

# Value of Alpha Interferon in Adjuvant Therapy for Melanoma: The Case Against

---

Lynn E. Spitler

A new staging system for melanoma went into effect in 2002 with the publication of the Sixth Edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual.<sup>1</sup> This new staging system is important because it allows precise determination of the prognosis for individual patients with melanoma and has been validated in more than 17,000 patients with melanoma (Table 19.1).<sup>2,3</sup> Under the new staging system, survival curves correlate well with stage of disease, and subgroupings within each stage allow further refinement of prognosis. This allows the physician to determine with precision which patients are at high risk for recurrence and to counsel patients about their prognosis and therapeutic options.

On the basis of this important information, the dilemma being faced by the treating physicians and patients with melanoma is what, if any, surgical adjuvant therapy they should choose. The problem arises in part because studies of adjuvant therapy for melanoma are not as advanced as those on other cancers, and no therapeutic agent has yet been identified that shows unequivocal efficacy combined with an acceptable safety profile. In breast cancer, for example, tens of thousands of women have participated in randomized controlled clinical trials of

**TABLE 19.1 Staging of Malignant Melanoma**

Stage	Characteristics	5-Year Survival
I	No nodal or distant metastases and	
A	Primary tumor $\leq 1$ mm in thickness without ulceration	95%
B	Primary tumor $\leq 1$ mm in thickness with ulceration or level IV or V or primary tumor 1.01–2 mm in thickness without ulceration	90%
II	No nodal or distant metastases and	
A	Primary tumor 1.01–2 mm in thickness with ulceration or primary tumor 2.01–4 mm in thickness without ulceration	78%
B	Primary tumor 2.01–4 mm in thickness with ulceration or primary tumor $> 4$ mm in thickness without ulceration	64%
C	Primary tumor $> 4$ mm in thickness with ulceration	45%
III		
A	1–3 nodes with micrometastasis and no ulceration of the primary tumor	67%
B	1–3 nodes with micrometastasis and ulceration of the primary tumor or 1–3 nodes with macrometastasis and no ulceration of the primary tumor or in transit met(s)/satellite(s) without metastatic nodes with or without ulceration of the primary	52%
C	1–3 nodes with macrometastasis and ulceration of the primary tumor or 4 or more nodes with micro- or macrometastasis with or without ulceration of the primary tumor or satellite or in-transit metastases with metastatic nodes with or without ulceration of the primary	27%
IV		
M1a	Skin and/or subcutaneous metastasis, normal LDH	19%
M1b	Lung metastasis, normal LDH	7%
M1c	Other visceral metastasis or any distant site of metastasis and elevated LDH	9%

Abbreviations: LDH, lactate dehydrogenase.

Modified from Balch et al.<sup>2</sup>

tamoxifen as surgical adjuvant therapy, and this agent offers a clear survival benefit with acceptable toxicity. A meta-analysis of 28 randomized trials of tamoxifen as surgical adjuvant therapy in 16,581 patients with stage I and II breast cancer showed a reduction of 16% ( $\pm$  3%) in the odds of death in women of all ages who were assigned to receive tamoxifen. The analysis also showed a clear reduction of mortality in women 50 years of age and older for whom tamoxifen reduced the annual odds of death during the first 5 years by about 20% ( $P < 0.0001$ ).<sup>4</sup>

Clinical trials of adjuvant therapy for melanoma have focused mainly on patients with stage III disease, probably because these patients have a poor prognosis ( $< 50\%$  5-year survival) and are represented in greater numbers than patients with stage IV disease that was surgically excised, who have an even worse prognosis. In the United States and Canada, but not in other countries, high-dose interferon (HDI) is approved for adjuvant therapy for “high-risk” melanoma, although many patients refuse this therapy after being informed of the risk/benefit ratio. There is no approved adjuvant therapy for patients in other categories, so such patients can receive standard therapy (observation) after surgery, participate in a clinical trial, or receive off-label treatment. It is clear that alternative therapies with greater efficacy and less toxicity are needed, but, so far, all phase 3 trials of alternative treatments have failed to show a difference between the control and treatment arms, i.e., they have been negative studies.

## INTERFERON

The results of multicenter randomized controlled trials for interferon for the adjuvant treatment of melanoma have been the subject of several reviews.<sup>5-8</sup> The reviews included between 8 and 12 of the following trials: Austrian,<sup>9</sup> Eastern Cooperative Oncology Group (ECOG) 1684,<sup>10</sup> ECOG 1690,<sup>11</sup> ECOG 1694,<sup>12,13</sup> EORTC 18871,<sup>14</sup> EORTC 18952,<sup>14</sup> French,<sup>15</sup> NCCTG 83705,<sup>16</sup> Scottish MG,<sup>17</sup> UKCCCR,<sup>18</sup> and WHO 16.<sup>19,20</sup> The trials varied in terms of the product used (interferon- $\alpha$ 2b [Intron<sup>®</sup> A] or interferon  $\alpha$ 2a [Roferon<sup>®</sup>]), dose (high or low), duration of interferon administration, and stage of disease.

Even though the trials were heterogeneous and the reviews each included different trials, the conclusions of the reviews were remarkably similar in the following ways: They agreed that the administration of interferon alfa (IFN- $\alpha$ ) as adjuvant therapy for melanoma does prolong disease-free survival (DFS) but not overall survival (OS):

1. “In our review, results from included RCTs (randomized clinical trials) demonstrated no clear benefit of IFN- $\alpha$  therapy on OS in melanoma patients.”<sup>8</sup>
2. “With mature data on OS of some trials using this schedule still pending, high-dose IFN- $\alpha$  does prolong DFS but not OS in patients with stage IIB-III melanoma. Given the toxicity of this treatment, the standard use of adjuvant high-dose IFN- $\alpha$  currently cannot be recommended.”<sup>7</sup>

3. "For DFS, there was clear benefit for IFN: odds ratio (OR) = 0.84, 95% confidence interval (CI) = 0.77-0.92,  $P = .0001$ . The advantage was less clear for OS (OR = 0.76, CI = 0.82-1.00,  $P = .05$ ) and the upper CI is compatible with no survival benefit . . . decisions on the use of IFN for melanoma will need to be based on considerations such as the relative importance of benefits on DFS compared to OS, patient quality of life and financial cost."<sup>21</sup>
4. ". . . all estimates of odds ratios are  $< 1.0$ , indicating survival benefit. However, only one of these estimates appears to be significantly different from 1.0 [Kirkwood et al<sup>10</sup>]. The upper bounds of all other 95% confidence intervals include 1.0. The estimate of odds ratio for all studies is 0.88 (95% CI: 0.77, 1.03;  $P = .065$ )."<sup>5</sup>

### HIGH-DOSE INTERFERON

---

The only drug approved for the postsurgical adjuvant treatment of melanoma in the United States and Canada is interferon- $\alpha 2b$ . The labeled indication on the package insert states: "INTRON<sup>®</sup>A Interferon alfa-2b, recombinant for injection is indicated as adjuvant to surgical treatment in patients 18 years of age or older with malignant melanoma who are free of disease but at high risk for systemic recurrence within 56 days of surgery." The meaning of "high risk" is not defined on the product label, so it is not clear exactly what this approval covers. However, the clinical trials on which this approval was based included patients with primary melanoma  $> 4$  mm in thickness (stage II, T4) and melanoma metastatic to regional nodes (part of the stage III classification). One assumes that this is the population that the approval covers, but that has not been made clear. The clinical trials did not include patients with in transit or satellite metastases (another part of the stage III classification) or patients with stage IV disease that has been surgically excised, who are also at very high risk for recurrence.

