

ORIGINAL ARTICLE

Prognostic Significance of Autoimmunity during Treatment of Melanoma with Interferon

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ABSTRACT

BACKGROUND

Immunotherapy for advanced melanoma induces serologic and clinical manifestations of autoimmunity. We assessed the prognostic significance of autoimmunity in patients with stage IIB, IIC, or III melanoma who were treated with high-dose adjuvant interferon alfa-2b.

METHODS

We enrolled 200 patients in a substudy of a larger, ongoing randomized trial. Blood was obtained before the initiation of intravenous interferon therapy, after 1 month of therapy, and at 3, 6, 9, and 12 months. Serum was tested for antithyroid, antinuclear, anti-DNA, and anticardiolipin autoantibodies, and patients were examined for vitiligo.

RESULTS

The median duration of follow-up was 45.6 months. Relapse occurred in 115 patients, and 82 patients died. The median relapse-free survival was 28.0 months, and the median overall survival was 58.7 months. Autoantibodies and clinical manifestations of autoimmunity were detected in 52 patients (26 percent). The median relapse-free survival was 16.0 months among patients without autoimmunity (108 of 148 had a relapse) and was not reached among patients with autoimmunity (7 of 52 had a relapse). The median survival was 37.6 months among patients without autoimmunity (80 of 148 died) and was not reached among patients with autoimmunity (2 of 52 died). In univariate and multivariate regression analyses, autoimmunity was an independent prognostic marker for improved relapse-free survival and overall survival ($P < 0.001$).

CONCLUSIONS

The appearance of autoantibodies or clinical manifestations of autoimmunity during treatment with interferon alfa-2b is associated with statistically significant improvements in relapse-free survival and overall survival in patients with melanoma.

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ADJUVANT THERAPY WITH INTERFERON alfa-2b (after surgery for deep primary or regionally metastatic melanoma) has clinically significant benefits for patients with melanoma who are at high risk for relapse or death. Patients with American Joint Committee on Cancer (AJCC) stage IIB, IIC, or III melanoma have risks of relapse and death exceeding 40 percent at five years and are candidates for interferon alfa-2b therapy.¹ Several large cooperative-group trials have evaluated adjuvant therapy with high-dose interferon alfa-2b in patients at high risk and have consistently demonstrated statistically significant prolongation of relapse-free survival with adjuvant therapy as compared with observation.²⁻⁴ Two of these trials also demonstrated a statistically significant improvement in overall survival,^{2,4} although a pooled analysis of four Eastern Cooperative Oncology Group and intergroup trials did not show a survival benefit.⁵ The treatment tested in these trials consisted of an intravenous induction phase at the maximum tolerated dose (20 million international units [IU] per square meter of body-surface area per day) 5 days per week for the initial 4 weeks, followed by subcutaneous therapy at a dose of 10 million IU per square meter of body-surface area three times per week for 11 months. Analyses of the relapse-free and overall survival curves from the initial trial (EST 1684) revealed early separation between the group assigned to high-dose interferon alfa-2b and the group assigned to observation.² Therefore, the one-month intravenous induction phase of the regimen may be necessary and sufficient to reduce the risk of recurrence, and several prospective trials designed to test this hypothesis are ongoing.

Acceptance of treatment with high-dose interferon alfa-2b by physicians and patients has been limited because of its toxicity and cost as well as evidence suggesting that only a subgroup of patients benefit from it. Most patients have fatigue, fever, arthralgias, anorexia, and toxic hepatic effects; some have severe depression. A better understanding of the mechanism of action of interferon alfa-2b and identification of predictive markers that would permit selection of patients most likely to benefit would therefore be beneficial.

