Active Specific Immunotherapy Phase III Trials for Malignant Melanoma: Systematic Analysis and Critical Appraisal

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The incidence of malignant melanoma is increasing faster than that of any other cancer. In men, it is the fifth most common cancer, accounting for 5% of new cancer cases. In women, it is the sixth most common cancer, with 4% of new cancer cases; only the frequency of lung cancer is rising faster than that of melanoma.[1,2]

Although early stage lesions are curable with adequate surgical resection, postsurgical management of advanced malignant melanoma continues to be a challenge. The 5-year survival rate for patients with local stages of melanoma is 98.3%, compared with 63.8% and 16% for patients with regional or distant metastases, respectively.[2]

There is no effective treatment for metastatic melanoma AJCC stage IV); patients with AJCC stage IIB (T4N0M0) and AJCC stage III (T1–4N1M0) are at high risk of recurrence after definitive surgery. Multiple chemotherapeutic regimens have been used to treat advanced stage melanoma patients, but little effect on overall survival has been found.[3,4]

Although adjuvant therapy with interferon-α-2b was approved by the FDA for patients with stage III melanoma, it is associated with considerable side effects and costs.[5] Similarly, the use of FDA-approved high-dose interleukin (IL)-2 is associated with systemic toxicities.

So it is important to investigate alternative treatment options, including the possibility of inducing an immune response against tumor-associated antigens (TAA) by vaccination, the so-called active specific immunotherapy (ASI).

Tumors express antigens (TAA) are recognized by the immune system as foreign, so they induce a T-cell mediated immune response. The molecular description of the first human TAA was published in 1991 and was a breakthrough in tumor immunology.[6] Since then a large number of these TAA have been described. They are recognized by CD8+ cytolytic T lymphocytes as peptides derived from proteins processed in the cytosol and displayed on the cell surface bound to class I major histocompatibility complex molecules of antigen-presenting cells. But, this immune response frequently is unable to prevent tumor growth because of tumor escape mechanisms, high tumor burden, and weak antigenicity.

Immunotherapy aims at actively enhancing the immune response or at passively delivering immunity. Examples of passive immunotherapy are adoptive cellular therapy, consisting of the transfer of cultured antitumor reactive immune cells, or administration of tumor-specific monoclonal antibodies. Active immunotherapy may be achieved either by specific stimulation, the so-called active specific immunotherapy (ASI), or by nonspecific stimulation of the immune system, eg, by injecting proinflammatory substances such as bacillus Calmette-Guérin or by administration of cytokines and costimulators. ASI enhances the host's immune response by immunization with either killed tumor cells or tumor cell lysates possibly expressing multiple TAA, or with defined tumor antigens. Examples of vaccines using defined tumor antigens are the direct injection of peptides with or without adjuvants or cytokines, the ex vivo loading of antigen-presenting cells (APC) such as dendritic cells with peptides before reinjection, or the use of recombinant virus encoding TAAs and capable of infecting antigen-presenting cells, mimicking the physiologic pathway.

The objective of this review was to critically appraise the current status of ASI for treatment of melanoma. Although there are numerous phase I and II trials and case series, only a few phase III randomized controlled clinical trials were performed. We focused on two endpoints: disease-free survival (DFS) because it is the outcome showing most directly the efficacy of the treatment under investigation, and overall survival (OS) because it is the most important outcome to patients and the outcome best defined and least subject to investigator bias.

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Abbreviations and Acronyms

ASI = active specific immunotherapy
BCG = bacillus Calmette-Guérin
DFS = disease-free survival
DTIC = dacarbazine
GM2 = ganglioside
GMK = GM2 keyhole limpet hemocyanin
HR = hazard ratio
OS = overall survival
TAA = tumor-associated antigen
VMCL= vaccinia melanoma cell lysate
VMO = vaccinia melanoma oncolysate.

METHODS

Inclusion criteria

Randomized-controlled phase III trials (RCT), published until December 2006, addressing active specific immunotherapy for melanoma patients were included in this review. Phase I and II trials, case series, and retrospective analyses were not considered. Other modalities of immunotherapy, such as passive (use of monoclonal antibodies or adoptive transfer therapy) and nonantigen-specific immunotherapies (cytokines, bacillus Calmette-Guérin, etc) were also excluded.

Search strategy

The review of all relevant articles was based on a Medline search on "melanoma," "immunotherapy, and "phase III," with the limits "clinical trial" and "randomized controlled trial," until December 2006. No time limit was added, so articles published at any date in the past were taken into account, including articles published more than 20 years ago. Additional articles were identified through cross-referencing of the studies retrieved. Ongoing studies were identified by the NIH Website, www.clinicaltrials.gov, using the key words *melanoma*, *immunotherapy*, and *phase III*.

Data analysis

All identified trials were critically appraised for study design, disease-free survival (DFS), and overall survival (OS).

RESULTS

Eight phase III trials were identified.[7-14] All trials provided detailed information about outcomes after ASI. But the design of the studies showed considerable variations. Table 1 summarizes the trials included, and Table 2 provides an overview of the results.

