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A multicentre study of naevus-associated melanoma vs. *de novo* melanoma, tumour thickness and body site differences*

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Summary

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Conflicts of interest

J.E.G. has served as a consultant or advisory board member for Merck, Bristol Myers Squibb, Novartis, Syndax and Regeneron, unrelated to the content of this work. J.F.T. has received honoraria for advisory board participation from Bristol Myers Squibb Australia and MSD Australia. He has received honoraria and travel support from GlaxoSmithKline and Provectus Inc. R.A.S. has received fees for professional services from MSD Australia, GlaxoSmithKline Australia, Bristol Myers Squibb, Novartis, Myriad, NeraCare and

Background Whether melanoma in histological contiguity with a naevus [naevus-associated melanoma (NAM)] is distinctly different from melanoma arising *de novo* remains unclear.

Objectives To determine whether the characteristics of *de novo* melanoma differ from NAM and are not due to naevus obliteration in thicker tumours.

Methods We conducted a multicentre retrospective study of *de novo* melanoma and NAM in seven referral centres in Europe, Australia and the USA between 2006 and 2015.

Results In a total of 9474 localized melanomas, *de novo* melanoma was associated with thicker tumours and body site differences compared with NAM. In the subset of T₁ melanomas (n = 5307), similar body site differences were found in multivariate analysis by body site. When compared with NAM, *de novo* melanoma was more likely to affect older individuals (≥ 70 years) when located on the head/neck [odds ratio (OR) 4.65, 95% confidence interval (CI) 2.55–8.46], the trunk (OR 1.82, 95% CI 1.40–2.36) or the upper extremity (OR 1.69, 95% CI 1.14–2.50), was more likely to affect female patients when located on the lower extremities (OR 1.36, 95% CI 1.03–1.80), and was more likely to be of the nodular melanoma subtype (OR 2.23, 95% CI 1.14–4.35) when located on the trunk. *De novo* melanoma was less likely to have regression present compared with NAM.

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C.G. and A.J.S. contributed equally as senior authors.

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Conclusions Clinicopathological and body site differences between *de novo* melanoma and NAM support the divergent pathway model of development. These differences were also found in thin melanomas, suggesting that *de novo* melanomas are different from NAM and their differences are not due to the obliteration of naevus remnants in thicker tumours.

What is already known about this topic?

- Cutaneous melanoma appears *de novo* in approximately 70% of cases and has adjacent naevus remnants on histological examination [naevus-associated melanoma (NAM)] in the remaining 30% of cases.
- Whether melanoma arising *de novo* is a biological type distinct from NAM remains unclear.
- Robust conclusions on the differences between *de novo* melanoma and NAM have been hindered by the possible obliteration of naevus in thicker melanomas.

What does this study add?

- In 9474 patients with localized melanoma there were body site differences for *de novo* melanoma compared with NAM, supporting the divergent pathway model of melanoma development.
- In 5307 T₁ melanomas there were similar body site associations for *de novo* melanoma and older age at diagnosis, female sex, the nodular subtype and absence of regression.
- These findings suggest that *de novo* melanomas are different from NAM and the differences are not due to the obliteration of naevus remnants in thicker tumours.

Cutaneous melanoma has been reported to appear *de novo* in approximately 70% of cases, whereas it has an adjacent naevus remnant on histological examination in the remaining 30% of cases [naevus-associated melanoma (NAM)].¹ The interest in studying NAM stems from the possibility of naevi serving as precursor lesions in the genetic evolution of cutaneous melanomas, most being underpinned by BRAF^{V600E} or NRAS^{Q61} mutations.² Understanding the origins of melanoma may shed light on the biology of the tumour and assist in the design of prevention strategies.

Whether melanoma arising *de novo* is a biological type distinct from NAM remains unclear. It has been reported that melanoma located on the trunk is more likely to be NAM than *de novo* melanoma^{3,4} and more likely to harbour a BRAF mutation.^{5,6} The body site of melanoma as a proxy of sun-exposure patterns provides evidence in support of the divergent pathway model of melanoma development. One pathway described melanoma affecting body sites with considerable cumulative sun exposure (head/neck), in older individuals (> 55 years) with few concomitant melanocytic naevi, which were of the nodular melanoma (NM) subtype and associated with NRAS mutations. Another pathway described melanoma on areas with noncumulative sun exposure (trunk), in younger individuals with multiple concomitant naevi, that were of the superficial spreading melanoma (SSM) subtype, with more frequent BRAF mutations.^{7–9} Additional clinical, pathological and molecular data have accumulated and nine distinct pathways to melanoma have been described, defining

distinct melanoma subtypes.^{10,11} Robust conclusions on the characteristics of *de novo* melanoma and NAM have been hindered by the heterogeneity of studies including *in situ* and invasive melanomas, or not accounting for the stage at diagnosis, and the possible underestimation of NAM in thicker melanomas.^{12,13}

