Title

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Journal

Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 31(33)

ISSN

0732-183X

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Publication Date

2013-11-01

DOI

10.1200/jco.2013.51.3002

VOLUME 31 · NUMBER 33 · NOVEMBER 20 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Tumor-Infiltrating Lymphocyte Grade in Primary Melanomas Is Independently Associated With Melanoma-Specific Survival in the Population-Based Genes, Environment and Melanoma Study

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Published online ahead of print at www.jco.org on October 14, 2013.

Written on behalf of the Genes, Environment and Melanoma (GEM) Study Group.

Supported by Grants No. R01CA112243, R01CA112524, R01CA112243-05S1, R01CA112524-05S2, CA098438, U01CA83180, and P30CA016086 from the National Cancer Institute; the University of Sydney Medical Foundation Program (B.K.A.); and by the Michael Smith Foundation for Health Research Infrastructure Award (R.P.G.).

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/13/3133w-4252w/$20.00

DOI: 10.1200/JCO.2013.51.3002

ABSTRACT

Purpose
Although most hospital-based studies suggest more favorable survival with tumor-infiltrating lymphocytes (TILs) present in primary melanomas, it is uncertain whether TILs provide prognostic information beyond existing melanoma staging definitions. We addressed the issue in an international population-based study of patients with single and multiple primary melanomas.

Patients and Methods
On the basis of the Genes, Environment and Melanoma (GEM) study, we conducted follow-up of 2,845 patients diagnosed from 1998 to 2003 with 3,330 invasive primary melanomas centrally reviewed for TIL grade (absent, nonbrisk, or brisk). The odds of TIL grades associated with clinicopathologic features and survival by TIL grade were examined.

Results
Independent predictors ($P < .05$) for nonbrisk TIL grade were site, histologic subtype, and Breslow thickness, and for brisk TIL grade, they were age, site, Breslow thickness, and radial growth phase. Nonbrisk and brisk TIL grades were each associated with lower American Joint Committee on Cancer (AJCC) tumor stage compared with TIL absence ($P_{trend} < .001$). Death as a result of melanoma was 30% less with nonbrisk TIL grade (hazard ratio [HR], 0.7; 95% CI, 0.5 to 1.0) and 50% less with brisk TIL grade (HR, 0.5; 95% CI, 0.3 to 0.9) relative to TIL absence, adjusted for age, sex, site, and AJCC tumor stage.

Conclusion
At the population level, higher TIL grade of primary melanoma is associated with a lower risk of death as a result of melanoma independently of tumor characteristics currently used for AJCC tumor stage. We conclude that TIL grade deserves further prospective investigation to determine whether it should be included in future AJCC staging revisions.

J Clin Oncol 31:4252-4259. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Classification of melanoma stage is based on pathology features of the primary tumor and spread of disease at the time of diagnosis. Stage is a major contributor to treatment decision making. The primary tumor characteristics used in the most recent version of the AJCC/UICC-TNM staging system (American Joint Committee on Cancer/International Union Against Cancer–Tumor-Node-Metastasis) for melanoma are Breslow thickness, ulceration, and mitoses.[1] Presence of tumor-infiltrating lymphocytes (TILs) in the primary melanoma is not currently included in AJCC tumor staging. TILs are lymphocytes that infiltrate tumors and disrupt the tumor cells, and they are scored as absent, nonbrisk, or brisk.[2-4] Although several hospital- or clinic-based studies[3,5-9] indicate that TILs in primary melanomas predict better patient survival, it is uncertain whether TILs add information to present melanoma staging.

A higher TIL grade has been found to be more common in younger patients[10] and thinner melanomas,[2,5,8,10,11] but less common with higher mitotic rate or ulceration,[8,9] although others found no correlation with mitoses or ulceration.[11] One population-based study of primary melanomas reported no melanoma-specific survival advantage for

Table 1. Characteristics of 3,330 Primary Invasive Cutaneous Melanomas Centrally Scored for TIL Grade From 2,845 Patients

Characteristic	All SPMs and MPMs (n = 3,330)		SPMs (n = 1,919)		Index MPMs (n = 673)		Previous MPMs (n = 738)	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	1,940	58	982	51	464	69	494	67
Female	1,390	42	937	49	209	31	244	33
Age at diagnosis, years								
Median	60		55		67		64	
IQR	48-71		44-68		57-75		53-72	
TIL grade*								
Absent	696	21	424	22	135	20	137	19
Nonbrisk	2,124	64	1,227	64	424	63	473	64
Brisk	510	15	268	14	114	17	128	17

NOTE. Patients who had TIL grades scored in their melanoma(s) were included.
Abbreviations: IQR, interquartile range; MPM, multiple primary melanoma; SPM, single primary melanoma; TIL, tumor-infiltrating lymphocyte.
*TIL grades were scored on standardized review of slides stained with hematoxylin and eosin.

the presence of TILs.[12] Several hospital- or clinic-based studies found TILs associated with better overall[5,7] and melanoma-specific survival[3,6,8,9] adjusted for other prognostic factors; however, all were selected on the basis of referral to a particular center, and most applied additional selection criteria, such as excluding patients with melanomas less than 0.75 mm thick,[6-8] including only patients in clinical trials,[5-7] or including only patients age 65 years or older at diagnosis.[9] The majority included only vertical growth phase melanomas.[3,5-7,9-11]

We examined TIL grade in the large, population-based Genes, Environment and Melanoma (GEM) study of patients with single primary melanomas (SPMs) and multiple primary melanomas (MPMs). GEM study patients were diagnosed from 1998 to 2003 and had centralized review of histopathology characteristics of their melanomas, including TIL grade, and follow-up for survival.[13-15] The GEM study has allowed us to characterize more clearly the pathology features and AJCC tumor staging associated with TIL grades and to compare survival in patients whose tumors had absent, nonbrisk, or brisk TIL grades.

