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Presence and Prognostic Significance of Melanoma-Associated Antigens CYT-MAA and HMW-MAA in Serum of Patients with Melanoma

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With the goal of finding serological markers to monitor patients with early- as well as late-stage melanoma, we compared the levels of the cytoplasmic melanoma-associated antigens (CYT-MAA) and high-molecular-weight melanoma-associated antigen (HMW-MAA) in the sera of melanoma patients and controls. Using double-sandwich ELISA, we measured levels of both antigens in 117 patients and in 62 age- and sex-matched controls. Patients were stratified into four risk group based on stage of the disease. Serum levels of both markers were significantly higher in melanoma patients than in controls. CYT-MAA was the more sensitive marker, with 61% of patients showing elevated levels regardless of the stage of disease. HMW-MAA was elevated in 29%. Elevated CYT-MAA was also significantly correlated with poorer clinical outcome. By multivariate analysis (adjusting for stage and age), patients who had elevated CYT-MAA were 81% more likely to recur than patients with undetectable levels (hazard ratio = 1.81, 95% CI = [1.07, 3.06], p-value = 0.03). Elevated levels of HMW-MAA did not correlate with poor prognosis. These results suggest that both CYT-MAA and HMW-MAA are serum markers for residual melanoma in patients with resected disease. Furthermore, CYT-MAA appears to be a prognostic marker of clinical outcome in melanoma vaccine-treated patients.

Key words: biomarkers/cancer vaccine/tumor antigens
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Measurement of tumor markers in serum is increasingly being used in the clinical management of several malignancies; the most frequently assessed are serum carcinoembryonic antigen in colorectal cancer (Goldenberg *et al*, 1981) and prostate-specific antigen in prostate cancer (Stamey *et al*, 1987). A number of proteins have also been investigated as markers in the serum of patients with melanoma. Those most studied are S100B (Deichmann *et al*, 1999; Krähn *et al*, 2001; Bottoni *et al*, 2003), LDH (Franzke *et al*, 1998; Deichmann *et al*, 1999; Krähn *et al*, 2001), and melanoma inhibitory activity (Krähn *et al*, 2001; Matsushita *et al*, 2002; Faries *et al*, 2003). Most often, these markers have been investigated in patients with stage IV disease. In addition, there have been few comparisons of their presence in age- and sex-matched normal individuals. There is a need to find serum markers that can serve as indicators in patients with less advanced melanoma and their presence and level must be compared with that in age- and sex-matched controls in order to establish meaningful sensitivity and specificity.

A number of antigens are expressed by both malignant cells and normal tissues but in greater amounts by cancer cells. Cancer cells can also shed some of these antigens (Bystryn *et al*, 1981; Natali *et al*, 1982; Giacomini *et al*, 1984), resulting in their appearance in serum of patients with cancer (Natali *et al*, 1983; Giacomini *et al*, 1984). We have developed sensitive and quantitative assays to measure serum levels for two such antigens. One is the high-molecular-weight melanoma-associated antigen (HMW-MAA), a membrane proteoglycan consisting of two subunits of 280 and 440 kDa, expressed in a high proportion of melanomas. Its expression correlates with poorer clinical outcome in acral lentiginous melanoma (Wilson *et al*, 1981; Giacomini *et al*, 1984; Kageshita *et al*, 1993). The other one is the cytoplasmic melanoma-associated antigen (CYT-MAA), which is composed of four polypeptides of 94, 75, 70, and 25 kDa. It is expressed in the cytoplasm of normal cells but to a higher degree in melanoma and other malignant cell types. Both antigens are shed by melanoma cells in culture. Previous studies have found both HMW-MAA and CYT-MAA in the sera of normal individuals and patients with melanoma (Natali *et al*, 1983; Giacomini *et al*, 1984).

Here we report the results of a pilot study in which we compared the measurements of CYT-MAA and HMW-MAA in the sera of patients with different stages of melanoma with normal age- and sex-matched controls. Furthermore, we investigated whether the presence of either of these antigens was associated with clinical outcome.