We conducted a large multicentre study of localized invasive melanomas, including a subgroup of thin melanomas, to investigate whether NAM has different epidemiological, clinical, histological and prognostic characteristics compared with *de novo* melanoma.

Patients and methods

Study patients

This was a retrospective study using the European Association of Dermato-Oncology (EADO) Melanoma Collaborative study database containing deidentified data for cases of invasive melanoma (as previously described)¹⁴ in a collaborative network within the 15 centres of the EADO. In addition, the Melanoma Institute Australia in Sydney, Australia, and the University of Texas MD Anderson Cancer Center (MD Anderson) in Houston, TX, USA, provided epidemiological, clinical, histological and survival data collected from 2006 to 2015. Cases included patients older than 16 years of age with a diagnosis of primary invasive cutaneous melanoma of the NM or SSM subtype. Melanomas *in situ* were not included. Institutional ethics

and/or review board approval was obtained by each participating centre.

Variables of interest

Variables of interest at initial diagnosis included patient age and sex, the anatomical site of the tumour, and histological characteristics including Breslow thickness, the histological presence of an associated naevus, ulceration, regression, NM or SSM subtype, presence of mitoses, and tumour spread (coded as localized melanoma only, or with satellite/in-transit, or regional nodal micrometastases or regional nodal macrometastases or distant metastasis. Only the highest classification was coded for each case). Only patients with localized primary melanoma at the time of diagnosis (i.e. with no satellite/in-transit, regional nodal or distant metastasis), and with data on the presence or absence of a histologically identified naevus remnant, were eligible for inclusion in this study. To obtain further confirmation of the correct classification of T₂–T₄ clinically node-negative melanomas as localized [as individual patient data for sentinel lymph node biopsy (SLNB) were not included in the Excel file provided], all centres confirmed the routine use of SLNB for all T₂–T₄ melanomas, except for Bucharest and a group of patients with T₄ melanoma from Turin, as previously published.¹⁵ All centres confirmed that they routinely report whether melanoma is *de novo* or naevus-associated as part of the histopathology report. Breslow thickness was recalculated to the nearest 0.1 mm according to the current 8th American Joint Committee on Cancer staging system.¹⁶ Survival data included status at last observation (alive or dead), follow-up duration, and melanoma-specific mortality. Complete data were available for naevus remnant, sex, age, melanoma subtype and Breslow thickness. Owing to a high rate in the variable of mitoses, (30.4%) this variable was not used in any multivariate analysis.

Statistical analysis

Characteristics associated with *de novo* melanoma and NAM were investigated in exploratory analyses using Student's *t*-test for continuous data and χ^2 -test or Fisher's exact test for categorical variables. The effect of greater Breslow thickness was explored in multinomial multivariate logistic regression analysis, comparing T₂, T₃ or T₄ cases with T₁ (reference) for the presence or absence of naevus remnants. As the divergent melanoma pathway model proposes different melanoma characteristics depending on the body site of melanoma, a multivariate logistic regression analysis stratified by the body site of the melanomas was carried out. Also, these analyses were conducted separately for T₁ melanomas.

The melanoma-specific survival (MSS) of *de novo* melanoma compared with NAM was investigated using the Kaplan–Meier estimator and the log-rank test. Patient survival time was calculated as the time from the date of the primary tumour diagnosis to the date of melanoma-related death or last follow-up visit. A shared frailty model was used to assume that the proportional hazards assumption holds conditional on an

unobserved cluster centre-specific random effect for the seven centres.¹⁷ All P-values were two-sided and the significance level was < 0.05. Analyses were carried out using Stata, version 13 (StataCorp, College Station, TX, USA).

Results

Participating centres and included cases

Among 17 centres participating in the EADO Melanoma Collaboration, seven centres had fewer than 15% of cases with a missing value for the naevus remnant variable of interest and only patients from these centres were eligible for inclusion in this study (Tübingen, Kiel, Zurich, Coimbra, Graz, Sydney and MD Anderson). Among the 10 366 eligible patients with localized melanoma, 892 (8.6%) were excluded owing to missing naevus remnant data, leaving a total of 9474 patients with localized melanoma (Figure 1).