PATIENTS AND METHODS

Study Population

The GEM study included patients from Australia, Italy, Canada, and the United States who had single and multiple primary cutaneous melanomas.[13-15] Patients with an SPM were diagnosed in 2000 and those with MPMs were diagnosed with a second or higher-order invasive or in situ melanoma from 1998 to 2003 (these were the index melanomas); MPMs were over-represented by design for purposes other than this analysis. In addition to the index melanomas, we searched for the previous (usually the first) melanoma for patients with MPMs in local cancer registry records. The institutional review boards at the coordinating center, Memorial Sloan-Kettering Cancer Center, and at each participating institution approved the study protocol. Physician approval was obtained before contacting eligible participants, and all study participants provided informed consent for obtaining diagnostic slides of their index and previous melanoma(s) (for patients with MPMs) for centralized review.

In GEM, there were 3,578 participants with a total of 4,784 primary melanomas. In situ melanomas were eligible as index MPMs when the patient had a previous invasive melanoma, but were excluded (n = 302) from this study because we sought to investigate TILs in relationship to the clinicopathologic features of and survival from invasive melanomas. This analysis included only primary invasive melanomas centrally scored for TIL grade by study dermatopathologists. They comprised 1,919 index SPMs (81% of 2,372), 673 index MPMs (74% of 904), and 738 previous MPMs (61% of 1,206)—3,330 melanomas in all (74% of 4,482) diagnosed in 2,845 (80% of 3,578) GEM participants (Table 1). The 673 index MPMs and 738 previous MPMs had occurred in 926 index patients with MPMs (77% of 1,206): 188 with pathology reviewed for only the index MPM, 253 for only the previous MPM, and 485 for both.

Pathology Review

Patients' age and sex and body sites of the melanomas were extracted from pathology reports and confirmed during patient interview; histologic subtype and Breslow thickness were also extracted from pathology reports. Standardized pathology review of the melanoma slides recorded histologic subtype, Breslow thickness, mitoses, ulceration, and radial or vertical growth phase. Melanomas were classified according to published histopathology criteria.[16,17] Mitoses were recorded as present or absent.[18]

TIL grade was scored as absent, nonbrisk, or brisk by using a previously defined grading system.[2-4] The reviewers were blind to melanoma outcome. In a test set of 19 sections scored for TIL grade by the three dermatopathologists who reviewed the GEM melanomas, the κ statistic for agreement on nominal response between the pathologists was 0.77, which indicates good agreement.

All data items were available for the T classification describing the state of the primary tumor in the AJCC TNM (tumor, regional nodes, distant metastasis) melanoma staging system[1]; data on regional nodal and distant metastases were not available.

Information about deaths from melanoma or other causes was obtained for all participants from National Death Indexes, cancer registries, and municipal records. Patient follow-up for vital status was complete to the end of 2007 in most centers and to the end of 2008 in British Columbia, Canada, and Torino, Italy.

Statistical Analysis

We examined the associations of clinical and pathologic characteristics with TIL grade for melanomas in an analysis that included both SPMs and MPMs. We used multinomial logistic generalized estimating equation regression models specifying an exchangeable working correlation structure to account for the clustering of melanomas for patients with MPMs. Robust variance was used to obtain the CIs. TIL grade was treated as a nominal response variable, and brisk and nonbrisk were simultaneously compared with absent TIL grade. All models included study center and lesion status (SPM, index MPM, or previous MPM), the design variables. Statistical significance was assessed by using Wald tests. We also report results from similar models examining the association of TIL grade with AJCC tumor

stage. Linear trend was tested by using the Wald statistic when tumor stage was treated as a single ordinal variable. Statistical analysis was performed by using R software (version 2.15.0; R Development Core Team, Vienna, Austria) with package multgee.

The association of TIL grade with survival was examined in all patients, including those with SPMs and MPMs. For patients with MPMs with review data for both the index and a previous MPM, we used the pathology characteristics of the tumor with the greatest Breslow thickness in the analysis. When thickness was the same for both melanomas, we used the characteristics of the index MPM, and if one MPM had thickness missing, the pathology characteristics of the other were used. Since the parent study involved population-based ascertainment of incident SPMs and MPMs, survival time was accumulated from the diagnosis date of the index lesion, whether SPM or MPM. The end point was date of death or the end of complete follow-up (censored patients). For melanoma-specific survival, patients were censored at the time of death as a result of any cause other than melanoma.