Abbreviations: CYT-MAA, cytoplasmic melanoma-associated antigen; HMW-MAA, high-molecular-weight melanoma-associated antigen; mAb, monoclonal antibody; ROC, receiver operating characteristic

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Results

Patient characteristics The study was conducted on sera collected from 117 randomly selected patients, 68% males and 32% females, with malignant melanoma who were enrolled into melanoma vaccine trials. The sera were collected within 2 wk before the initiation of treatment. The median age (range) was 55 (16–77) y. Thirty had resected American Joint Committee on Cancer (AJCC) stage IIb or IIIa, 30 had resected stage IIc, IIIb, or IIIc, 30 had resected stage IV, and 27 had measurable stage IV melanoma. Sixty-two control sera were sex matched to the melanoma patients, and matched in 5-y intervals by age.

Linearity and reproducibility of double-sandwich ELISA for CYT-MAA and HMW-MAA To determine the linearity of the standard curves, serial dilutions of a standard lot of melanoma cell lysate known to express CYT-MAA and HMW-MAA were assayed in triplicate on 3 d. Each assay was quantitative, showing a linear relation between the amount of antigen present and the read-out of the assay (data not shown). The correlation coefficients (r) were close to 1.00 for all assays, with r-values ranging from 0.97 to 0.99 for all curves for both antigens. The repeatability or precision with the same operator for replicate patients' samples (three replicates) tested on 4 d had coefficients of variation of less than 10% (7.7% and 4.8% for CYT-MAA and HMW-MAA, respectively). The reproducibility of the assay was determined by repeating the baseline readings of 117 vaccine-treated patients on 2 different days. A regression curve was constructed using the two readings of every patient. The fit of the line was close to 1.00 ($R^2 = 0.993$). The slope was 0.98 and the y-intercept was close to zero (0.184).

Presence and level of CYT-MAA and HMW-MAA in the sera of patients with melanoma and controls As shown in Fig 1 and summarized in Table I, the median serum levels of both antigens were significantly higher in melanoma patients than in control sera. The median level of CYT-MAA was 4.8 U in melanoma patients *versus* 2.6 U in controls ($p < 0.01$), and HMW-MAA was 4.1 *vs* 1.2 U ($p = 0.03$).

To determine the serum antigen level that should be considered elevated, the cutoff levels were calculated for each antigen using receiver operating characteristic (ROC) curves. The optimal level of each antigen that best separated melanoma patients from controls based on the highest point attained on the ROC curve for CYT-MAA was $\geqslant 1.0$ U (ROC curve value = 0.967), and for HMW-MAA was $\geqslant 1.0$ U (ROC curve value = 0.998) (data not shown). Based on these criteria, both antigens were found to be elevated, with significantly higher frequency in the sera of melanoma patients than in those of controls (see Table I). CYT-MAA was elevated in 71 (61%) of melanoma patients *versus* 7 (11%) of controls ($p < 0.01$), and HMW-MAA was elevated in 34 (29%) patients *versus* 2 (3%) of controls ($p < 0.01$). Sera from melanoma patients were 12.1 times more likely to be elevated for CYT-MAA (95% CI = 5.1, 28.9) and 12.3 times for HMW-MAA (95% CI = 2.9, 52.8) compared with controls.

Relation between the stage of disease and serum marker levels As shown in Fig 2, median CYT-MAA levels were equally high in all stages of melanoma. Although patients with stage IV measurable disease had the highest median serum level of 6.8 U, patients in the other three risk groups had only slightly lower median levels ranging from 4.5 to 4.7 U. Likewise, median levels of HMW-MAA were also similar in all stages of disease. Although the serum levels of HMW-MAA were higher in patients with resected stage IV disease than in the other patients, the difference did not reach the level of statistical significance.