Immunotherapy trials based on whole cell vaccines

Vaccinia virus melanoma oncolysate versus control vaccinia virus

Wallack and colleagues[15-17] performed phase I and II trials to evaluate toxicity and the effective dose of a vaccinia virus-augmented polyvalent melanoma oncolysate vaccine. Based on their promising findings, they randomized 250 stage III melanoma patients (intention-to-treat); 33 of these patients were excluded because they were inadequately operated on, they presented more than one primary melanoma, they had extranodal spread, or they did not have a melanoma. The eligible patients were the remaining 217, who received vaccinia melanoma oncolysate (VMO) or control vaccinia virus.[7]

A polyvalent VMO derived from four allogeneic mela-

Table 1. Summary of Studies Included in this Report

First author,y	Vaccine	Control	n*	Stage†	Followup, mo
Whole cell vaccines					
Wallack, 1998[7]	VMO	V	217 (250)	III	46.3 median
Mitchell, 1997[8]	Melacine	Chemotherapy	106 (140)	IV	–
Sondak, 2002[9]	Melacine	Observation	600 (689)	T3N0M0	67.2 median
Hersey, 2002[10]	VMCL	Observation	675 (700)	IIB/III	96 median
Morton, 2006[11]	Canvaxin	Observation	(1,160)	III	69 median
			(496)	IV	69
Ganglioside vaccines					
Livingston, 1994[12]	GM2/ BCG	BCG	122 (123)	III	63 median
Kirkwood, 2001[13]	GMK	IFNα2b	774 (880)	IIB/III	16 median
Epitope-specific vaccines					
Schadendorf, 2006[14]	DC	DTIC	104 (108)	IV	22.2 median

BCG, bacillus Calmette-Guérin; DC, autologous peptide-pulsed dendritic cells; DTIC, dacarbazine; GM2, ganglioside GM2; GMK, GM2 keyhole limpet hemocyanin-QS-21; IFN, interferon; V, control vaccinia virus; VMCL, vaccinia melanoma cell lysates; VMO, vaccinia melanoma oncolysate.
*Number of eligible patients (intention to treat).
†Stage according to American Joint Committee on Cancer or TNM classification.

Table 2. Results of Included Studies

First author	Time*	Vaccine	Control	Vaccine		Control		p Value	
				DFS	OS	DFS	OS	DFS	OS
Whole cell vaccines									
Wallack[7]	5 y	VMO	V	41.7%	48.6%	40.4%	48.2%	0.61	0.79
Mitchell[8]	MS	Melacine	Chemotherapy	—	9.4 mo	—	12.3 mo	—	0.16
Sondak[9]	5 y	Melacine	Observation	65%	—	63%	—	0.51	—
Hersey[10]	MS	VMCL	Observation	6.98 y	>8.45 y	4.37 y	7.34 y	0.27	0.11
Morton[11]		Canvaxin	Observation						
Stage III	5 y			47.2%	59.1%	52.1%	67.7%	0.047	0.040
Stage IV	5 y			27.4%	39.6%	20.9%	44.9%	0.418	0.245
Ganglioside vaccines									
Livingston[12]	51 mo	GM2/ BCG	BCG	48%	57%	30%	46%	0.09	0.22
Kirkwood[13]	2 y	GMK	IFNα2b	49%	73%	62%	78%	0.0015	0.009
Epitope-specific vaccines									
Schadendorf[14]	22.2 mo	DC	DTIC	—	26.4%	—	31.0%	—	0.48

Disease-free survival, overall survival in patients as randomized (Mitchell, Sondak, Hersey, Morton, Livingston, Schadendorf); in eligible patients (Wallack, Kirkwood).

BCG, bacillus Calmette-Guérin; DC, autologous peptide-pulsed dendritic cells; DFS, disease-free survival; DTIC, dacarbazine; GM2, ganglioside GM2; GMK, GM2 keyhole limpet hemocyanin-QS-21; MS, median survival; OS, overall survival; V, control vaccinia virus; VMCL, vaccinia melanoma cell lysates; VMO, vaccinia melanoma oncolysate.

*Time, actual survival at the indicated time-point (Morton, Livingston, Schadendorf); estimated survival at timepoint (Wallack, Sondak, Kirkwood); median survival (Mitchell, Hersey).

