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Favourable prognostic role of histological regression in stage III positive sentinel lymph node melanoma patients

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Background: Sentinel lymph node (SLN)-positive melanoma patients are a heterogeneous group of patients with survival rates ranging from ∼20 to over 80%. No data are reported concerning the role of histological regression on survival in stage III melanoma.

Methods: The study included 365 patients with positive SLN from two distinct hospitals. The model was developed on patients from 'AOU Città della Salute e della Scienza di Torino', and externally validated on patients from IRCCS of Candiolo. Survival analyses were carried out according to the presence of regression and adjusted for all other prognostic factors.

Results: Among patients followed at 'AOU Città della Salute e della Scienza di Torino' ($n = 264$), the median follow-up time to death or censoring (whatever two events occurred earlier) was 2.7 years since diagnosis (interquartile range: 1.3–5.8). In all, 79 patients died from melanoma and 11 from other causes. Histological regression ($n = 43$) was associated with a better prognosis (sub-HR = 0.34, CI 0.12–0.92), whereas the other factors above showed an inverse association. In the external validation, the concordance index was 0.97 at 1 year and decreased to 0.66 at 3 years and to 0.59 at 5 years. Adding histological regression in the prognostic model increased the discriminative ability to 0.75 at 3 years and to 0.62 at 5 years. Finally, using a cutoff of 20% for the risk of death led to a net re-classification improvement of 15 and 11% at 3 and 5 years after diagnosis, respectively.

Conclusions: Histological regression could lead to an improvement in prognostic prediction in patients with stage III-positive SLN melanoma.

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Histological regression in primary melanoma is defined as replacement of tumour cells by lymphocytic inflammation, as well as attenuation of the epidermis and non-laminated dermal fibrosis with inflammatory cells, melanophagocytosis and telangiectasia (College of the American Pathologist Protocol, 2015).

Its prognostic role has been a matter of debate for many years. Previously, it has been considered a negative prognostic factor, as it may lead to an underestimation of thickness measurement. Agreement about the worst prognostic factor is only reported in totally regressed melanoma (Bartlett et al, 2016; Mihic-Probst et al, 2016).

Shaw et al (1989) hypothesised that the presence of metastatic melanoma in a regional lymph node might stimulate an immune response resulting in regression of the primary lesion. However, other studies proved that regression does not increase the risk of nodal metastases (Ma et al, 2012; Savoia et al, 2012). Kaur et al, (2008) considered primary regression as a positive prognostic feature in melanoma patients and showed no association with a higher risk of metastatic sentinel lymph node (SLN).

Previous studies have shown a favourable prognostic role of histological regression in stage I and II melanomas (Traves et al, 2012; Ribero et al, 2013a, b). A meta-analysis has recently demonstrated an inverse association between histological regression and SLN status (Ribero et al, 2015). No prognostic data are published in stage III melanoma patients with positive SLN. Therefore, we performed a study to asses the prognostic role of histological regression in primary tumours on overall survival in stage III melanomas with positive SLN.

MATERIALS AND METHODS

Data. Clinical data from 264 consecutive patients with a positive SLN for melanoma (Stage III at diagnosis according to AJCC (American Joint Committee on Cancer)) (Balch et al, 2009) were collected in this study. All patients were diagnosed and followed-up at the Department of Surgical Dermatology of the 'AOU Città della Salute e della Scienza di Torino' from 1 January 1999 to 31 December 2014. The study protocol was approved by the internal ethics committee. Patients were classified on the basis of AJCC criteria and treated and followed-up according to standard guidelines (Cochran et al, 2000; Garbe et al, 2010; Rossi et al, 2014). Sentinel lymph node biopsy (SLNB) was performed in the presence of: (1) melanoma of >1 mm thickness or thinner in case of ulceration and/or mitotic rate >1/mm^2 (stage T1b disease); (2) other potential significant predictors of SLN positivity (as younger age, mitotic rate, vertical growth phase); and (3) histological regression of the primary tumour diagnosed between 1999 and 2008. After 2008, regression was no longer considered as an indication for SLNB because of the lack of evidence confirming the usefulness of this procedure in thin melanomas and in accordance with the European consensus-based interdisciplinary guidelines for diagnosis and treatment of melanoma patients (Garbe et al, 2010). The SLN evaluation protocol was in accordance with Cook and Di Palma (2008). The SLN tumour burden has been evaluated measuring the maximum diameter of the largest metastatic deposit in SLN/SLNs according with van Akkooi et al (2006).

