Prognostic impact of regression in patients with primary cutaneous melanoma >1 mm in thickness

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Background: The impact of histologic regression on sentinel lymph node biopsy (SLNB) status and on clinical outcome is uncertain.

Objective: To investigate whether and to what extent regression <75% is able to predict SLNB status and clinical outcome of patients with melanoma >1-mm thick.

Methods: The study included patients with diagnoses given at 4 centers of the Italian Melanoma Intergroup. Univariate and multivariate Cox proportional hazard models stratified by center were used to analyze the effect of regression on disease-free interval and melanoma-specific survival.

Results: Out of 1182 patients given primary cutaneous melanoma diagnoses during 1998-2015 with a Breslow thickness >1 mm, 954 (304 with and 650 without regression) were included in the analysis. The proportion of patients with a positive SLNB was lower in patients with regression than without (24.4% vs 31.6%, chi-squared test $P = .0368$). At multivariate analysis, no association was detected between regression and disease-free interval (hazard ratio 1.11, 95% confidence interval 0.85-1.46; $P = .4509$) or melanoma-specific survival (hazard ratio 1.05, 95% confidence interval 0.77-1.44; $P = .7600$).

Limitation: Retrospective analysis.

Conclusion: In our series, regression was not an independent prognostic factor in primary cutaneous melanoma patients with Breslow thickness >1 mm whereas it was associated with a lower incidence of SLNB positivity. (J Am Acad Dermatol 2019;80:99-105.)

Key words: cutaneous melanoma; disease-free interval; melanoma-specific survival; outcome; prognosis; regression; sentinel lymph node.

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Melanoma is one of the most immunogenic tumors. Histologic regression[1] is defined as the replacement of tumor cells by lymphocytic inflammation with attenuation of the epidermis and nonlaminated dermal fibrosis with inflammatory cells, melanophagocytosis, and telangiectasia.[2] Histologic regression is a parameter typically included in pathology reports upon diagnosis of a primary cutaneous melanoma (PCM), although this is not specifically requested for current staging according to the American Joint Committee on Cancer classification.

Questions have been raised about its prognostic role, and for many years, divergent conclusions have been reached by several authors, probably due to the lack of agreement on its histopathologic definition.[3] In the past, histologic tumor regression was considered a negative prognostic factor, since it might lead to an underestimation of measurement of tumor thickness. However, other studies have proven that regression is not correlated to an increased risk for nodal metastases.[4-6]

Previous studies have shown a favorable prognostic role of histologic regression in stage I, II, and III melanomas.[7-9] A recent meta-analysis has confirmed its protective role relating to the clinical outcome.[10] Taken together, the studies investigating the prognostic significance of regression have provided controversial findings. Furthermore, in most studies so far reported, the independent role of regression was not adjusted for some of the most important prognostic biomarkers, including mitotic rate and tumor-infiltrating lymphocytes (TILs).

In the current study, we aimed to assess the prognostic role of histologic regression in terms of disease-free interval (DFI) and melanoma-specific survival (MSS) in PCM patients with Breslow thicknesses >1 mm.

CAPSULE SUMMARY

- The prognostic role of regression in melanoma is uncertain
- In our cohort, regression was not an independent prognostic factor in patients with melanoma >1-mm thick, whereas it was associated with a lower incidence of sentinel lymph node positivity.
- Regression does not predict melanoma-specific survival in patients with melanomas >1 mm thick.

METHODS

Approval to conduct this study was obtained from the local ethical committees of the participating centers. The study included all consecutive patients with PCM diagnosed, treated, and followed up prospectively in 4 Italian Melanoma Intergroup centers during 1998-2015. The following histologic criteria were considered in defining histologic regression: replacement of tumor cells by predominantly lymphocytic inflammation or disappearance of melanoma cells in a circumscribed or more diffuse tumor area, attenuation of the epidermis, dermal fibrosis associated with inflammatory cells (mainly lymphocytes), melanophagocytosis, and telangiectasia. These parameters define tumor regression in the CAP-approved protocol[2] and are categorized as not identified; present, involving <75% of lesion; present, involving ≥75% of lesion; and indeterminate. Data collection and inclusion criteria are described in the Supplementary Appendix (available at http://www.jaad.org).

Statistical methods

Continuous variables were summarized with the mean and standard deviation (SD), whereas for categorical variables, the frequency and percentage were provided. The characteristics at diagnosis were compared by using the chi-squared test for categorical variables and the *t* test or analysis of variance test for continuous variables.