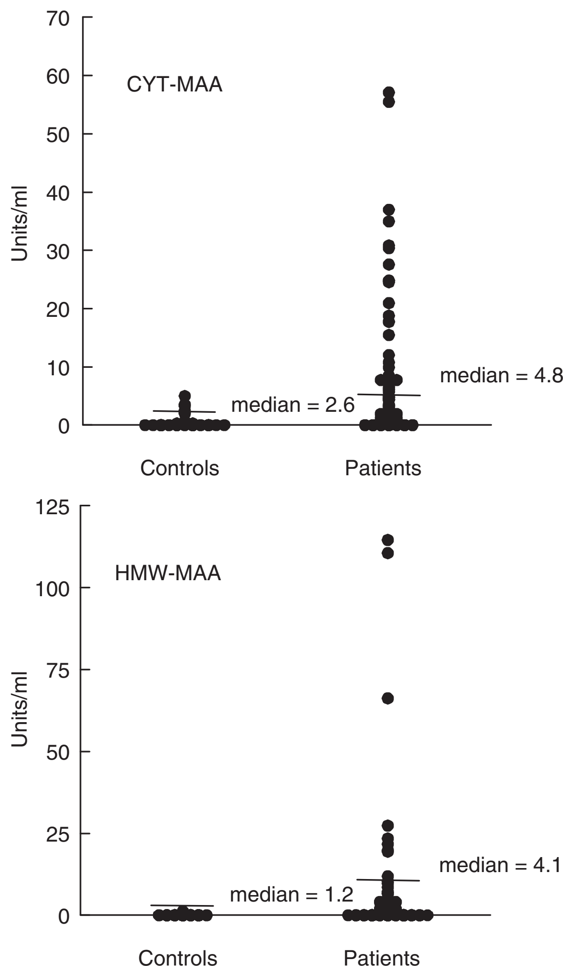

Figure 1
Serum levels (U per mL) of cytoplasmic melanoma-associated antigens (CYT-MAA) and high-molecular-weight melanoma-associated antigen (HMW-MAA) in 117 patients with melanoma and 62 controls. Each point is the median of triplicate samples. All three antigens were significantly higher in sera of patients with melanoma than in controls.

The frequency of patients who had elevated serum levels for both antigens is summarized in Table II. CYT-MAA was found to be the most frequently elevated marker regardless of the disease stage. Sixteen (53%), 20 (67%), 19 (63%), and 16 (59%) of the patients with resected stage IIb or IIIa, resected stage IIc, IIIb, or IIIc, resected stage IV, and measurable stage IV melanoma, respectively, had elevated levels. HMW-MAA was elevated in 7 (23%), 10 (33%), 11 (37%), and 6 (22%) of the patients with resected stage IIb or IIIa, resected stage IIc, IIIb, or IIIc, resected stage IV, and measurable stage IV melanoma, respectively.

Effect of measuring both markers on sensitivity and specificity of detecting a melanoma antigen in serum As

Table I. Median levels of CYT-MAA and HMW-MAA in serum of patients with melanoma and controls without melanoma

Antigen	Patients n (%) (n = 117)	Controls n (%) (n = 62)	OR [95% CI]	p-value
CYT-MAA				
Median serum level	4.8 U per mL	2.6 U per mL		<0.01[a]
% elevated	71 (61%)	7 (11%)	12.1 [5.1, 28.9]	<0.01[b]
HMW-MAA				
Median serum level	4.1 U per mL	1.2 U per mL		= 0.03[a]
% elevated	34 (29%)	2 (3%)	12.3 [2.9, 52.8]	<0.01[b]

[a]Student's *t* test.
[b]χ^2 test.
OR, odds ratio; CYT-MAA, cytoplasmic melanoma-associated antigens; HMW-MAA, high-molecular-weight melanoma-associated antigen.

there was considerable heterogeneity of the presence in the sera of any one melanoma antigen, we examined whether measuring both antigens in the same serum sample could enhance the sensitivity and specificity of the analysis. As shown in Table III, the highest specificity of 97% was observed when HMW-MAA was measured alone. The sensitivity, however, was low—only 29%. Measurement of both antigens simultaneously increased the sensitivity of detecting elevated levels in melanoma patients to 74%, an increase of 13% over measuring CYT-MAA alone, whereas the specificity remained high (86%).