Histological regression was evaluated on histological slides stained with haematoxylin and eosin from primary cutaneous melanoma tumoursby an experienced dermatopathologist in each centre. Based on our experience and literature data (Kang et al, 1993; Requena et al, 2009; Ribero et al, 2016a), the following histological criteria were considered in defining histological regression: replacement by lymphocytic inflammation or disappearance of melanoma cells in a circumscribed or more diffuse tumour area, as well as attenuation of the epidermis, dermal

fibrosis associated to inflammatory cells (mainly lymphocytes), melanophages and telangiectasia (Supplementary Figure 1). These parameters define the histological regression in the CAP protocol (College of the American Pathologist Protocol, 2015). None of the evaluated lesions were associated with previous inflammatory or infectious reactions, or previous treatments, that could justify the development of primary tumour regression. Cases of whole regression were not included in the study cohorts.

The validation cohort was composed of 101 positive SLN melanoma patients diagnosed and followed-up at the IRCCS of Candiolo from the 1 January 2001 to 31 December 2014. The surgical protocol for the SLN as well as the inclusion criteria and the follow-up were the same as those applied at the Department of Surgical Dermatology of the 'AOU Città della Salute e della Scienza di Torino'.

Follow-up data were available up to the end of June 2015. Patients who were not recorded as dying by the date of last registration at the General Registration Office were censored at this date. Overall survival from melanoma was the outcome of interest and death from other causes was considered a competing risk, that is, an event whose occurrence precluded the occurrence of the event of interest.

Statistical analysis. The potential prognostic factors investigated were gender, age, year at diagnosis, site (head, trunk, arm, leg), Breslow thickness, evidence of ulceration, histological regression, histotype (superficial spreading, nodular, lentigo maligna, acral lentiginous), mitotic rate, SLN tumour burden and total positive lymph node count. Multiple imputations by chained equations (MICE) approach were performed assuming the data were missing at random (MAR) (Marshall et al, 2009; Steyerberg, 2009; White and Royston, 2009). Univariable analysis was performed non-parametrically by representing the cumulative incidence curves, that is, the probability of dying from melanoma, according to each potential prognostic factor, and by testing differences by covariates value by Gray's test (Gray, 1988). Variable selection was carried out by both backward stepwise approach based on Akaike criterion (Wood et al, 2008) and the least absolute shrinkage selection operator (LASSO) method. Multivariable analyses were conducted by the Fine and Gray model allowing to directly assess the effect of each covariate on cumulative incidence function (Fine and Gray, 1999). By modelling the sub-distribution hazard function, that is, the instantaneous risk of the event of interest given that an individual has survived until that time without any event or has had the competing event before that time, the effect of each potential prognostic factor on the sub-distribution hazard was quantified by sub-hazard ratio (sub-HR) and hence a measure of association with the cumulative incidence function was obtained. Proportionality assumption on the sub-hazard distribution scale was checked by including the interaction between time (on logarithmic scale) and each variable included in the model. Continuous variables were modelled firstly as linear and then as restricted cubic splines with three knots fixed at tertiles of their distribution. If both conditions of no evidence of nonlinear trends and no increase in the predictive performance of the model were satisfied, the model with linear terms (and then with reduced *d.f.*) was chosen. External validation was followed by internal validation because of low number of events in the validation set. Specifically, internal cross-validation was conducted by bootstrap resampling. The prediction models were trained on 100 bootstrap samples drawn with replacement of the same size as the original data. Discriminative ability over time was assessed by time-dependent concordance index, which quantifies the ability of the model to correctly rank events of interest up to well-defined times and to discriminate them from competing events. To deal with right censored data, inverse probability of censoring weighted estimator of the concordance index was used (Wolbers et al, 2014).