DFI was defined as the time from diagnosis to the date of first relapse. MSS was defined as the time from the date of diagnosis to the date of death due to melanoma-related causes. Patients who were not reported as having died from a melanoma-related cause or relapsed were censored at their last available contact date.

The follow-up calculated with the reverse Kaplan-Meier method was summarized as median and interquartile range. Survival curves were estimated with the Kaplan-Meier method and compared by means of the log-rank test. The impact of probable confounders was explored by means of univariate and multivariate Cox proportional hazard models including clinical and biologic features as covariates. All the models were stratified by center to minimize the potential bias. The proportional hazards assumption was assessed for all the endpoints. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated.

Since a significant difference between the median follow-up of patients with and without regression was found (91.8 months for patients with and 69.4 months for patients without regression, log-rank $P = .0408$), a sensitivity analysis was performed setting 72 months as the maximum follow-up for each patient.

Abbreviations used:

CAP: College of American Pathologists
CI: confidence interval
DFI: disease-free interval
HR: hazard ratio
MSS: melanoma-specific survival
PCM: primary cutaneous melanoma
SD: standard deviation
SLN: sentinel lymph node
SLNB: sentinel lymph node biopsy
TIL: tumor-infiltrating lymphocytes

RESULTS

During 1998-2015, a total of 1182 patients were given diagnoses of PCM with Breslow thickness >1 mm with or without tumor regression <75% in 4 Italian Melanoma Intergroup centers. Tumor regression ≥75% according to the CAP definition was present in only ~4% of the cases, and these cases were excluded from the analysis. Among patients with regression <75%, 228 were excluded because complete clinical data were not available; therefore, 954 patients (mean age 58.1 [SD 16.5] years) were included in the final analysis. Overall, 304 (31.9%) and 650 (68.1%) patients did and did not have regression, respectively. A comparison among centers in terms of characteristics at diagnosis is provided in Supplemental Table I (available at http://www.jaad.org).

Patient characteristics at diagnosis according to regression status are summarized in Table I. The mean age was 60.4 (SD 16.0) years and 57.1 (SD 16.6) years for patients with and without regression, respectively (P = .0033). Moreover, the proportion of patients with a positive sentinel lymph node biopsy (SLNB) was lower in patients with regression (24.4% vs 31.6%, P = .0368).

After a median follow-up of 77.0 (interquartile range 48.6-133.0) months, 120 (39.5%) patients with regression and 248 (38.2%) patients without regression relapsed. Moreover, 96 (31.6%) and 181 (27.8%) patients died due to melanoma-related causes in the regression and no regression groups, respectively. The survival curves of DFI and MSS are shown in Figs 1 and 2. The log-rank test found no significant differences between the curves for both DFI (P = .745) and MSS (P = .799). The proportional hazards assumption was satisfied for both DFI and MSS. Univariate and multivariate analysis results are summarized in Table II for the DFI and in Table III for the MSS. No significant effect of regression was detected on DFI (adjusted HR 1.11, 95% CI 0.85-1.46, P = .4509) or MSS (adjusted HR 1.05, 95% CI 0.77-1.44, P = .7600).

At multivariate analysis, the effects of age, Breslow thickness, ulceration, and SLNB were confirmed. Specifically, a 1-year increase in age at diagnosis was associated with an HR of 1.01 (95% CI 1.01-1.02, P = .0008) for DFI and an HR of 1.03 (95% CI 1.02-1.04, P < .0001) for MSS. Compared with a Breslow thickness of ≤2.00, a Breslow thickness of 2.01-4.00 mm was associated with an HR of 1.91 (95% CI 1.38-2.64, P < .0001) for DFI and an HR of 1.79 (95% CI 1.22-2.63, P = .0030) for MSS; a Breslow thickness of 4.01-8.00 mm was associated with an HR of 4.16 (95% CI 2.84-6.10, P < .0001) for DFI and an HR of 3.01 (95% CI 1.92-4.70, P < .0001) for MSS; and a Breslow thickness >8.00 mm was associated with an HR of 4.20 (95% CI 2.52-7.01, P < .0001) for DFI and an HR of 3.24 (95% CI 1.80-5.83, P < .0001) for MSS. Last, ulceration and a positive SLNB were confirmed as risk factors for a shorter DFI (HR for ulceration 1.56, 95% CI 1.19-2.04, P = .0014; HR for positive SLNB 2.46, 95% CI 1.90-3.18, P < .0001) and MSS (HR for ulceration 2.04, 95% CI 1.48-2.81, P < .0001; HR for positive SLNB 2.13, 95% CI 1.57-2.88, P < .0001).