noma cell lines from four patients with primary or secondary melanoma, potentially containing a variety of TAA, was used. The cell lines were infected with vaccinia virus and lysed before injection. Treatment was administered weekly for 13 weeks and then every other week during 12 months, or until recurrence. Median followup was 46.3 months. This multicenter trial was initiated in June 1988. The first interim analysis in May 1994 showed no significant difference in DFS or OS.[18] Subset analysis, however, showed survival benefit in male patients overall (17% OS difference), in male patients younger than 57 years old with one to five positive lymph nodes (30%), and in clinical stage I, but pathologic stage II, patients after prophylactic lymph node dissection (23%).[19] In the second interim analysis, performed in June 1995, a subset of male patients between 44 and 57 years old, with 1 to 5 positive nodes (active treatment, n = 20; control, n = 18), and the subset of patients with clinical stage I (active treatment, n = 20; control, n = 23) were found to have a statistically significant survival benefit ($p = 0.037$ and $p = 0.05$, respectively).[20] The final results of this trial were published in 1998.[7] The active treatment increased neither the disease-free interval (active treatment, median 20.7 months; control, 26.9 months, $p = 0.61$) nor the overall survival (active treatment, 50.2 months; control, 41.3 months; $p = 0.79$). With active treatment, 47.8%, 43.8%, and 41.7% of the patients were disease-free after 2, 3, and 5 years, respectively, as compared with 51.2%, 44.8%, and 40.4%, respectively, of the patients treated with the control vaccinia virus. Concerning overall survival after 2, 3, and 5 years, 70.0%, 60.0%, and 48.6% survived as compared with 65.4%, 55.6%, and 48.2%, respectively, in the control group. But male patients aged 44 to 57 years, with 1 to 5 positive nodes treated with VMO (n = 20), showed 18.9%, 26.8%, and 21.3% ($p = 0.046$) improvements in overall survival after 2, 3, and 5 years, respectively, as compared with the corresponding control group (n = 18), as shown by a retrospective subset analysis. No analysis of vaccine or tumor-specific immune response was attempted.

Melanoma oncolysate Melacine (Corixa Corp) versus chemotherapy

In a phase III trial published only in abstract form in 1997, Mitchell and associates [8] compared Melacine, an allogeneic cell lysate derived from a mixture of two human melanoma cell lines possibly expressing multiple TAA, coadministered with Detox, to a multiagent chemotherapy. This vaccine had previously shown evidence of antitumor activity in phase I and II clinical trials.[21,22] Detox (detoxified Freund's adjuvant; Corixa Corp) is an immunologic adjuvant consisting of monophosphoryl lipid A and purified mycobacterial cell-wall skeleton.

Seventy stage IV melanoma patients were enrolled in each treatment group; 54 patients in the Melacine group and 52 patients in the chemotherapy group were eligible.[8] Melacine was injected intramuscularly weekly in weeks 1 to

5 and 8 to 12. In weeks 1 and 8, cyclophosphamide was administered 3 days before the vaccination. The control arm consisted of dacarbazine, cisplatin, carmustine, and tamoxifen. Additional treatment was given to patients with objective tumor responses. There was no placebo-controlled arm. There was no significant difference in median survival time, either on an intention-to-treat basis (9.4 months in the Melacine group versus 12.3 months in the chemotherapy group, $p = 0.16$) or in a "per protocol" analysis (11 versus 12.4 months, $p = 0.37$). In the Melacine group, there were fewer objective tumor responses (2 complete responses, 3 partial responses, 5 with stable disease) as compared with the chemotherapy group (2 complete responses, 5 partial responses, 19 with stable disease). The quality of life, however, was significantly higher in the Melacine group ($p = 0.008$, multivariate test), probably because of the lower toxicity, with one grade 3 or 4 drug-associated adverse event in the Melacine-treated group as compared with 86 events in the chemotherapy arm. The treatment induced humoral immune responses in 65% of patients and skin test positivity in 6 of 70 patients.

Melanoma oncolysate Melacine versus observation

The Southwest Oncology Group (SWOG) conducted a randomized phase III trial (SWOG-9035) comparing the same allogeneic melanoma lysate vaccine, Melacine, to observation in patients undergoing resection for intermediate thickness (T3N0M0) melanoma.[9] In this phase III randomized clinical trial published in 2002, 689 patients were enrolled. Eighty-nine of them were ineligible, largely ($n = 76$) because central pathology review found that the primary lesion was not intermediate thickness melanoma as defined in the eligibility criteria. Other reasons for ineligibility were satellitosis, lymph node metastases, inadequate operation, and high serum alkaline phosphatase. Stratified randomization was performed based on gender, tumor thickness, and lymph node staging.

Median followup was 5.6 years, with a minimum of 4 years. The treatment protocol included two intramuscular injections of 1.0 mL of Melacine cell lysate and 0.25 mL of Detox adjuvant split between two injection sites. Four 6-months cycles of 10 treatments (20 injections) were administered weekly for 4 weeks, then biweekly for 4 weeks, then monthly for 4 months, followed by a 3-week rest period.

In the treatment group, 107 tumor recurrences or deaths were reported, as compared with 114 in the observation group (hazard ratio [HR] = 0.92 for eligible /0.84 for intention-to-treat; Cox-adjusted $p = 0.51$[eligible]/ 0.17[intention-to-treat]). Five-year estimated DFS was 65% (eligible)/66% (intention-to-treat) in the vaccine group and 63% (eligible)/62% (intention-to-treat) in the observation group. There was no significant difference in vaccine efficacy among patients with tumors ≤ 3 mm or $>$ 3 mm thick. The authors concluded that there was no evidence of improved DFS among patients receiving the vaccine, possibly because of the insufficient statistical power of their study. Clinical differences, however, were indeed modest and of questionable relevance. No evidence of vaccine or melanoma-specific immune response was reported.