All immunotherapies that confer a survival benefit in patients with advanced melanoma induce the collateral appearance of autoimmunity. Hypothyroidism, hyperthyroidism,⁶⁻¹⁰ the antiphospholipid-antibody syndrome,¹¹ and vitiligo^{10,12} have

been cited as early correlates of benefit from high-dose interleukin-2. Indeed, the appearance of paraneoplastic and presumably autoimmune vitiligo was considered a favorable prognostic factor in patients with melanoma even before the advent of interleukin-2 therapy.¹³⁻¹⁵ Increased levels of autoantibodies, including antithyroid antibodies (microsomal antigens and thyroglobulin) and antinuclear, anti-DNA, antiplatelet, and anti-islet-cell antibodies, all of which may persist for several months, have been well documented in patients receiving interferon alfa for hematologic cancers or chronic viral hepatitis.¹⁶⁻²¹

In this report we present the results of a prospective evaluation of the incidence of autoantibody detection and clinically apparent autoimmune disorders in patients with melanoma who received adjuvant therapy with high-dose interferon alfa-2b. The patients included in this analysis were a subgroup of patients enrolled in an ongoing, randomized, phase 3 trial being conducted by the Hellenic Cooperative Oncology Group to evaluate intravenous induction therapy with interferon alfa-2b for 4 weeks as compared with the same regimen followed by 11 months of adjuvant interferon alfa-2b therapy.

METHODS

PATIENTS

Participants were enrolled in trial 13A/98, a prospective, multicenter, randomized, phase 3 trial conducted at 13 institutions by the Hellenic Cooperative Oncology Group. In this trial, 364 patients with histologically documented AJCC stage IIB, IIC, or III primary cutaneous melanoma were enrolled between 1998 and 2004. Stage was defined pathologically by sentinel-lymph-node dissection. Any patient with a positive sentinel lymph node was required to undergo complete lymphadenectomy. All patients were randomly assigned to receive protocol-directed treatment within 2 months after the initial surgery or within 1.5 months after lymph-node dissection. We gave interferon alfa-2b according to a modification of the EST 1684 regimen.²² Patients in one group received induction therapy with interferon alfa-2b (15 million IU per square meter per day, intravenously, five days per week for four weeks) followed by observation. Patients in a second group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks. The primary end

points for the core protocol were relapse-free survival and overall survival.

The autoimmunity substudy reported here was conducted prospectively at a single institution (the First Department of Medicine, University of Athens) but included 15 patients from two collaborating centers. This substudy had separate institutional review board approval, and patients provided written informed consent. Blood samples for the evaluation of autoantibodies were drawn at the same time as samples for standard follow-up tests. The first 10 ml of blood collected was used for standard biochemical analyses and blood-cell counts; the second 10 ml was used for autoantibody testing. Blood samples were obtained before treatment, after 1 month of intravenous interferon alfa-2b therapy, and at 3, 6, 9, and 12 months. Patients who tested positive for autoantibodies or who had evidence of vitiligo-like skin depigmentation before the initiation of treatment were not included in the analysis.

Patients were followed prospectively for clinical outcome with the use of standardized testing. Clinical staging consisted of medical history taking, physical examinations, blood-cell counts, blood biochemical analyses at three-month intervals, and chest radiography and liver ultrasonography at six-month intervals.

SEROLOGIC ASSAYS

Blood samples were tested by enzyme-linked immunosorbent assays (Quanta Lite, Inova Diagnostics) for antinuclear antibodies (a positive result was defined as a titer of $\geq 1:40$), anti-DNA antibodies (a positive result was defined as a titer of $\geq 1:40$), antithyroglobulin antibodies (a positive result was defined as a titer of $\geq 1:100$), antimicrosomal antibodies (a positive result was defined as a titer of $\geq 1:100$), and anticardiolipin antibodies (IgM and IgG; a positive result was defined as a titer of $\geq 1:100$). When a blood sample was positive for antinuclear antibodies, it was also tested for antibodies against extractable nuclear antigens. Thyroid-stimulating hormone (normal range, 0.34 to 3.5 IU per milliliter), thyroxine (normal range, 4.5 to 13.0 μg per deciliter [57.9 to 167.3 nmol per liter]), and triiodothyronine (normal range, 0.8 to 1.8 ng per milliliter [1.2 to 2.8 nmol per liter]) levels were also measured.