Results of the sensitivity analysis were similar to those obtained, considering the available follow-up data. The median follow-up was 69.4 months for patients without regression and 72.0 months for patients with regression. Relapse was reported for 108 (35.5%) of patients with regression and 226 (34.8%) patients without regression, whereas death from melanoma was reported in 76 (25.0%) and 150 (23.1%) patients in the regression and no regression groups, respectively. The absence of an effect of regression on DFI (log-rank P = .738, adjusted HR 1.11, 95% CI 0.83-1.49, P = .4654, Supplemental Table II; available at http://www.jaad.org) and MSS (log-rank P = .932, adjusted HR 1.01, 95% CI 0.71-1.44, P = .9465, Supplemental Table III; available at http://www.jaad.org) was confirmed.

DISCUSSION

Predicting prognosis on the basis of the histopathologic parameters is crucial to determine the need for further investigation and patient counselling and can be used as a guide to appropriate management. According to CAP[2] and American Joint Committee on Cancer,[11] regression is one of the recommended elements for pathology reporting of PCM and should be included in tumor registries, although whether this histopathologic feature affects SLNB status or clinical outcome is still an open issue. To investigate the clinical impact of histologic regression, an accurate evaluation using standardized CAP criteria is needed.

Table I. Characteristics of melanoma patients with Breslow thickness >1 mm at diagnosis

Characteristic	No regression, N = 650	Regression, N = 604	Overall, N = 954	*t* test or chi-squared *P* value
Center				
Unit of Medical Oncology, Papa Giovanni XXIII Hospital, Bergamo	206 (31.7)	121 (39.8)	327 (34.3)	
Department of Oncology, Santa Croce e Carle Hospital, Cuneo	63 (9.7)	27 (8.9)	90 (9.4)	
Department of Dermatology, University of Florence	106 (16.3)	53 (17.4)	159 (16.7)	
Section of Dermatology, Medical Sciences Department, University of Turin	275 (42.3)	103 (33.9)	378 (39.6)	
Age at diagnosis, y, mean (SD)	57.1 (16.6)	60.4 (16.0)	58.1 (16.5)	.0033
Sex				.4333
Male	348 (53.5)	171 (56.3)	519 (54.4)	
Female	302 (46.5)	133 (43.8)	435 (45.6)	
Breslow thickness, mm, mean (SD)	3.3 (2.5)	3.3 (4.8)	3.3 (3.4)	.8834
Breslow thickness category, mm				.5697
1.01-2.00	278 (42.8)	141 (46.4)	419 (43.9)	
2.01-4.00	210 (32.3)	99 (32.6)	309 (32.4)	
4.01-8.00	127 (19.5)	49 (16.1)	176 (18.4)	
>8.00	35 (5.4)	15 (4.9)	50 (5.2)	
Ulceration				.9285
Present	262 (40.5)	122 (40.8)	384 (40.6)	
Absent	385 (59.5)	177 (59.2)	562 (59.4)	
Missing	3	5	8	
Tumor-infiltrating lymphocytes				.2515
Absent	266 (48.9)	111 (42.9)	377 (46.9)	
Present				
Nonbrisk	210 (38.6)	109 (42.1)	319 (39.7)	
Brisk	68 (12.5)	39 (15.1)	107 (13.3)	
Missing	106	45	151	
Mitotic rate				.0961
$<1/mm^2$	77 (11.9)	48 (15.8)	125 (13.1)	
$\geq 1/mm^2$	571 (88.1)	256 (84.2)	827 (86.9)	
Missing	2	0	2	
Mitotic rate, mean (SD)	6.0 (6.2)	5.3 (5.5)	5.8 (6.0)	.0926
Missing	1	0	1	
Sentinel lymph node biopsy				.0368*
Positive	184 (31.6)	62 (24.4)	246 (29.4)	
1	144 (78.7)	53 (86.9)	197 (80.7)	
2	33 (18.0)	7 (11.5)	40 (16.4)	
3	6 (3.3)	1 (1.6)	7 (2.9)	
Missing	1	1	2	
Negative	399 (68.4)	192 (75.6)	591 (70.6)	
Missing	67	50	117	
No. positive lymph nodes at lymphadenectomy, mean (SD)	0.8 (1.8)	0.7 (1.2)	0.8 (1.6)	.4324
Stage				
I	178 (30.4)	82 (31.4)	260 (30.7)	.3379†
Ib	178 (31.4)	82 (33.3)	260 (32.0)	
Missing	0	0	0	
II	217 (37.1)	107 (41.0)	324 (38.3)	
IIa	104 (18.3)	53 (21.5)	157 (19.3)	
IIb	78 (13.8)	34 (13.8)	112 (13.8)	
IIc	35 (6.2)	18 (7.3)	53 (6.5)	
Missing	0	2	2	