Calibration, indicating agreement between observed outcomes and predictions, was represented by cumulative incidence estimate computed within percentiles of predicted risk, against the average predicted risk within the same percentiles of the events of interest at several time-points. Finally, to assess the incremental value of the regression as marker, we compared two models, with and without regression, by C-index and the net reclassification improvement (NRI) over time (Pencina *et al*, 2011). The latter shows how many subjects are re-classified conditional on the outcome by adding a marker in the model and is given by the difference of the expected numbers of events reclassified upwards and downwards and the expected numbers of non-events reclassified downwards and upwards divided by the total expected cases of events and non-events.

Details on multiple imputation model, variable selection and the inverse probability of censoring weighted estimator of the concordance index are reported in Supplementary Material.

All analyses were performed using R version 3.2.3 (www.r-project.org).

RESULTS

Clinical data. Out of 264 patients followed-up at the Department of Surgical Dermatology of the 'AOU Città della Salute e della Scienza di Torino', 57.6% (152 out of 264) were males with a median age of 57 years at diagnosis (interquartile range (IQR): 43–68). Superficial spreading melanoma was the most common histotype (68%), and the most common body sites were the trunk (47%) and leg (39%), respectively. Ulceration and histological regression were described in 102 (40%) and 43 (16%) patients, respectively. Median Breslow thickness was 3.3 mm (IQR: 2.0–5.0), median mitoses rate was 4.0 (IQR: 2–6), median SLN tumour burden was 1.8 mm (IQR: 0.6–4.1) and the median positive SLN count ranged from 1 to 3 (median 1). Median number of positive lymph node considering SLN and CLND was 1 (IQR:1–2). In all, 66 (25%) experienced first metastasis in regional skin (60) or regional lymph nodes (6) and 48 (18%) in a distant site. The variable distribution was similar in patients followed at the IRCCS of Candiolo except for the presence of ulceration, mitoses rate and SLN tumour burden ($P<0.05$ by χ^2 test for categorical variables and by Wilcoxon test for continuous variables). Potential prognostic factors are reported in Table 1 and Supplementary Table 1.

Survival study. Among patients followed at 'AOU Città della Salute e della Scienza di Torino', the median follow-up time to death or censoring, whatever two events occurred earlier, was 2.7 years since diagnosis (IQR: 1.3–5.8); 79 patients (30%) died from melanoma and 11 (4%) from other causes. Among patients followed at IRCCS of Candiolo, the median follow-up time to death or censoring was 3.3 years since diagnosis (IQR: 1.4–5.9); 21 (21%) patients died from melanoma and 5 (5%) from other causes.

The prognostic model was developed on patients followed at 'AOU Città della Salute e della Scienza di Torino', excluding those with lentigo maligna melanoma and other rare histotypes of melanoma ($n=5$). Cumulative incidence curves stratified according to each prognostic factor are represented in Figure 1. Gray's test did not highlight any difference in the probability of dying from melanoma by gender or body site ($P>0.05$). Consistently with the crude analysis, the selection procedure led to the selection of variables for the predictive model as age at diagnosis, melanoma histotype, Breslow thickness, ulceration, histological regression, SLN tumour burden and positive lymph nodes count. In the multivariable analysis, continuous variables were modelled as linear. There was no evidence of non-proportionality when interactions between the prognostic factors and the time were