STATISTICAL ANALYSIS

The primary end points for all the patients were relapse-free survival and overall survival from

the time of study entry. Relapse-free survival was calculated from the initiation of treatment to the date on which relapse was first documented or on which death without documented relapse occurred. Follow-up data were updated on April 1, 2005, and data from patients who had not had a relapse by that date were censored at the time of the last clinic visit. Overall survival was calculated from the initiation of treatment to the date of death or last contact. Development of autoimmunity was defined as either a positive test for autoantibodies or presentation with a clinical manifestation of autoimmunity in the 12-month period during which blood samples were analyzed for autoantibodies.

Univariate hazard ratios were calculated with 95 percent confidence intervals with use of the Cox proportional-hazards model.²³ Probabilities of relapse-free and overall survival were estimated by the Kaplan–Meier method, and the log-rank test or the Wald test from the corresponding Cox models was used to compare time-to-event distributions.²⁴ The simultaneous prognostic effect of various factors was determined in a multivariate analysis with use of the Cox proportional-hazards model (forward selection of variables). Landmark analysis at 3 and 12 months was performed for relapse-free and overall survival according to autoimmune status. Autoimmune status was included in the models as a time-dependent variable. Other covariates in the univariate and multivariate models included age (<52 years vs. ≥ 52 years), interferon alfa-2b treatment group (induction therapy only vs. extended therapy), sex (male vs. female), Breslow thickness (0 to 2.0 vs. 2.1 to 4.0 vs. >4.0 mm), Clark level (II or III vs. IV or V), vascular invasion (yes vs. no), ulceration (yes vs. no), regression (yes vs. no), stage (IIB or IIC or unspecified II vs. III), and lymph-node involvement (yes vs. no). Fisher's exact and chi-square tests were used to compare groups with or without autoantibodies or autoimmune manifestations. All reported P values are two-sided. No interim analyses were performed.

RESULTS

PATIENTS

Blood samples were obtained from 203 patients; 3 had autoantibodies at baseline and were excluded from the study. Table 1 shows the baseline characteristics of the remaining 200 patients. Of these, 96 were randomly assigned to intravenous induc-

tion therapy only and 104 to intravenous induction therapy plus 48 weeks of subcutaneous therapy. The median age of the patients was 52.7 years (range, 19.4 to 73.6). At study entry, 55 patients (28 percent) had AJCC stage II disease, 138 (69 percent) had stage III disease, and 7 (4 percent) had unknown lymph-node status. In all patients except the 7 with unknown lymph-node status and 26 with no information concerning ulceration

of the skin lesion, the disease was restaged according to the revised AJCC staging criteria.¹

Patients were followed for a median of 45.6 months (95 percent confidence interval, 39.5 to 51.6); 115 patients (58 percent) had a recurrence, and 2 died without a documented recurrence. Staging workups in these two patients performed one month before death in one patient and two months before death in the other revealed no evi-

Table 1. Demographic and Baseline Characteristics of the Patients.