Continued

Table I. Cont'd

Characteristic	No regression, N = 650	Regression, N = 604	Overall, N = 954	*t* test or chi-squared *P* value
III	190 (32.5)	72 (27.6)	262 (31.0)	
IIIa	77 (13.6)	30 (12.2)	107 (13.2)	
IIIb	73 (12.9)	25 (10.2)	98 (12.1)	
IIIc	22 (3.9)	4 (1.6)	26 (3.2)	
Missing	18	13	31	
Missing	65	43	108	

SD, Standard deviation.
**P* value for the comparison of the sentinel lymph node biopsy results, distributions classified as positive and negative.
†*P* value for the stage distribution comparison classified as stage I, stage II, and stage III melanoma according to the American Joint Committee on Cancer version 7.

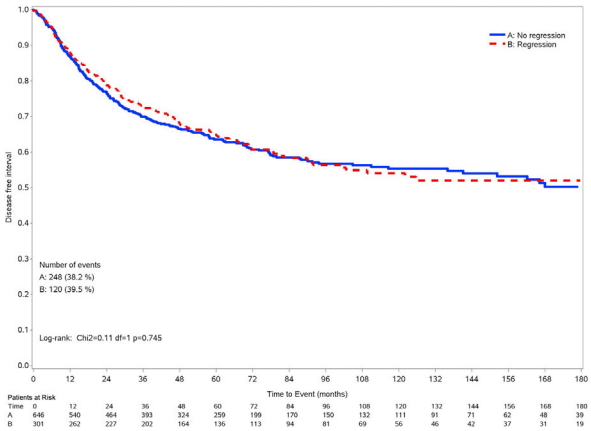

Fig 1. Kaplan-Meier curves of disease-free interval in patients with melanomas >1-mm thick with or without regression.

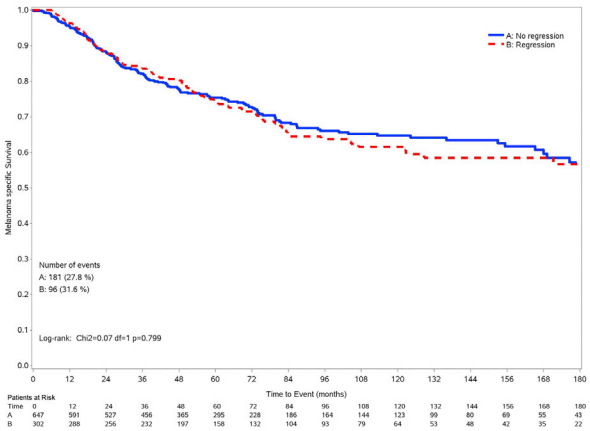

Fig 2. Kaplan-Meier curves of melanoma-specific survival in patients with melanomas >1-mm thick with or without regression.

Herein, we have prospectively retrieved a relatively large number of melanoma patients with a Breslow thickness >1 mm to evaluate the prognostic variables of the primary tumor that could predict DFI and MMS. In our series, the first interesting observation was that regression ≥75% in horizontal extension according to the CAP definition[2] is present in only ~4% of cases; hence, extensive regression is exceedingly rare in thick melanomas. For this reason, we focused our analysis on the prognostic role of tumor regression <75% according to the CAP definition. This analysis is relevant for clinicians because it is unclear whether a portion of the PCM undergoing regression, thus leading to a mere reduction of tumor volume, has prognostic significance. In our study, the prevalence of regression was 31.9% in the entire cohort, in agreement with a recent review that reported regression as a relatively common event in melanoma.[3]

In this study, complete information on stages of regression (early vs intermediate vs late, as previously defined)[1,3] was not available for all cases. All late stage regression cases (characterized by complete absence of melanocytes) fulfill, by definition, the criteria for horizontal extension ≥75% according to CAP. In our experience, the intermediate stage of regression represents by large the most prevalent form of regression that pathologists face in routine activity. It should be noted that the distinction between the active, early (inflammatory) stage of regression and TILs is questionable. By the widely used definition, TILs should be assessed only during the vertical growth phase,[12] and early-stage regression is associated with a mononuclear inflammatory cell infiltrate immediately adjacent to melanocytes and disruption of the dermal and, rarely, the junctional melanocytic component (some loss of vertical and radial growth phases).[3] Much work is still needed to verify reproducibility in the distinction of TILs from early regression due to highly heterogeneous patterns and dynamic cell interaction in the individual patient.