Earlier work by Mitchell and coworkers[21,22] showed that melanoma lysate vaccination was able to induce cytotoxic T-lymphocyte immune responses, and that an increase in cytotoxic T-lymphocyte precursors correlated with better clinical outcomes. So they investigated the association of HLA phenotype with response to the vaccine.[23] Expression of HLA class I alleles A2, A28, B12, and its split B44 and B45 and C3 was associated with clinical remission. They found a 38% response rate in patients with two or three of these alleles as compared with 20% in the group as a whole. Sosman and colleagues,[24] whose trial enrolled patients from April 1992 until November 1996, amended the protocol in September 1994 to obtain HLA-A/B/C serotyping from all patients subsequently enrolled; HLA class I typing of previously enrolled patients was obtained if possible. In this analysis, HLA typing of 553 (80% of 689) patients was registered. A significantly improved effect of the vaccine treatment was found in patients expressing two or more alleles from a group of five, including HLA-A2, HLA-A28, HLA-B44, HLA-B45, and HLA-C3. In these patients, a 5-year relapse-free survival of 83% versus 59% ($p = 0.0005$) was observed. Patients positive for HLA-A2, HLA-C3, or both, showed a 5-year relapse-free survival of 77% in the vaccine group as compared with 64% in the control group ($p = 0.004$). The authors concluded that HLA-A2 and HLA-C3 may present processed melanoma peptides found in Melacine, and they proposed to conduct a phase III trial enrolling only patients with HLA-A2 or HLA-C3 positivity, or both. To our knowledge, this study has not yet been initiated.

Vaccinia virus melanoma oncolysate versus observation

The randomized trial of Hersey and associates published in 2002[10] was based on phase II studies showing an improved overall survival in patients receiving vaccinia viral lysates as compared with historical controls.[25,26] Immunogenic preparation consisted of a vaccine prepared from a vaccinia virus-infected and lysed allogeneic melanoma cell line, capable of inducing specific immune responses, named vaccinia melanoma cell lysate (VMCL). A previous report had shown increased cell-mediated immunity to an autologous melanoma vaccine after pretreatment with cyclophospha-

mide.[27] But the effects of vaccinia melanoma cell lysates in a subsequent trial were not found to be enhanced by such pretreatment.[26] In the actual trial, VMCL was administered over a 2-year period after definitive surgery in patients with stages IIB and III melanoma.[10] A total of 700 patients were randomized in this phase III, unblinded, multicenter study to receive either VMCL (n = 353) or no immunization (n = 347). Stratification into five groups was undertaken depending on the primary tumor thickness, number of lymph nodes, and time point of lymph node dissection. VMCL injections were administered intradermally every 2 weeks for the first four injections, every 3 weeks for the next six injections, then monthly for 18 months. Of the 700 patients, 675 were eligible. Median followup was 8 years. The authors reported a median overall survival of 88 months in the control versus 151 months in the treated group (hazard ratio [HR] = 0.81; p = 0.068) and a median disease-free survival of 43 months in the controls versus 83 months (HR = 0.86; p = 0.17) in the treated group. At 5 years, the overall survival rate for treated patients was 60.6% versus 54.8% in untreated patients, and disease-free survival was 50.9% versus 46.8%, respectively. At 10 years, the survival rate was 53.4% for treated patients versus 41% for untreated patients, and the recurrence-free survival was 45.4% versus 42.5%, respectively.

In an intention-to-treat analysis, the 5-year overall survival was 59.6% for treated patients versus 55.1% for untreated patients, with a median overall survival of 8.45 years versus 7.34 (HR = 0.83; p = 0.11), respectively. Relapse-free survival was 51.8% for treated patients versus 48.3% for untreated patients, with a median relapse-free survival of 6.98 years versus 4.37 years, respectively (HR = 0.89; p = 0.27). Subset analyses failed to show a benefit from treatment in any stratum. Immune responsiveness eventually induced by vaccination was not evaluated.

Melanoma oncolysate Canvaxin (CancerVax Corp) versus observation

In 2002, Morton and colleagues[28] presented a large case series of 2,602 stage III melanoma patients treated after complete surgical resection of regional nodal metastases. The study compared the polyvalent vaccine Canvaxin (n = 935) to observation (n = 1,667). The decision to receive the vaccination or not was the individual patient's choice. Canvaxin is a polyvalent vaccine derived from entire melanoma cells from three cell lines known to express multiple TAA, which were cultured, pooled, irradiated, and cryopreserved. The vaccine was administered intradermally. The authors found a significantly increased median overall survival of 56.4 months in vaccinated patients as compared with 31.9 months in the control group (p = 0.0001).[28] To control for selection bias, a computerized random matching of vaccinated and nonvaccinated patients was performed, and 739 patients could be matched for 6 prognostic factors. Still, patients receiving Canvaxin had a significantly (p = 0.0001) higher median overall survival (55.3 months versus 31.6 months) than the control group did.[28]