Characteristic	Value (N = 200)*	Characteristic	Value (N = 200)*
Age — yr		Clark level — no. (%)	
Median	52.7	II or III	42 (21)
Range	19.4–73.6	IV or V	138 (69)
Treatment group — no. (%) †		Unknown	20 (10)
Induction therapy	96 (48)	Lymph-node involvement — no. (%)	
Extended therapy	104 (52)	No	55 (28)
Sex — no. (%)		Yes	138 (69)
Male	104 (52)	Unknown	7 (4)
Female	96 (48)	Vascular invasion — no. (%)	
Site of primary tumor — no. (%)		No	111 (56)
Head	34 (17)	Yes	58 (29)
Limbs	88 (44)	Unknown	31 (16)
Trunk	67 (34)	Ulceration — no. (%)	
Mucosa	4 (2)	No	39 (20)
Unknown	7 (4)	Yes	130 (65)
AJCC stage — no. (%) ‡		Unknown	31 (16)
II unspecified	4 (2)	Regression — no. (%)	
IIB	6 (3)	No	117 (59)
IIC	45 (23)	Yes	52 (26)
III unspecified	22 (11)	Unknown	31 (16)
IIIA	20 (10)	Histology — no. (%)	
IIIB	63 (32)	Superficial spreading melanoma	94 (47)
IIIC	33 (17)	Nodular melanoma	53 (27)
Unknown	7 (4)	Not otherwise specified	11 (6)
Breslow thickness — no. (%)		Mucosal melanoma	4 (2)
0–2.0 mm	30 (15)	Acral lentiginous melanoma	10 (5)
2.1–4.0 mm	47 (24)	Lentigo maligna melanoma	5 (3)
>4.0 mm	107 (54)	Other	7 (4)
Unknown	16 (8)	Unknown	16 (8)

* Because of rounding, not all percentages total 100.

† Patients in the induction-therapy group received interferon alfa-2b (15 million IU per square meter of body-surface area per day, intravenously, five days per week for four weeks) followed by observation. Patients in the extended-therapy group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks.

‡ AJCC denotes American Joint Committee on Cancer.

dence of tumor recurrence. Median relapse-free survival for the entire population was 28.0 months (range, 0.3 to 84.7; 95 percent confidence interval, 18.2 to 37.7). At last follow-up, 82 patients (41 percent) had died. The median survival was 58.7 months (range, 3.8 to 91.8; 95 percent confidence interval, 42.8 to 74.6).

DETECTION OF AUTOANTIBODIES OR AUTOIMMUNE MANIFESTATIONS

As shown in Table 2, autoantibodies or clinical manifestations of autoimmunity were detected in 52 patients (26 percent) (23 in the induction-therapy group and 29 in the extended-therapy group, P=0.63); multiple manifestations of autoimmunity developed in 16 patients (8 percent) (1 and 15, respectively). Antithyroid antibodies were detected in 43 patients (22 percent) (16 in the induction-therapy group and 27 in the extended-therapy group, P=0.12). In general, autoantibodies were observed more frequently in the group of patients receiving therapy for one year. Vitiligo developed in 11 patients (6 percent) (5 in the induction-therapy group and 6 in the extended-therapy group), and 19 patients (2 and 17, respectively) had other clinical manifestations attributed to autoimmunity. Of these 19, hypothyroidism developed in 11 patients (2 in the induction-therapy group and 9 in the extended-therapy group), thyrotoxicosis was found in 2, autoimmune thrombocytopenic purpura with antiplatelet antibodies occurred in

1, and retinopathy was found in 1. In addition, two patients presented with myalgias and arthralgias, and signs and symptoms of rheumatoid arthritis developed in two other patients. In one patient with myalgias and arthralgias, tests for antinuclear antibodies and rheumatoid factor became positive, suggesting a systemic syndrome similar to lupus erythematosus.

The median time to the detection of autoantibodies after the start of interferon alfa-2b treatment was three months, and the median time to the development of clinical manifestations was nine months. The onset of vitiligo occurred between 3 and 12 months after the initiation of interferon alfa-2b treatment. The time to the development of autoantibodies or autoimmune manifestations did not differ between the treatment groups.