Survival curves by TIL grade were constructed by using the Kaplan-Meier method and compared by using a log-rank test. Hazard ratios (HRs) and 95% CIs for melanoma-specific survival by TIL grade were estimated in Cox regression models. For 96 patients enrolled as patients with SPMs who experienced a subsequent melanoma during the period of participant recruitment, a time-dependent covariate for MPM status was included in the Cox models at the date of diagnosis of the subsequent melanoma. Linear trend was tested by using the Wald statistic in which TIL grade was treated as a single ordinal variable. An initial Cox model was adjusted for study center and whether SPM or MPM, and another model also included age, sex, site, and tumor stage. Scalp/neck and face/ears were included as separate covariates because previous studies found that patients with scalp/neck melanomas had poorer survival than patients with melanoma of other sites, including extremities, trunk, face, and ears.[19-21] The likelihood ratio test with an a priori alpha of .2[22] was used to test interactions, comparing a model with main effects to a model with main effects and interaction terms. Reanalysis was performed including only melanomas in vertical growth phase and, separately, including only T1b or higher-stage melanomas. A separate fully adjusted Cox model for overall survival by TIL grade is also presented in Table 2.

Tests based on Schoenfeld residuals and graphical methods using Kaplan-Meier curves showed no evidence that proportional hazards assumptions were violated for TIL grade. All significance tests were two-sided. SAS version 9.3 (SAS Institute, Cary, NC) was used for all survival analyses except for Kaplan-Meier curves and likelihood ratio test, which were implemented in STATA/IC 12.1 (STATA, College Station, TX).

RESULTS

Clinicopathologic Features

The patients' age, sex and TIL frequencies for the 3,330 melanomas scored for TIL grade are shown in Table 1. TIL grade was classified as absent in 21%, nonbrisk in 64%, and brisk in 15% of melanomas.

We examined clinicopathologic characteristics in relationship to TIL grade, simultaneously comparing nonbrisk and brisk to absent TILs for 3,207 SPMs and MPMs with complete data for all variables in Appendix Table A1 (online only) and Table 2. Nonbrisk and brisk TIL grades were each positively associated ($P < .05$) with trunk/pelvis or upper extremities, superficial spreading or lentigo maligna subtype, thinner lesions, and absent mitoses, but not sex, adjusted for the design variables of study center and lesion status (SPM, index MPM, or previous MPM; Appendix Table A1). Brisk TIL grade, but not nonbrisk grade, was also positively associated (each $P < .05$) with younger age, absent ulceration, and radial growth adjusted for design variables.

When all variables were included in the model (Table 2), independent predictors for nonbrisk TIL grade were trunk/pelvis or upper extremity location, histologic subtypes other than unclassified/other, and thinner lesions (each $P < .05$). Independent predictors for brisk TIL grade were younger age, trunk/pelvis or upper extremity location, thinner lesions, and radial growth phase (each $P < .05$). Breslow thickness in the full model accounted for the effects of mitoses for nonbrisk and brisk TIL grades and also ulceration for brisk TIL grade. Age and Breslow thickness accounted for the effects of histologic subtype for brisk TIL grade.

The relationship between TIL grade and individual AJCC tumor stage categories was also examined (Table 3), adjusting for factors known to be important for survival (age, sex, anatomic site) and the design variables. Nonbrisk TIL grade was generally more likely among lower-stage tumors, with odds ratios (ORs) of 1.1 for T1b and 1.4 for T2b but ORs of 0.8 for T2a and T3b and 0.6 for T3a, T4a, and T4b relative to T1a tumors ($P_{trend} = .001$). Brisk TIL grade was also more likely among lower-stage tumors, with ORs of 0.9 for T1b, 0.5 for T2a and T2b, 0.3 for T3a, 0.2 for T3b, and 0.1 for T4a ($P_{trend} < .001$); no T4b melanomas had brisk TILs.

Melanoma-Specific Survival

There were 208 melanoma deaths in 2,728 GEM patients with complete AJCC tumor stage information; the median follow-up time was 7.6 years. The 5-year survival was 93% (95% CI, 91% to 95%) with absent, 94% (95% CI, 93% to 95%) with nonbrisk, and 97% (95% CI, 95% to 99%) with brisk TIL grade (log-rank test $P < .001$; Fig 1). The HRs for melanoma death were 0.7 (95% CI, 0.5 to 0.9) for nonbrisk and 0.3 (95% CI, 0.2 to 0.5) for brisk relative to absent TIL grade in a Cox regression model including only design variables (study center and whether SPM or MPM; $P_{trend} < .001$; not shown in tables). This survival advantage with higher TIL grade persisted with the addition of tumor stage, age, sex, and site to the model (HR, 0.7 for nonbrisk and 0.5 for brisk relative to absent TIL grade; $P_{trend} < .009$; Table 4). No significant interactions were found between TIL grade and age, sex, site or stage in the fully adjusted model. Younger age, location other than the scalp/neck, higher TIL grade, and lower tumor stage each remained significantly associated with survival in the fully adjusted model ($P < .05$), although sex did not ($P = .15$).

The majority of other studies have excluded radial growth phase (RGP) melanomas from analyses examining TIL grades in relationship to survival. Thus, we repeated our analysis with only the 1,741 patients with vertical growth phase melanomas who experienced 187 melanoma deaths, excluding 979 participants with RGP melanomas and eight patients with missing data for growth phase (not shown in tables). Again there was a melanoma survival advantage for nonbrisk (HR, 0.7; 95% CI, 0.5 to 1.0) and brisk (HR, 0.5; 95% CI, 0.3 to 1.0) TIL grade ($P_{trend} = .02$) in a model with age, sex, site, tumor stage, study center, and whether SPM or MPM. Among RGP melanoma participants, there were 21 melanoma-related deaths, too few to conduct a separate survival analysis; 2% (n = 4) with absent; 3% (n = 14) with nonbrisk, and 1% (n = 3) with brisk TIL grade died from melanoma.