Table 1. Potential prognostic factors in the study data sets

Characteristics	Molinette (n = 264) n (%)	Candiolo (n = 101) n (%)
Gender		
Male	152 (57.6)	57 (56.4)
Female	112 (42.4)	44 (43.6)
Missing	0	0
Age at diagnosis (years)		
⩽40	48 (18.2)	23 (22.8)
41–60	105 (39.8)	36 (35.7)
>60	111 (42.4)	42 (41.6)
Missing	0	0
Year at diagnosis (years)		
⩽2006	80 (30.3)	27 (26.7)
2007–2009	64 (24.2)	27 (26.7)
2010–2012	69 (26.1)	25 (24.7)
>2013 Missing	51 (19.3)0	22 (21.8)0
Body site 0		
Head	13 (4.9)	11 (10.9)
Trunk	123 (46.6)	50 (49.5)
Arm	24 (9.1)	10 (9.9)
Leg	104 (39.4)	30 (29.7)
Missing	0	0
Breslow AJCC		
1	8 (3.0)	5 (4.9)
2	62 (23.5)	21 (20.8)
3	94 (35.6)	53 (52.5)
4	100 (37.9)	22 (21.8)
Median (IQR) (mm)	3.3 (2.0–5.0)	3.0 (2.0–4.0)
Missing	0	1
Ulceration		
No	153 (60.0)	32 (41.0)
Yes	102 (40.0)	46 (59.0)
Missing	9	23
Histological regression		
No	221 (83.7)	80 (79.2)
Yes	43 (16.3)	21 (20.8)
Missing	0	0
Histotype		
Superficial spreading melanoma	178 (67.7)	70 (69.3)
Nodular melanoma	49 (18.6)	24 (23.8)
Lentigo maligna melanoma	3 (1.1)	/
Acral lentiginous melanoma	29 (11.0)	7 (6.9)
Other	4 (1.5)	/
Missing	1	0
Mitotic rate (1/mm^2)		
0	10 (8.8)	6 (7.0)
⩾1	104 (91.2)	79 (9.3)
Median (IQR)	4.0 (2.0–6.0)	8.0 (5.0–12.0)
Missing	150	16
Positive lymph nodes		
1	148 (57.1)	50 (54.9)
2	65 (25.1)	26 (28.6)
⩾3	46 (17.7)	9 (16.5)
Median (IQR)	1 (1–2)	1 (1–2)
Missing	5	10
SLN tumour burden (mm)		
⩽0.10	11 (4.4)	12 (12.6)
0.10–1.00	79 (31.5)	31 (32.6)
>1.00	161 (64.1)	52 (54.7)
Median (IQR)	1.8 (0.6–4.1)	1.4 (0.4–2.5)
Missing	13	6