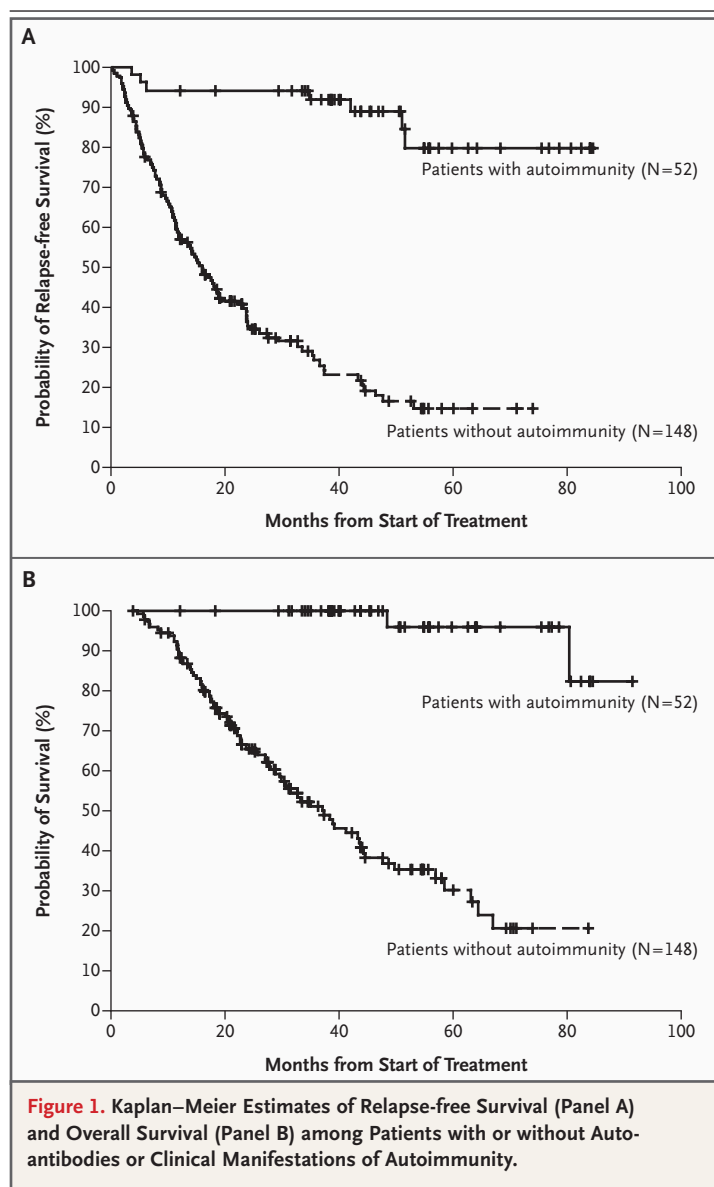
CLINICAL OUTCOME ACCORDING TO AUTOIMMUNITY

At a median follow-up of 45.6 months, only 7 of the 52 patients (13 percent) in whom autoantibodies or clinical manifestations of autoimmunity developed had had a recurrence. In contrast, 108 of the 148 patients (73 percent) with no evidence of autoimmunity had had a relapse. Figure 1A shows Kaplan–Meier estimates of relapse-free survival. Median relapse-free survival among patients without evidence of autoimmunity was 16.0 months (range, 0.3 to 74.3; 95 percent confidence

Table 2. Autoantibodies or Manifestations of Autoimmunity in Patients Treated with Interferon Alfa-2b.*

Autoantibodies or Manifestations of Autoimmunity	All Patients (N = 200)	Induction-Therapy Group (N = 96)	Extended-Therapy Group (N = 104)
		<i>no. of patients (%)</i>	
Autoantibodies or autoimmune disorders	52 (26)	23 (24)	29 (28)
Antithyroid antibodies	43 (22)	16 (17)	27 (26)
Antinuclear antibodies	12 (6)	2 (2)	10 (10)
Anticardiolipin antibodies	10 (5)	2 (2)	8 (8)
Vitiligo	11 (6)	5 (5)	6 (6)
Clinical manifestations	19 (10)	2 (2)	17 (16)
With autoantibodies	16 (8)	2 (2)	14 (13)
Without autoantibodies (vitiligo)	3 (2)	1 (1)	2 (2)
Multiple manifestations of autoimmunity	16 (8)	1 (1)	15 (14)