Table II. Disease-free interval among melanoma patients with Breslow thickness >1 mm by using Cox proportional hazard models stratified by center

	Univariate analysis		Multivariate analysis	
Characteristic	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Regression	0.96 (0.77-1.20)	.7489	1.11 (0.85-1.46)	.4509
Female sex	0.85 (0.69-1.04)	.1186	1.00 (0.78-1.28)	.9949
Age at diagnosis, 1-year increase	1.03 (1.02-1.04)	<.0001	1.01 (1.01-1.02)	.0008
Breslow thickness, mm (reference 1.01-2.00)		<.0001		<.0001
2.01-4.00	2.97 (2.25-3.90)	<.0001	1.91 (1.38-2.64)	<.0001
4.01-8.00	6.00 (4.49-8.01)	<.0001	4.16 (2.84-6.10)	<.0001
>8.00	7.28 (4.85-10.92)	<.0001	4.20 (2.52-7.01)	<.0001
Ulceration	2.55(2.07-3.15)	<.0001	1.56 (1.19-2.04)	.0014
TILs (reference absent)		.0185		.5678
Nonbrisk	0.75 (0.59-0.96)	.0217	0.89 (0.67-1.19)	.4258
Brisk	0.64 (0.44-0.94)	.0231	0.81 (0.52-1.26)	.3524
Positive sentinel lymph node biopsy	3.08 (2.43-3.90)	<.0001	2.46 (1.90-3.18)	<.0001
Mitotic rate >1/mm^2	2.55 (1.67-3.89)	<.0001	1.35 (0.81-2.23)	.2472

CI, Confidence interval; *HR*, hazard ratio; *TIL*, tumor-infiltrating lymphocytes.

Table III. Melanoma specific-survival among patients with Breslow thickness >1 mm by using Cox proportional hazard models stratified by center

	Univariate analysis		Multivariate analysis	
Characteristic	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Regression	0.99 (0.77-1.27)	.9512	1.05 (0.77-1.44)	.7600
Female sex	0.79 (0.62-1.01)	.0585	0.94 (0.70-1.26)	.6949
Age at diagnosis, 1-year increase	1.04 (1.03-1.04)	<.0001	1.03 (1.02-1.04)	<.0001
Breslow thickness, mm (reference 1.01-2.00)		<.0001		<.0001
2.01-4.00	3.03 (2.21-4.16)	<.0001	1.79 (1.22-2.63)	.0030
4.01-8.00	5.50 (3.93-7.70)	<.0001	3.01 (1.92-4.70)	<.0001
>8.00	6.82 (4.29-10.82)	<.0001	3.24 (1.80-5.83)	<.0001
Ulceration	2.91 (2.28-3.72)	<.0001	2.04 (1.48-2.81)	<.0001
TILs (reference absent)		.0025		.3092
Nonbrisk	0.70 (0.53-0.92)	.0108	0.92 (0.66-1.29)	.6271
Brisk	0.48 (0.30-0.78)	.0028	0.65 (0.37-1.13)	.1255
Positive sentinel lymph node biopsy	2.90 (2.20-3.83)	<.0001	2.13 (1.57-2.88)	<.0001
Mitotic rate >1/mm^2	2.64 (1.55-4.50)	.0004	1.57 (0.80-3.06)	.1876

CI, Confidence interval; *HR*, hazard ratio; *TIL*, tumor-infiltrating lymphocytes.

The second finding of this study was that, despite the lack of a difference in Breslow thickness between regressed and nonregressed melanomas, the latter had a higher likelihood to be associated with a positive SLNB, thus confirming the results of a previous meta-analysis.[13] Our findings are in agreement with previous reports,[14] although 2 small studies did not find any correlation between regression and SLNB positivity, likely due to the relatively small cohort investigated.[15,16] Although there was an inverse correlation between regression and SLNB positivity in our series, DFI and MSS were similar in regressed and not regressed melanomas after adjusting for all the well-known prognostic markers, including Breslow thickness, ulceration, TILs, mitotic rate, and SLNB status.

The strengths of our study include a) the relatively large cohort of patients evaluated; b) data prospectively collected into specific databases that included information on demographics, previous medical history, diagnosis, surgical procedures, pathologic features, systemic therapies, and follow-up; and c) Italian Melanoma Intergroup pathologists with expertise in melanoma diagnosis who assessed all cases according to the CAP guidelines. However, our study has some limitations, including the retrospective nature of our analysis, which cannot exclude patient selection bias and lack of central pathology review. Nevertheless, despite the fact that in 2 centers (Torino and Bergamo) statistically different regression rates were found, the potential bias due to differences between centers was statistically

controlled for by performing a stratified analysis by center. Moreover, a competing risk analysis was not performed because data about deaths from causes not related to melanoma were not available from all 4 centers.