Relation between elevated serum antigen level and clinical outcome Cox proportional hazards models were used to test whether elevation in the level of either marker correlated with recurrence or progression-free survival. Seventy-one out of 117 (61%) of patients had recurrence during follow-up. The median time to recurrence or progression for all patients was 315 d; the median time to recurrence or progression among the 61% of the patients who had an event was 196 d.

A preliminary univariate analysis revealed statistically significant associations for stage, age, and elevated CYT-MAA; the results are shown in Table IV. Patients who had elevated CYT-MAA prior to treatment had an 80% increased risk of progression of melanoma during follow-up relative to patients in whom CYT-MAA was undetectable (hazard ratio = 1.80, 95% CI = [1.07, 3.03], p-value = 0.03).

For the multivariate analyses, the relationship between CYT-MAA or HMW-MAA and recurrence or progression was adjusted for age and stage of disease, including separate staging for patients who had measurable stage IV disease. The relation between CYT-MAA and recurrence or progression remained significant; patients with elevated CYT-MAA were at 81% increased risk of recurrence or progression relative to CYT-MAA-negative patients (hazard ratio = 1.81, 95% CI = [1.07, 3.06], p-value = 0.03). Interactions between elevated marker and stage were not significant (>0.10), indicating that the observed adjusted relationship between CYT-MAA or HMW-MAA and the risk of recurrence or progression did not depend on stage. Thus, CYT-MAA appeared to be an independent predictor of early recurrence or progression. The Kaplan–Meier survival estimates for CYT-MAA are summarized in Fig 3.

Elevated HMW-MAA was not associated with early progression in either univariate or multivariate analyses. Furthermore, the prognostic value of the detection of CYT-MAA in serum of patients with melanoma was not improved by combining the results related to the detection of HMW-

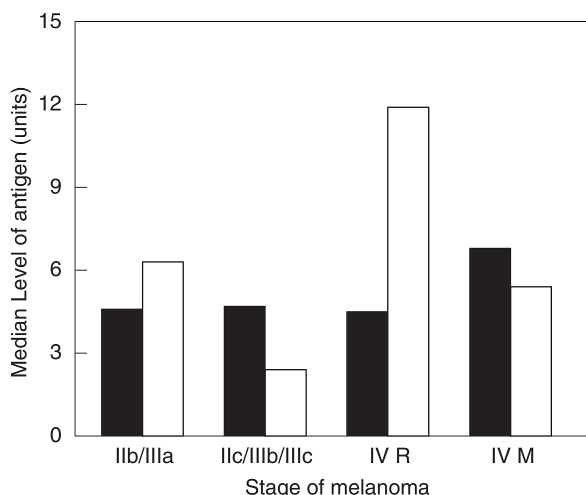

Figure 2
Median serum levels (U per mL) of cytoplasmic melanoma-associated antigens (■), and high-molecular-weight melanoma-associated antigen (□) in different stages of melanoma. The levels of both antigens were equally high in all stages of melanoma.

Table II. Relation between the stage of melanoma and detection of serum CYT-MAA and HMW-MAA

	% positive (n)	
Melanoma stage	CYT-MAA	HMW-MAA
IIb or IIIa (n = 30)	53% (16)	23% (7)
IIc, IIIb, or IIIc (n = 30)	67% (20)	33% (10)
IV resected (n = 30)	63% (19)	37% (11)
IV measurable (n = 27)	59% (16)	22% (6)
All stages (n = 117)	61% (71)	29% (34)
Controls (n = 62)	11% (7)	3% (2)

CYT-MAA, cytoplasmic melanoma-associated antigens; HMW-MAA, high-molecular-weight melanoma-associated antigen.