* Patients in the induction-therapy group received interferon alfa-2b (15 million IU per square meter of body-surface area per day, intravenously, five days per week for four weeks) followed by observation. Patients in the extended-therapy group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks.



interval, 12.5 to 19.3), whereas the median relapse-free survival was not reached during follow-up among patients in whom autoimmunity developed (range, 3.5 to 84.7 months). A univariate analysis of relapse-free survival showed a statistically significant association between absence of recurrence and the development of autoantibodies or autoimmune manifestations (or both) as a time-varying covariate ($P<0.001$), between recurrence and vascular invasion ($P<0.001$), and between recurrence and regional lymph-node involvement ($P=0.02$) (Table 3). Patients with regional lymph-node involvement or vascular invasion at baseline had

a shorter median relapse-free survival. Analysis of disease stage among patients with evidence of autoimmunity and those without evidence of autoimmunity showed no statistically significant differences between these groups that could explain the observed differences in clinical outcome ($P=0.46$ by the two-sided Pearson chi-square test).

At last follow-up, only 2 of 52 patients (4 percent) in whom autoantibodies were detected or who had manifestations of autoimmunity had died. In contrast, 80 of 148 patients (54 percent) who had no evidence of autoimmunity had died. Figure 1B shows Kaplan–Meier estimates of overall survival. The median overall survival among patients who did not have autoimmunity was 37.6 months (range, 3.8 to 74.3; 95 percent confidence interval, 28.9 to 46.3), whereas the median survival was not reached among patients with autoimmunity (range, 12.2 to 91.8 months). In findings similar to those in the evaluation of relapse-free survival, univariate analysis showed a significant association between overall survival and the presence of autoimmunity as a time-varying covariate ($P<0.001$), between death and regional lymph-node involvement ($P=0.01$), and between death and vascular invasion ($P=0.02$). Patients with regional lymph-node involvement and vascular invasion at baseline had the shortest median overall survival.

In the multivariate analysis, with the use of a Cox model adjusted according to treatment group and with autoantibody expression as a time-varying covariate, the only statistically significant, independent prognostic factors for relapse-free and overall survival were manifestations of autoimmunity and regional lymph-node involvement. The effect of treatment group and the corresponding interactions were not significant. The development of autoimmunity correlated with longer relapse-free survival (hazard ratio, 0.12; 95 percent confidence interval, 0.05 to 0.25; $P<0.001$) and longer overall survival (hazard ratio, 0.02; 95 percent confidence interval, <0.01 to 0.15; $P<0.001$). Lymph-node involvement correlated with shorter relapse-free survival (hazard ratio, 1.87; 95 percent confidence interval, 1.20 to 2.93; $P=0.007$) and shorter overall survival (hazard ratio, 2.36; 95 percent confidence interval, 1.29 to 4.31; $P=0.005$).

Landmark analyses at 3 and 12 months (Table 4) showed a persistently strong association between autoimmune manifestations and both relapse-free survival and overall survival ($P<0.001$ for both). Of note, by 12 months, all 52 patients

Table 3. Univariate Cox Regression Models of Relapse-free Survival and Overall Survival.*