De novo melanoma was found in 70.3% of cases, while NAM was present in 29.7%. Sydney (Australia) had significantly higher rates of NAM than EADO (Europe) and MD Anderson (USA) (Figure 1), which were present across all age categories. Case-control studies previously reported that Australians had about three times as many naevi as those living in the UK.^{18,19} When the effect of the centre (Australia, Europe, USA) was included in the analyses in order to account for the absence of information on naevus counts, there were no differences in the logistic regression results and the models without the centre variable were selected.

The proportion of naevus-associated melanoma differs with Breslow thickness

The proportions of *de novo* melanoma and NAM were significantly different when stratified by Breslow thickness. For example, for T₁ melanomas, 65.3% (*n* = 3465) were *de novo* melanomas and 34.7% (*n* = 1842) were NAM, whereas for T₄ melanomas, 87.4% (*n* = 599) were *de novo* melanomas and 12.6% (*n* = 86) were NAM (*P* < 0.001) (Table 1).

In multinomial multivariate logistic regression analysis, after adjustment for confounders (age, sex, ulceration, regression, subtype), *de novo* melanoma was associated with thicker tumours compared with NAM; *de novo* melanoma was more likely to be T₃ [odds ratio (OR) 1.36, 95% confidence interval (CI) 1.13–1.62] or T₄ (OR 1.78, 95% CI 1.35–2.36) vs. T₁ (reference), whereas there was no association of naevus remnants for T₂ vs. T₁ melanomas (Table S1; see Supporting Information).

Clinical and histological characteristics of naevus-associated melanoma

Of 9474 localized melanomas, there were 6657 cases of *de novo* melanoma (70.3%) and 2817 cases of NAM (29.7%). Logistic regression analysis adjusted for age and sex was performed. Compared with patients < 40 years of age, *de novo* melanoma (vs. NAM) was more likely to affect patients aged 60 years and