Because clinical decisions typically depend on tumor staging,[23] we also performed an analysis limited to the 1,423 patients with T1b or higher tumor stage who also experienced 187 melanoma deaths, excluding 1,305 patients with T1a melanomas who had 21 melanoma-related deaths (not shown in tables). Among patients with T1b or higher tumor stage, there remained a survival advantage for nonbrisk (HR, 0.8; 95% 0.6 to 1.1) and brisk (HR, 0.4; 95% CI 0.2 to 0.9) TIL

Table 2. Relationship Between TIL Grade and Clinical and Pathologic Features for 3,307 Primary Invasive Melanomas From 2,827 Patients

Characteristic	TIL Grade						Compared With Absent TIL Grade					
	Absent (n = 690)		Nonbrisk (n = 2,108)		Brisk (n = 509)		Fully Adjusted Nonbrisk TIL Grade*			Fully Adjusted Brisk TIL Grade*		
	No.	%	No.	%	No.	%	OR	95% CI	P†	OR	95% CI	P†
Sex									.41			.38
Male	381	55	1,233	58	316	62	Reference	—		Reference	—	
Female	309	45	875	42	193	38	0.9	0.8 to 1.1		0.9	0.7 to 1.2	
Age at diagnosis, years									.47			.009
< 50	183	27	601	29	144	28	Reference	—		Reference	—	
50-69	293	42	878	42	241	47	0.9	0.7 to 1.1		0.8	0.6 to 1.1	
> 70	214	31	629	30	124	24	0.9	0.7 to 1.1		0.6	0.4 to 0.8	
Anatomic site									.02			< .001
Trunk/pelvis	257	37	943	45	277	54	Reference	—		Reference	—	
Scalp/neck	57	8	123	6	27	5	0.6	0.4 to 0.9		0.5	0.3 to 0.8	
Face/ears/other‡	92	13	243	12	29	6	0.7	0.5 to 1.0		0.3	0.2 to 0.5	
Upper extremities	117	17	371	18	100	20	0.9	0.7 to 1.2		0.9	0.6 to 1.2	
Lower extremities	167	24	428	20	76	15	0.7	0.6 to 0.9		0.4	0.3 to 0.6	
Histologic subtype									.03			.10
Superficial spreading	433	63	1,466	70	380	75	Reference	—		Reference	—	
Nodular	76	11	193	9	28	6	0.9	0.6 to 1.3		1	0.6 to 1.8	
Lentigo maligna	89	13	286	14	76	15	1.1	0.8 to 1.4		1.4	0.9 to 2.0	
Unclassified/other§	92	13	163	8	25	5	0.6	0.5 to 0.9		0.6	0.4 to 1.0	
Breslow thickness, mm									.04			.003
0.01-1.00	403	58	1,400	66	407	80	Reference	—		Reference	—	
1.01-2.00	151	22	407	19	74	15	0.8	0.6 to 1.0		0.7	0.5 to 1.1	
2.01-4.00	90	13	206	10	26	5	0.6	0.4 to 0.9		0.5	0.3 to 0.9	
> 4.00	46	7	95	5	2	0	0.6	0.4 to 1.0		0.1	0.0 to 0.4	
Ulceration									.05			.62
Absent	623	90	1,910	91	492	97	Reference	—		Reference	—	
Present	67	10	198	9	17	3	1.4	1.0 to 1.9		0.9	0.5 to 1.6	
Mitoses									.24			.50
Absent	367	53	1,240	59	367	72	Reference	—		Reference	—	
Present	323	47	868	41	142	28	0.9	0.7 to 1.1		0.9	0.6 to 1.3	
Growth phase									.11			.009
Radial growth phase	255	37	807	38	321	63	Reference	—		Reference	—	
Vertical growth phase	435	63	1,301	62	188	37	1.2	1.0 to 1.6		0.6	0.5 to 0.9	

NOTE. Invasive single melanomas and multiple melanomas (index and previous) were included. Melanomas with one or more data points missing for Breslow thickness (n = 3), ulceration (n = 11), mitoses (n = 1), and growth phase (n = 8) were excluded. We used multinomial logistic generalized estimating equation regression models specifying an exchangeable working correlation structure to account for the clustering of melanomas for patients with multiple primary melanomas (MPMs). TIL grade was treated as a nominal response variable, and the brisk and nonbrisk TIL grades were simultaneously compared with the reference (absent TIL grade).

Abbreviations: OR, odds ratio; TIL, tumor-infiltrating lymphocyte.

*Included all variables in the table and adjusted for study center and lesion status (single primary melanoma, index MPM, or previous MPM).

†*P* values were calculated in the generalized estimating equation models.

‡Other includes melanomas on the head that are not otherwise specified.

§Other includes spindle cell, nevoid, and Spitzoid melanomas.

grade (P_{trend} = .01), adjusting for age, sex, site, tumor stage, study center, and whether SPM or MPM. Among patients with T1a melanoma, 2% (n = 5) with absent, 1% (n = 12) with nonbrisk, and 2% (n = 4) with brisk TIL grade died from melanoma.

Overall Survival

Higher TIL grade was also associated with a lower risk of overall death in a fully adjusted Cox model (P_{trend} = .009; Appendix Table A2, online only).