Further studies should focus on a more precise understanding of the molecular mechanisms of regression at the late phase, as reported here, as well as the immunophenotyping of infiltrating immune cells at the early phase and a biomolecular classification of histologic regression, instead of merely describing the epiphenomenon of these pathobiologic processes. Furthermore, in the era of immune checkpoint therapy, the phenomenon of regression in melanoma is potentially of considerable potential value. In summary, in our cohort of melanoma patients >1 mm, the classical prognostic factors (Breslow thickness, ulceration, age, SLNB status) have an impact on prognosis, while regression still needs to be better defined regarding its role as a prognostic factor.

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SUPPLEMENTARY APPENDIX

METHODS

The study included all consecutive patients with primary cutaneous melanoma diagnosed, treated, and followed up prospectively in 4 Italian Melanoma Intergroup centers (Dermatologic Clinic of the University of Turin, Papa Giovanni XXIII Cancer Center Bergamo, Department of Dermatology of the University of Florence, Santa Croce e Carle Cuneo Hospital, Italy) during 1998-2015.

Data were prospectively collected into specific databases with information on demographics, previous medical history, diagnosis, surgical procedures, pathologic features, systemic therapies, and follow-up. The databases were used to identify all consecutive patients who came to our attention with histologically confirmed melanoma, clinically negative lymph nodes, and no evidence of distant disease.

In general, as for Italian Melanoma Intergroup rules, sentinel lymph node biopsy was offered to patients with lesions that were >1.0 mm in thickness or to patients with vertical growth phase melanoma that were 1.0 mm or less in thickness if adverse histopathologic features of the primary tumor were present, such as ulceration or Clark level IV and V, as previously published.[S1] Age >75 years and clinically significant comorbidities were exclusion criteria for offering sentinel lymph node biopsyies. A total-body computed tomography scan or an ultrasound of the regional basin was performed in all patients to exclude the presence of distant metastases before sentinel lymph node biopsy or locoregional lymphadenectomy procedures. All patients underwent wide local excision with free margins of at least 1 cm for melanomas with a Breslow thickness of 1 mm or less, 1-2 cm for a Breslow thickness of 1-2 mm, and at least 2 cm for a Breslow thickness of ≥2 mm. Patients with positive sentinel lymph nodes were eventually offered radical lymphadenectomy according to the guidelines.[S2] Postoperative follow-up was uniform in participating centers and consisted of physical examination, abdominal ultrasound, chest X-ray, and determinations of lactate dehydrogenase levels for pathologic stage II disease. Further investigations, including computed tomography, magnetic resonance imaging, and positron emission tomography, were also selectively performed to investigate abnormal clinical findings suspicious for metastatic melanoma. Routine surveillance was planned every 4 months for the first 2 years, every 6 months for years 3-5, and annually thereafter. Clinical factors evaluated were the age of the patient, sex, and site of the primary lesion. The anatomical site was classified as axial (comprising truncal, head and neck, volar and subungual lesions) or extremities. Candidate histopathologic prognostic factors were the Breslow thickness (measured in millimeters), ulceration, mitotic rate, tumor-infiltrating lymphocytes, sentinel lymph node and non−sentinel lymph node biopsy status. The following histologic criteria were considered in defining histologic regression: replacement of tumor by predominantly lymphocytic inflammation or disappearance of melanoma cells in a circumscribed or more diffuse tumor area, attenuation of the epidermis, dermal fibrosis associated with inflammatory cells (mainly lymphocytes), melanophagocytosis, and telangiectasia. These parameters define tumor regression in the College of the American Pathologists approved protocol and are categorized as not identified; present, involving <75% of the lesion; present, involving ≥75% of the lesion; and indeterminate.

SUPPLEMENTAL REFERENCES

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Supplemental Table I. Characteristics of melanoma patients with Breslow thickness >1 mm at diagnosis and comparison between centers