Table III. Sensitivity and specificity of measuring single markers or multiple markers concurrently

Antigenic marker	Sensitivity (%)[a]	Specificity (%)[b]
CYT-MAA	61	89
HMW-MAA	29	97
CYT-MAA or HMW-MAA	74	86

[a]% of all those with disease who had a positive test result.
[b]% of those without the disease who had a negative test result.
CYT-MAA, cytoplasmic melanoma-associated antigens; HMW-MAA, high-molecular-weight melanoma-associated antigen.

MAA. It should be noted that no patients died prior to a recurrence or progression event. Therefore, competing risks does not pose a challenge in the interpretation of these results.

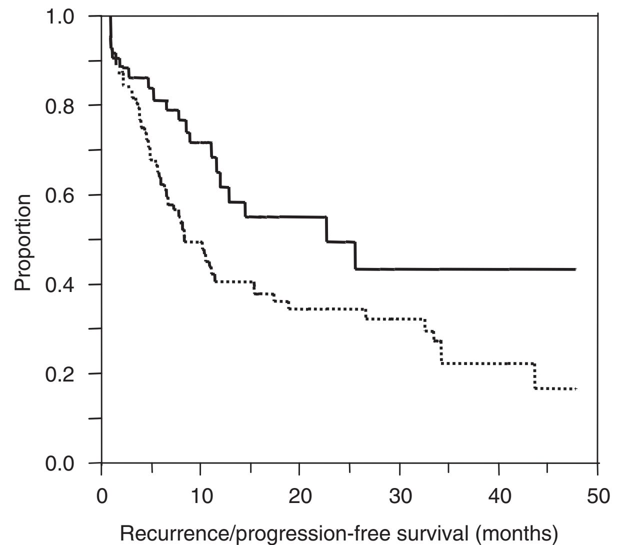

Figure 3
Kaplan–Meier analysis of recurrence or progression-free survival of patients with non-detectable cytoplasmic melanoma-associated antigens (CYT-MAA) (*solid line*) *versus* those with detectable CYT-MAA (*dotted line*). Patients with CYT-MAA were significantly more likely to recur or progress.

Discussion

Both HMW-MAA and CYT-MAA can be present in the sera of healthy donors as well as patients with melanoma. But they are both expressed to a much higher degree in melanoma cells. In one early study, one of us found no difference in levels between normal and patients' sera (Natali *et al*, 1982). One of the differences between the previous study and the present one is that the dilution of serum used in the former study was 1:50. In this study, we found that if sera is diluted 1:1600, there is a clear difference between normal and melanoma patient sera. Therefore, it is important to evaluate a broad range of serum dilutions when setting up assays.

Although CYT-MAA appears to be the most promising marker in our study, to further develop and standardize an assay for this antigen, the cutoff that we established using ROC analysis will have to be confirmed with a second data set with melanoma patients and control sera in future studies. Furthermore, it would be desirable to have a recombinant antigen to be used as a standard for assays. We believe that that CYT-MAA may be the same as the protein cloned and sequenced by Koths *et al* (1993), which is also known as Mac-2 BP, a galactin-binding protein. This protein has been reported to be a potent immune stimulator (Ullrich *et al*, 1994) and associated with many different types of cancer (Grassadonia *et al*, 2004) as well as a marker for melanoma (Cesinaro *et al*, 2002). We plan to confirm that CYT-MAA is the same as Mac-2 BP and produce the recombinant protein in order to confirm the usefulness of this protein as a marker for melanoma and other malignancies in larger prospective studies. Since the percent of patients showing elevated levels of serum CYT-MAA in this study

Table IV. Cox proportional hazards analysis of time to progression or recurrence of melanoma