Variable	Relapse-free Survival			Overall Survival		
	Rate	Median Duration (95% CI)	P Value†	Rate	Median Duration (95% CI)	P Value†
	<i>no. of events/ no. of patients</i>	<i>mo</i>		<i>no. of events/ no. of patients</i>	<i>mo</i>	
Age (yr)			0.71			0.71
<52	59/98	31.3 (14.3–48.3)		44/98	63.3 (41.6–85.0)	
≥52	56/102	28.0 (17.9–38.0)		38/102	58.7 (NE)	
Group‡			0.94			0.82
Induction therapy	54/96	24.0 (6.4–41.7)		39/96	58.7 (40.0–77.5)	
Extended therapy	61/104	32.9 (21.2–44.6)		43/104	63.3 (39.5–87.2)	
Sex			1.00			0.58
Male	61/104	28.0 (13.8–42.1)		45/104	57.0 (34.9–79.2)	
Female	54/96	27.7 (13.3–42.1)		37/96	58.7 (40.5–76.9)	
Breslow thickness (mm)			0.33			0.90
0–2.0	16/30	18.6 (NE)		11/30	80.8 (NE)	
2.1–4.0	31/47	23.7 (8.0–39.5)		21/47	43.8 (NE)	
>4.0	59/107	35.7 (20.4–51.0)		43/107	58.7 (40.0–77.5)	
Clark level			0.22			0.12
II or III	19/42	NR (NE)		13/42	80.8 (NE)	
IV or V	83/138	26.1 (14.2–38.1)		60/138	47.9 (27.7–68.2)	
Vascular invasion			<0.001			0.02
No	54/111	43.8 (27.3–60.3)		39/111	80.8 (51.6–110.0)	
Yes	42/58	16.0 (8.7–23.2)		29/58	37.6 (18.1–57.1)	
Ulceration			0.61			0.48
No	22/39	35.7 (11.1–60.3)		17/39	57.0 (31.7–82.3)	
Yes	74/130	32.9 (19.1–46.7)		51/130	64.6 (45.8–83.3)	
Regression			0.39			0.77
No	69/117	23.8 (9.2–38.4)		49/117	63.6 (45.3–81.4)	
Yes	27/52	36.6 (18.9–54.3)		19/52	NR (NE)	
Lymph-node involvement			0.02			0.01
No	25/55	51.1 (37.7–64.8)		13/55	NR (NE)	
Yes	84/138	19.0 (12.5–25.4)		63/138	48.6 (31.5–65.7)	
Autoimmunity			<0.001§			<0.001§
No	108/148	16.0 (12.5–19.3)		80/148	37.6 (28.9–46.3)	
Yes	7/52	NR (NE)		2/52	NR (NE)	

* CI denotes confidence interval, NE not evaluable, and NR not reached.

† P values were calculated with the use of the Wald test.

‡ Patients in the induction-therapy group received interferon alfa-2b (15 million IU per square meter of body-surface area per day, intravenously, five days per week for four weeks) followed by observation. Patients in the extended-therapy group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks.

§ The P value is for autoimmunity status as a time-varying covariate.

Table 4. Landmark Analyses of Relapse-free and Overall Survival According to Autoimmunity Status.*

Time Point and Autoimmunity Status	Relapse-free Survival			Overall Survival				
	Rate no. of events/ no. of patients	Median Duration (95% CI) mo	Hazard Ratio (95% CI)	P Value†	Rate no. of events/ no. of patients	Median Duration (95% CI) mo	Hazard Ratio (95% CI)	P Value†
At 3 mo				<0.001				<0.001
No autoimmunity	96/152	20.8 (13.5–28.1)	0.15 (0.06–0.37)		80/166	40.8 (32.5–49.1)	0.07 (0.02–0.28)	
Autoimmunity	5/34	NR (NE)			2/34	NR (NE)		
At 12 mo				<0.001				<0.001
No autoimmunity	46/83	21.6 (14.4–28.8)	0.08 (0.03–0.22)		64/127	29.4 (23.1–35.7)	0.02 (<0.01–0.15)	
Autoimmunity	4/48	NR (NE)			2/52	NR (NE)		

* CI denotes confidence interval, NR not reached, and NE not evaluable.
 † P values were calculated with the use of the Wald test.

with autoimmunity were alive, and 48 of them had not had a relapse.