DISCUSSION

Melanoma location on the trunk and upper extremities and thinner melanomas were each independently associated with nonbrisk and brisk TIL grade in melanomas in the GEM study. Histologic subtypes other than unclassified/other were independently associated with nonbrisk TIL grade, and younger age and radial growth were independently associated with brisk TIL grade. Nonbrisk and brisk TIL grades were each more frequent among melanomas diagnosed with lower AJCC tumor stage. Melanoma-specific fatality was 30% lower when nonbrisk and 50% lower when brisk TIL grade was present after adjustment for age, sex, site, tumor stage, center, and whether SPM or MPM. Independent predictors for better melanoma-specific survival were higher TIL grade, younger age, location other than scalp/neck, and lower tumor stage; survival was the same in men and women in the adjusted model.

The association of brisk TIL grade with younger age in GEM is in concordance with Mandala et al[10] and could be a result of innate

Table 3. Relationship Between TIL Grade and AJCC Tumor Stage for 3,317 Primary Invasive Melanomas From 2,836 Patients

	TIL Grade						Compared With Absent TIL Grade					
	Absent (n = 694)		Nonbrisk (n = 2,114)		Brisk (n = 509)		Adjusted Nonbrisk TIL Grade†			Adjusted Brisk TIL Grade†		
AJCC Tumor Stage*	No.	%	No.	%	No.	%	OR	95% CI	P_{trend}‡	OR	95% CI	P_{trend}‡
T1a	324	47	1,108	52	335	66	Reference	—	.001	Reference	—	< .001
T1b	82	12	295	14	72	14	1.1	0.8 to 1.4		0.9	0.6 to 1.3	
T2a	140	20	351	17	67	13	0.8	0.6 to 1.0		0.5	0.3 to 0.7	
T2b	12	2	58	3	7	1	1.4	0.7 to 2.7		0.5	0.2 to 1.4	
T3a	58	8	128	6	20	4	0.6	0.5 to 0.9		0.3	0.2 to 0.6	
T3b	32	5	79	4	6	1	0.8	0.5 to 1.2		0.2	0.1 to 0.5	
T4a	26	4	51	2	2	0	0.6	0.4 to 1.0		0.1	0.02 to 0.3	
T4b	20	3	44	2	0	—	0.6	0.4 to 1.1		—	—	

NOTE. Invasive single melanomas and multiple melanomas (index and previous) except for 13 melanomas with missing data for stage were included. We used multinomial logistic generalized estimating equation regression models specifying an exchangeable working correlation structure to account for the clustering of melanomas for patients with multiple primary melanomas (MPMs). TIL grade was treated as a nominal response variable, and the brisk and nonbrisk TIL grades were simultaneously compared with the reference (absent TIL grade).

Abbreviations: AJCC, American Joint Committee on Cancer; OR, odds ratio; TIL, tumor-infiltrating lymphocyte.

*T1a, Breslow thickness ≤ 1.0 mm and absence of ulceration or mitoses; T1b, Breslow thickness ≤ 1.0 mm and presence of ulceration or mitoses; T2a, Breslow thickness 1.01-2.0 mm without ulceration; T2b, Breslow thickness 1.01-2.0 mm with ulceration; T3a, Breslow thickness 2.01-4.0 mm without ulceration; T3b, Breslow thickness 2.01-4.0 mm with ulceration; T4a, Breslow thickness > 4.0 mm without ulceration; T4b, Breslow thickness > 4.0 mm with ulceration.

†Adjusted for age (< 50, 50-69, > 70 years), sex, anatomic site (scalp/neck, face/ears/other, trunk/pelvis, upper extremities, lower extremities), study center, and lesion status (single primary melanoma, index MPM, or previous MPM).

‡Linear trend was tested using the Wald statistic when AJCC tumor stage was treated as a single ordinal variable.

immune system defects reported as part of the aging process.[24] In contrast to our finding of more frequent brisk and nonbrisk TILs in trunk/pelvis and upper extremity melanomas, previous studies have reported no association of TIL grade with melanoma site, but these studies aggregated data from different sites.[10,11] We report an independent inverse association of nonbrisk TILs with unclassified/other melanoma that remains to be confirmed.

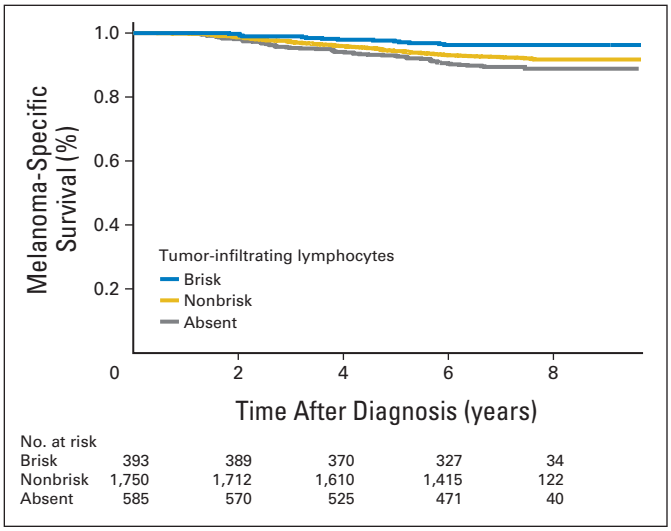

Fig 1. Kaplan-Meier melanoma-specific survival probabilities by tumor-infiltrating lymphocyte grade are shown for patients with melanomas (n = 2,728) with median follow-up of 7.6 years. Patients with a single primary melanoma were diagnosed in 2000 and those with multiple primary melanomas were diagnosed from 1998 to 2003. Patient follow-up for vital status was complete to the end of 2007 in most centers and to the end of 2008 in British Columbia, Canada, and Torino, Italy. Thus, only patients with multiple primary melanomas would have been followed longer than 8 years, which explains the decrease in the number of participants at risk of death after 8 years in the table.