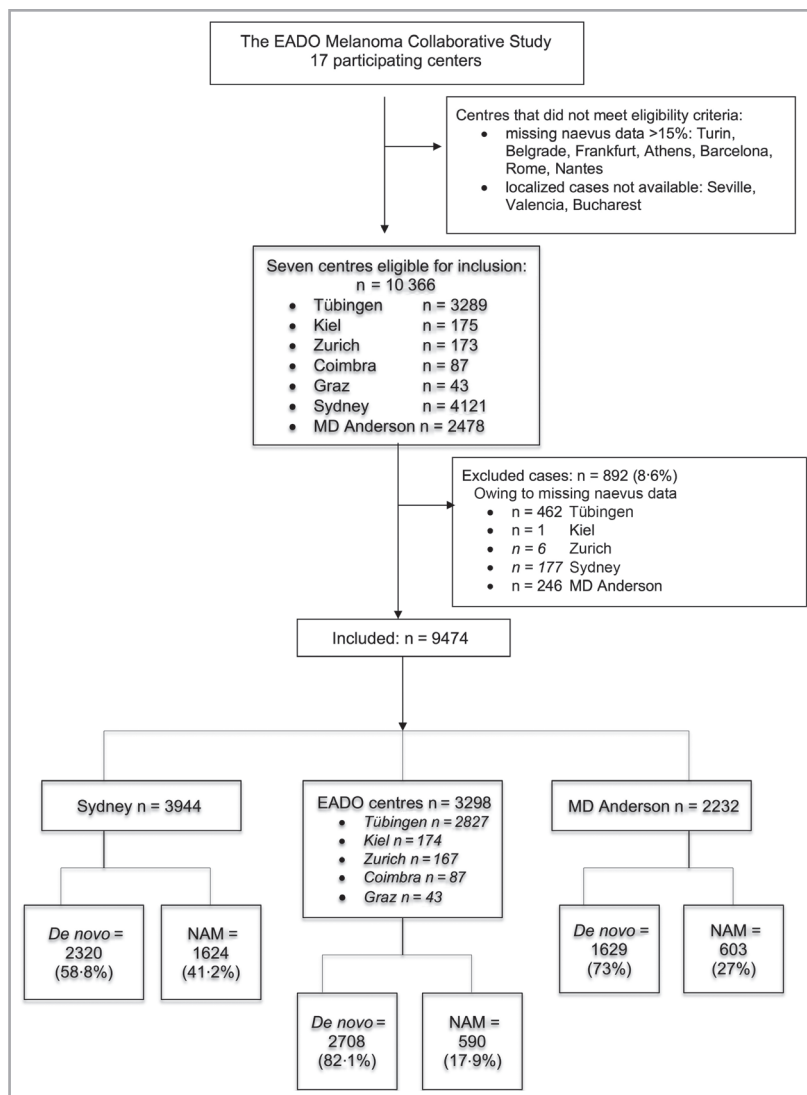


Figure 1 Flowchart of patients with localized invasive melanoma who were included in the study. EADO, European Association of Dermato-Oncology; NAM, naevus-associated melanoma.

older [age 60–69 years (OR 1.62, 95% CI 1.40–1.87) and for age ≥ 70 years (OR 2.39, 95% CI 2.06–2.78)] and female patients (OR 1.28, 95% CI 1.17–1.40). *De novo* melanoma was more likely than NAM to be located on the head/neck (OR 1.57, 95% CI 1.35–1.82) or the upper (OR 1.48, 95% CI 1.31–1.67) or lower extremity (OR 1.93, 95% CI 1.71–2.18) compared with the trunk. Thicker melanomas were more likely to be *de novo* melanoma vs. NAM. In addition, *de novo* melanoma was more likely to be associated with the presence of ulceration (OR 1.82, 95% CI 1.58–2.10) and NM (vs. SSM) subtype (OR 2.36, 95% CI 2.07–2.68), while it was less likely to have histological regression present (OR 0.59, 95% CI 0.53–0.66) (Table 1).

Clinical and histological characteristics of naevus-associated melanoma in thin (T_1) melanomas

To address whether *de novo* melanoma has truly different characteristics from NAM, and is not a result of naevus obliteration

in thicker tumours, a subgroup study of T_1 melanoma ($n = 5307$) was conducted. In logistic regression analysis adjusted for age and sex, the differences between *de novo* and NAM persisted. Compared with patients < 40 years of age, *de novo* melanoma (vs. NAM) was more likely to affect patients aged 60 years and older [for age 60–69 years, OR 1.38 (95% CI 1.15–1.65) and for age ≥ 70 years, OR 1.90 (95% CI 1.56–2.33)] and female patients (OR 1.37, 95% CI 1.22–1.54). *De novo* melanoma was more likely than NAM to be located on the upper (OR 1.29, 95% CI 1.11–1.50) or lower extremity (OR 2.07, 95% CI 1.77–2.43) compared with the trunk. Compared with NAM, *de novo* melanoma was less likely to have a thickness of 0.8–1.0 mm than a thickness of < 0.8 mm (OR 0.87, 95% CI 0.77–0.99) or to have regression present (OR 0.66, 95% CI 0.58–0.75). There was no association of the presence of a naevus remnant with ulceration or melanoma subtype in thin melanomas (Table S2; see Supporting Information).

Table 1 Clinical and histological characteristics of localized *de novo* melanoma and naevus-associated melanoma (NAM) (n = 9474)

