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Histological regression in melanoma: impact on sentinel lymph node status and survival

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Regression in melanoma is an immunological phenomenon that results in partial or complete replacement of the tumor with variably vascular fibrous tissue, often accompanied by pigment-laden macrophages and chronic inflammation. In some cases, tumor-infiltrating lymphocytes (TILs) may represent the earliest phase of this process. The prognostic significance of regression has long been a matter of debate, with inconsistent findings reported in the literature to date. This study sought to determine whether regression in primary cutaneous melanomas predicted sentinel lymph node (SLN) status and survival outcomes in a large cohort of patients managed at a single centre. Clinical and pathological parameters for 8,693 consecutive cases were retrieved. Associations between regression and SLN status, overall survival (OS), melanoma-specific survival (MSS) and recurrence-free survival (RFS) were investigated using logistic and Cox regression. Histological evidence of regression was present in 1958 cases (22.5%). Regression was significantly associated with lower Breslow thickness, lower mitotic rate, and absence of ulceration ($p < 0.0001$). Multivariable analysis showed that regression in combination with TILs independently predicted a negative SLN biopsy (OR 0.33; 95% C.I. 0.20–0.52; $p < 0.0001$). Patients whose tumors showed both regression and TILs had the highest 10-year OS (65%, 95% C.I. 59–71%), MSS (85%, 95% C.I. 81–89%), and RFS (60%, 95% C.I. 54–66%). On multivariable analyses, the concurrent presence of regression and TILs independently predicted the lowest risk of death from melanoma (HR 0.69; 95% C.I. 0.51–0.94; $p = 0.0003$) as well as the lowest rate of disease recurrence (HR 0.71; 95% C.I. 0.58–0.85; $p < 0.0001$). However, in contrast, in the subgroup analysis of Stage III patients, the presence of regression predicted the lowest OS and RFS, with MSS showing a similar trend. Overall, these findings indicate a prognostically favorable role of regression in primary cutaneous melanoma. However, in Stage III melanoma patients, regression may be a marker of more aggressive disease.

INTRODUCTION

It has long been recognised that the interplay between tumor cells and the host immune system has a key role in the modification of disease progression and survival outcomes in patients with melanoma [1] and other cancer types [2, 3]. Regression in primary cutaneous melanoma is an immunological phenomenon that results in partial or complete disappearance of the tumor [4]. This process can be divided into three temporal phases, each with a distinct histological appearance (Fig. 1). The initiation of regression is heralded by tumor-infiltrating lymphocytes (TILs), which permeate the tumor and disrupt nests or directly appose tumor cells (Fig. 1A). As such, TILs represent the earliest phase of regression in many cases. In intermediate regression, there are areas of immature fibroblasts and newly formed blood vessels resembling granulation tissue (Fig. 1B). The final phase is late regression, in which tumor cells are at least partially replaced by mature fibrosis that is often associated with pigment incontinence and flattening of the overlying epidermis with loss of rete ridges

(Fig. 1C). The phases of regression are not independent of each other and can co-exist to varying degrees in a single tumor.

Currently, Breslow thickness (BT), tumor mitotic rate and ulceration are considered the most important prognostic characteristics of a primary cutaneous melanoma, and these features are also helpful for predicting sentinel lymph node (SLN) status [5]. Recent work from our institution has shown that the presence of microsatellites and lymphovascular invasion are also associated with SLN positivity [6]. The latter, in turn, is the most powerful predictor of melanoma survival [7] and is of great importance for selecting patients who may benefit from adjuvant drug therapy and determining clinical trial eligibility [8, 9].

In contrast, the prognostic significance of histological regression in primary melanoma has been a matter of debate for decades. Whilst some studies reported an association with improved survival and a reduced likelihood of SLN positivity, others did not find any significant association, and some even reported worse outcomes in patients whose tumors showed regression.

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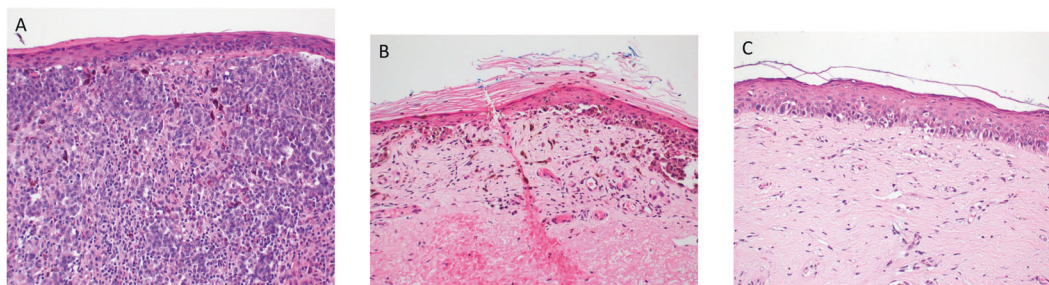
Fig. 1 Histological appearance of immune response in cutaneous melanoma. A Tumor-infiltrating lymphocytes. **B** Intermediate regression. **C** Late regression.

Recently, we showed that regression was a favorable prognostic factor for patients with Stage I and II cutaneous melanoma in two large cohorts from The Netherlands and Australia [10]. These findings prompted us to conduct a more detailed inquiry into the effect of various phases of regression as well as the interplay between them. In the present study, we sought to determine the impact of regression and TILs on SLN status and prognosis in a large cohort of patients treated at Melanoma Institute Australia (MIA). We also evaluated whether the extent (width) or depth of regression impacted patient outcomes. Finally, we investigated the association of regression with survival outcomes in patients whose tumor had already spread to SLNs. To our knowledge, this is the largest study of its kind undertaken to date.