HDI is an imperfect solution for adjuvant therapy for melanoma because most studies show improvement in DFS, but survival benefit in the treated patients is controversial and toxicity is considerable. The details of the clinical trials of HDI supporting this conclusion have been extensively reported and debated and are summarized in Table 19.2.

### Clinical Benefit, Overall Survival

**E1684**<sup>10</sup> The ECOG completed a prospective, randomized, controlled study of interferon- $2\alpha b$  versus observation as surgical adjuvant therapy in 287 patients with melanoma. As reported, with a median follow-up time of 6.9 years, the median OS was increased by 1 year in the treatment arm as compared with the ob-

**TABLE 19.2 ECOG Trials of HDI as Adjuvant Therapy of Melanoma**

Trial	Design	Outcome	Toxicity	Comments
E1684 <sup>10</sup>	252 eligible patients (280 ITT) randomly assigned to high-dose interferon or observation; 11% node negative.	Publication reports significant improvement in disease-free survival in patients receiving high-dose interferon. Survival at 5 years: 46% in patients receiving interferon, 37% in observation group. Median follow-up = 6.9 years.	67% of patients had severe (grade 3) toxicity at some point during the year of treatment, 9% had life-threatening toxicity, and two had lethal hepatic toxicity.	The most important prognostic indicator for this patient population, number of positive nodes, <sup>3,67</sup> was not recorded; therefore, it is not known whether the treated and observation patient populations were balanced with regard to this indicator. The overall survival benefit was lost with longer follow-up (median, 145 months; $P = .09$ ) <sup>7</sup>
E1690 <sup>11</sup>	608 eligible patients (642 ITT) randomly assigned to high-dose or low-dose interferon or observation; 25% had nodes clinically or pathologically negative.	Significant improvement in disease-free survival in patients receiving high-dose interferon but no difference in overall survival ( $P = 0.99$ ). Median follow-up = 4.3 years.	The manuscript does not state the percentage of patients with grade 3 or 4 toxicity but states that "Treatment with HDI was associated with significant toxicity consistent with the adverse events that have been reported by others and those observed in trial E1684." There were no treatment-related deaths in the HDI arm.	Patients randomly assigned to the observation who experienced relapse and then received interferon-containing salvage therapy had an apparent postrelapse survival advantage compared with patients who did not receive interferon-containing salvage therapy, indicating that interferon treatment is beneficial when it is given immediately after lymphadenectomy or after relapse.
E1694 <sup>12</sup>	774 eligible patients (880 ITT) randomly assigned to high-dose interferon or GMK melanoma vaccine; no observation group; 23% had nodes clinically or pathologically negative.	Significant improvement in disease-free and overall survival in patients receiving high-dose interferon as compared with those receiving vaccine. Median follow-up = 16 months.	The manuscript does not state what percentage of patients had grade 3 or 4 toxicity but does state there were no treatment-related deaths.	Median follow-up relatively short.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HDI, high-dose interferon; ITT, intent-to-treat.

ervation arm, and the 5-year survival was increased by 9% from 37% in patients randomly assigned to observation to 46% in patients receiving HDI ( $P = .0237$ , one-sided log-rank test). The analysis performed by the U.S. Food and Drug Administration (FDA) also concluded that the improvement in median OS was 12 months (3.8 years vs 2.8 years) but the  $P$  value did not meet the standard measure of significance ( $P = .06$  unstratified log-rank test, two-sided log-rank test;  $P = .045$  stratified log-rank test). In a later analysis by Cole et al<sup>22</sup> using the same data set, there was an improvement of 7.0 months of OS, and the difference between the treatment arm and observation arm did not reach the level of statistical significance when a two-sided test was applied ( $P = .07$ ). Similarly, Lens and Dawes<sup>8</sup> reviewed this trial and calculated the number needed to treat for OS and found that it did not reach statistical significance. Recent long-term follow-up data at a median follow-up of 145 months shows no OS benefit, with 95 deaths in the control arm and 93 deaths in the HDI arm ( $P = .09$ ).<sup>7</sup>

**E1690**<sup>11</sup> In follow-up, a large, prospective, randomized trial was performed on 608 eligible patients who were randomly assigned to one of three arms: HDI, low-dose interferon, or observation. There was no survival benefit in patients receiving HDI compared with those receiving low-dose interferon (LDI) or observation ( $P = 0.99$ ). In Europe, after the publication of these data, reimbursement for HDI as surgical adjuvant therapy for melanoma was no longer offered, and the use of this modality virtually ceased. Another observation in this study was that patients who were randomly assigned to the observation arm who experienced relapse and then received interferon-containing salvage therapy had an apparent postrelapse survival advantage compared with those who did not receive interferon-containing salvage therapy, indicating that timing of the interferon treatment is not important—it can be given immediately after lymphadenectomy or after relapse.

**E1694**<sup>12</sup> The study included 774 eligible patients who were randomly assigned to HDI or to the GM2/KLH vaccine with QS 21 (called GMK). There was progression-free and OS benefit in patients receiving HDI compared with those receiving GMK vaccine, but the median follow-up was only 16 months, and there was no untreated control group. Because there was no untreated control arm, it is not possible to rule out the possibility that the GMK vaccine had a deleterious effect on survival. It is noteworthy that the adjuvant was changed from bacille Calmette-Guérin (BCG), which was used in the phase II trial,<sup>23</sup> to QS21 in the phase III trial. This could have produced a higher level of gamma G immunoglobulin antibodies, which might have adversely affected the outcome.

The value of HDI in improving OS was discussed in detail at the meeting of the Oncologic Drugs Advisory Committee on February 27, 2002. Although the committee did not vote on the issues, the consensus was that HDI has not been shown to provide benefit in OS and that phase III trials of adjuvant therapy for

high-risk melanoma are not required to include a HDI treatment arm. It would not be appropriate to include quotes on this subject by the committee members out of context, but the transcript of their deliberations is available on-line.<sup>24</sup>

### **Toxicity**

- 67% of the patients in E1684 had grade 3 toxicity; 9% had grade 4 toxicity, and two deaths occurred as a result of hepatotoxicity.<sup>10</sup>
- Comparable figures are not given for E1690 or E1694, except to state that no deaths occurred as a result of toxicity.<sup>11,12</sup>
- The product label includes toxicity data only from 143 patients included in the first trial (E1684); it has not been updated with data from the other trials or with phase IV data.
- The manufacturer states that they are not collecting postmarketing toxicity data.
- The FDA prepared a lengthy and detailed analysis of the results of the trials on the use of interferon as adjuvant therapy for melanoma, but this analysis does not include any mention of toxicity.<sup>5</sup>
- The toxicity of the regimen must be viewed in the context of the patients' clinical status. Although these patients are at high risk for recurrence, they have been treated with surgery, the best treatment available for melanoma, and they are clinically tumor free at the time of treatment with HDI; therefore, some may already have been cured of their disease by surgery.