Similarly, 107 completely resected stage IV patients receiving Canvaxin within phase II studies were compared with 107 computer-matched stage IV patients without adjuvant active immunotherapy. Vaccinated patients showed a median overall survival of 38 months as compared with 19 months for patients in the control group (p = 0.0009).[29]

Based on the very promising results of these large case series,[28] the authors conducted two phase III randomized clinical trials of Canvaxin versus placebo with identical design except for stage and stratification factors. Data of these two trials were presented at the 2006 Society of Surgical Oncology (SSO) meeting and data for stage IV melanoma patients were included in the corresponding abstract.[11] In one trial, stage III melanoma patients without evidence of residual disease after operation were randomized to Canvaxin versus observation. Stratification factors were the number of tumor-involved lymph nodes and whether the nodes were palpable or not. A similar trial was performed for completely resected stage IV melanoma patients, with the stratification factors being number of individual metastatic lesions and site of metastases.[11] In these studies, 1,160 stage III and 496 stage IV patients were randomized to receive Canvaxin or placebo. Either preparation was administered intradermally every 2 weeks for the first five injections, monthly during the first treatment year, every other month during the second year, and every 3 months during the next 3 years. Together with the first two injections of Canvaxin or placebo, bacillus Calmette-Guérin (BCG) was administered as an immunologic adjuvant. For stage III patients, the intention-to-treat analysis showed a median disease-free survival of 42.6 months in the Canvaxin group (n = 579) versus a disease-free survival of greater than 60 months in the placebo group (n = 581; p = 0.047) and a median overall survival greater than 69 months in both groups, with a p value of 0.040 in favor of placebo. But according to the statistical plan, the boundary of statistical significance required a value of 0.01 at the third interim analysis.

For stage IV patients, the authors found a median disease-free survival of 8.3 months and a median overall survival of 31.5 months in the Canvaxin group (n = 246) versus a 7.2-month disease-free survival and a 38.7-month overall survival in the placebo group (n = 250; p = 0.418 and p = 0.0245, respectively). Most adverse events were of

grades 1 and 2, similar in both study arms, and likely from BCG. Seven stage III patients and three stage IV patients discontinued the treatment because of adverse events related to the study drug. In the stage III patients, three related, unexpected serious adverse events occurred: one BCG abscess in the placebo group, one myelodysplastic syndrome in the Canvaxin group in a patient with a history of benzene exposure, and one unnecessary neck dissection because of reactive lymph nodes with a false positive positron emission tomography scan in the placebo group. The delayed-type hypersensitivity responder status was associated with an increased overall survival in stage IV, but not in stage III patients, with a median survival of 37.2 months in responders ($n = 115$) versus 24.9 months in nonresponders ($n = 125$; $p = 0.029$).

The study was terminated after the third interim analysis for stage III patients and at the second interim analyses for stage IV patients, based on recommendations of the independent Data and Safety Monitoring Board, unrelated to safety concerns or adverse effects, but because of the low probability of showing a significant survival benefit if the study was continued. No data on vaccine-specific immune responsiveness were provided.

Immunotherapy trials based on ganglioside vaccines

BCG/ganglioside vaccine versus BCG alone

GM2 represents a well-defined melanoma associated antigen of the ganglioside group, so it has been used for antigen-specific immunotherapy.[30,31]

Livingston and associates[32,33] showed that immunization with the purified GM2 ganglioside and BCG, after pretreatment with low-dose cyclophosphamide, induced IgM antibodies in a high percentage of melanoma patients and that patients producing GM2 antibodies after immunization displayed significantly longer DFS and OS than patients who did not.

To confirm the beneficial effects of the vaccine-induced GM2 antibody production, a randomized-controlled trial was performed comparing the administration of 200 μg GM2 combined with 10^7 viable units of BCG with a treatment with BCG alone. In patients with a positive purified protein derivative skin test, only 3×10^6 viable units of BCG were used.[12] One hundred twenty-two patients with AJCC stage III melanoma were randomized after surgery. Fifty-eight patients received the combination of GM2 with BCG and 64 received BCG alone. All patients were pretreated with low-dose cyclophosphamide (IV 200 mg/m^2) before the first and fourth vaccine injections. The vaccination was administered intradermally in an extremity with intact lymphatic drainage, and it was repeated twice at 14-day intervals. After 2 and 5 months, booster immunizations were performed. The minimum followup period was 51 months. The authors detected GM2-specific antibodies in 50 of 58 patients treated with GM2 and BCG and in 7 of 64 of those treated with BCG alone. In the 57 antibody-positive patients, a highly significant increase in DFS ($p = 0.004$) and a 17% increase in OS ($p = 0.02$) were found, confirming their previous experience. Preexisting GM2 antibodies were found in one patient in the GM2 and BCG group and in five patients in the BCG-only group. When these patients were excluded, the increase was 23% in DFS ($p = 0.02$) and 14% in OS ($p = 0.15$). But the comparison of all patients as randomized, showed a nonsignificant difference of 18% in DFS and 11% in OS in favor of the GM2 and BCG group as compared with the BCG group, presenting a DFS of 30% and an OS of 46% ($p = 0.09$ and $p = 0.22$, respectively). Stratifying the two treatment groups for number of positive lymph nodes showed that in patients with only one positive node, immunization had less impact on DFS; patients with two or more positive nodes showed a significant DFS benefit after vaccination with GM2 and BCG ($p = 0.02$), with a similar trend in overall survival ($p = 0.08$). In summary, the study demonstrated a nonsignificant, clinically relevant trend toward a DFS benefit after vaccination with GM2 combined with BCG.