PATIENTS AND METHODS
Data collection
The study cohort consisted of consecutive patients with primary cutaneous melanoma treated between 1 January 1999 and 31 December 2014 at MIA (a subset of this cohort, namely 4,980 Stage I and II cases treated between 1 January 2000 and 31 December 2014, formed part of the study population in a previous publication by our group [10]). For each case, clinical parameters were recorded, including age at diagnosis, sex and primary melanoma anatomic site. Investigation and treatment data included whether a SLN biopsy was performed, SLN status, any subsequent lymph node dissection, date and type of first recurrence (local, regional, in-transit or distant), date of last follow-up and status at that time. Histology slides of all primary tumors were reviewed at the time of referral by MIA-affiliated pathologists at the Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney. The "reviewer" pathology reports were analyzed for this study (the original pathology slides were not re-reviewed). Documented pathological characteristics of the primary tumor included tumor subtype, BT, Clark level, presence or absence of ulceration, dermal mitotic rate (per square mm), presence or absence of microsatellites and presence or absence of a host immune response. The latter was sub-categorized as TILs (which in some cases represent early regression), intermediate regression or late regression. Where multiple phases of immune response (TILs, intermediate regression and/or late regression) were present, each was recorded. Intermediate regression was defined as areas of immature scar and angiogenesis with or without superimposed chronic inflammation. Late regression was defined as replacement of all or part of the tumor with mature dermal fibrosis, often associated with pigment-laden macrophages, flattening of epidermis and/or loss of rete ridges. In cases where residual in-situ and/or invasive tumor cells were present above or below the area of regressive fibrosis, regression was recorded as present. TILs were reported as present if lymphocytes had infiltrated the tumor to some extent, causing at least partial disruption of tumor nests, whereas a peritumoral lymphocytic infiltrate alone was considered insufficient. Cases with missing data or ambiguous findings ($n = 69$) were excluded. Where available, the maximal depth of regression ($n = 254$) and width (diameter) of regression ($n = 269$) were also recorded. The depth of regression was measured from the top of the granular layer to the deepest point of fibrosis, ignoring any surrounding inflammation. The diameter of regression was taken as the horizontal extent of fibrosis (or the sum of all horizontal measurements, if more than one area of fibrosis was present). Other measurements of regression extent, such as percentage of tumour replaced by fibrosis, were

not available. The clinical outcome measures were SLN status, overall survival (OS), melanoma-specific survival (MSS) and recurrence-free survival (RFS). RFS was defined as time from primary melanoma diagnosis to recurrence of any kind (local, regional or distant) or death.

Statistical analysis
Clinical and histopathological features collected at the time of melanoma diagnosis were summarised using standard statistics and stratified by type of host immune response (classified as "no host response," "regression only," "TILs only," or "regression and TILs"). Categorical variables were described using frequency and proportion while continuous variables were summarised using median and range. Differences between the groups were tested using either chi-square test or Wilcoxon signed rank test as appropriate. The effect of host response on SLN positivity was evaluated using univariable and multivariable logistic regression. Kaplan-Meier curves, stratified by type of host response for OS, MSS and RFS, were calculated along with the survival rates at 3-, 5- and 10-year landmark time points. Survival differences between groups were assessed using the log-rank test. Univariable and multivariable Cox regression were used to test the associations between the type of host response and OS, MSS or RFS. Survival analyses were also carried out on specific subgroups, including patients with a positive SLN biopsy and patients with intermediate vs late regression. The extent of regression was also evaluated by stratifying groups according to the regression depth (lower median versus upper median) and diameter (lower median versus upper median).

RESULTS
The clinical and pathological characteristics of the study population are presented in Table 1, stratified by the presence of TILs and regression. The intermediate and late phases of regression were grouped together for analyses. In total 8693 patients were available for analysis. The median age was 58 years (range, 7–102) and the male to female ratio was 1.4:1. Median follow-up was 40.7 months (95% C.I., 39–43). A total of 3680 (42%) patients underwent a SLN biopsy and, of those, 697 (19%) were SLN-positive. An infiltrate of TILs was present in 5502 tumors (63.3%). Histological evidence of regression was present in 1958 cases (22.5%), comprising cases with intermediate, late or both phases of regression. The majority of tumors were located on the extremities (44% of all cases), followed by the trunk (39%).

The presence of a host immune response was significantly associated with several favorable histological parameters. Tumors showing TILs were found to have a lower median BT than tumors lacking this feature, while tumors with established regression were associated with the lowest median BT, regardless of whether or not TILs were also present (median BT of 1.8, 1.3, and 0.8 mm in tumors lacking any host response, those with TILs only, and those with regression with or without TILs, respectively; $p < 0.0001$). Mitotic rates were lower in tumors that had evidence of regression (46.8% of tumors with regression had a mitotic count of 0/mm², vs 18.2% of tumors with no host response). Likewise, the finding of ulceration inversely correlated with the presence of regression, with the lowest proportion of ulcerated tumors found in the group showing both regression and TILs (10.4%, compared with 23.5% in tumors lacking any host response and 21.0% in tumors with TILs

Table 1. Clinicopathological characteristics of the overall cohort, stratified by type of immune response present in the primary tumor.