DISCUSSION

Autoimmune phenomena have been associated with improved outcome in patients with cancer in general, and in those with melanoma in particular,^{6-12,25} suggesting that a robust antitumor immune response may prolong survival but can also lead to autoimmunity. Notably, vitiligo-like skin depigmentation was recognized more than 20 years ago in patients with melanoma, when paraneoplastic depigmentation was reported to be associated with prolonged survival apart from any specific therapeutic intervention.¹³⁻¹⁵ However, to our knowledge, a prospective analysis of autoantibodies or autoimmune manifestations in patients with melanoma receiving adjuvant interferon alfa-2b therapy has not been reported.

In our study of 200 patients with high-risk melanoma who received adjuvant high-dose interferon alfa-2b (intravenous induction therapy for 4 weeks with or without 48 weeks of subcutaneous therapy), we found that the development of autoantibodies or clinical manifestations of autoimmunity occurred in about one quarter of the patients and was associated with statistically significant improvements in both relapse-free survival and overall survival. This relation between autoimmunity and outcome was demonstrated in univariate and multivariate Cox proportional-hazards models and in an analysis of disease stage (according to the current AJCC staging criteria) among patients with evidence of autoimmunity and those without it. The observed differences in clinical outcome are not attributable to any other statistically significant differences between these groups. Models in which autoantibody formation was used as a time-dependent variable yielded similar results.

Analysis of autoantibody formation and autoimmune manifestations according to treatment group showed a trend toward more frequent development of autoimmunity among patients receiving interferon alfa-2b for one year than among those receiving it for only four weeks. Although the overall incidence of autoantibodies or autoimmune manifestations among patients receiving therapy for one year was only slightly higher than that among the patients receiving only induction therapy (28 percent vs. 24 percent, respec-

tively), the frequency of antinuclear antibodies, anticardiolipin antibodies, clinical manifestations of autoimmunity, and multiple manifestations of autoimmunity was much higher in the group receiving therapy for one year. Antithyroid antibodies were the most frequently observed autoantibodies, occurring in 22 percent of the patients (17 percent in the induction-therapy group and 26 percent in the extended-therapy group). Clinical evidence of hypothyroidism or thyrotoxicosis was found in approximately 6 percent of the patients overall, a slightly lower fraction than previously reported.²⁶ Notably, hypothyroidism developed in only two patients in the induction-therapy group as compared with nine in the extended-therapy group, although the frequency of antithyroid antibodies was only slightly higher in the latter group. Risk factors for the development of thyroid dysfunction during interferon alfa-2b therapy include administration of the cytokine at increased doses or for extended periods of time, combination with other agents (especially interleukin-2), and female sex.²⁷ Likewise, in the current study, all the patients who had clinical manifestations of rheumatologic illnesses were among those receiving one year of treatment with interferon alfa-2b.

The association between autoimmune phenomena and prolonged survival has been demonstrated in recent studies of treatment of melanoma with anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibodies,²⁸⁻³¹ which appear to block T-cell regulatory functions of the immune system and thereby unleash a wide range of autoimmune reactions,

ranging from enteritis and hepatitis to thyroiditis, hypophysitis, and dermatitis. In these studies, the occurrence of autoimmune responses appeared to be linked to antitumor responses. Although the reported observations of autoimmune phenomena in retrospective series of patients treated with interleukin-2 or anti-CTLA-4 antibody are compelling, they have not been validated in prospective studies.

Our observations suggest opportunities to guide and improve the therapeutic index of adjuvant high-dose interferon alfa-2b therapy. For example, it may be possible to use autoimmune responses as surrogate markers to evaluate new treatments quickly. In conclusion, we have shown that the appearance of autoantibodies and clinical signs of autoimmunity are strongly associated with improved relapse-free and overall survival in patients with melanoma who are receiving adjuvant therapy with high-dose interferon alfa-2b. Serologic and clinical manifestations of autoimmunity are easily observed during the course of adjuvant interferon alfa-2b therapy and may provide clinicians and investigators with useful surrogate markers for monitoring the activity of adjuvant interferon treatment in patients with stage IIB, IIC, or III melanoma.

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This article is dedicated to the memory of Professor John Ioannovich.

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