Ganglioside vaccine versus interferonα2b

The Eastern Cooperative Oncology Group Trial EST 1684 had shown prolonged relapse-free interval and overall survival in high-risk resected American Joint Committee on Cancer stage IIb and III melanoma patients on IV treatment with interferon (IFN)α2b at a maximum tolerated dose of 20 MU/m^2/day, administered at 10 MU/m^2 three times per week for 1 month and subcutaneously during 48 weeks, as compared with observation.[5]

In the Intergroup Trial E1694/S9512/C509801, Kirkwood and colleagues[5] randomized patients receiving high-dose IFNα2b for 1 year as standard adjuvant treatment versus vaccination with the GM2 keyhole limpet hemocyanin-QS-21 (GMK) vaccine. This vaccine formulation contained the ganglioside GM2 conjugated to keyhole limpet hemocyanin carrier and was administered together with QS-21 adjuvant weekly for a total of four injections, then every 12 weeks for eight more injections in 96 weeks. Vaccines were administered in 1-mL volumes by deep subcutaneous injection.[13] Keyhole limpet hemocyanin (KLH) is a large protein produced by a shelled sea creature that causes an immune response and acts as carrier for cancer associated antigens. QS-21 is an immunostimulating plant extract. Patients with resected stage IIB/III melanoma were included in the trial. Eight hundred eighty patients were randomized (440 per treatment arm) and 774

were eligible. One hundred six patients were ineligible because of inappropriate stage of disease or surgery, an interval of more than 70 days from biopsy, comorbidity or second malignancy, inappropriate or missing laboratory values, or earlier chemotherapy or radiotherapy. After an interim analysis after a median followup of 16 months, the trial was closed because of the superiority of IFNα2b. In the GMK group, the authors found a significantly higher risk of relapse for eligible patients (HR for DFS, 1.47; $p = 0.0015$; Cox-regression analysis adjusting for gender, age, performance status, and nodal category $p = 0.0027$) and a higher hazard of death (HR for OS = 1.52; $p = 0.009$; Cox-regression analysis $p = 0.0147$) and corresponding results for intention-to-treat (HR for DFS, 1.49; $p = 0.00045$; Cox-regression analysis $p = 0.0007$) and a higher hazard of death (HR for OS = 1.38; $p = 0.023$; Cox-regression analysis $p = 0.035$). The estimated 2-year DFS rate for eligible patients was 62% in the IFNα2b group as compared with 49% in the GMK group; the estimated 2-year overall survival rate for eligible patients was 78% in the IFNα2b group and 73% in the GMK group. High-dose IFN administration was associated with a treatment benefit in all subsets of patients irrespective of the number of lymph nodes involved. The greatest benefit was observed in node-negative patients (DFS HR, 2.07; OS HR, 2.71). Concerning GM2, antibody responses with titers $\geq$ 1:80 at days 29, 85, 365, and 720 were associated with a trend toward improved DFS and OS. Five grade IV toxicities were found in the GMK group as opposed to 52 grade IV toxicities in the IFNα2b group (of which 34 corresponded to granulocytopenia or leukopenia).

Epitope-specific immunotherapy

The molecular characterization of the first human TAA, published in 1991,[6] was followed by a number of phase I/II trials, using TAA-derived peptides or recombinant viruses as specific immunogens.[4,34,35] Based on the findings of phase I/II clinical trials taking advantage of dendritic cell (DC) administration,[36] a randomized phase III trial was performed.

Dacarbazine versus autologous peptide-pulsed dendritic cell vaccine

One hundred eight stage IV melanoma patients were randomized to receive either dacarbazine (DTIC), 850 mg/m^2 IV every 4 weeks (n = 55) or a vaccine consisting of autologous, antigenic peptide-pulsed, monocyte-derived, dendritic cells, injected subcutaneously every 2 weeks for 10 weeks and every 4 weeks thereafter (n = 53).[14] Patients were eligible if they presented at least one measurable target lesion, Karnofsky index > 70%, and HLA-A1/2/3/24 or B44 positivity, or both. Patients with central nervous system metastases were excluded. Four patients in the vaccination group were ineligible because of central nervous system metastases, HLA mismatch, or because they didn't present stage IV disease. Eight patients in the vaccination arm either refused treatment, or did not receive treatment because of peripheral blood mononuclear cell contamination, or they were excluded because they refused assessment, had no stage IV bone metastases, or received less than two vaccinations. Three patients in the DTIC arm refused treatment. So the response was assessed in 52 patients in the DTIC group and in 41 patients in the vaccine group. After a median followup of 22.2 months, in the intention-to-treat population, no significant differences in median progression-free survival (DTIC, 2.8 months; vaccine, 3.2 months) and median overall survival (DTIC, 11.6 months; vaccine, 9.3 months; $p = 0.48$) were found. There was no significant difference in objective response (DTIC, 5.5%; vaccine, 3.8%). Evaluation on a per-protocol basis showed similar results concerning duration of survival and objective response rate. Grade 3 or 4 toxicities were found in seven patients in each treatment arm. The study was closed after the first interim analysis based on recommendation of the Data Monitoring and Safety Board because of the low probability of demonstrating superiority of the vaccine.