Characteristics	Overall (N = 8693)	No host response (N = 2128)	Regression only (N = 1063)	TILs only (N = 4607)	Regression + TILs (N = 895)	p value
SNB conducted						
No	5013 (57.7%)	1096 (51.5%)	763 (71.8%)	2521 (54.7%)	633 (70.7%)	<0.0001
Yes	3680 (42.3%)	1032 (48.5%)	300 (28.2%)	2086 (45.3%)	262 (29.3%)	
SNB status						
Negative	2983 (81.1%)	769 (74.5%)	253 (84.3%)	1725 (82.7%)	236 (90.1%)	<0.0001
Positive	697 (18.9%)	263 (25.5%)	47 (15.7%)	361 (17.3%)	26 (9.9%)	
Gender						
Female	3623 (41.7%)	962 (45.2%)	384 (36.1%)	1935 (42.0%)	342 (38.2%)	<0.0001
Male	5070 (58.3%)	1166 (54.8%)	679 (63.9%)	2672 (58.0%)	553 (61.8%)	
Age at diagnosis (years)						
Median (Range)	58 (7, 102)	60 (12, 98)	61 (16, 102)	57 (7, 95)	58 (17, 91)	<0.0001
Primary melanoma site						
Arm	1696 (19.5%)	400 (18.8%)	180 (16.9%)	977 (21.2%)	139 (15.5%)	<0.0001
Head and Neck	1448 (16.7%)	472 (22.2%)	108 (10.2%)	801 (17.4%)	67 (7.5%)	
Leg	2135 (24.6%)	658 (30.9%)	247 (23.2%)	1064 (23.1%)	166 (18.5%)	
Trunk	3414 (39.3%)	598 (28.1%)	528 (49.7%)	1765 (38.3%)	523 (58.4%)	
Breslow thickness (mm)						
Median (Range)	1.2 (0.0, 50.0)	1.8 (0.1, 40.0)	0.8 (0.0, 50.0)	1.3 (0.1, 35.0)	0.8 (0.0, 20.0)	<0.0001
Breslow thickness (categorised) (mm)						
<=1.0	3642 (42.1%)	629 (29.7%)	636 (61.1%)	1830 (39.8%)	547 (61.5%)	<0.0001
1.01–2.0	2271 (26.3%)	539 (25.4%)	211 (20.3%)	1307 (28.4%)	214 (24.0%)	
2.01–4.0	1697 (19.6%)	554 (26.1%)	116 (11.1%)	935 (20.3%)	92 (10.3%)	
>4.0	1038 (12.0%)	399 (18.8%)	78 (7.5%)	524 (11.4%)	37 (4.2%)	
Mitoses (n/mm^2)						
Median (Range)	2.0 (0.0, 98.0)	3.0 (0.0, 98.0)	1.0 (0.0, 45.0)	2.0 (0.0, 60.0)	1.0 (0.0, 44.0)	<0.0001
Mitoses (categorised)						
0	2253 (27.2%)	373 (18.2%)	459 (46.8%)	1065 (24.0%)	356 (42.6%)	<0.0001
1-2	2365 (28.5%)	573 (28.0%)	238 (24.3%)	1297 (29.3%)	257 (30.8%)	
3-4	1271 (15.3%)	351 (17.2%)	116 (11.8%)	717 (16.2%)	87 (10.4%)	
>=5	2405 (29.0%)	748 (36.6%)	168 (17.1%)	1354 (30.5%)	135 (16.2%)	
Ulceration						
No	6875 (80.7%)	1607 (76.5%)	906 (89.0%)	3573 (79.0%)	789 (89.6%)	<0.0001
Yes	1646 (19.3%)	493 (23.5%)	112 (11.0%)	949 (21.0%)	92 (10.4%)	
Melanoma subtype						
ALM	145 (1.8%)	61 (3.2%)	10 (1.0%)	66 (1.5%)	8 (1.0%)	<0.0001
DM	650 (8.1%)	229 (11.9%)	49 (5.1%)	334 (7.7%)	38 (4.5%)	
LMM	330 (4.1%)	76 (3.9%)	54 (5.7%)	175 (4.0%)	25 (3.0%)	
NM	1851 (23.0%)	609 (31.6%)	109 (11.4%)	1040 (24.0%)	93 (11.1%)	
SSM	5053 (62.8%)	938 (48.7%)	732 (76.6%)	2708 (62.5%)	675 (80.5%)	
Other	23 (0.3%)	14 (0.7%)	1 (0.1%)	8 (0.2%)	0 (0.0%)	
Regression diameter (mm)						
Median (Range)	3.0 (0.5, 136.0)	NA	3.0 (0.5, 136.0)	NA	4.0 (0.5, 26.0)	0.7085
Regression depth (mm)						
Median (Range)	0.5 (0.1, 7.5)	NA	0.5 (0.1, 7.5)	NA	0.6 (0.1, 6.0)	0.9339

TILs tumour-infiltrating lymphocytes, *SNB* sentinel lymph node biopsy, *ALM* acral lentiginous melanoma, *DM* desmoplastic melanoma, *LMM* lentigo maligna melanoma, *NM* nodular melanoma, *SSM* superficial spreading melanoma.

only; $p < 0.0001$). With regard to melanoma subtype, superficial spreading melanoma was most frequently associated with regression, with 28% of cases showing evidence of this feature, whereas only 11% of nodular melanomas showed evidence of regression, the least of any tumor subtype.

Factors impacting sentinel node positivity

Of the patients who underwent SLN biopsy ($n = 3680$), both regression and TILs were associated with a lower likelihood of SLN positivity, with the lowest positive biopsy rate found when both TILs and regression were present

Table 2. Logistic regression of sentinel lymph node status in the overall cohort.