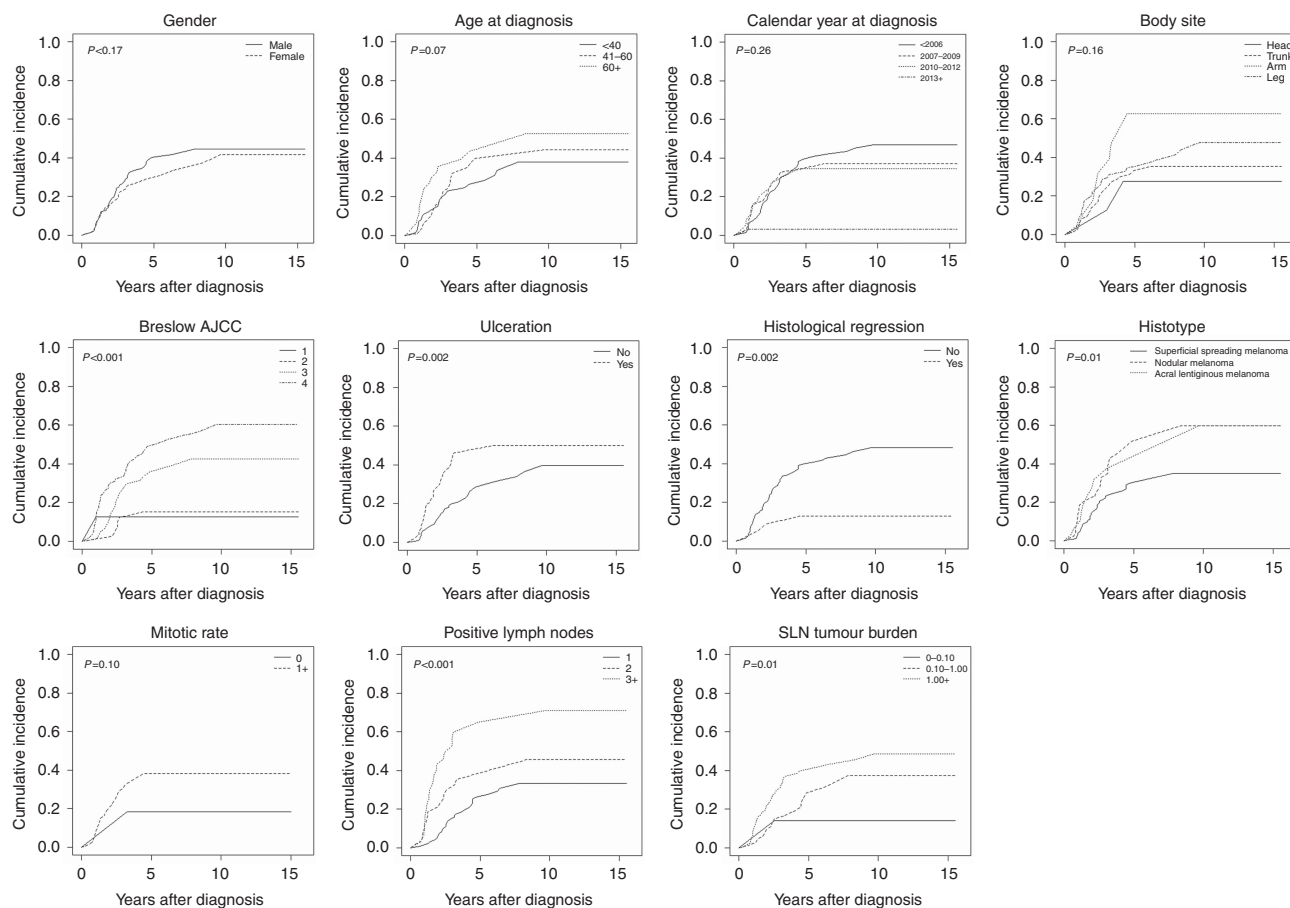Abbreviations: AJCC = American Joint Committee on Cancer; IQR = interquartile range; SLN = sentinel lymph node.

Figure 1. Non-parametric cumulative incidence curves by potential prognostic factors. *P*-value calculated by Gray's test.

included in the model. As the parameters estimated by Fine and Gray model measure the association between each factor and the cumulative incidence function, an increase in the risk of dying from melanoma was observed among older patients, nodular melanomas, thicker melanomas, ulcerated melanomas, higher positive lymph node count and increased SLN tumour burden (Table 2). Histological regression at diagnosis was associated with a decreased risk of death from melanoma in both univariable and multivariable analyses (Table 2). When analyses were performed on patients from IRCCS of Candiolo, the direction of the association was maintained but the confidence interval was much larger and containing the null value (sub-HR = 0.73, 95% CI: 0.11–4.93). The C-index, ranging from 0.5 when the model has no ability to discriminate between low- and high-risk subjects and 1 when the model perfectly discriminates between the two groups, was 0.79 at 1 year after diagnosis and decreased to 0.73 at 3 and 5 years after diagnosis in the training set. In the external validation, C-index was 0.97 at 1 year after diagnosis and decreased to 0.66 at 3 years after diagnosis and to 0.59 at 5 years after diagnosis. In the internal cross-validation, C-index was 0.74 at 1 year after diagnosis and decreased to 0.69 at 3 and 5 years after diagnosis (Figure 2) and the calibration at different time-points indicated that low predictions were slightly too low and high predictions were slightly too high (Figure 3).

Clinical implications. Predictive probability of death from melanoma at 3 and 5 years after diagnosis for 14 hypothetical clinical scenarios is reported in Supplementary Table 2. For example, the cumulative incidence at 3 and 5 years for a patient diagnosed at 65 years of age with superficial spreading melanoma, ulceration, one positive lymph node, 1 mm of Breslow thickness,

positive SLN (0.5 mm in maximum diameter) and no evidence of histological regression was 17.1% and 25.8% respectively. It decreased to 6.1% at 3 years and to 9.6% at 5 years after diagnosis in a patient with the same characteristics as above but evidence of histological regression. When comparing the discriminative ability of the models without and with histological regression, the C-index increased from 0.70 to 0.73 at 3 and 5 years after diagnosis. In the validation set, the C-index increased from 0.66 to 0.75 and from 0.59 to 0.62 at 3 and 5 years after diagnosis, respectively. Using a cutoff of 20% for the risk of dying from melanoma led to classification of 120 and 124 patients at 3 years and 203 and 195 at 5 years after diagnosis at high risk of death from melanoma according to the model without and with histological regression, respectively. The NRI was 14.8% and 11.2% at 3 and 5 years after diagnosis, respectively.