Characteristic	Unit of Medical Oncology, Bergamo, N = 327	Santa Croce e Carle hospital, Cuneo, N = 90	University of Florence, N = 159	University of Turin, N = 378	Overall, N = 954	ANOVA or chi-squared *P* value
Regression						.0463
No	206 (63.0)	63 (70.0)	106 (66.7)	275 (72.8)	650 (68.1)	
Follow-up, median (IQR)	163.4 (124.2-194.2)	50.5 (36.4-66.7)	88.2 (59.8-121.0)	53.0 (38.9-72.3)	71.1 (46.5-131.8)	
Yes	121 (37.0)	27 (30.0)	53 (33.3)	103 (27.2)	304 (31.9)	
Follow-up, median (IQR)	166.3 (121.5-184.1)	83.4 (49.0-115.5)	83.8 (54.2-120.6)	63.9 (44.8-89.9)	97.6 (54.8-161.3)	
Year of diagnosis						<.0001
1998-2005	210 (64.2)	6 (6.7)	30 (18.9)	0 (0)	246 (25.8)	
2006-2010	68 (20.8)	23 (25.6)	67 (42.1)	135 (35.7)	293 (30.7)	
2011-2012	23 (7.0)	23 (25.6)	34 (21.4)	114 (30.2)	194 (20.3)	
2012-2015	26 (8.0)	38 (42.2)	28 (17.6)	129 (34.1)	221 (23.2)	
Age at diagnosis, y, mean (SD)	55.2 (17.4)	63.6 (13.5)	65.2 (16.3)	56.4 (15.2)	58.1 (16.5)	<.0001
Sex						.4139
Male	168 (51.4)	54 (60.0)	91 (57.2)	206 (54.5)	519 (54.4)	
Female	159 (48.6)	36 (40.0)	68 (42.8)	172 (45.5)	435 (45.6)	
Breslow thickness mm, mean (SD)	3.2 (4.7)	3.7 (3.2)	3.1 (2.7)	3.3 (2.4)	3.3 (3.4)	.5557
Ulceration						.0026
Present	124 (38.6)	53 (58.9)	65 (40.9)	142 (37.8)	384 (40.6)	
Absent	197 (61.4)	37 (41.1)	94 (59.1)	234 (62.2)	562 (59.4)	
Missing	6	0	0	2	8	
TILs						<.0001
Absent	158 (49.2)	8 (9.1)	61 (38.4)	150 (63.8)	377 (46.9)	
Present						
Nonbrisk	137 (42.7)	52 (59.1)	76 (47.8)	54 (23.0)	319 (39.7)	
Brisk	26 (8.1)	28 (31.8)	22 (13.8)	31 (13.2)	107 (13.3)	
Missing	6	2	0	143	151	
Mitotic rate						<.0001
$<1/mm^2$	17 (5.2)	5 (5.6)	11 (6.9)	92 (24.3)	125 (13.1)	
$\geq 1/mm^2$	308 (94.8)	85 (94.4)	148 (93.1)	286 (75.7)	827 (86.9)	
Missing	2	0	0	0	2	
Mitotic rate						
Mean (SD)	5.8 (5.7)	5.6 (4.6)	7.1 (8.0)	5.1 (5.3)	5.8 (6.0)	.0117
Median (IQR)	4.0 (2.0-8.0)	4.0 (3.0-8.0)	5.0 (2.0-9.0)	3.0 (2.0-6.0)	4.0 (2.0-8.0)	
Range	1.0-33.0	1.0-22.0	1.0-57.0	1.0-35.0	1.0-57.0	
Missing	0	1	0	0	1	
SLNB						.0004*
Positive	60 (21.1)	20 (27.4)	29 (28.4)	137 (36.2)	246 (29.4)	
1	53 (88.3)	15 (75.0)	26 (96.3)	103 (75.2)	197 (80.7)	

Continued

Supplemental Table I. Cont'd

Characteristic	Unit of Medical Oncology, Bergamo, N = 327	Santa Croce e Carle hospital, Cuneo, N = 90	University of Florence, N = 159	University of Turin, N = 378	Overall, N = 954	ANOVA or chi-squared *P* value
2	6 (10.0)	4 (20.0)	1 (3.7)	29 (21.2)	40 (16.4)	
3	1 (1.7)	1 (5.0)	0 (0)	5 (3.6)	7 (2.9)	
Missing	0	0	2	0	2	
Negative	224 (78.9)	53 (72.6)	73 (71.6)	241 (63.8)	591 (70.6)	
Missing	43	17	57	0	117	
No. positive lymph nodes at lymphadenectomy, mean (SD)	1.1 (2.1)	0.8 (1.5)	0.3 (0.6)	0.7 (1.5)	0.8 (1.6)	.1223
Stage						
I	99 (34.1)	15 (19.5)	46 (44.7)	100 (26.6)	260 (30.7)	<.0001†
Ib	99 (35.7)	15 (21.1)	46 (50.0)	100 (26.8)	260 (32.0)	
II	121 (41.7)	37 (48.1)	27 (26.2)	139 (37.0)	324 (38.3)	
IIa	61 (22.0)	15 (21.1)	14 (15.2)	67 (18.0)	157 (19.3)	
IIb	42 (15.2)	16 (22.5)	7 (7.6)	47 (12.6)	112 (13.8)	
IIc	16 (5.8)	6 (8.5)	6 (6.5)	25 (6.7)	53 (6.5)	
Missing	2	0	0	0	2	
III	70 (24.1)	25 (32.5)	30 (29.1)	137 (36.4)	262 (31.0)	
IIIa	29 (10.5)	3 (4.2)	11 (12.0)	64 (17.2)	107 (13.2)	
IIIb	23 (8.3)	14 (19.7)	8 (8.7)	53 (14.2)	98 (12.1)	
IIIc	7 (2.5)	2 (2.8)	0 (0.0)	17 (4.6)	26 (3.2)	
Missing	11	6	11	3	31	
Missing	37	13	56	2	108	