Our study results support previous findings that higher TIL grade is associated with thinner melanomas.[5,8,10,11] We also report an association of brisk TIL grade with radial growth phase independent of thickness. As previously suggested, it is impossible to determine the temporality of these associations by using cross-sectional data available from pathology records[11]; the findings could be due to the release by TILs of factors that inhibit tumor growth or lead to tumor regression or to thicker melanomas eliciting local or systemic immunosuppression.

One population-based study found no correlation between TILs and melanoma-specific survival.[12] However, that study compared TILs simply as present versus absent,[12] in contrast to most other studies, including ours, which report presence of TILs as either nonbrisk or brisk. In concordance with our results, several studies[3,6,8,9] reported that higher TIL grades were independently associated with better melanoma-specific survival. Previous studies have suggested that survival differences based on TIL grade are mainly in melanomas with vertical growth.[25] This also appeared to be the case in our study, and these differences were limited to T1b or higher-stage tumors. Few deaths occurred in RGP only or T1a melanomas, each of which had a high proportion of melanomas with higher TIL grades.

Major advantages of our study are its large sample size, which allowed us to examine TIL grades separately, population-based case ascertainment, comparatively long follow-up period, and standardized pathology review. In addition, our observational period ended before the US Food and Drug Administration approvals of new melanoma therapies vemurafenib and ipilimumab in 2011.[26,27] Because *BRAF*-mutant primary melanomas have been reported to more frequently have tumor lymphocytic infiltrates,[28] any future survival study of TIL grade in relationship to survival could be confounded by *BRAF*-mutant targeted treatment.[27]

Observer error could have led to TIL grade misclassification, but TIL grading has previously been found to have high interobserver

Table 4. HRs for Melanoma-Specific Death According to TIL Grade Among Patients With Primary Melanomas (n = 2,728)

Characteristic	Censored (n = 2,520)		Death As a Result of Melanoma (n = 208)		Fully Adjusted TIL Grade*			
	No.	%	No.	%	HR	95% CI	*P*	P_{trend}
TIL grade								.009
Absent	524	90	61	10	Reference	—		
Nonbrisk	1,617	92	133	8	0.7	0.5 to 1.0		
Brisk	379	96	14	4	0.5	0.3 to 0.9		
Age, years							.006	
Increase in 10-year increments	—		—		1.2	1.0 to 1.3		
Sex							.15	
Male	1,385	90	151	10	Reference	—		
Female	1,135	95	57	5	0.8	0.6 to 1.1		
Anatomic site							.004	
Trunk/pelvis	1,129	93	89	7	Reference	—		
Scalp/neck	127	81	29	19	1.8	1.1 to 2.7		
Face/ears/other†	252	89	32	11	1.1	0.8 to 1.7		
Upper extremities	470	94	29	6	0.7	0.5 to 1.1		
Lower extremities	542	95	29	5	0.7	0.4 to 1.1		
AJCC tumor stage‡							< .001	
T1a	1,284	98	21	2	Reference	—		
T1b	373	97	13	3	2.1	1.1 to 4.2		
T2a	465	90	52	10	6.0	3.6 to 10.0		
T2b	56	80	14	20	13.4	6.8 to 26.4		
T3a	165	82	37	18	10.0	5.8 to 17.2		
T3b	78	72	30	28	17.1	9.7 to 30.3		
T4a	58	76	18	24	12.1	6.3 to 23.1		
T4b	41	64	23	36	22.6	12.3 to 41.5		

NOTE. Of the 2,845 patients included in this study, patients with multiple primary melanomas (MPMs) who were missing TIL grade (n = 109) or AJCC tumor stage (n = 8) for their selected (usually thicker) melanoma were excluded. Patients who entered the study as patients with single primary melanoma who developed a subsequent melanoma were treated as time dependent.

Abbreviations: AJCC, American Joint Committee on Cancer; HR, hazard ratio; TIL, tumor-infiltrating lymphocyte.

*The Cox model included TIL grade (absent, nonbrisk, brisk), age (continuous), sex, anatomic site, AJCC tumor stage, study center, and whether single primary melanoma or MPM.

†Other includes melanomas on the head that are not otherwise specified.