DISCUSSION

Here we report that stage III melanoma patients, defined as having positive SLNB, showed a better prognosis when histological regression was present in the primary tumour, independently from other prognostic factors. In addition, the developed prognostic model confirms the relevance of the three classical prognostic parameters in stage III melanoma (Breslow thickness, ulceration and positive lymph nodes count) and proves that SLN tumour burden, histotype and age also bear prognostic significance.

Many parameters have previously been analysed to find out new prognostic parameters for melanoma patients. Age at diagnosis, primary cutaneous tumour site and number of lymphatic basin

Table 2. Melanoma-specific sub-hazard ratio (sub-HR) estimated by Fine and Gray model with 95% confidence interval (95% CI) in 'Città della Salute e della Scienza di Torino'

Variable	Univariable		Multivariable	
	Sub-HR	95% CI	Sub-HR	95% CI
Gender				
Male	1.00	Ref.	/	/
Female	0.80	0.50–1.27	/	/
Age at diagnosis				
Unit increase (year)	1.02	1.00–1.04	1.01	1.00–1.03
Year at diagnosis				
Unit increase (year)	0.98	0.92–1.03	/	/
Body site				
Head	1.00	Ref.	/	/
Trunk	1.43	0.38–5.39	/	/
Arm	2.64	0.65–10.74	/	/
Leg	1.85	0.50–6.92	/	/
Breslow				
Unit increase (mm)	1.22	1.13–1.30	1.13	1.04–1.22
Ulceration				
No	1.00	Ref.	1.00	Ref.
Yes	1.95	1.24–3.06	1.22	0.73–2.03
Histological regression				
No	1.00	Ref.	1.00	Ref.
Yes	0.26	0.09–0.71	0.34	0.12–0.92
Histotype				
Superficial spreading melanoma	1.00	Ref.	1.00	Ref.
Nodular melanoma	2.00	1.22–3.29	1.38	0.82–2.32
Acral lentiginous melanoma	1.70	0.85–3.40	0.63	0.27–1.45
Mitotic rate				
Unit increase (1/mm^2)	1.04	0.99–1.08	/	/
Positive lymph nodes				
Unit increase	1.40	1.26–1.57	1.28	1.15–1.44
SLN tumour burden				
Unit increase (mm)	1.09	1.05–1.12	1.04	1.00–1.09

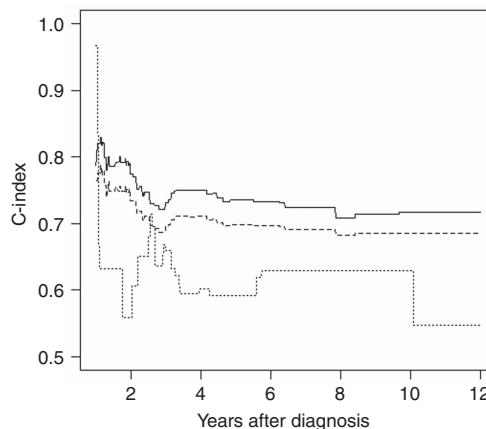Abbreviations: Ref = reference value; SLN = sentinel lymph node.