Here is a description written by a patient taking interferon and published in "Rays of Wisdom," a publication of the American Melanoma Foundation: ". . . most of the time I make it into the bathroom before my world spins to black. The cool of the porcelain feels good against my cheek, and I rest there, on the floor, dizzy from the exertion that has gotten me here. And now, the next decision comes. Do I use the sink to pull myself off the floor, knowing the explosive and instant pain it will bring, or do I stay resting, soothing my face on the coolness, and moving as little as possible?" This patient had a recurrence of her melanoma, and the same publication states, "When her doctor prescribed more interferon, Linn told him, unequivocally, that she would rather die."

### **LOW-DOSE INTERFERON**

---

The use of LDI for patients with stage II melanoma has been approved by the European Medicine Evaluation Agency, but not by the U.S. FDA. The approval was based on two trials. Low-dose interferon was evaluated in a large, randomized study on adjuvant therapy in 499 patients with stage II melanoma (primary melanoma greater than 1.5 mm in thickness with clinically negative nodes).<sup>25</sup> The results in this patient population seemed promising; even though the low-dose

regimen does not seem effective in higher-risk patients; there was benefit in progression-free survival in the patients who were randomly assigned to LDI. There was also benefit in terms of OS at a median follow-up of 5 years, but not at a median follow-up of 8 years.<sup>7,15</sup> In a second randomized trial of LDI in 311 patients with resected primary stage II melanoma, prolonged DFS was also demonstrated.<sup>9</sup> The results of the LDI on OS in this trial are unknown. A third trial included patients with stage II and III disease who were randomly assigned to LDI or observation; this trial demonstrated no difference between the treatment arms.<sup>17</sup>

## ALTERNATIVE APPROACHES

### Negative Phase 3 Trials

Numerous therapeutic agents other than interferon have completed randomized phase 3 trials as adjuvant therapy for melanoma, and none of the trials have shown a survival benefit (Table 19.3).

These trials included evaluation of four vaccines. In one study, 217 eligible subjects with stage III melanoma were randomly assigned to receive vaccinia melanoma oncolysate or vaccinia alone. There was no difference in the DFS

**Agents that Have Been Tested as Adjuvant Therapy for Melanoma in Randomized Trials and Have Been Shown Not to Affect Overall Survival**

**TABLE 19.3**

Agent	Reference, Comments
Vaccinia melanoma oncolysate (VMO)	Wallack et al <sup>26</sup>
Vaccinia melanoma cell lysate (VMCL)	Hersey et al <sup>27,28</sup>
Melacine	Sondak et al <sup>29</sup> ; subjects of specified HLA type had improved disease-free survival. <sup>30</sup>
GMK vaccine	Kirkwood et al <sup>12</sup>
Dacarbazine (DTIC)	Hill et al <sup>31</sup>
Bacille Calmette-Guérin (BCG)	Pinsky et al <sup>33</sup> ; Cunningham, T.J. et al <sup>32</sup>
Levamisole	Quirt et al <sup>34</sup> ; Spittle et al <sup>35</sup> ; the study by Quirt et al reported clinical benefit in the treated patients, and on this basis, levamisole was approved as adjuvant therapy for melanoma in Canada but is no longer available.

Abbreviation: HLA, human leukocyte antigen.

( $P = 0.61$ ) or the OS ( $P = 0.79$ ).<sup>26</sup> In a similar study using different melanoma cell lines, 675 eligible patients with stage IIB and III melanoma were randomly assigned to vaccinia melanoma cell lysate or observation. After a median follow-up of 8 years, the median OS was 88 months in the control arm and 151 months in the treated arm ( $P = 0.068$ ).<sup>27,28</sup> The Southwest Oncology Group (SWOG) conducted a randomized trial (SWOG-9035) of Melacine versus observation in 600 eligible patients with stage II melanoma.<sup>29</sup> The results showed no difference in the DFS rates in the treatment arms; there were 65 deaths in the vaccine arm and 71 deaths in the treatment arm. A further analysis of this study was performed on the basis of the patients' human leukocyte antigen (HLA) type.<sup>30</sup> There were 97 vaccine-treated patients and 78 observation patients whose HLA type matched  $\geq$  two of the M5 HLA class I antigens, and these vaccine-treated patients had a significantly better relapse-free survival than the observation patients (5-year relapse-free survival was 83% vs 59%;  $P = 0.0002$ ). Finally, a combined intergroup protocol by three cooperative groups (E1694/S9512/C50981) was conducted in which 774 eligible patients with high-risk melanoma were randomly assigned to the GM2/KLH vaccine with QS 21 (called GMK) versus high-dose interferon.<sup>12</sup> The results showed that patients receiving the HDI had better OS and DFS than in patients who received the vaccine.

Other agents that have been tested and failed as adjuvant therapy for high-risk melanoma include dacarbazine (DTIC)<sup>31</sup> and BCG.<sup>32,33</sup> In a large, prospective, randomized trial, levamisole was reported to be effective in the adjuvant therapy of high-risk melanoma,<sup>34</sup> and on the basis of that study, levamisole was approved for this purpose in Canada, but not in the United States. A similar, prospective, randomized, placebo-controlled trial failed to show a benefit of le-vamisole in the adjuvant treatment of melanoma, but the dose used was slightly different.<sup>35</sup> The manufacturer has ceased production of levamisole, so it is no longer available in the United States.

## Vaccines

Numerous vaccines have completed phase 2 trials with encouraging results, but as of August 2002, only three of them (a multiepitope peptide vaccine, Melacine, and the GMK vaccine) were in phase 3 trials open to patient accrual (Table 19.4). CancerVax was developed by Donald Morton at the John Wayne Cancer Institute and consists of an irradiated live-cell preparation of three allogeneic melanoma cell lines chosen for their high content of immunogenic melanoma-associated and common tumor-associated antigens, administered with BCG as an adjuvant.<sup>36</sup> In phase 2 clinical trials conducted worldwide, the vaccine has been administered to more than 1600 patients with advanced-stage melanoma, and the results document the safety of the vaccine and its potential efficacy, as shown by a significant increase in 5-year survival rates and in OS time when compared with historical controls.<sup>37,38</sup> CancerVax is now in phase 3 trials in separate

**TABLE 19.4** Noninterferon Agents in Phase 2 or 3 Clinical Trials as Adjuvant Therapy for Malignant Melanoma

Agent	Stage	Phase	Comments
<sup>a</sup> Peptide vaccine, GM-CSF vs placebo	III, IV	3	Randomized intergroup trial (E1697)
<sup>a</sup> GMK vs observation	II	3	EORTC trial
<sup>a</sup> Melacine + LDI vs HDI	III	3	
CancerVax vs observation	III	3	Study on indefinite hold for patient accrual.
CancerVax vs observation	IV	3	Study on indefinite hold for patient accrual.
Melacine(®) vs observation	II	3	Study not yet open for accrual.
<sup>a</sup> Biochemotherapy vs HDI	III	3	Bedikian <sup>68</sup>
TriGem + HDI vs HDI	III	3	Study not yet open for accrual.
<sup>a</sup> GM-CSF	II(T4), III, IV	2	
<sup>a</sup> Peptide vaccine + adjuvants	IIB, IIC, III, IV	2	Conducted by Dr. J. Weber, pers. comm. 2002
<sup>a</sup> Peptide vaccine + Montanide ISA-51	I-IV	2	Patients must be HLA-A1, A3, or O201 positive. <sup>69</sup> Accrual for types HLA-A24 and HLA-A31 is closed.
<sup>a</sup> Autologous tumor cells plus autologous dendritic cells	III, IV	2	Conducted by Dr. R.O. Dillman, pers. comm. 2002
<sup>a</sup> Neoadjuvant biochemotherapy	III, IV	2	

Abbreviations: GM-CSF, granulocyte macrophage colony stimulating factor; LDI, low-dose interferon; HDI, high-dose interferon; EORTC, European Organization for Research and Treatment of Cancer; HLA, human leukocyte antigen.