	De novo melanoma (n = 6657, 70.3%), n (%)	NAM (n = 2817, 29.7%), n (%)	Total (n = 9474), n (%)	P-values	Unadjusted OR (95% CI) for de novo melanoma vs. NAM	P-values	Age- and sex- adjusted OR (95% CI) for de novo melanoma vs. NAM	P-values
Age, years								
< 40	905 (13.6)	528 (18.7)	1433 (15.1)	< 0.001 ^a	Ref		Ref	
40–49	1205 (18.1)	631 (22.4)	1836 (19.4)		1.11 (0.96–1.29)	0.142	1.14 (0.99–1.32)	0.068
50–59	1269 (19.1)	614 (21.8)	1883 (19.9)		1.21 (1.04–1.39)	0.011	1.26 (1.09–1.46)	0.002
60–69	1498 (22.5)	579 (20.6)	2077 (21.9)		1.51 (1.31–1.74)	< 0.001	1.62 (1.40–1.87)	< 0.001
≥ 70	1780 (26.7)	465 (16.5)	2245 (23.7)		2.23 (1.93–2.59)	< 0.001	2.39 (2.06–2.78)	< 0.001
Sex								
Male	3567 (53.6)	1612 (57.2)	5179 (54.7)	0.001 ^a	Ref		Ref	
Female	3090 (46.4)	1205 (42.8)	4295 (45.3)		1.16 (1.06–1.27)	0.001	1.28 (1.17–1.40)	< 0.001
Body site of melanoma								
Trunk	2540 (38.2)	1453 (51.6)	3993 (42.2)	< 0.001 ^a	Ref		Ref	
Head/neck	864 (13.0)	294 (10.4)	1158 (12.2)		1.68 (1.45–1.95)	< 0.001	1.57 (1.35–1.82)	< 0.001
Upper extremity	1527 (22.9)	561 (19.9)	2088 (22.0)		1.56 (1.39–1.75)	< 0.001	1.48 (1.31–1.67)	< 0.001
Lower extremity	1713 (25.7)	506 (18.0)	2219 (23.4)		1.94 (1.72–2.18)	< 0.001	1.93 (1.71–2.18)	< 0.001
Missing values	13 (0.2)	3 (0.1)	16 (0.2)					
Breslow thickness, mm (T category)								
≤ 1.0 (T ₁)	3465 (52.1)	1842 (65.4)	5307 (56.0)	< 0.001 ^a	Ref		Ref	
1.1–2.0 (T ₂)	1479 (22.2)	627 (22.3)	2106 (22.2)		1.25 (1.12–1.40)	< 0.001	1.21 (1.09–1.35)	0.001
2.1–4.0 (T ₃)	1114 (16.7)	262 (9.3)	1376 (14.5)		2.26 (1.95–2.62)	< 0.001	2.05 (1.76–2.38)	< 0.001
> 4.0 (T ₄)	599 (9.0)	86 (3.1)	685 (7.2)		3.70 (2.93–4.67)	< 0.001	3.24 (2.56–4.11)	< 0.001
Ulceration								
Present	1215 (18.3)	278 (9.9)	1493 (15.8)	< 0.001 ^a	2.04 (1.77–2.34)	< 0.001	1.82 (1.58–2.10)	< 0.001
Absent	5368 (80.6)	2500 (88.8)	7868 (83.1)		Ref		Ref	
Missing values	74 (1.1)	39 (1.4)	113 (1.2)					
Regression								
Present	1357 (20.4)	818 (29.0)	2175 (23.0)	< 0.001 ^a	0.62 (0.56–0.68)	< 0.001	0.59 (0.53–0.66)	< 0.001
Absent	5187 (77.9)	1926 (68.4)	7113 (75.1)		Ref		Ref	
Missing values	113 (1.7)	73 (2.6)	186 (2.0)					
Melanoma subtype								
NM	1678 (25.2)	330 (11.7)	2008 (21.2)	< 0.001 ^a	2.54 (2.24–2.89)	< 0.001	2.36 (2.07–2.68)	< 0.001
SSM	4979 (74.8)	2487 (88.3)	7466 (78.8)		Ref		Ref	

OR, odds ratio; CI, confidence interval; NM, nodular melanoma; SSM, superficial spreading melanoma. ^aP-values were calculated using a two-sided χ^2 -test.

Distribution of naevus-associated melanoma according to histological subtype and primary tumour body sites

Although SSM subtype was the most frequent subtype in all cases, NM was more common in *de novo* melanoma vs. NAM across all body sites ($P < 0.001$); in particular, 43.9% of *de novo* melanomas on the head/neck were NM (Table 2).

A multivariate logistic analysis stratified by anatomical site was carried out. There were differences in the characteristics associated with *de novo* melanoma vs. NAM by body site, supporting an underlying naevus-associated divergent melanoma pathway model. In older individuals (≥ 70 years), the melanoma was 4.43 times more likely to be *de novo* melanoma when located on the head/neck (OR 4.43, 95% CI 2.98–6.59), and 1.86 times more likely when located on the trunk (OR 1.86, 95% CI 1.52–2.27), compared with patients < 50 years of age. *De novo* melanoma was more likely

to occur in female patients (vs. male patients) only for melanomas located on the lower extremities (OR 1.41, 95% CI 1.14–1.74). Regression was associated with a lower likelihood of *de novo* melanoma for melanomas located on the trunk, upper or lower extremities. *De novo* melanoma was more likely to be of the NM subtype (vs. SSM) for melanomas located on the head/neck, trunk or lower extremities (Table 3).