Figure 2. C-index in the training set (solid line), in the internal validation set (dash line), and in the external validation set (dotted line) over time.
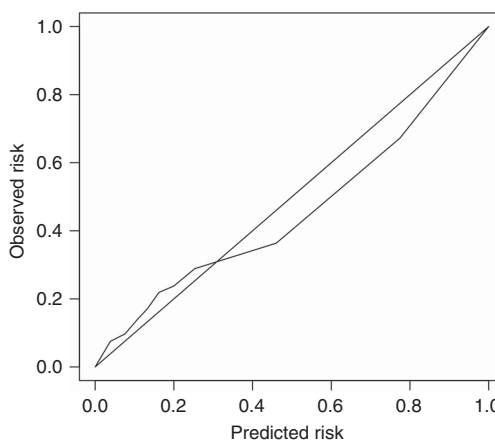

Figure 3. Plot of cumulative incidence estimate computed within percentiles of predicted risk, against the average predicted risk within the same percentiles of the events of interest at 3 years after diagnosis.

drainage have been proposed even if they have not been included yet in the AJCC classification (Balch *et al*, 2013; Pasquali *et al*, 2014; Sanlorenzo *et al*, 2015).

Histological regression in primary cutaneous melanoma occurs in 10–35% of cases (Blessing *et al*, 1990; Sanlorenzo *et al*, 2015). In our series, regression was found in 16% of stage III melanoma patients. Histological regression has traditionally been considered as a marker of poor prognosis, mainly in thin melanomas, as it hampers a real evaluation of the initial thickness of the tumour. However, the majority of these studies are based on small series of patients, and have been performed in single institutions (Ronan *et al*, 1987; Slingluff and Seigler, 1992; Ribero *et al*, 2016b). The introduction of SLNB has changed the clinical approach to melanoma patients in the past 20 years (Morton *et al*, 2006) A recent meta-analysis in more than 10 000 patients eligible for SLN biopsy showed that SLN *status* is inversely correlated with histological regression (Ribero *et al*, 2015): therefore, histological regression seems to be inversely associated with the risk of lymph node metastases.

A study on 1600 patients in stage I–II melanoma showed a protective role of histological regression on survival despite all adjustments (Ribero *et al*, 2013b), and confirms results previously

reported by other authors through a univariable analyses. Similarly, a recent meta-analysis on survival of >8500 melanoma patients reported a lower relative risk of death (RR 0.772, 95% CI, 0.612–0.973) for patients with histological regression than patients without (Gualano *et al*, 2017). A host immunological response to the primary tumour is presumed to be at the basis of histological regression, and likely reflects an active immunological response considered as prognostically favourable. In fact, Ma *et al*, (2012) reported that the presence of primary tumour histological regression results from a T cell immune response. No data had been reported on the role of histologic regression in the stratification of survival in stage III melanoma patients. The reported prognostic model in the study reported here was developed and externally validated using two distinct Italian cohorts of 264 and 101 patients diagnosed at stage III melanoma with positive SNL. These patients were followed-up in two hospitals adopting the same SLN surgical and follow-up protocols. The prognostic model was formulated by considering the main aspects of survival data, right censoring and competing risks. The former was taken into account by including appropriate weights in calculating the model's performance and the latter was considered by indirectly modelling cumulative incidence function. Model's performance, measured by time-dependent C-index and calibration plot, was discrete in the first years after diagnosis but then worsened over time when fewer events were observed due to short

follow-up of patients. However, the inclusion of histological regression in the prognostic model increased its discriminative ability in both the training and validation sets over time and led to a reclassification of 15% and 11% of patients, respectively, at 3 and 5 years after diagnosis when the cutoff for the risk of dying from melanoma was fixed at 20%. Hence, the inclusion of histological regression in the risk equation may lead to more precise classification of patients' risk of death, helping in the decisional process for a clinical setting in stage III melanoma patients.

Because of the relatively recent (1999) introduction of the SLNB technique in both institutions in this study, the follow-up time is relatively short. This could affect the number of observed events and consequently the power of the study. In particular, the predictive ability of the model was affected, in terms of magnitude and high variability, by the small size of validation set.

In conclusion, this study demonstrates that histological regression is independently associated with a lower death rate in stage III melanoma patients and that its inclusion in the prognostic model may improve the prognostic classification of patients at higher risk of dying from melanoma. Further research is required to improve the model's performance over longer follow-up.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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