‡T1a, Breslow thickness ≤ 1.0 mm and absence of ulceration or mitoses; T1b, Breslow thickness ≤ 1.0 mm and presence of ulceration or mitoses; T2a, Breslow thickness 1.01-2.0 mm without ulceration; T2b, Breslow thickness 1.01-2.0 mm with ulceration; T3a, Breslow thickness 2.01-4.0 mm without ulceration; T3b, Breslow thickness 2.01-4.0 mm with ulceration; T4a, Breslow thickness > 4.0 mm without ulceration; T4b, Breslow thickness > 4.0 mm with ulceration.

agreement,[29] which we confirmed among our review pathologists. However, we did not examine specific subpopulations of TILs or markers of immune cell activation that appear promising for outcome prediction.[25,30,31] TILs, as we scored them, might have included cell populations with both positive and negative effects on tumor immunity.[25] Further, we did not assess expression of costimulatory molecules or their ligands, such as PD-1/PD-L1, which could allow melanomas with TILs to escape immune destruction.[32]

We found TIL grade to predict survival independently of age, sex, tumor site, and tumor stage, suggesting that TIL grade provides additional information. Survival differences based on TIL grade are mainly for melanomas with T1b or higher tumor stage. The biologic mechanisms underlying our observed associations of nonbrisk and/or brisk TIL grades with age, site, lesion thickness, histologic subtype, and growth phase are currently not well described. The importance of improving our understanding of TILs is underscored by emerging evidence that TILs may predict responses to immunotherapies, including ipilimumab and PD-1/PD-L1 antibodies and cancer vaccines.[32-34] We conclude that TIL grade deserves further prospective investigation to determine whether it should routinely be included in primary melanoma pathology reports and future AJCC staging revisions.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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GLOSSARY TERMS

AJCC/UICC-TNM staging: The TNM Classification of Malignant Tumours (TNM) is a cancer staging system that describes the extent of cancer in a patient's body. * T describes the size of the tumor and whether it has invaded nearby tissue, * N describes regional lymph nodes that are involved, * M describes distant metastasis (spread of cancer from one body part to another). TNM is developed and maintained by the International Union Against Cancer (UICC) to achieve consensus on one globally recognized standard for classifying the extent of spread of cancer. The TNM classification is also used by the American Joint Committee on Cancer (AJCC). In 1987, the UICC and AJCC staging systems were unified into a single staging system. Prognosis of a patient is defined by the TNM Classification.

Appendix

The following are participants in the Genes, Environment and Melanoma (GEM) Study Group: Coordinating Center, Memorial Sloan-Kettering Cancer Center, New York, NY: Marianne Berwick (principal investigator [PI]), currently at the University of New Mexico), Colin B. Begg (co-PI), Irene Orlow (co-investigator), Klaus J. Busam (dermatopathologist), Anne S. Reiner (biostatistician), Pampa Roy (laboratory technician), Ajay Sharma (laboratory technician). University of New Mexico, Albuquerque, NM: Marianne Berwick (PI), Li Luo (biostatistician), Kirsten White (laboratory manager), Susan Paine (data manager). *Study centers included the following:* The University of Sydney and The Cancer Council New South Wales, Sydney, Australia: Bruce K. Armstrong (PI), Anne Kricker (co-PI), Anne Cust (co-investigator); Menzies Research Institute Tasmania, University of Tasmania, Hobart, Australia: Alison Venn (current PI), Terence Dwyer (PI, currently at International Agency for Research on Cancer, Lyon, France), Paul Tucker (dermatopathologist); British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada: Richard P. Gallagher (PI), Donna Kan (coordinator); Cancer Care Ontario, Toronto, Ontario, Canada: Loraine D. Marrett (PI), Elizabeth Theis (co-investigator), Lynn From (dermatopathologist); Center for Prevention in Oncology, Center for Cancer Prevention, Torino, Italy: Roberto Zanetti (PI), Stefano Rosso (co-PI); University of California, Irvine, CA: Hoda Anton-Culver (PI), Argyrios Ziogas (statistician); University of Michigan, Ann Arbor, MI: Stephen B. Gruber (PI, currently at University of Southern California [USC], Los Angeles, CA), Timothy Johnson (director of Melanoma Program), Shu-Chen Huang (co-investigator, joint at USC-University of Michigan); New Jersey Department of Health and Senior Services, Trenton, NJ: Judith Klotz (PI, currently retired), Homer Wilcox (co-PI, currently retired); University of North Carolina, Chapel Hill, NC: Nancy E. Thomas (PI), Robert C. Millikan (previous PI, deceased), David W. Ollila (co-investigator), Kathleen Conway (co-investigator), Pamela A. Groben (dermatopathologist), Sharon N. Edmiston (research analyst), Honglin Hao (laboratory specialist), Eloise Parrish (laboratory specialist), Jill S. Frank (research assistant); University of Pennsylvania, Philadelphia, PA: Timothy R. Rebbeck (PI), Peter A. Kanetsky (co-investigator); ultraviolet data consultants: Julia Lee Taylor and Sasha Madronich, National Centre for Atmospheric Research, Boulder, CO.

Table A1. Relationship Between TIL Grade and Clinical and Pathologic Features for 3,307 Primary Invasive Melanomas from 2,827 Patients