Covariates	Univariable		Multivariable	
	OR	P value	OR	P value
Regression				
No host response	1	<0.0001	1	<0.0001
Regression only	0.54 (0.39, 0.76)		0.67 (0.46, 0.98)	
TILs only	0.61 (0.51, 0.73)		0.59 (0.48, 0.72)	
Regression + TILs	0.32 (0.21, 0.49)		0.33 (0.20, 0.52)	
Gender				
Female	1	0.7724		
Male	1.03 (0.87, 1.21)			
Age at diagnosis	0.98 (0.98, 0.99)	<0.0001	0.98 (0.97, 0.98)	<0.0001
Breslow thickness (mm)	1.15 (1.12, 1.19)	<0.0001	1.14 (1.09, 1.18)	<0.0001
Primary melanoma site				
Head and Neck	1	<0.0001	1	<0.0001
Arm	0.55 (0.41, 0.76)		0.62 (0.44, 0.87)	
Leg	1.23 (0.94, 1.60)		1.18 (0.87, 1.59)	
Trunk	1.07 (0.83, 1.37)		1.22 (0.93, 1.62)	
Melanoma subtype				
SSM	1	<0.0001	1	<0.0001
ALM	3.36 (2.11, 5.35)		2.50 (1.47, 4.24)	
DM	0.37 (0.25, 0.55)		0.30 (0.19, 0.47)	
LMM	0.47 (0.19, 1.20)		0.60 (0.23, 1.59)	
NM	1.16 (0.96, 1.39)		0.73 (0.60, 0.90)	
Other	1.42 (0.38, 5.26)		0.90 (0.24, 3.45)	
Ulceration				
No	1	<0.0001	1	<0.0001
Yes	2.13 (1.79, 2.54)		1.59 (1.30, 1.95)	
Mitoses (categorised) (n/mm^2)				
0	1	<0.0001	1	<0.0001
1-2	1.91 (1.16, 3.15)		2.13 (1.19, 3.81)	
3-4	3.01 (1.82, 4.95)		3.16 (1.76, 5.67)	
>=5	4.57 (2.82, 7.38)		4.00 (2.24, 7.13)	

OR odds ratio, *TILs* tumour-infiltrating lymphocytes, *ALM* acral lentiginous melanoma, *DM* desmoplastic melanoma, *LMM* lentigo maligna melanoma, *NM* nodular melanoma, *SSM* superficial spreading melanoma.

(9.9% compared with 25.5% in tumors lacking any host response, $p < 0.0001$).

The impact of various histological parameters on SLN status is shown in Table 2. On univariable logistic regression analysis, all combinations of TILs and regression were significantly associated with a lower rate of SLN positivity. This association held true in the multivariable model, corresponding toodds ratios (ORs) of 0.67 (95% C.I. 0.46–0.98) for regression only, 0.59 (95% C.I. 0.48–0.72) for TILs only, and 0.33 (95% C.I. 0.20–0.52) for regression in combination with TILs ($p < 0.0001$). Compared with superficial spreading melanoma, multivariable analysis showed a significantly increased likelihood of having a positive SLN if the primary tumor was of acral lentiginous subtype (OR 2.50; 95% C.I. 1.47–4.24; $p < 0.0001$), while desmoplastic melanoma showed the opposite association (OR 0.30; 95% C.I. 0.19–0.47; $p < 0.0001$). As expected, ulceration and higher mitotic rates were independent predictors of SLN positivity.

Factors impacting recurrence and survival
Kaplan–Meier curves of survival stratified by the presence and type of immune response are displayed in Fig. 2. Compared with patients whose tumor showed no regression or TILs, those with both features had the highest 5- and 10-year OS, MSS and RFS rates. Likewise, all types of survival at both 5 and 10 years were significantly improved in patients showing TILs alone (without concurrent regression). Patients whose tumors showed regression alone (without concurrent TILs) had improved MSS and RFS at 5 and 10 years, although no statistically significant effect on OS was seen (Supplementary Table 1).

Logistic regression analyses of OS, MSS and RFS are presented in Table 3. The presence of any pattern of immune response was associated with improvement of all outcomes in the univariable models. On multivariable analyses, the concurrent presence of regression and TILs independently predicted the lowest risk of death from melanoma (HR 0.69; 95% C.I. 0.51–0.94; $p = 0.0003$) as well as the lowest risk of disease recurrence (HR 0.71; 95% C.I. 0.58–0.85; $p < 0.0001$). The finding of regression alone showed a similar trend, but the associations did not reach statistical significance, with a HR of 0.93 (95% C.I. 0.73–1.19) for MSS and 0.89 (95% C.I. 0.76–1.04) for RFS.