Distribution of naevus-associated melanoma according to primary tumour body sites in T₁ melanomas

To address the controversy regarding whether *de novo* melanoma has truly different characteristics from NAM, and is not a result of naevus obliteration in thicker tumours, we analysed the subset of T₁ lesions (n = 5143) in multivariate analysis by body site.

Table 2 Distribution of *de novo* melanoma vs. naevus-associated melanoma (NAM) by melanoma subtype and primary tumour body site

	De novo	NAM	P-values ^a
Head/neck (n = 1158)			
NM	379 (43.9)	57 (19.4)	< 0.001
SSM	485 (56.1)	327 (80.6)	
Trunk (n = 3993)			
NM	535 (21.1)	135 (9.3)	< 0.001
SSM	2005 (78.9)	1318 (90.7)	
Upper extremity (n = 2088)			
NM	431 (28.2)	80 (14.3)	< 0.001
SSM	1096 (71.8)	481 (85.7)	
Lower extremity (n = 2219)			
NM	330 (19.3)	57 (11.3)	< 0.001
SSM	1383 (80.7)	449 (88.7)	

NM, nodular melanoma; SSM, superficial spreading melanoma.
^aP-values were calculated using a two-sided χ^2 -test for each comparison by body site.

In older individuals (≥ 70 years) *de novo* melanoma was more likely than NAM when located on the head/neck (OR 4.65, 95% CI 2.55–8.46), on the trunk (OR 1.82, 95% CI 1.40–2.36), or on the upper extremity (OR 1.69, 95% CI 1.14–2.50), compared with patients < 50 years of age. *De novo* melanoma was more likely to occur in female patients (vs. male patients) only for melanomas located on the lower extremities (OR 1.36, 95% CI 1.03–1.80). Breslow thickness

was an independent factor associated with *de novo* melanoma only for melanomas located on the head/neck or trunk. Regression was associated with a lower likelihood of *de novo* melanoma for melanomas located on the trunk (OR 0.77, 95% CI 0.64–0.92), upper extremities (OR 0.65, 95% CI 0.48–0.88) or lower extremities (OR 0.49, 95% CI 0.36–0.66). *De novo* melanoma was more likely to be of the NM subtype (vs. SSM) (OR 2.23, 95% CI 1.14–4.35) only for melanomas located on the trunk (Table 4).

Melanoma-specific survival for *de novo* melanoma compared with naevus-associated melanoma

The median follow-up was 32 months. MSS was not significantly different for *de novo* melanoma vs. NAM (3-year MSS, 96.4% vs. 97.3% and 5-year MSS 93.1% vs. 93.9%, respectively; $P = 0.091$) (Figure S1; see Supporting Information). Using Cox regression for survival analysis, MSS was not different for *de novo* melanoma vs. NAM after adjusting for age, sex, Breslow thickness, ulceration and frailty for centre (hazard ratio 1.02, 95% CI 0.80–1.31). The same results were noted after stratification analysis by melanoma body site, by melanoma thickness and by age (Table S3; see Supporting Information).

Discussion

This large multicentre study provides evidence that *de novo* melanomas (without histological presence of naevus) have

Table 3 Multivariate analysis for characteristics associated with localized *de novo* vs. naevus-associated melanoma, stratified by body site (n = 9179)

	Head/neck (n = 1127)	Trunk (n = 3878)	Upper extremity (n = 2023)	Lower extremity (n = 2151)
Age, years				
< 50	Ref	Ref	Ref	Ref
50–59	1.52 (1.01–2.28)	1.19 (0.99–1.42)	1.34 (1.01–1.77)	0.87 (0.66–1.15)
60–69	2.38 (1.57–3.60)	1.75 (1.45–2.10)	1.26 (0.96–1.66)	0.90 (0.68–1.20)
≥ 70	4.43 (2.98–6.59)	1.86 (1.52–2.27)	1.79 (1.33–2.40)	1.15 (0.85–1.56)
Sex				
Male	Ref	Ref	Ref	Ref
Female	1.03 (0.75–1.41)	1.08 (0.93–1.25)	1.18 (0.96–1.45)	1.41 (1.14–1.74)
Breslow thickness (mm)				
≤ 1.0	Ref	Ref	Ref	Ref
1.1–2.0	1.05 (0.72–1.55)	0.93 (0.78–1.11)	1.41 (1.08–1.83)	0.81 (0.63–1.05)
2.1–4.0	1.30 (0.80–2.12)	1.28 (0.98–1.68)	1.88 (1.27–2.78)	1.19 (0.78–1.79)
> 4.0	1.78 (0.93–3.40)	1.61 (1.06–2.43)	3.38 (1.66–6.91)	1.30 (0.67–2.54)
Ulceration				
Absent	Ref	Ref	Ref	Ref
Present	1.80 (1.14–2.83)	1.04 (0.81–1.34)	1.14 (0.79–1.63)	1.40 (0.96–2.05)
Regression				
Absent	Ref	Ref	Ref	Ref
Present	1.00 (0.68–1.48)	0.72 (0.63–0.84)	0.75 (0.58–0.95)	0.60 (0.47–0.78)
Melanoma subtype				
SSM	Ref	Ref	Ref	Ref
NM	1.85 (1.23–2.80)	1.87 (1.46–2.40)	1.31 (0.94–1.81)	1.67 (1.15–2.42)