<sup>a</sup>Study open to accrual.

studies in patients with stage III and in those with stage IV disease that has been surgically excised. These studies have been placed on indefinite hold for patient accrual by the FDA because of manufacturing issues.

Analysis of tumor-infiltrating lymphocytes obtained from patients with melanoma led to the cloning of the genes that encode proteins recognized by T cells with antitumor activity and to the subsequent identification and synthesis of the relevant peptides. This led to the synthesis of peptides designed to have

increased binding to the HLA-A2 molecule. The combination of these synthetic peptides resulted in the multiepitope peptide vaccine. The peptides included in this vaccine are gp 100 209-217 210M, MART-1 26-35 27L, and tyrosinase 368-376 370D. A trial (E1697) has been initiated that will include 600 patients with known HLA-A2 status. Eligibility is restricted to melanoma patients with locoregional recurrence after prior adjuvant interferon therapy, local recurrence after adequate surgical excision of the primary tumor, mucosal melanoma, stage IV melanoma, satellite or intransit disease, stage III disease with gross extracapsular extension, recurrence in a previously resected nodal basin, four or more involved lymph nodes, matted lymph nodes, or an ulcerated primary melanoma and any involved lymph nodes, and to those who are medically unfit for HDI. Patients must not have received prior treatment with granulocyte macrophage colony stimulating factor (GM-CSF) and must be rendered surgically free of disease. Eligible patients who are HLA-A2 positive will be randomly assigned to control (placebo), multiepitope peptide vaccine, GM-CSF, or GM-CSF and multiepitope peptide vaccine. Patients who are HLA-A2 negative will be randomly assigned to GM-CSF or placebo. Accrual to this study is slow, probably because of the restrictive eligibility criteria.

Melacine consists of lysed cells from two human melanoma cell lines combined with an adjuvant, DETOX, which consists of monophosphoryl lipid A and mycobacterial cell wall skeleton. The vaccine induced an increase in precursors of cytolytic T cells and objective responses in some patients with advanced melanoma.<sup>39,40</sup> This led to the design of a phase 3 randomized trial in patients with stage II melanoma, described earlier, in which there was no difference in outcome between patients randomized to the treatment or control arms, i.e. it was a negative study. The subgroup analysis showing clinical benefit for vaccinated patients who were positive for HLA-A2, HLA-C3, or both, prompted the owner, Corixa Corporation, to propose a second randomized pivotal trial of Melacine as adjuvant therapy for patients with stage II melanoma who exhibited those HLA types.<sup>24</sup> Melacine is also being evaluated in a phase III randomized multicenter trial of Melacine and LDI versus HDI as adjuvant therapy for patients with stage III melanoma (study #6875-01).

GM2 is a ganglioside that is overrepresented on melanoma cells and thus can be used in a vaccine in an effort to stimulate an immune response to melanoma. A double-blind, randomized trial was conducted of a GM2 ganglioside vaccine, administered with bacille Calmette-Guérin (GM2/BCG) versus BCG alone as adjuvant therapy in 122 patients with stage III melanoma.<sup>23</sup> The results showed improved survival in patients with GM2 antibodies over patients who did not have these antibodies. In a subsequent phase I trial, it was shown that conjugating the GM2 ganglioside with keyhole limpet hemocyanin and administering to it the QS-21 as adjuvant resulted in serologic responses against GM2 that were strikingly superior, quantitatively and qualitatively, to any seen with previously tested GM2 vaccines.<sup>41</sup> The resulting vaccine is named GMK, and it was evaluated in

the randomized trial described earlier, which showed that patients receiving HDI had a better OS and DFS than patients who received the vaccine.<sup>12</sup> The GMK vaccine is also being tested in Europe in ongoing studies by the EORTC in a randomized trial of the vaccine versus observation as adjuvant therapy for patients with stage II melanoma.<sup>42</sup>

Some vaccines have completed successful phase 2 trials but have not progressed to phase 3 trials and are not currently available. One of these is a polyvalent, shed-antigen vaccine prepared from material shed into the culture medium by three allogeneic and one xenogeneic melanoma cell line adapted to long-term growth in serum-free medium.<sup>43</sup> A double-blind, prospectively randomized, placebo-controlled trial of this vaccine was initiated.<sup>44</sup> Thirty-eight patients with resected melanoma to regional nodes (stage III) were randomly assigned in a 2:1 ratio to vaccine or placebo. With a median follow-up of 2.5 years, there was a statically significant prolongation of time to disease progression, but not of OS. Unfortunately, the study was stopped because of poor patient accrual.<sup>45</sup>

In another approach, patients were immunized with hapteneized autologous tumor cells.<sup>46</sup> Autologous tumor cells were conjugated to the hapten dinitrophenol and administered with BCG to patients who had been sensitized to dinitrophenol alone. In 214 patients with stage III disease who were rendered tumor free by surgery, with a median follow-up time of 4.4 years, the overall 5-year survival was 47% in patients treated with the vaccine with BCG, a result believed comparable to those obtained with HDI.<sup>47,48</sup> Unfortunately, there are no clinical trials of this vaccine currently under way.<sup>49</sup>

TriGem is an antiidiotypic monoclonal antibody that mimics the disialoganglioside GD2. It was evaluated in a multicenter evaluation in 69 patients with stage III melanoma, of whom 25 also received HDI. At a median follow-up of 2 years, the data suggested a clinical benefit of TriGem.<sup>50</sup>

Numerous other vaccines are in phase 1 clinical trials aimed at defining the safety and immunogenicity of these vaccines in patients with melanoma, but it will be some time before these products will be ready for phase 2 testing for the adjuvant therapy for melanoma. Two centers where these studies are being conducted are the University of Wisconsin (M.R. Albertini, personal communication, 2002) and the Memorial Sloan-Kettering Cancer Center.

### **GM-CSF (Leukine, Sargramostim)**

Many lines of evidence suggest that GM-CSF might have activity in the adjuvant therapy for cancer. Activated macrophages distinguish tumor cells from normal cells and kill only the tumor cells.<sup>51</sup> GM-CSF, which is approved for marketing for hematopoietic reconstitution and reversal of iatrogenic neutropenia, also has activity as a macrophage activator. GM-CSF stimulates peripheral blood monocytes in vitro to become cytotoxic for human melanoma cells.<sup>52,53</sup> Further, in vivo

administration of low doses of GM-CSF also results in monocyte activation, as shown by enhanced cytotoxicity.<sup>54,55</sup> GM-CSF also serves as the principal mediator of proliferation, maturation, and migration of dendritic cells,<sup>56-58</sup> antigen-presenting cells that play a major role in the induction of primary and secondary T-cell immune responses. Finally, GM-CSF increases production of angiostatin by the macrophages.<sup>59,60</sup>

We conducted a trial of GM-CSF as a surgical adjuvant treatment in patients who were at very high risk for recurrence of melanoma.<sup>61</sup> The patient population included 48 patients with stage III disease who were at very high risk for recurrence (more than four positive nodes) or with stage IV disease who were rendered clinically disease free by surgery before enrollment. The OS and the DFS were significantly prolonged in patients who received GM-CSF compared with matched historical controls. This means that the patients receiving GM-CSF had a longer median time to disease progression and a longer survival than the matched controls. At 2 years, the survival in the control group was 15%, and in the treatment group, it was 64%. The median survival was 37.5 months in the study patients, versus 12.2 months in the matched controls ( $P < 0.001$ ).