Subgroup analyses did not show any significant association between regression depth ($n = 254$) and survival or disease recurrence (cases were stratified as having either above or below the median thickness of regression; see Supplementary Fig. 1).
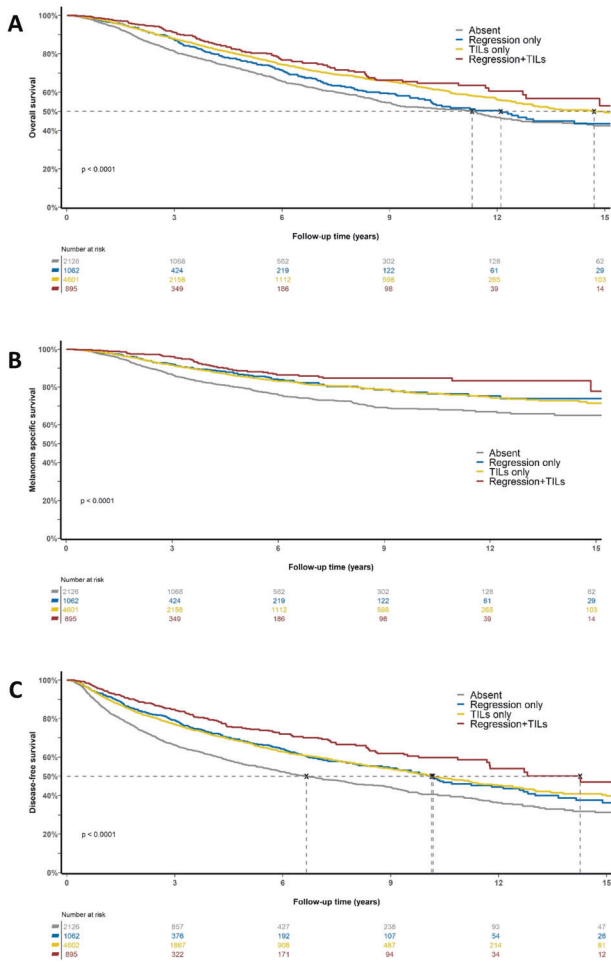
Fig. 2 Kaplan–Meier curves of survival stratified by the presence of regression and/or TILs. (A), melanoma-specific survival (**B**), and recurrence-free survival (**C**) in the overall cohort.

Similarly, analyses of regression diameter ($n = 269$) did not yield significant results (Supplementary Fig. 2).

Finally, we compared the relative effects of intermediate and late regression on patient outcomes (Supplementary Fig. 2). Patients selected for this analysis were stratified as having either intermediate or late regression in their primary tumor (without concurrent TILs), and compared with those who lacked any host response. Survival analyses showed no significant difference between the two phases of established regression.

Prognostic impact in stage III melanoma

Figure 3 shows the survival analyses in a subgroup of patients who had a lymph node metastases. The best outcomes were observed in patients who had TILs in their primary tumor, while the worst outcomes were seen in the patient group who had regression only. There was significant separation between the curves for OS, with corresponding 5-year survival fractions of 0.70 (95% C.I. 0.65–0.75) in the TILs group vs 0.44 (95% C.I. 0.31–0.63) in the regression-only group. Similarly, a significant difference in the 5-year RFS was observed, while the MSS curves were not significantly separated but showed the same trend.

DISCUSSION

The findings of this study demonstrate that histological regression in primary cutaneous melanoma is associated with favorable pathological features including lower BT, lower mitotic rate and lack of ulceration. Furthermore, we found that regression had a positive impact on clinical outcomes, predicting lower rates of SLN positivity and better survival compared with patients who lack this feature. Considering the individual contributions of established regression and TILs, there appeared to be an additive effect whereby the simultaneous presence of both features was independently associated with the longest MSS and RFS. Furthermore, regression independently predicted a negative SLN biopsy in our cohort, even in the absence of TILs.

The existing literature on the prognostic significance of regression shows conflicting results. Early studies reported an association between regression and worse outcomes including distant metastasis [11–15] and death [14, 16]. These studies were all limited to thinner melanomas (BT ≤ 1.5 mm). This led to the hypothesis that regression may be the biological phenomenon responsible for the metastatic potential of so-called "thin metastasizing melanomas", a subset of tumors that deviated from the usually favorable prognosis of such lesions. The proposed mechanism was underestimation of the true BT. To investigate this possibility, Traves et al conducted a study of 77 melanomas showing regression, comparing the BT with the depth of regression [17]. The authors found that regression depth exceeded BT only in melanomas <0.76 mm in thickness (10/18 cases), but the difference was small and would not have caused upstaging of the tumor had the regression thickness been taken into account. Furthermore, other studies demonstrated no effect of regression on outcomes [18–21], including some that focused exclusively on thin melanomas [22, 23]. In contrast, some studies found a positive prognostic impact [24–26]. One of the latter was a meta-analysis that included a total of 8,557 patients, which showed that there was a lower risk of death when regression was present in the primary tumor (RR 0.77, 95% C.I. 0.61–0.97). Osella-Abate et al. [27] found that regression predicted a better RFS. In that study, regression was quantified as the ratio between the linear extension of regression and tumor. Defined in this way, a 10% cut-off was established as the minimum amount of regression required for a favorable outcome. Although MSS was not significantly affected in that cohort, an important caveat that could have confounded the outcome was that cases with more than 75% regression were excluded from analysis.

Studies investigating the effect of regression on SLN status have been equally inconsistent. Concordant with our findings, several authors have reported a decreased likelihood of a positive SLN biopsy when regression is present [19, 21, 28, 29]. Most notably, a meta-analysis of 10,098 patients showed a markedly lower risk of SLN positivity with the presence of regression in the primary tumor (OR 0.56; 95% C.I. 0.41–0.77) [30]. On the other hand, a lack of significant effect has also been reported [18, 20]. In marked contrast, Olah et al. [31] found that in patients with melanomas less than 2 mm thick, the relative risk of SLN positivity for patients with regressing tumors was 9.78 (95% C.I. 3.56–26.86) compared to patients with nonregressing tumors. Similarly, Kocsis et al. [32] showed that patients with melanomas <1 mm thick had a 5.8-fold increase in the probability of a positive SLN when regression was present. More recent work by Maurichi et al. [33] has also identified extensive regression as a risk factor for SLN positivity in thin melanomas.