NM, nodular melanoma; SSM, superficial spreading melanoma. Multivariate analysis adjusted for all variables included in the table. Data are presented as odds ratio (95% confidence interval). Statistically significant results are presented in bold.

Table 4 Multivariate analysis for characteristics associated with localized T₁ *de novo* melanoma vs. naevus-associated melanoma, stratified by body site (n = 5143)

	Head/neck (n = 452)	Trunk (n = 2358)	Upper extremity (n = 1085)	Lower extremity (n = 1248)
Age, years				
< 50	Ref	Ref	Ref	Ref
50–59	1.19 (0.71–2.01)	1.02 (0.82–1.27)	1.24 (0.88–1.73)	0.86 (0.62–1.20)
60–69	2.99 (1.56–5.78)	1.49 (1.19–1.87)	1.18 (0.84–1.66)	0.86 (0.60–1.24)
≥ 70	4.65 (2.55–8.46)	1.82 (1.40–2.36)	1.69 (1.14–2.50)	1.45 (0.93–2.28)
Sex				
Male	Ref	Ref	Ref	Ref
Female	0.96 (0.63–1.47)	1.13 (0.94, 1.35)	1.03 (0.79–1.35)	1.36 (1.03–1.80)
Breslow thickness (mm)				
< 0.8	Ref	Ref	Ref	Ref
0.8–1.00	1.64 (1.05–2.57)	0.73 (0.60–0.88)	0.81 (0.61–1.07)	0.92 (0.68–1.24)
Ulceration				
Absent	Ref	Ref	Ref	Ref
Present	1.05 (0.37–3.00)	0.87 (0.47, 1.61)	1.05 (0.51–2.17)	1.91 (0.72–5.07)
Regression				
Absent	Ref	Ref	Ref	Ref
Present	1.19 (0.73–1.94)	0.77 (0.64–0.92)	0.65 (0.48–0.88)	0.49 (0.36–0.66)
Melanoma subtype				
SSM	Ref	Ref	Ref	Ref
NM	2.45 (0.78–7.69)	2.23 (1.14–4.35)	0.74 (0.38–1.47)	0.73 (0.32–1.67)

NM, nodular melanoma; SSM, superficial spreading melanoma. ^aMultivariate analysis adjusted for all variables included in the table. Data are presented as odds ratio (95% confidence interval). Statistically significant results are presented in bold characters.

different characteristics from melanomas arising in histological association with naevi (NAM), supporting the divergent pathway models of melanoma development. In our study, *de novo* melanoma was more likely than NAM to be associated with older age at diagnosis, female sex, thicker tumours, ulceration, NM subtype (compared with SSM subtype), and location on the head/neck or extremities compared with the trunk, whereas it was less likely to have regression present. Similar differences were present also in T₁ melanomas, suggesting that *de novo* melanoma is a distinct subtype compared with NAM. The inverse relationship of age with NAM may be due to an age-related involution of naevi³ or different age-related pathogenetic mechanisms.^{20,21} Previous reports have shown an association of *de novo* melanoma with the NM subtype,²² and suggested that naevi tend to give rise to the SSM subtype.^{3,5,6,23–26}

In our study, 29.7% of melanomas were histologically associated with naevi, conforming with the rate of 29.1% reported in a recent meta-analysis.¹ Also, we found that, compared with NAM, *de novo* melanoma was independently associated with thicker tumours, as previously reported.²² This finding has led to some controversy regarding whether differences between *de novo* melanoma and NAM may be attributed to the obliteration of the naevus component by the tumour, thus leading to an underestimation of NAM in thicker melanomas.^{27,28}