We have initiated a second trial to further evaluate this therapy in an expanded patient population. Eligible patients are those with stage II (T4), III, or IV melanoma that has been surgically excised. An interim analysis of this study was presented at the 4th International Conference on Adjuvant Therapy of Melanoma held at the Royal College of Physicians in London, March 2002.<sup>62</sup> Fifty patients were included in the analysis, and the results suggested a survival benefit for the patients treated with GM-CSF as compared with 1000 matched patients from the AJCC database who had been treated with surgery alone (Fig. 19.1).

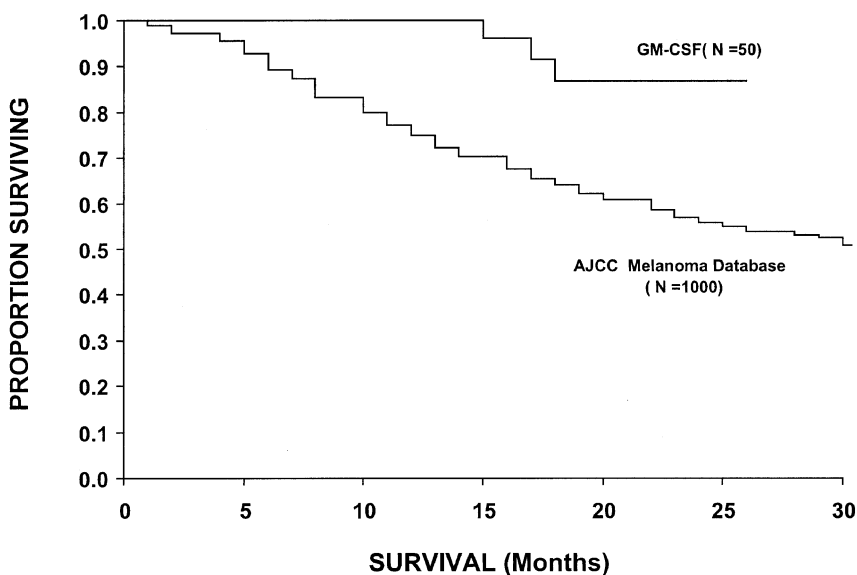
A prospective randomized trial to definitively evaluate efficacy of this approach has been initiated by a consortium of cooperative groups. This trial includes vaccine arms and is described in the Vaccines section.

### Other Biologic Approaches

Other biologic approaches for adjuvant therapy of melanoma are being pursued, including monoclonal antibodies, gene transfer, cellular therapies, and antiangiogenesis (Table 19.5). These agents are earlier in the course of development than other agents discussed in this chapter, but most have completed phase 1 trials that have shown safety and potential efficacy.

### Chemotherapy

In a series of phase 2 trials in patients with stage III node-positive melanoma, investigators found that patients receiving vindesine alone or in combination with DTIC or LDI had a statistically significant benefit when compared with the



**FIGURE 19.1** Survival in patients with high-risk melanoma treated with granulocyte macrophage colony stimulating factor (GM-CSF) or surgery alone (American Joint Committee on Cancer [AJCC] Melanoma Database).

untreated concurrent controls or the AJCC stage III patients enrolled in the Aim High trial, which was conducted in the United Kingdom.<sup>63,64</sup> Because of reports of the efficacy of biochemotherapy, especially CVD (cisplatin, vinblastine, dacarbazine) therapy for patients with metastatic melanoma<sup>65</sup>, a phase III randomized trial has been initiated consisting of biochemotherapy with CVD, interleukin-2, and interferon versus HDI for adjuvant therapy for melanoma patients with lymph node metastases that have been surgically resected (MDA-DM-95196, MDA-ID-95196, NCI-G96-1089). A pilot phase II study of neoadjuvant biochemotherapy was performed in 48 patients with stage III melanoma. The patients were given two cycles of biochemotherapy consisting of cisplatin, vinblastine, DTIC (CVD), interleukin-2, and interferon before and after lymph node dissection.<sup>66</sup> At a median follow-up of 31 months, 38 or the 48 patients (79%) were alive, and 31 patients (65%) remained free of disease progression.

## SUMMARY AND CONCLUSIONS

The FDA approved HDI for adjuvant therapy for high-risk melanoma on the basis of a trial (E1684) in which the survival benefit to patients did not reach the usually accepted level of statistical significance. Long-term follow-up of these patients showed less difference (not statistically significant, even by the one-tailed test used

**TABLE 19.5 Biologic Agents in Development as Adjuvant Therapy for Melanoma****Monoclonal Antibodies**

- Ch14.18: monoclonal antibody to the ganglioside GD2<sup>70</sup>  
 R24: monoclonal antibody to the disialoganglioside GD3<sup>71,72</sup>  
 ch14.18-IL-2: a fusion protein of the anti GD-2 antibody with IL-2<sup>73,74</sup>  
 Anti-CTLA4: antibody to the cytotoxic T lymphocyte antigen 4<sup>75</sup>  
 TriGem: anti-idiotypic antibody that mimics the disialoganglioside GD2<sup>50,76</sup>  
 (phase 3 trial planned)  
 BEC2: anti-idiotypic antibody that mimics the GD3 disialoganglioside<sup>77</sup>  
 (phase 3 trial planned in small-cell lung cancer)

**Gene Transfer**

- GM-CSF into melanoma cells<sup>78-80</sup>  
 IL-2 into melanoma cells<sup>81,82</sup>  
 IL-4 into melanoma cells [Maio, 2002 #751  
 IL-12 gene into autologous fibroblasts [Kang, 2001 #752]  
 IL-2 gene in a monkey fibroblast cell line (Vero)<sup>83</sup>  
 gp75 (TRP-1) xenogeneic DNA vaccine  
 Tyrosinase human vs xenogeneic DNA vaccine

**Cellular Therapies**

- Autologous dendritic cells pulsed with peptides representing epitopes of melanoma-associated antigens<sup>84-87</sup>  
 Autologous dendritic cells pulsed with tumor lysates<sup>88</sup>  
 Autologous dendritic cells transduced with autologous tumor RNA<sup>89</sup>  
 Autologous tumor cells plus autologous dendritic cells administered as a vaccine<sup>90</sup>

**Antiangiogenesis**

- Thalidomide with temozolomide, <sup>91-93</sup> REVIMID

Abbreviations: GM-CSF, granulocyte macrophage colony stimulating factor; IL, interleukin.

in the original analysis) in survival between treated and control subjects. A large, randomized, follow-on study (E1690) showed no survival benefit. Four reviews that analyzed results from eight to 12 randomized trials of interferon as adjuvant therapy for melanoma each concluded that survival benefit was not demonstrated. At its meeting on February 27, 2002, the Oncologic Drugs Advisory Committee also concluded that a survival benefit of HDI had not been demonstrated. However, a consensus supports the notion that HDI therapy increases DFS. What price is paid for such an increase? HDI is toxic, but detailed information on its toxicity is lacking. The product label describes only the toxicity observed in the 143 patients treated in E1684. The follow-on publications provide minimal information regarding toxicity. The manufacturer has not conducted postmarketing surveil-

lance regarding toxicity. Finally, the analysis prepared by the FDA for the meeting that occurred on February 27, 2002 did not consider the issue of toxicity. No report indicates how many HDI-treated patients have committed suicide or required psychiatric hospitalization, although most physicians who care for these patients know of such untoward events. Detailed information regarding toxicity is essential for the physicians who advise patients with high-risk melanoma concerning the risk/benefit ratio of HDI use to prolong DFS.