Model type	Pre-treatment antigen parameter	Hazard ratio [95% CI]	p-value
Univariate			
	CYT-MAA ⩾ 1.0	1.80 [1.07, 3.03]	0.03
	HMW-MAA ⩾ 1.0	0.96 [0.57, 1.62]	0.87
	CYT-MAA or HMW-MAA ⩾ 1.0	1.57 [0.87, 2.82]	0.13
	Stage IIc/IIIb/IIIc resected	2.45 [1.08, 5.57]	0.03
	Stage IV resected	3.39 [1.56, 7.34]	<0.01
	Stage IV measurable	5.33 [2.45, 11.61]	<0.01
	Age category	1.10 [1.00, 1.20]	0.05
	Gender	0.74 [0.44, 1.26]	0.27
Multivariate[a]			
	CYT-MAA ⩾ 1.0	1.81 [1.07, 3.06]	0.03
	HMW-MAA ⩾ 1.0	0.94 [0.55, 1.59]	0.81

[a]Models that included stage of melanoma and patient age at baseline.
CYT-MAA, cytoplasmic melanoma-associated antigens; HMW-MAA, high-molecular-weight melanoma-associated antigen.

was very high for all disease stages, this antigen has great potential as a prognostic marker for melanoma.

Materials and Methods

Patients and controls This study was conducted on the sera of 117 patients with melanoma who were enrolled in clinical trials of a polyvalent, shed antigen, melanoma vaccine. The sera were collected from all patients within 2 wk before the start of treatment. The patients were selected randomly from a group of over 500 vaccine-treated patients who had been stratified into four risk groups based on the stage of their disease according to the 2001 AJCC staging for cutaneous melanoma (Balch *et al*, 2001). Within each strata, 27–30 patients were selected using a random digit table. The patients included 30 with resected stage IIb or IIIa, 30 with resected stage IIc, stage IIIb, or IIIc, 30 with resected stage IV (distant metastasis) and 27 with measurable AJCC stage IV melanoma. The majority of resected patients had their surgery 2–4 mo before the start of the trials. All patients gave informed consent at the time of their vaccine trial for their sera to be used in future studies. All patient studies were conducted using adherence to Helsinki guidelines.

Sera from 58 healthy blood bank donors and four non-melanoma patients were used as controls. The non-melanoma patients were being treated for skin rashes. These sera were frequency matched to melanoma patients for sex and age (in 5 y age groups). All sera were coded and tested without knowledge of the patient's clinical status. The study was approved by the New York University School of Medicine Institutional Review Board of Research Associates.

Vaccine treatment and clinical outcome All patients were treated with the same polyvalent shed antigen melanoma vaccine administered intradermally every 2–3 wk $\times$ 4 monthly $\times$ 3, every 3 mo $\times$ 2, and then every 6 mo for a total of 2–5 y or until disease progression. Patients were monitored every 3 wk during the first 2 mo and then at intervals of 1–3 mo during the first year and every 3 mo thereafter. Laboratory studies (blood count and liver function tests) were performed every 3 mo, chest X-rays every 6 mo, and computer tomography scans of the brain, chest, and abdomen once a year or as clinically indicated.

Monoclonal antibodies (mAb) The mAb 465.125, which recognizes a repeating determinant of CYT-MAA, and the mAb TP41.2 and 763.74, which recognize distinct and spatially distant determinants of HMW-MAA, were developed and characterized as described (Natali *et al*, 1983; Giacomini *et al*, 1984). Because 465.125 recognizes a repeating epitope of CYT-MAA, it can be used in assays for both capture and detection of the antigen. For detection, mAb 465.12 and 763.74 were biotinylated with biotinamidocaproate *N*-hydroxysuccinimide ester (Sigma, St. Louis, Missouri) as described (Heitzmann and Richards, 1974).