The lack of consensus observed in the literature for the prognostic significance of regression may be partially explained by inconsistent histological criteria for reporting regression. A clear definition of regression was first proposed by Clark et al. in 1989, and was similar to that used in our study, except that it required a complete absence of in-situ or invasive melanoma cells in the area of regression [34]. Defined in this way, regression was found to have an unfavorable association with survival in that study by Clark et al. However, the restrictive definition may have led to a number of partially regressed melanomas being inappropriately classified as having absent regression. This

Table 3. (**A**) Cox proportional hazard regression of overall survival. (**B**) Cox proportional hazard regression of melanoma-specific survival. (**C**) Logistic regression of recurrence-free survival.

Variable	Univariable		Multivariable	
	HR	*P* value	HR	*P* value
(a) Cox proportional hazard regression of overall survival				
Regression				
No host response	1		1	<0.0001
Regression only	0.84 (0.71, 0.99)	<0.0001	1.03 (0.86, 1.23)	
TILs only	0.71 (0.64, 0.79)		0.78 (0.69, 0.87)	
Regression + TILs	0.60 (0.49, 0.73)		0.82 (0.66, 1.02)	
Gender				
Female	1		1	<0.0001
Male	1.62 (1.46, 1.80)	<0.0001	1.26 (1.13, 1.41)	
Age at diagnosis	1.05 (1.04, 1.05)	<0.0001	1.04 (1.03, 1.04)	<0.0001
Breslow thickness (mm)	1.11 (1.10, 1.12)	<0.0001	1.06 (1.05, 1.08)	<0.0001
Primary melanoma site				
Head and Neck	1		1	0.0097
Arm	0.63 (0.54, 0.74)	<0.0001	0.83 (0.70, 0.97)	
Leg	0.65 (0.57, 0.75)		0.80 (0.69, 0.94)	
Trunk	0.74 (0.65, 0.84)		0.97 (0.84, 1.11)	
Melanoma subtype				
SSM	1		1	0.0037
ALM	2.79 (2.14, 3.63)	<0.0001	1.47 (1.11, 1.96)	
DM	1.50 (1.26, 1.79)		0.78 (0.65, 0.95)	
LMM	1.58 (1.21, 2.06)		1.12 (0.84, 1.50)	
NM	1.97 (1.77, 2.20)		0.98 (0.87, 1.11)	
Other	1.56 (0.74, 3.29)		0.67 (0.30, 1.50)	
Ulceration				
No	1		1	<0.0001
Yes	2.80 (2.54, 3.09)	<0.0001	1.59 (1.42, 1.79)	
Mitoses (categorised) (n/mm^2)				
0	1		1	<0.0001
1-2	1.58 (1.31, 1.92)	<0.0001	1.45 (1.18, 1.79)	
3-4	2.48 (2.05, 3.02)		1.97 (1.59, 2.44)	
>=5	4.02 (3.39, 4.77)		2.35 (1.92, 2.89)	
(b) Cox proportional hazard regression of melanoma-specific survival				
Regression				
No host response	1		1	0.0003
Regression only	0.64 (0.51, 0.81)	<0.0001	0.93 (0.73, 1.19)	
TILs only	0.68 (0.59, 0.78)		0.74 (0.64, 0.86)	
Regression + TILs	0.44 (0.33, 0.59)		0.69 (0.51, 0.94)	
Gender				
Female	1		1	0.0008
Male	1.54 (1.35, 1.76)	<0.0001	1.28 (1.11, 1.48)	
Age at diagnosis	1.02 (1.01, 1.02)	<0.0001	1.01 (1.00, 1.01)	0.0018
Breslow thickness (mm)	1.12 (1.11, 1.13)	<0.0001	1.07 (1.06, 1.09)	<0.0001
Primary site				
Head and Neck	1		1	0.0003
Arm	0.53 (0.42, 0.66)	<0.0001	0.66 (0.52, 0.84)	
Leg	0.80 (0.66, 0.96)		0.88 (0.71, 1.08)	
Trunk	0.91 (0.77, 1.08)		1.05 (0.88, 1.27)	
Melanoma subtype				

Table 3 continued

SSM	1		1	<0.0001
ALM	3.36 (2.44, 4.61)	<0.0001	1.93 (1.37, 2.71)	
DM	1.13 (0.88, 1.47)		0.65 (0.50, 0.86)	
LMM	0.60 (0.34, 1.04)		0.69 (0.40, 1.22)	
NM	2.15 (1.87, 2.47)		0.95 (0.82, 1.11)	
Other	2.35 (1.05, 5.27)		1.04 (0.43, 2.53)	
Ulceration				
No	1		1	<0.0001
Yes	3.34 (2.94, 3.79)	<0.0001	1.78 (1.54, 2.06)	
Mitoses (categorised) (n/mm^2)				
0	1		1	<0.0001
1-2	3.24 (2.28, 4.61)	<0.0001	3.02 (2.04, 4.45)	
3-4	6.24 (4.40, 8.83)		5.05 (3.43, 7.45)	
>=5	10.09 (7.26, 14.01)		6.23 (4.26, 9.11)	