As with HDI, no randomized trial of alternative therapies for the adjuvant treatment of melanoma has shown survival benefit. These include four recent trials of vaccines and earlier trials of DTIC, BCG, and levamisole. Nonetheless, several agents (vaccines, GM-CSF, and biochemotherapy) that have completed phase 2 trials show promise, and some of these have advanced to phase 3 trials. Additional agents are being evaluated in phase 1 and 2 trials. HDI provides an imperfect solution for the adjuvant therapy of melanoma. Trials of alternative agents are needed to find more effective and less toxic therapies.

## REFERENCES

1. Greene FL, Page DL, Fleming ID et al, eds. American Joint Committee on Cancer Cancer Staging Manual, 6th ed. New York: Springer-Verlag 2002.
2. 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.
3. Balch CM, Soong SJ, Gershenwald JE et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001;19:3622–3634.
4. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer: an overview of 61 randomized trials among 28,896 women. Early Breast Cancer Trialists' Collaborative Group. *N Engl J Med* 1988;319:1681–1692.
5. FDA: Issues in the clinical development of investigational agents being evaluated for the post-surgical adjuvant treatment of high-risk (stages IIb and III) melanoma. <http://www.fda.gov/ohrms/dockets/ac/02/briefing/3838bl.htm>. February 27, 2002.
6. Wheatley K, Ives N. Adjuvant interferon for melanoma. *Ann Oncol* 2002;13:1319–1920.
7. Punt CJA, Eggermont AMM. Adjuvant interferon-alfa for melanoma revisited: news from old and new studies. *Ann Oncol* 2001;12:1663–1666.
8. Lens MB, Dawes M. Interferon alfa therapy for malignant melanoma: a systematic review of randomized controlled trials. *J Clin Oncol* 2002;20:1818–1825.
9. Pehamberger H, Soyer HP, Steiner A et al. Adjuvant interferon alfa-2a treatment in resected primary stage II cutaneous melanoma. Austrian Malignant Melanoma Cooperative Group [see comments]. *J Clin Oncol* 1998;16:1425–1429.
10. Kirkwood JM, Strawderman MH, Ernstoff MS et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684 [see comments]. *J Clin Oncol* 1996;14:7–17.
11. Kirkwood JM, Ibrahim JG, Sondak VK et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of Intergroup Trial E1690/S9111/C9190. *J Clin Oncol* 2000;18:2444–2458.

12. Kirkwood JM, Ibrahim JG, Sosman JA et al. High-dose interferon alfa-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.
13. Kirkwood JM, Ibrahim J, Lawson DH et al. High-dose interferon alfa-2b does not diminish antibody response to GM2 vaccination in patients with resected melanoma: results of the Multicenter Eastern Cooperative Oncology Group Phase II Trial E2696. *J Clin Oncol* 2001;19:1430–1436.
14. Eggermont AMM, Kleeberg UR, Ruiter DJ et al. The European Organization for Research and Treatment of Cancer Melanoma Group Trial experience with more than 2000 patients, evaluating adjuvant therapy treatment with low or intermediate doses of interferon alpha-2b. *Am Soc Clin Oncol* 2001 Educational Book. Alexandria, VA, 2001:88–93.
15. Grob JJ, Dreno B, Delaunay M et al. Long-term results of adjuvant therapy with low dose IFN-g2A in resected primary melanoma thicker than 1.5 mm without clinically detectable node metastases. *Proc ASCO* 1998:1983a.
16. Creagan ET, Dalton RJ, Ahmann DL et al. Randomized, surgical adjuvant clinical trial of recombinant interferon alfa-2a in selected patients with malignant melanoma. *J Clin Oncol* 1995;13:2776–2783.
17. Cameron DA, Cornbleet MC, Mackie RM et al. Adjuvant interferon alpha 2b in high risk melanoma: the Scottish study. *Br J Cancer* 2001;84:1146–1149.
18. Hancock BW, Wheatley K, Harrison G et al. Aim high: adjuvant interferon in melanoma (high risk), a United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) randomized study of observation vs. adjuvant low dose extended duration interferon alfa-2a in high risk resected malignant melanoma [abstract]. *Proc Am Soc Clin Oncol* 2001;20:1393.
19. Cascinelli N, Bufalino R, Morabito A et al. Results of adjuvant interferon study in WHO melanoma programme. *Lancet* 1994;343:913–914.
20. Cascinelli N, Belli F, MacKie RM et al. Effect of long-term adjuvant therapy with interferon alpha-2a in patients with regional node metastases from cutaneous melanoma: a randomised trial. *Lancet* 2001;358:866–869.
21. Wheatley K, Hancock B, Gore M et al. Interferon-alpha as adjuvant therapy for melanoma: a meta-analysis of the randomised trials. *Proc ASCO* 2001:1394a.
22. Cole BF, Gelber RD, Kirkwood JM et al. Quality-of-life-adjusted survival analysis of interferon alfa-2b adjuvant treatment of high-risk resected cutaneous melanoma: an Eastern Cooperative Oncology Group study. *J Clin Oncol* 1996;14:2666–2673.
23. Livingston PO, Wong GY et al. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. *J Clin Oncol* 1994;12:1036–1044.
24. FDA: Oncologic Drugs Advisory Committee, Trial design considerations and appropriate patient populations for studies of investigational agents for adjuvant therapy of melanoma given the availability of an approved agent for this indication. <http://www.fda.gov/ohrms/dockets/ac/02/transcripts/3838t1.htm>, February 27, 2002
25. Grob JJ, Dreno B, de la Salmoniere P et al. Randomised trial of interferon alpha-2a as adjuvant therapy in resected primary melanoma thicker than 1.5 mm without clinically detectable node metastases. *French Cooperative Group on Melanoma. Lancet* 1998;351:1905–1910.
26. Wallack MK, Sivanandham M, Balch CM et al. Surgical adjuvant active specific immunotherapy for patients with stage III melanoma: the final analysis of data from a phase III, randomized, double-blind, multicenter vaccinia melanoma oncolysate trial. *J Am Coll Surg* 1998;187:69–77; discussion 77–79.