Preparation of standard antigen lysate The amount of CYT-MAA and HMW-MAA was calculated by reference to a standard curve generated by preparing serial dilutions of a standard lot of melanoma cell lysate prepared from the melanoma cell line, HT-144 (ATCC, Manassas, Virginia), which expresses both antigens. The lysate was prepared by gently grinding on ice for 20 min, 1×10^8 HT-144 cells resuspended in 0.5% NP-40 in PBS with protease inhibitors in a Tenbroeck tissue grinder. After a further 20 min incubation on ice, the preparation was centrifuged for 15 min at $10{,}000 \times g$. The supernatant was collected, and the protein concentration was determined by the BCA assay (Pierce, Rockford, Illinois).

Assays of serum levels of CYT-MAA and HMW-MAA: The two antigens were measured by double-sandwich ELISA. Plates (Maxisorp, Nunc, Rochester, New York) were coated with 0.01 μg per mL anti-CYT-MAA mAb 465.125 or anti-HMW-MAA mAb TP41.2 and incubated overnight at 4°C. They were then washed 3 $\times$ with 0.05% Tween/PBS and blocked for 1 h with 0.3% Tween/PBS. Sera diluted in blocking buffer 1:1600 for CYT-MAA and 1:50 for HMW-MAA were added and incubated overnight at 4°C. These dilutions were determined in preliminary studies as optimal for yielding readings that were within the quantitative range of each assay. After washing 3 $\times$ as above, biotinylated anti-CYT-MAA mAb 465.125 or anti-HMW-MAA mAb 763.74 was added and incubated for an additional 2 h at 37°C. The plates were washed 3 $\times$ and incubated with horseradish peroxidase–streptavidin conjugate (Sigma) for 1 h, then washed and developed with TMB peroxidase substrate (KPL, Gaithersburg, Maryland). Serum levels of each antigen were calculated from the standard curve and expressed in "units", where 1 U equaled the amount of CYT-MAA and HMW-MAA in 1 μg per mL of the standard cell lysate. Serum from each patient was assayed for both antigens on the same day. All assays were performed in triplicate, and the median value for each of the triplicates was used for statistical analysis. A standard curve was included on every assay plate.

Statistical analysis To determine the cutoff level of each antigen to be considered elevated, ROC curves were constructed (Zweig and Campbell, 1993) using readings for melanoma and control sera. The number and percent of patients and controls above or below the cutoff was determined. The median levels for cases and controls were compared by Student's *t* test. The frequencies of elevated sera in melanoma patients and controls were compared by the χ^2 test. Odds ratios were calculated for the odds of melanoma cases being above the cutoff point for each marker relative to the controls. Sensitivity was calculated from true positives/(true positives + false negatives), and specificity from true negatives/(true negatives + false positives). Among the patients with melanoma, Cox univariate and multivariate proportional hazards models were constructed to evaluate the association between the presence of elevated marker and other risk factors (stage, age, and sex) and time to recurrence or progression of melanoma. Recurrence or progression-free survival was calculated from the start of vaccine treatment to the time of first evidence of recurrence or disease progression.

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References

Balch CM, Buzaid AC, Soong S-J, *et al*: Final version of the American Joint Committee on cancer staging system for cutaneous melanoma. J Clin Oncol 19:3635–3648, 2001

Bottoni U, Izzo P, Richetta A, *et al*: S100 serum level: A tumor marker for metastatic melanoma. Melanoma Res 13:427–429, 2003

Bystryn J-C, Tedholm CA, Heaney-Kieras J: Release of surface macromolecules by human melanoma and normal cells. Cancer Res 41:910–914, 1981

Cesinaro AM, Natoli C, Grassadonia A, Tinari N, Iacobelli S, Trentini GP: Expression of the 90K tumor-associated protein in benign and malignant melanocytic lesions. J Invest Dermatol 119:187–190, 2002

Deichmann M, Brenner A, Bock M, Jäckel A, Uhl K, Waldmann V, Näher H: S100-beta, melanoma-inhibiting activity, and lactose dehydrogenase discrim-

inate progressive from nonprogressive American Joint Committee on cancer stage IV melanoma. J Clin Oncol 17:1891–1896, 1999