(c) Logistic regression of recurrence-free survival				
Regression				
No host response	1		1	<.0001
Regression only	0.70 (0.61, 0.81)	<0.0001	0.89 (0.76, 1.04)	
TILs only	0.70 (0.64, 0.77)		0.76 (0.69, 0.84)	
Regression + TILs	0.50 (0.42, 0.59)		0.71 (0.58, 0.85)	
Gender				
Female	1		1	0.0007
Male	1.46 (1.34, 1.60)	<0.0001	1.18 (1.07, 1.29)	
Age at diagnosis	1.04 (1.03, 1.04)	<0.0001	1.03 (1.02, 1.03)	<.0001
Breslow thickness (mm)	1.13 (1.12, 1.14)	<0.0001	1.08 (1.06, 1.09)	<.0001
Primary melanoma site				
Head and Neck	1		1	0.0008
Arm	0.60 (0.52, 0.68)	<0.0001	0.75 (0.66, 0.87)	
Leg	0.69 (0.62, 0.78)		0.89 (0.78, 1.01)	
Trunk	0.67 (0.60, 0.75)		0.85 (0.75, 0.95)	
Melanoma subtype				
SSM	1		1	0.0272
ALM	2.66 (2.10, 3.38)	<0.0001	1.26 (0.98, 1.63)	
DM	1.54 (1.33, 1.78)		0.81 (0.69, 0.95)	
LMM	1.40 (1.11, 1.76)		1.08 (0.84, 1.38)	
NM	2.08 (1.89, 2.28)		0.98 (0.89, 1.09)	
Other	1.62 (0.87, 3.02)		0.72 (0.37, 1.40)	
Ulceration				
No	1		1	<.0001
Yes	2.90 (2.66, 3.16)	<0.0001	1.59 (1.44, 1.75)	
Mitoses (categorised) (n/mm^2)				
0	1		1	<.0001
1-2	1.70 (1.45, 2.00)	<0.0001	1.51 (1.27, 1.80)	
3-4	2.79 (2.37, 3.29)		2.14 (1.79, 2.56)	
>=5	4.62 (3.99, 5.34)		2.64 (2.23, 3.14)	

HR hazard ratio, *TILs* tumour-infiltrating lymphocytes, *ALM* acral lentiginous melanoma, *DM* desmoplastic melanoma, *LMM* lentigo maligna melanoma, *NM* nodular melanoma, *SSM* superficial spreading melanoma.

viewpoint is supported by many clinical examples of melanoma in which there is clear clinical or dermatoscopic evidence of partial tumor loss and concurrent histological findings of regressive fibrosis with overlying melanoma in-situ and in some cases residual dermal invasive melanoma. Indeed, patients occasionally present with metastatic melanoma and are subsequently discovered to have melanoma in-situ with underlying dermal regression [35].
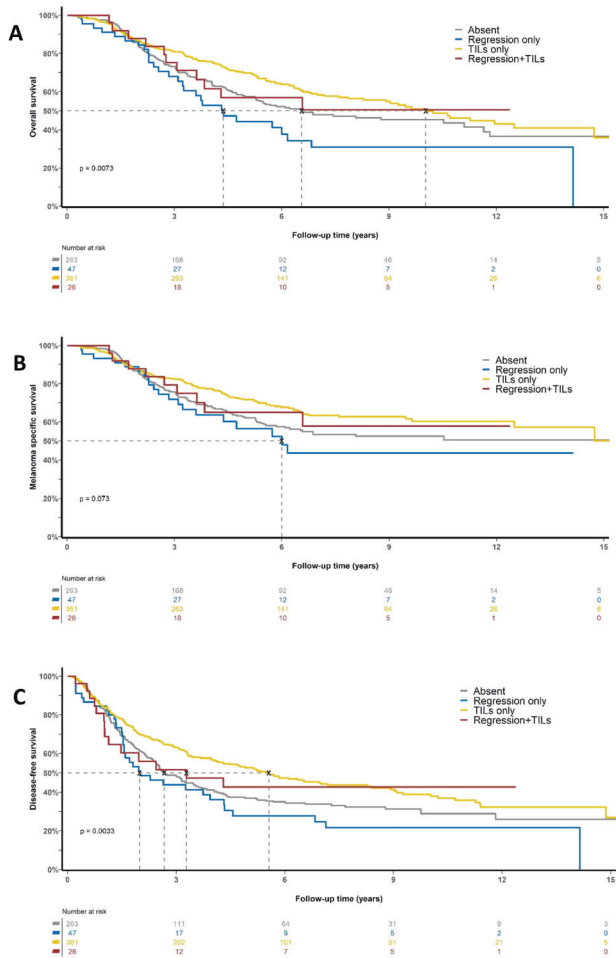
Fig. 3 Kaplan-Meier curves of survival in melanoma patients with lymph node metastases. (**A**), melanoma-specific survival (**B**), and recurrence-free survival (**C**) in sentinel lymph node-positive patients.

Today, the most commonly used definition of regression is complete or partial replacement of tumor cells by lymphocytic inflammation, as well as epidermal attenuation and nonlaminated dermal fibrosis accompanied by inflammatory cells, melanophages, and telangiectasia. This definition is in line with the College of American Pathologists (CAP) reporting protocol [36], which also recommends quantifying the amount of regression as less than or more than 75% of the lesion. Despite this, substantial variability persists in the literature [4]. Some studies do not specify how regression was defined, while others establish threshold values below which regression is considered absent. Additionally, interpretation of any given case is subjective, and it can sometimes be difficult to distinguish between late regression and biopsy-related scar or a non-regressive fibrous stromal response. Similarly, there is some histological overlap between TILs and the inflammatory infiltrate often observed in intermediate regression. Many authors did not control for the presence of TILs in their analyses, whereas others specifically excluded TILs as a criterion [28]. In fact, whether or not TILs equate with an early phase of regression is still a matter of debate. Kang et al [37] first described the three phases of regression, where the early phase was represented by a dense lymphocytic infiltrate admixed with nests of melanocytes. However, whilst a cytotoxic T cell-mediated antitumor response is thought to have a significant role in tumor regression, the process is complex and multifactorial. Most authors consider TILs alone insufficient for defining regression, as it is unclear whether the initiation of an immune response necessarily

results in effective clearing of the tumor cells [38]. Indeed, there is evidence that different T-cell immunophenotypes predominate in regressed versus non-regressed melanomas, such that the immune cell milieu in non-regressed tumors favors immune tolerance and suppression of the antitumor response [27]. Nevertheless, lymphocytes clearly have a vital role in the initiation of regression, as underscored by the clinical efficacy of immunotherapy-based treatments.