**408 PROGRESS IN ONCOLOGY 2003**

27. Hersey P, Coates A, McCarthy WH et al. Adjuvant immunotherapy of patients with high risk melanoma using vaccinia viral lysates of melanoma: results of a randomized trial. *J Clin Oncol* 2002;20:4181-4190.
28. Hersey P, Coates A, McCarthy WH et al. Adjuvant immunotherapy of patients with high risk melanoma with vaccinia viral lysates of melanoma. 4th International Conference on The Adjuvant Therapy of Malignant Melanoma. Royal College of Physicians, London, United Kingdom, 2002:15 (I-25a).
29. Sondak VK, Liu PY, Tuthill RJ et al. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: overall results of a randomized trial of the Southwest Oncology Group. *J Clin Oncol* 2002;20:2058-2066.
30. Sosman JA, Unger JM, Liu PY et al. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: impact of HLA class I antigen expression on outcome. *J Clin Oncol* 2002;20:2067-2075.
31. Hill GJ, Moss SE, Golomb FM et al. DTIC and combination therapy for melanoma: III. DTIC (NSC 45388) Surgical Adjuvant Study COG PROTOCOL 7040. *Cancer* 1981;47:2556-2562.
32. Cunningham TJ, Schoenfeld D, Nathanson L et al. A controlled ECOG study of adjuvant therapy (BCG, BCG-DTIC) in patients with stage I and II malignant melanoma, Second International Conference on the Immunotherapy of Cancer; Present Status of Trials in Man. Bethesda, MD, 1980.
33. Pinsky CM, Hirshaut Y, Wanebo HJ et al. Randomized trial of Bacillus Calmette-Guerin (percutaneous administration) as surgical adjuvant immunotherapy for patients with stage-II melanoma. *Ann NY Acad Sci* 1976;277:187-194.
34. Quirt IC, Shelley WE, Pater JL et al. Improved survival in patients with poor-prognosis malignant melanoma treated with adjuvant levamisole: a phase III study by the National Cancer Institute of Canada Clinical Trials Group [see comments]. *J Clin Oncol* 1991;9:729-735.
35. Spittler LE. A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma. *J Clin Oncol* 1991;9:736-740.
36. Morton DL, Foshag LJ, Hoon DS et al. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine [published erratum appears in *Ann Surg* 1993;217:309]. *Ann Surg* 1992;216:463-482.
37. Morton DL. Current Status of Vaccines for Melanoma. Baltimore, MD, Lippincott Williams & Wilkins, 2000.
38. Chan AD, Morton DL. Active immunotherapy with allogeneic tumor cell vaccines: present status. *Semin Oncol* 1998;25:611-622.
39. Mitchell MS, Kan-Mitchell J, Kempf RA et al. Specific immunotherapy for melanoma: phase I trial of allogeneic lysates and a novel adjuvant. *Cancer Res* 1988;48:5883-5893.
40. Mitchell MS, Harel W, Kempf RA et al. Active-specific immunotherapy for melanoma. *J Clin Oncol* 1990;8:856-869.
41. Livingston PO, Adluri S, Helling F et al. Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-keyhole limpet haemocyanin conjugate vaccine in patients with malignant melanoma. *Vaccine* 1994;12:1275-1280.
42. Eggermont AM. Phase III randomized study of postoperative GM2-KLH and QS21 vaccination versus observation in patients with primary cutaneous stage II melanoma. [http://www.cancer.gov/clinical\\_trials/view\\_clinicaltrials.aspx?version=health+professional&args=8;a66fcf71-4563-4bda-8cfa-eb848d77dcd1](http://www.cancer.gov/clinical_trials/view_clinicaltrials.aspx?version=health+professional&args=8;a66fcf71-4563-4bda-8cfa-eb848d77dcd1), 2002

43. Bystryn JC, Oratz R, Roses D et al. Relationship between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. *Cancer* 1992;69:1157–1164.
44. Bystryn JC, Zeleniuch-Jacquotte A, Oratz R et al. Double-blind trial of a polyvalent, shed-antigen, melanoma vaccine. *Clin Cancer Res* 2001;7:1882–1887.
45. Livingston P. The unfulfilled promise of melanoma vaccines. *Clin Cancer Res* 2001;7:1837–1838.
46. Berd D, Murphy G, Maguire HC Jr et al. Immunization with hapteneized, autologous tumor cells induces inflammation of human melanoma metastases. *Cancer Res* 1991;51:2731–2734.
47. Berd D. Autologous, haptene-modified vaccine as a treatment for human cancers. *Vaccine* 2001;19:2565–2570.
48. Berd D. M-Vax: an autologous, haptene-modified vaccine for human cancer. *Expert Opin Biol Ther* 2002;2:335–342.
49. AVAX Technologies I. 2002. <http://www.avax-tech.com/mvax.html>.
50. Safa M, Lutzky J, Chatterjee M et al. Trigem anti-idiotypic (Anti-Id) monoclonal antibody (MAb) treatment for stage III melanoma: results of a multicenter phase II trial. *Proc Am Soc Clin Oncol* 2001;20:1008a.
51. Fidler IJ, Kleinerman ES. Lymphokine-activated human blood monocytes destroy tumor cells but not normal cells under cocultivation conditions. *J Clin Oncol* 1984;2:937–943.
52. Grabstein KH, Urdal DL, Tushinski RJ et al. Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. *Science* 1986;232:506–508.
53. Thomassen MJ, Barna BP, Rankin D et al. Differential effect of recombinant granulocyte macrophage colony-stimulating factor on human monocytes and alveolar macrophages. *Cancer Res* 1989;49:4086–4089.
54. Wing EJ, Magee DM, Whiteside TL et al. Recombinant human granulocyte/macrophage colony-stimulating factor enhances monocyte cytotoxicity and secretion of tumor necrosis factor alpha and interferon in cancer patients. *Blood* 1989;73:643–646.
55. Chachoua A, Oratz R, Hoogmoed R et al. Monocyte activation following systemic administration of granulocyte-macrophage colony-stimulating factor. *J Immunother Emphasis Tumor Immunol* 1994;15:217–224.
56. Young JW, Szabolcs P, Moore MA. Identification of dendritic cell colony-forming units among normal human CD34+ bone marrow progenitors that are expanded by c-kit-ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony-stimulating factor and tumor necrosis factor alpha [published erratum appears in *J Exp Med* 1996;183:1283]. *J Exp Med* 1995;182:1111–1119.
57. Szabolcs P, Moore MA, Young JW. Expansion of immunostimulatory dendritic cells among the myeloid progeny of human CD34+ bone marrow precursors cultured with c-kit ligand, granulocyte-macrophage colony-stimulating factor, and TNF-alpha. *J Immunol* 1995;154:5851–5861.
58. Szabolcs P, Avigan D, Gezelter S et al. Dendritic cells and macrophages can mature independently from a human bone marrow-derived, post-colony-forming unit intermediate. *Blood* 1996;87:4520–4530.
59. Kumar R, Dong Z, Fidler IJ. Differential regulation of metalloelastase activity in murine peritoneal macrophages by granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor. *J Immunol* 1996;157:5104–5111.
60. Dong Z, Kumar R, Yang X et al. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* 1997;88:801–810.