Faries MB, Gupta RK, Ye X, Hsueh EC, Morton DL: Melanoma-inhibiting activity assay predicts survival in patients receiving a therapeutic cancer vaccine after complete resection of American Joint Committee on Cancer stage III melanoma. Ann Surg Oncol 11:85–93, 2003

Franzke A, Probst-Kepper M, Buer J, *et al*: Elevated pretreatment serum levels of soluble vascular cell adhesion molecule 1 and lactate dehydrogenase as predictors of survival in cutaneous metastatic malignant melanoma. Br J Cancer 78:40–45, 1998

Giacomini P, Veglia F, Cordiali Fei P, Rehle T, Natali PG, Ferrone S: Level of membrane-bound high-molecular-weight melanoma-associated antigen and a cytoplasmic melanoma-associated antigen in surgically removed tissues and sera from patients with melanoma. Cancer Res 44:1281–1287, 1984

Goldenberg DM, Neville AM, Carter AC, *et al*: CEA (carcinoembrionic antigen): Its role as a marker in the management of cancer. J Cancer Res Clin Oncol 101:239–242, 1981

Grassadonia A, Tinary N, Iurisci I, *et al*: 90K (Mac-2 BP) and galectins in tumor progression and metastasis. Glycoconjugate J 19:551–556, 2004

Heitzmann H, Richards FM: Use of the avidin–biotin complex for specific staining of biological membranes in electron microscopy. Proc Natl Acad Sci USA 71:3537–3541, 1974

Kageshita T, Kuriya N, Ono T, Horikoshi T, Takahashi M: Association of high molecular weight melanoma-associated antigen expression in primary acral lentiginous melanoma lesions with poor prognosis. Cancer Res 53:2830–2833, 1993

Koths K, Taylor E, Halenbeck R, Casipit C, Wang A: Cloning and characterization of a human Mac-2 binding protein, a new member of the superfamily defined by the macrophage scavenger receptor cysteine-rich domain. J Biol Chem 268:14245–14249, 1993

Krähn G, Kaskel P, Sander S, *et al*: S100B is a more reliable tumor marker in peripheral blood for patients with newly occurred melanoma metastasis compared with MIA, albumin, and lactate-dehydrogenase. Anticancer Res 21:1311–1316, 2001

Matsushita Y, Hatta N, Wakamatsu K, Takehara K, Ito S, Takata M: Melanoma inhibitory activity (MIA) as a serum marker for early detection of post-surgical relapse in melanoma patients: Comparison with 5-S-cysteinyldopa. Melanoma Res 12:319–323, 2002

Natali PG, Giacomini P, Russo C, Steinbach G, Fenoglio C, Ferrone S: Antigenic profile of human melanoma cells: Analysis with monoclonal antibodies to histocompatibility antigens and to melanoma-associated antigens. J Cutaneous Pathol 10:225–237, 1983

Natali PG, Wilson BS, Imai K, Bigotti A, Ferrone S: Tissue distribution, molecular profile, and shedding of a cytoplasmic antigen identified by the monoclonal antibody 465.12S to human melanoma cells. Cancer Res 42: 583–589, 1982

Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E: Prostate specific-antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 317:909–916, 1987

Ullrich A, Sures I, D'Egidio M, *et al*: The secreted tumor-associated antigen 90K is a potent immune stimulator. J Biol Chem 269:18401–18407, 1994

Wilson BS, Imai K, Natali PG, Ferrone S: Distribution and molecular characterization of a cell-surface and a cytoplasmic antigen detectable in human melanoma cells with monoclonal antibodies. Int J Cancer 28:293–300, 1981

Zweig MH, Campbell G: Receiver operator characteristic (ROC) plots; a fundamental evaluation tool in clinical medicine. Clin Chem 39:561–577, 1993