ANOVA, Analysis of variance; *IQR*, interquartile range; *SD*, standard deviation; *SLNB*, sentinel lymph node biopsy; *TILs*, tumor-infiltrating lymphocytes.
**P* value for the comparison of the SNLB results distributions classified as positive and negative.
†*P* value for the stage distribution comparison classified as stage I, stage II and stage III.

Supplemental Table II. Sensitivity analysis of disease-free interval among melanoma patients with Breslow thickness >1 mm by using Cox proportional hazard models stratified by center

	Univariate analysis		Multivariate analysis	
Characteristic	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Regression	0.96 (0.76-1.21)	.7124	1.11 (0.83-1.49)	.4654
Female sex	0.86 (0.69-1.07)	.1683	0.99 (0.76-1.29)	.9533
Age at diagnosis, 1-year increase	1.03 (1.02-1.03)	<.0001	1.01 (1.00-1.02)	.0177
Breslow thickness, mm (reference 1.01-2.00)		<.0001		<.0001
2.01-4.00	3.39 (2.51-4.59)	<.0001	2.27 (1.59-3.24)	<.0001
4.01-8.00	6.77 (4.95-9.27)	<.0001	4.84 (3.20-7.32)	<.0001
>8.00	8.85 (5.80-13.50)	<.0001	5.13 (3.01-8.74)	<.0001
Ulceration	2.70 (2.16-3.36)	<.0001	1.55 (1.17-2.07)	.0026
TILs (reference absent)		.0267		.6735
Nonbrisk	0.74 (0.57-0.95)	.0201	0.87 (0.64-1.19)	.3883
Brisk	0.67 (0.45-0.99)	.0426	0.89 (0.57-1.40)	.6172
Positive sentinel lymph node biopsy	3.25 (2.54-4.15)	<.0001	2.58 (1.97-3.37)	<.0001
Mitotic rate > 1/mm^2	2.59 (1.66-4.03)	<.0001	1.34 (0.79-2.28)	.2801

CI, Confidence interval; *HR*, hazard ratio; *TIL*s, tumor-infiltrating lymphocytes.

Supplemental Table III. Sensitivity analysis of melanoma-specific survival among melanoma patients with Breslow thickness >1 mm by using Cox proportional hazard models stratified by center

	Univariate analysis		Multivariate analysis	
Characteristic	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Regression	0.97 (0.74-1.28)	.8447	1.01 (0.71-1.44)	.9465
Female sex	0.84 (0.64-1.09)	.1864	0.95 (0.69-1.33)	.7805
Age at diagnosis, 1-year increase	1.03 (1.02-1.04)	<.0001	1.02 (1.01-1.03)	.0006
Breslow thickness, mm (reference 1.01-2.00)		<.0001		<.0001
2.01-4.00	3.59 (2.46-5.25)	<.0001	2.21 (1.39-3.51)	.0008
4.01-8.00	6.77 (4.58-10.01)	<.0001	3.87 (2.30-6.51)	<.0001
>8.00	9.54 (5.80-15.70)	<.0001	4.51 (2.38-8.57)	<.0001
Ulceration	3.03 (2.30-3.98)	<.0001	1.92 (1.34-2.75)	.0004
TILs (reference absent)		.0003		.1518
Nonbrisk	0.63 (0.46-0.86)	.0035	0.78 (0.53-1.16)	.2219
Brisk	0.36 (0.21-0.64)	.0004	0.55 (0.29-1.05)	.0696
Positive sentinel lymph node biopsy	3.17 (2.34-4.29)	<.0001	2.20 (1.57-3.06)	<.0001
Mitotic rate >1/mm^2	3.34 (1.75-6.38)	.0002	1.64 (0.74-3.64)	.2252

CI, Confidence interval; *HR*, hazard ratio; *TILs*, tumor-infiltrating lymphocytes.