TILs represented the commonest form of host immune response observed in our cohort (present in 63% of all cases). In tumors lacking evidence of established regression, the presence of TILs independently predicted improved OS, MSS and RFS, consistent with the hypothesis that TILs reflect effective tumor clearance by the immune system in a proportion of cases. These results are in line with previous work from our institution [39], which showed that, in melanomas > 0.75 mm in thickness, higher TIL grades correlated with better MSS. The findings of that study corroborated previous reports of a similar nature [40]. More recent work has shown that applying a simple TIL percentage scoring system allows significant separation of survival curves, with higher TIL percentages predicting better OS, RFS and MSS [41]. A recent meta-analysis of 41 studies of TILs and their role in prognostication has confirmed the favorable association with survival [42].

Our sub-group analyses of intermediate vs late regression did not show significant differences in their prognostic effects. This may be because the two phases are essentially the same process observed at different time points in its evolution. In both intermediate and late regression, there has been a loss of neoplastic cells, inciting a host response of healing and repair. In addition, as regression is a dynamic process, there is a substantial degree of overlap between the two phases, leading to difficult distinction between them in some cases.

In our study, patients whose melanoma had spread to a SLN had worse survival outcomes if their primary tumor showed evidence of regression. Considering that regression predicted improved outcomes in the overall cohort, this was an unexpected finding. Interestingly, TILs retained their favorable prognostic effect in SLN-positive patients. One possible explanation for this observation is that the corresponding primary tumors with regression in patients with SLN metastases are inherently more aggressive, and metastasize despite the presence of regression. There are two possible mechanisms at play in this scenario. The first is that nodal spread occurs before the immune response is able to clear the primary tumor. Alternatively, the primary tumor may be genetically heterogeneous, such that a proportion of cells are cleared while the less immunogenic clones escape immune attack and seed to regional lymph nodes. Only one previous study has examined the prognostic effects of regression in Stage III patients [25], with findings that contradict ours. In that cohort of patients with positive SLNs, evidence of regression in the primary tumor independently predicted a lower death rate from melanoma (sub-HR 0.34; 95% C.I. 0.12–0.92). However, the authors of that paper did not control for the presence of TILs, which may have been the reason for the observed positive association with outcome. The interactions between tumor cells, various T cell subsets, and the tumor microenvironment are the subject of ongoing research that may enhance our understanding of these processes.

The main strength of this study is the cohort size; to our knowledge, it is the largest study to date examining the prognostic implications of histological regression in melanoma. A limitation is that many of the pathology reports were issued before widespread use of synoptic report templates. For these cases, data were extracted from free text and thus subject to interpretation. A further problem is potential interobserver variability. Kang et al. [37] noted that, despite rigid criteria, interobserver agreement was suboptimal when a three-tier classification of regression was used. Grouping intermediate and

late regression as one variable, as well as the single-institution study design and the power afforded by the large cohort size, should have ameliorated the impact of some of these issues. Finally, quantification of TIL density was not taken into account in this study. Whilst the effects of regression diameter and depth were analysed, no significant differences in survival were seen. It is possible that a more meaningful measure would be the relative extent of regression as a percentage of the tumor. Given mounting evidence that both TIL grade [39, 40] and percentage of fibrosis [27] may have discriminative value, there is potential for further research to address the impact of these parameters.

In conclusion, we found that the presence of histological regression in primary melanomas was associated with lower rates of SLN positivity and better survival outcomes. Of all the patterns of immune response, the combination of regression and TILs had the strongest protective effect on SLN status, MSS and RFS, even when controlling for other prognostic variables. Despite not being part of the AJCC staging system for cutaneous melanoma, the presence or absence of regression should form part of the minimal data set in melanoma synoptic report templates [43]. Furthermore, information about the regression status of the primary tumor may be useful to clinicians when deciding on optimal management strategies and should be evaluated as a parameter for possible inclusion in prognostic nomograms.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article and its supplementary information files.

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AUTHOR CONTRIBUTIONS

Conceptualization: KA, RAS; Data acquisition and interpretation: All authors; Project Administration and support: JFT, RAS; Supervision: RAS; Writing of Initial Draft of Manuscript: KA; Manuscript Review and Approval: All authors.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the ethical approval from the Sydney Local Health District Human Research Ethics Committee (Protocol No. X15-0454 and HREC/11/RPAH/444).

COMPETING INTERESTS

RAS has received fees for professional services from Evaxion, Provectus Biopharmaceuticals Australia, Qbiotics, Novartis, Merck Sharp & Dohme, NeraCare, AMGEN Inc., Bristol-Myers Squibb, Myriad Genetics, GlaxoSmithKline. JFT has received honoraria for advisory board participation from Bristol-Myers Squibb, Merck Sharp & Dohme Australia, GlaxoSmithKline and Provectus Inc, and travel support from GlaxoSmithKline and Provectus Inc. RPMS has received honoraria for advisory board participation from MSD, Novartis and Qbiotics and speaking honoraria from BMS. All other authors have no relevant disclosures.

ADDITIONAL INFORMATION

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