To address this controversy, we studied T1 melanomas separately. In our large subset of thin melanomas comprising 5307 cases, we confirmed that there were body site differences for *de novo* melanoma compared with NAM; *de novo* melanoma was more likely to affect older patients when located on

the head/neck or on the trunk, more likely to affect female patients only for melanomas on the lower extremities, and more likely to be of the NM subtype when located on the trunk, whereas it was less likely to have regression present. A cancer registry study reported that melanoma occurred more frequently on the lower limbs in women than in men in all populations at all ages, and suggested sex-specific implications in the melanoma divergent pathway hypothesis.²⁹ These results underscore the need to study *de novo* melanoma and NAM taking the body site of melanoma into account, in order to draw robust conclusions on the various characteristics associated with naevus remnants in the divergent pathways of melanoma development. To our knowledge, there is only one other study that investigated characteristics of NAM stratified by body site and it did not show any differences for NAM vs. *de novo* melanoma; however, it was noted that the analysis lacked statistical power owing to the limited number of NAM cases included.³⁰ The body site of melanoma may play a role in the pathways of melanomagenesis. Transformation of naevi to melanoma (NAM) may occur more commonly in non-chronically sun-damaged (non-CSD) skin, such as on the trunk and extremities.^{9,23} Furthermore, Bastian and colleagues have proposed that melanomas arising in CSD skin and non-CSD skin are fundamentally different, based on different mutational profiles.^{9–11,31,32}

In our study, when compared with NAM, *de novo* melanoma was not associated with a higher risk for melanoma-specific death. There are reports of no differences in MSS for NAM compared with *de novo* melanoma,^{33,34} whereas in the study by Cymerman et al. *de novo* melanoma was an independent factor

predicting worse overall survival in one of the two patient cohorts studied.²² A possible key link between NAM and survival may lie in the number of melanocytic naevi, which may warrant further study. Higher total naevus counts have been associated with NAM compared with *de novo* melanoma.^{30,34,35} Naevus counts have been associated with melanoma on occasionally sun-exposed body sites (trunk and legs)³⁶ and Ribero *et al.* found that higher naevus counts were associated with higher MSS.³⁷

There are limitations to our study. Firstly, our study included SSM and NM subtypes based on a previous study that investigated the characteristics of NM.¹⁴ Therefore, our results should not be extrapolated to other melanoma subtypes. Nevertheless, SSM, NM and lentigo maligna melanoma (LMM) represent the vast majority of invasive melanoma subtypes, and LMM is rarely associated with a naevus. Secondly, individual patient data on SLNB for pathological staging for T₂–T₄ cases were not included in the database in order to verify the classification of localized melanomas provided by participating centres; however, nearly all centres confirmed that they had performed SLNB for all T₂–T₄ cases. Thirdly, the absence of survival differences between *de novo* melanoma and NAM in localized melanomas should be interpreted with caution, taking into account the retrospective study design and the short median follow-up of 32 months, which may limit the value and generalizability of the results. Fourthly, details of patient sun exposure and phenotypic characteristics were not collected. Sun-exposure patterns have been associated with risk of melanoma on different body sites³⁸ and our analyses were performed separately for body site, which may serve as a proxy for patterns of sun exposure. A considerable strength of our study is the large number of invasive localized melanomas, which allowed for the analysis of melanoma stratified by body site. Also, the large subset of thin (T₁) melanomas was analysed as a surrogate for the possibility of the obliteration of naevus components in thicker melanomas.

In conclusion, our large retrospective study reports clinicopathological and body site differences between *de novo* melanoma and NAM, which were also found in thin melanomas. These findings suggest that *de novo* melanomas are different from NAM and their differences are not due to the obliteration of naevus remnants in thicker melanomas. Understanding the causes of these differences may further elucidate the biology of the tumours.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Characteristics of thicker melanoma compared with thin melanoma for localized melanomas analysed using multivariate multinomial logistic regression analysis ($n = 9194$).

Table S2 Clinical and histological characteristics of localized T₁ de novo melanoma and naevus-associated melanoma (NAMs) ($n = 5307$).

Table S3 Cox multivariate regression analysis for the risk of melanoma-specific death for localized de novo melanoma vs. naevus-associated melanoma (NAM).

Figure S1 Kaplan–Meier plots of melanoma-specific survival for de novo melanoma and naevus-associated melanoma (NAM).