To investigate the lack of vaccine efficacy, several posthoc analyses were conducted. Patients with normal serum lactate dehydrogenase or stage M1a/b (skin, lymph node, lung metastases), or both, were found to survive longer than patients presenting elevated lactate dehydrogenase or stage M1c (metastases to other organs) in either arm. In the vaccine arm, patients with a Karnofsky index of 100 or HLA-A2+/HLAB44 haplotype presented significantly longer survival. Importantly, cellular immune responsiveness to the antigenic peptides used in the vaccine preparation was not assessed.

DISCUSSION

Tumor immunology is typically characterized by waves of enthusiasm and disillusionment. Interestingly, these emotions stem largely from the analysis of numerous small phase I or II studies, only addressing, by definition, safety and, possibly, biologic activity of given immunostimulatory protocols. To provide solid foundations to our clinical research studies, we felt compelled to critically evaluate randomized-controlled studies performed in the area of active TAA-specific immunotherapy of melanoma.

In the past two decades substantial progress has been made in the understanding of the cellular and molecular bases of immune responses, resulting in new approaches to cancer immunotherapy, but the number of phase III clini-

cal trials remains dismally low. This is because these randomized trials not only require adequate preclinical investigations and preliminary phase I and II trials, but are time consuming and very expensive. In a majority of them, the scientific and technologic background appears to be relatively outdated when considered in the context of the accelerating pace of immunologic research. In this context, negative results largely prevail.

Two of the phase III trials have been published in abstract form only.[8,11] Data from the phase III trials of Morton and colleagues[11] mentioned in the Results section are based on their presentation at the 2006 Society of Surgical Oncology (SSO) meeting and the corresponding abstract. Data from the phase III trial of Mitchell and Von Eschen[8] presented are based on the abstract only.

In a large case series, Morton and associates[28] provided compelling evidence of a survival benefit of Canvaxin versus no vaccination. But a phase III trial of Canvaxin versus placebo was terminated after the third interim analysis for stage III patients and at the second interim analysis for stage IV patients because of the low probability of demonstrating a significant overall survival improvement.[11] Similarly, none of the other phase III active specific immunotherapy (ASI) protocols demonstrated significant disease-free or overall survival improvements.

An important limitation of these studies is frequently represented by the low number of participants. Especially if small but clinically relevant differences are supposed to be demonstrated, high participant numbers are required.[37] So even if a trend toward better outcomes in the ASI group using GM2 ganglioside was shown by Livingston and coworkers,[12] results were nonsignificant, possibly because of a lack of power. In this study, preexisting GM2 antibodies were found in five patients in the control group, as opposed to only one in the vaccination group. In eight vaccinated patients, no antibody response was found. Because antibody positivity was associated with better outcomes, the nonsignificant result of the two groups, as randomized, may have been biased toward no difference by not excluding patients with preexisting antibodies. This hypothesis is supported by the fact that on exclusion of these patients, in a post hoc analysis, a significant increase in DFS ($p = 0.02$) was observed.

Hersey and coauthors[10] also showed a trend toward overall survival improvement after a vaccinia melanoma cell lysate treatment, but again, the difference was nonsignificant. This was observed despite the fact that followup was 8 years and the number of participants was large ($n = 675$). The therapeutic effect was expected to be much higher based on nonrandomized phase II studies. The authors concluded that given the negligible toxicity of the vaccine, a clinical benefit is possible.

Similarly, Sondak and colleagues[9] concluded from their study that there was no evidence of improved DFS among patients receiving an allogeneic melanoma vaccine, although they judged the power to detect a small but clinically relevant difference to be too low.

Lack of an observation arm may impair the significance of some studies. For instance, the second ganglioside trial by Kirkwood and associates[13] comparing GM2 and high-dose IFNα2b was closed after an interim analysis indicating inferiority of GM2, although the absence of an observation arm prevented comparison of GM2 with observation. In the study by Wallack and coworkers,[7] the lack of a no-treatment arm potentially biased results, given the fact that biologically active vaccinia virus used as a control arm may have had a therapeutic effect in and of itself. In the study by Mitchell and colleagues,[8] a no-treatment arm was missing as well. But the comparison of active specific immunotherapy to chemotherapy allowed demonstration of a significantly higher quality of life in the active specific immunotherapy group.

