumor-involved lymph nodes in AJCC stage III melanoma patients receiving CancerVax versus other postoperative adjuvant therapies.

cinia virus alone (n=113) for one year. After a mean follow-up of 30 months, interim analysis showed no significant difference in disease-free and overall survival (Fig. 7). Subset analysis revealed an improvement of 17 percent in overall four-year survival for male patients treated with VMO (p=0.19), which increased to 30 percent for males younger than 57 years with one to five tumor-positive lymph nodes (p=0.06). A second interim analysis with longer follow-up is expected in the near future.

We recently reviewed the updated results of our adjuvant phase II trial of CancerVax for resected AJCC stage III melanoma. CancerVax was administered intradermally in a dose of 24 million living irradiated cells, as previously described.<sup>31</sup> Five years after lymphadenectomy, 51 percent of the 283 melanoma patients receiving CancerVax were disease free compared with 36 percent of 1,474 historic control patients receiving other adjuvant therapies (Fig. 8). Median disease-free survival was 24.3 months for the control group versus greater than 90

months for patients receiving CancerVax (p=0.0002). Although we could not completely eliminate the selection bias inherent in an historical data base, we minimized its effects by studying a large number of patients and by matching vaccine and control groups according to the number of involved lymph nodes, which is the most important prognostic factor in AJCC stage III melanoma (Fig. 9). The results were highly suggestive of a therapeutic benefit. Immunologic studies revealed that a delayed-type hypersensitivity reaction and/or increased cytotoxic T-cell activity were positive predictors of long-term survival.<sup>38,70</sup> As a result of these promising phase II results, a multicenter, randomized phase III trial of adjuvant therapy will shortly be initiated to compare CancerVax against IFN alfa-2b.

## Conclusion

The evolution of vaccine strategies for malignant melanoma parallels the development of modern tumor immunology and molecular biology. Although re-

markable advances have been made, many questions remain. Among these are the clinical relevance of certain MAA, the optimal immunization strategy, the best adjuvant, and the role of the various cytokines.


A particularly controversial issue is whether a melanoma vaccine should be a purified preparation of a single determinant or a polyvalent mixture of living whole cells. Polyvalent cell-based vaccines such as CancerVax contain a wide spectrum of MAA, some known and others unknown, which usually include many antigens present on cells of the patient's tumor. Standardized production and quality control of cell-based vaccines are delicate but feasible. However, assessment of specific immune responses requires tests against multiple MAA. When there is an HLA match, living cell vaccines have the great advantage of being able to present their antigens to the patient's T cells directly, as well as indirectly through antigen-presenting cells.<sup>35</sup>

Single-determinant vaccines are well defined and can usually be produced in large quantities without difficulty. Quality control and assessment of a specific immune response pose less of a problem. However, not all human melanomas will express a single, specific melanoma antigen or contain a certain HLA type, and therefore only a fraction of all melanoma patients may benefit from such a vaccine.

Although the data from vaccine studies indicate that outcome seems worse in patients who do not develop a cellular or humoral immune response and better in those who develop one or both types of response, it is still unclear whether an optimal vaccine should pri-

marily induce a cellular response, a humoral response, or both. Only randomized trials will provide answers to these important issues.

Over the last 25 years, active specific immunotherapy with vaccines has evolved as a promising new modality in the treatment of malignant melanoma. Phase II studies have established the feasibility and suggested the efficiency of vaccine approaches in the adjuvant and metastatic setting. Although early results from phase III trials have thus far not demonstrated an unequivocal survival benefit for adjuvant vaccine therapy, this failure possibly reflects inadequate sample size. Therefore, the results at present should be considered a stimulus rather than a deterrent to the design of larger trials that have the sensitivity to detect a small therapeutic benefit. Even a minimal therapeutic benefit would be fully justified by the low toxicity of cancer vaccines.

The future for melanoma and other cancer vaccines is bright because of their low toxicity. Novel approaches using optimized peptide vaccines<sup>71</sup>; concomitant cytokine administration<sup>72</sup>; or vaccine cell lines that have been transfected with an allogeneic HLA gene,<sup>73</sup> the gene of a cytokine,<sup>74-78</sup> or the costimulatory signal B7<sup>79,80</sup> have now entered clinical trials. These developments might further increase the impact of melanoma vaccine therapy and provide new hope for patients with this fatal disease. After many years without significant progress, we are now truly at the "end of the beginning" and can be optimistic about the future of therapeutic vaccines for melanoma and other types of cancer. 

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