**410 PROGRESS IN ONCOLOGY 2003**

61. Spittler LE, Grossbard ML, Ernstoff MS et al. Adjuvant therapy of stage III and IV malignant melanoma using granulocyte-macrophage colony-stimulating factor [see comments]. *J Clin Oncol* 2000;18:1614-1621.
62. Spittler LE, Weber R, Rose M et al. Adjuvant Therapy of Stage II (T4), III and IV Malignant Melanoma using GM-CSF. 4th International Conference on The Adjuvant Therapy of Malignant Melanoma. London, March 15-16, 2002 10 (I-08).
63. Retsas S, MacRae K, Henry K. Adjuvant treatment for clinically apparent regional lymph node metastases [ $>N1b$ ] from melanoma: single-institution experience from a cohort of 318 patients. 4th International Conference on The Adjuvant Therapy of Malignant Melanoma. Royal College of Physicians, London, March 15-16, 2002 1-26.
64. Retsas S, Quigley M, Pectasides D et al. Clinical and histologic involvement of regional lymph nodes in malignant melanoma: adjuvant vindesine improves survival. *Cancer* 1994;73:2119-2130.
65. Legha SS, Ring S, Eton O et al. Development of a biochemotherapy regimen with concurrent administration of cisplatin, vinblastine, dacarbazine, interferon alfa, and interleukin-2 for patients with metastatic melanoma. *J Clin Oncol* 1998;16:1752-1759.
66. Gibbs P, Anderson C, Pearlman N et al. A phase II study of neoadjuvant biochemotherapy for stage III melanoma. *Cancer* 2002;94:470-476.
67. Balch CM, Soong SJ, Murad TM et al. A multifactorial analysis of melanoma: III. Prognostic factors in melanoma patients with lymph node metastases (stage II). *Ann Surg* 1981;193:377-388.
68. Bedikian AY. Phase III randomized adjuvant study of interferon alfa-2b (IFN-A) Alone vs biochemotherapy using cisplatin, vinblastine, dacarbazine (DTIC), IFN-A, and interleukin-2 (IL-2) in melanoma patients with regional lymph node metastases. [http://www.cancer.gov/clinical\\_trials/view\\_clinicaltrials.aspx?version=health+professional&args=4;e14d7b24-9181-4fa6-b429-50ef712e4fe7](http://www.cancer.gov/clinical_trials/view_clinicaltrials.aspx?version=health+professional&args=4;e14d7b24-9181-4fa6-b429-50ef712e4fe7), 2002.
69. Rosenberg SA. Phase II randomized study of gp100:209-217 (210M) or gp100:17-25 antigen and tyrosinase:368-376 (370D), tyrosinase:240-251 (244S), tyrosinase:206-214, or tyrosinase-related protein-1 (ORF3):1-9 peptide emulsified in Montanide ISA-51 in patients with melanoma at high risk for recurrence. [http://www.cancer.gov/clinical\\_trials/view\\_clinicaltrials.aspx?version=health+professional&args=2;feedefa3-7625-401b-bc66-b8eaf9ec8521](http://www.cancer.gov/clinical_trials/view_clinicaltrials.aspx?version=health+professional&args=2;feedefa3-7625-401b-bc66-b8eaf9ec8521), 2002.
70. Kendra K, Malkovska V, Allen M et al. In vivo binding and antitumor activity of Ch14.18. *J Immunother* 1999;22:423-430.
71. Maguire HC Jr, Berd D, Lattime EC et al. Phase I study of R24 in patients with metastatic melanoma including evaluation of immunologic parameters. *Cancer Biother Radiopharm* 1998;13:13-23.
72. Nasi ML, Meyers M, Livingston PO et al. Anti-melanoma effects of R24, a monoclonal antibody against GD3 ganglioside. *Melanoma Res* 1997;7[suppl 2]:S155-S162.
73. Lode HN, Xiang R, Duncan SR et al. Tumor-targeted IL-2 amplifies T cell-mediated immune response induced by gene therapy with single-chain IL-12. *Proc Natl Acad Sci USA* 1999;96:8591-8596.
74. Lode HN, Reisfeld RA. Targeted cytokines for cancer immunotherapy. *Immunol Res* 2000;21:279-288.
75. Fallarino F, Fields PE, Gajewski TF B7-1 engagement of cytotoxic T lymphocyte antigen 4 inhibits T cell activation in the absence of CD28. *J Exp Med* 1998;188:205-210.
76. Foon KA, Lutzky J, Baral RN et al. Clinical and immune responses in advanced melanoma patients immunized with an anti-idiotype antibody mimicking disialoganglioside GD2. *J Clin Oncol* 2000;18:376-384.

77. McCaffery M, Yao TJ, Williams L et al. Immunization of melanoma patients with BEC2 anti-idiotypic monoclonal antibody that mimics GD3 ganglioside: enhanced immunogenicity when combined with adjuvant. *Clin Cancer Res* 1996;2:679–686.
78. Soiffer R, Lynch T, Mihm M et al. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 1998;95:13141–13146.
79. Kusumoto M, Umeda S, Ikubo A et al. Phase I clinical trial of irradiated autologous melanoma cells adenovirally transduced with human GM-CSF gene. *Cancer Immunol Immunother* 2001;50:373–381.
80. Rankin EM, Spits H, Orsini D et al. A phase I study of vaccination with autologous, GM-CSF-transduced and irradiated tumour cells in patients with advanced melanoma. *Proc Am Soc Clin Oncol* 1995;14:226.
81. Belli F, Arienti F, Sule-Suso J et al. Active immunization of metastatic melanoma patients with interleukin-2-transduced allogeneic melanoma cells: evaluation of efficacy and tolerability. *Cancer Immunol Immunother* 1997;44:197–203.
82. Osanto S, Schiphorst PP, Weijl NI et al. Vaccination of melanoma patients with an allogeneic, genetically modified interleukin 2-producing melanoma cell line. *Hum Gene Ther* 2000;11:739–750.
83. Rochlitz C, Dreno B, Jantschke P et al. Immunotherapy of metastatic melanoma by intratumoral injections of Vero cells producing human IL-2: phase II randomized study comparing two dose levels. *Cancer Gene Ther* 2002;9:289–295.
84. Gajewski TF, Fallarino F, Ashikari A et al. Immunization of HLA-A2+ melanoma patients with MAGE-3 or MelanA peptide-pulsed autologous peripheral blood mononuclear cells plus recombinant human interleukin 12. *Clin Cancer Res* 2001;7:895s–901s.
85. Panelli MC, Wunderlich J, Jeffries J et al. Phase I 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.
86. Banchereau J, Palucka AK, Dhodapkar M et al. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res* 2001;61:6451–6458.
87. Schuler-Thurner B, Schultz ES, Berger TG et al. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. *J Exp Med* 2002;195:1279–1288.
88. Nestle FO, Aljagid S, Gilliet M et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998;4:328–332.
89. Nair SK, Morse M, Boczkowski D et al. Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. *Ann Surg* 2002;235:540–549.
90. Dillman RO, Schiltz PM, Selvan R et al. Patient-specific cancer vaccine of cultured autologous tumor cells and autologous dendritic cells. *J Immunother* 2001;24:55.
91. Hwu WJ. New approaches in the treatment of metastatic melanoma: thalidomide and temozolomide. *Oncology (Huntingt)* 2000;14:25–28.
92. Hwu WJ, Krown SE, Panageas KS et al. Temozolomide plus thalidomide in patients with advanced melanoma: results of a dose-finding trial. *J Clin Oncol* 2002;20:2610–2615.
93. Hwu WJ, Krown SE, Panageas KS et al. Treatment of Metastatic melanoma in the brain with temozolomide and thalidomide. *Lancet Oncol* 2001;2:634–635.