Blinding may represent another problematic issue in some of these trials. For instance, the study by Hersey and colleagues[10] was performed in an unblinded fashion, and the study by Kirkwood and associates[13] was unblinded 2 months after the third interim analysis showing inferiority of GM2.

Interestingly, retrospective subset analyses within these phase III trials demonstrated statistically significant survival benefits in the active specific immunotherapy groups, suggesting that patients with specific, well-defined characteristics should be included in future trials.[7,12] Indeed, Wallack and coauthors[7] found a significant improved survival in a subset analysis of male patients with one to five positive nodes treated with VMO versus control vaccinia virus.

Although these findings are interesting, it must be emphasized that they are based on post hoc subset analyses and, as such, they may lead to both false-positive and false-negative findings.[38,39] So the conclusions made in the article have been criticized based on the analysis of data-derived subgroups, which were established post hoc, including an age cut at 57 years.[40]

Livingston and associates[12] found a significantly longer disease-free survival in immunized patients after stratifying the two treatment arms by the number of positive nodes, again however, looking at a subset of the entire sample. Importantly, Sosman and colleagues[24] amended their protocol during the course of their trial to mandate blood sample collection for serotyping of HLA-A/B/C and found a significantly higher DFS in vaccinated patients who were

positive for HLA-A2, HLA-C3, or both. These data suggest that clinically relevant tumor-specific responses restricted by these alleles could have actually been induced by vaccination. These interesting findings clearly deserve further investigation in a phase III trial enrolling only patients with HLA-A2 or HLA-C3 positivity or both.

Today's published data of phase III clinical trials are based on immunology knowledge from more than a decade ago. But in the rapidly evolving field of immunology research, as yet, only a few aspects of immune-escape, role of adjuvants, vaccination schedules, and rules governing the duration of immune response are reasonably clarified.[35,41-44]

Notably, cloning of the first human TAA was published 15 years ago,[6] and the first phase I/II clinical trial taking advantage of dendritic cell administration was published 8 years ago.[36] But strikingly, only one phase III clinical trial[14] has been performed using peptides alone or in combination with dendritic cells, despite large numbers of phase I/II studies.[35,41] This phase III report suggests that overall general conditions of patients, as delineated by a high Karnofsky index, might represent the indispensable biologic background for a clinically effective immunotherapy. This finding suggests that vaccination therapy should be administered as early as possible.

Most important, in only one trial were skin tests performed to assess delayed type hypersensitivity.[8] On the other hand, no phase III trial published so far has attempted to detect cellular immune responsiveness ex vivo or in vitro, or to characterize its specificity. So clinical effectiveness, or the lack of it, could not be reliably correlated to the induction of antigen-specific immunity. Indeed, monitoring of cellular immune response still suffers from poor standardization, and its technologic complexity is hardly amenable to the analysis of large groups of patients within multicenter studies.[34] Establishment of simple but reliable techniques that adequately monitor T-cell responsiveness to tumor-associated antigens is emerging as a critical issue in cancer immunotherapy.

A search on www.clinicaltrial.gov showed that two phase III active specific immunotherapy trials are no longer recruiting patients. These include a study comparing the effect of immunization with GM2-KLH and QS-21 to observation of patients with stage II melanoma (NCT00005052, EORTC-18961) and a study of heat shock protein-peptide complex versus interleukin-2 and/or dacarbazine/temozolamide-based therapy and/or complete tumor resection in stage IV melanoma. Publication of these studies is eagerly awaited.

Although these data might suggest an overall negative experience in the active specific immunotherapy of melanoma, fast, unwise conclusions should be avoided. The molecular characterization of human TAA in the past decade has provided solid evidence of a class I restricted responsiveness against autologous tumors, but the evaluation of immunization procedures aimed at the induction of specific cytotoxic T lymphocytes is still in its infancy. Notably, even for vaccinations administered to prevent infectious diseases, cytotoxic T lymphocyte generation still represents a critical problem. Ongoing efforts to develop adequate adjuvants should bring rapid progress in this area. In addition, advances in the study of the regulation of CD8 T-cell function by cytokines[45] or by surface receptors including PD-1[46,47] and CTLA-4[48] may soon lead to clinically relevant breakthroughs.

In this context, reevaluation of past protocols might also take place. For instance, recent evidence clearly indicates that gangliosides can be targeted by T cells in addition to humoral responses.[49] In addition, the clinical effectiveness of anti HER2 antibody treatment in HER2 positive patients suggests that the selection of adequate patient populations might represent a decisive key to therapeutic success.[50,51]

In conclusion, there is currently no evidence of the efficacy of active specific immunotherapy in terms of overall and disease-free survivals. But trends emerging from retrospective subset analyses of previous specific immunotherapy studies suggest that future trials should enroll patients with well-defined characteristics. Also, trials must be adequately powered to detect small but clinically relevant differences. Incorporating recent and ongoing progress in tumor immunology may increase the likelihood of positive results.

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