Characteristic	Nonbrisk TIL Grade*			Brisk TIL Grade*		
	OR	95% CI	*P*†	OR	95% CI	*P*†
Sex			.33			.24
Male	Reference	—		Reference	—	
Female	0.9	0.8 to 1.1		0.9	0.7 to 1.1	
Age at diagnosis, years			.29			< .001
< 50	Reference	—		Reference	—	
50-69	0.9	0.7 to 1.1		0.8	0.6 to 1.1	
> 70	0.8	0.6 to 1.1		0.5	0.4 to 0.7	
Anatomic site			.001			< .001
Trunk/pelvis	Reference	—		Reference	—	
Scalp/neck	0.6	0.4 to 0.8		0.4	0.3 to 0.7	
Face/ears/other‡	0.7	0.5 to 0.9		0.3	0.2 to 0.5	
Upper extremities	0.9	0.7 to 1.1		0.8	0.6 to 1.1	
Lower extremities	0.7	0.5 to 0.9		0.4	0.3 to 0.5	
Histologic subtype			< .001			< .001
Superficial spreading	Reference	—		Reference	—	
Nodular	0.8	0.6 to 1.0		0.4	0.2 to 0.6	
Lentigo maligna	0.9	0.7 to 1.2		0.9	0.6 to 1.2	
Unclassified/other§	0.5	0.4 to 0.7		0.3	0.2 to 0.5	
Breslow thickness, mm			< .001			< .001
0.01-1.00	Reference	—		Reference	—	
1.01-2.00	0.8	0.6 to 1.0		0.5	0.4 to 0.7	
2.01-4.00	0.6	0.5 to 0.8		0.3	0.2 to 0.4	
> 4.00	0.6	0.4 to 0.8		0.04	0.01 to 0.17	
Ulceration			.89			< .001
Absent	Reference	—		Reference	—	
Present	1.0	0.7 to 1.3		0.3	0.2 to 0.5	
Mitoses			.01			< .001
Absent	Reference	—		Reference	—	
Present	0.8	0.7 to 0.9		0.4	0.3 to 0.6	
Growth phase			.36			< .001
Radial growth phase	Reference	—		Reference	—	
Vertical growth phase	0.9	0.8 to 1.1		0.4	0.3 to 0.5	

NOTE. Invasive single melanomas and multiple melanomas (index and previous) were included. Melanomas with one or more data points missing for Breslow thickness (n = 3), ulceration (n = 11), mitoses (n = 1), growth phase (n = 8), pigmentation (n = 9), solar elastosis (n = 98), or co-existing nevus (n = 9) were excluded. We used multinomial logistic generalized estimating equation regression models specifying an exchangeable working correlation structure to account for the clustering of melanomas for patients with multiple primary melanomas (MPMs). TIL grade was treated as a nominal response variable, and the brisk and nonbrisk TIL grade were simultaneously compared with the reference (absent TIL grade).

Abbreviations: OR, odds ratio; TIL, tumor-infiltrating lymphocyte.

*Compared with absent TIL grade; adjusted for design variables of study center and lesion status (single primary melanoma, index MPM, or previous MPM) only.

†*P* values were calculated in the generalized estimating equation models.

‡Other includes melanomas on the head that are not otherwise specified.

§Other includes spindle cell, nevoid, and Spitzoid melanomas.

Table A2. HRs for Overall Death According to TIL Grade Among Patients With Primary Melanomas (n = 2,728)

Characteristic	Censored (n = 2,269)		Overall Deaths (n = 459)		Fully Adjusted TIL Grade*			
	No.	%	No.	%	HR	95% CI	*P*	P_{trend}
TIL grade								.009
Absent	470	80	115	20	Reference	—		
Nonbrisk	1,448	83	302	17	0.8	0.7 to 1.1		
Brisk	351	89	42	11	0.6	0.4 to 0.9		
Age, years							< .001	
Increase in 10-year increments	—		—		1.6	1.5 to 1.8		
Sex							.006	
Male	1,196	78	340	22	Reference	—		
Female	1,073	90	119	10	0.7	0.6 to 0.9		
Anatomic site							.002	
Trunk/pelvis	1,005	83	213	17	Reference	—		
Scalp/neck	109	70	47	30	1.3	0.9 to 1.8		
Face/ears/other†	208	73	76	27	1.0	0.7 to 1.3		
Upper extremities	429	86	70	14	0.8	0.6 to 1.0		
Lower extremities	518	91	53	9	0.6	0.4 to 0.8		
AJCC tumor stage‡							< .001	
T1a	1,176	90	129	10	Reference	—		
T1b	351	91	35	9	1.0	0.7 to 1.4		
T2a	419	81	98	19	1.8	1.4 to 2.4		
T2b	45	64	25	36	3.5	2.3 to 5.4		
T3a	141	70	61	30	2.7	2.0 to 3.7		
T3b	61	56	47	44	3.9	2.8 to 5.5		
T4a	48	63	28	37	2.9	1.9 to 4.5		
T4b	28	44	36	56	4.8	3.3 to 7.0		

NOTE. Of the 2,845 patients included in this study, patients with multiple primary melanomas (MPMs) who had missing TIL grade (n = 109) or AJCC tumor stage (n = 8) for their selected (usually thicker) melanoma were excluded. Patients who entered the study as patients with single primary melanomas who developed a subsequent melanoma were treated as time dependent.

Abbreviations: AJCC, American Joint Committee on Cancer; HR, hazard ratio; TIL, tumor-infiltrating lymphocyte.

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†Other includes melanomas on the head that are not otherwise specified.

‡T1a, Breslow thickness ≤ 1.0 mm and absence of ulceration or mitoses; T1b, Breslow thickness ≤ 1.0 mm and presence of ulceration or mitoses; T2a, Breslow thickness 1.01-2.0 mm without ulceration; T2b, Breslow thickness 1.01-2.0 mm with ulceration; T3a, Breslow thickness 2.01-4.0 mm without ulceration; T3b, Breslow thickness 2.01-4.0 mm with ulceration; T4a, Breslow thickness > 4.0 mm without ulceration; T4b, Breslow thickness > 4.0 mm with ulceration.