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Pembrolizumab versus placebo as adjuvant therapy in resected stage IIB or IIC melanoma: Outcomes in histopathologic subgroups from the randomized, double-blind, phase 3 KEYNOTE-716 trial

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ABSTRACT

Background Adjuvant pembrolizumab significantly improved recurrence-free survival (RFS) and distant metastasis-free survival (DMFS) versus placebo in the phase 3 KEYNOTE-716 study of resected stage IIB or IIC melanoma. At the prespecified third interim analysis (data cut-off, January 4, 2022), the HR for RFS in the overall population was 0.64 (95% CI, 0.50 to 0.84) and the HR for DMFS was 0.64 (95% CI, 0.47 to 0.88). We present a post hoc analysis of efficacy by subtypes defined by histopathologic characteristics.

Methods Patients aged ≥12 years with newly diagnosed, resected stage IIB or IIC melanoma were randomly assigned (1:1) to pembrolizumab 200 mg every 3 weeks (2 mg/kg up to 200 mg for pediatric patients) or placebo. The primary end point was RFS per investigator review; DMFS per investigator review was secondary. Subgroups of interest were melanoma subtype (nodular vs non-nodular), tumor thickness (≤4 mm vs >4 mm), presence of ulceration (yes vs no), mitotic rate (<5 per mm^2 (median) vs ≥5 per mm^2), and presence of tumor-infiltrating lymphocytes (TILs; absent vs present).

Results Between September 23, 2018, and November 4, 2020, 976 patients were assigned to pembrolizumab (n=487) or placebo (n=489). Median follow-up was 27.4 months (range, 14.0–39.4). The HR (95% CI) for RFS was 0.54 (0.37 to 0.79) for nodular and 0.77 (0.53 to 1.11) for non-nodular melanoma; 0.57 (0.37 to 0.89) for thickness ≤4 mm and 0.69 (0.50 to 0.96) for >4 mm; 0.66 (0.50 to 0.89) for ulceration and 0.57 (0.32 to 1.03) for no ulceration; 0.57 (0.35 to 0.92) for mitotic rate <5 per mm^2 and 0.57 (0.40 to 0.80) for ≥5 per mm^2; and 0.89 (0.52 to 1.54) for TILs absent and 0.51 (0.34 to 0.76) for TILs present. DMFS results were similar. In a Cox multivariate analysis, treatment arm, tumor thickness, and mitotic rate were significant independent factors for RFS, and treatment arm and mitotic rate were significant independent factors for DMFS.

Conclusions In this post hoc analysis, the benefit of pembrolizumab was largely consistent with the overall study population regardless of histopathologic

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Adjuvant pembrolizumab was shown to significantly improve recurrence-free survival and distant metastasis-free survival compared with placebo in patients with completely resected stage IIB or IIC cutaneous melanoma in the phase 3 KEYNOTE-716 study. Although these results generally support the use of adjuvant pembrolizumab for patients with stage IIB or IIC melanoma, outcomes can vary depending on the histopathologic features of an individual's disease.

WHAT THIS STUDY ADDS

⇒ The results of this post hoc analysis of KEYNOTE-716 showed that the benefit of adjuvant pembrolizumab was largely consistent across histopathologic subgroups, including melanoma subtype, tumor thickness, presence of ulceration, mitotic rate, and presence of tumor-infiltrating lymphocytes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this study add to the body of evidence supporting the use of pembrolizumab as adjuvant therapy for patients with resected stage IIB or IIC melanoma.

characteristics. These results support the use of adjuvant pembrolizumab in patients with resected stage IIB or IIC melanoma.
Trial registration number ClinicalTrials.gov, NCT03553836.

BACKGROUND

Historically, the standard of care for stage II melanoma following resection was observation or participation in a clinical trial of adjuvant therapy. However, patients with stage IIB or IIC melanoma are at high risk of recurrence, with survival outcomes similar to patients with stage IIIA and IIIB disease.[1–3] Based on the results of the phase 3 KEYNOTE-716 study, the programmed death 1 (PD-1) inhibitor pembrolizumab is now approved for the adjuvant treatment of pathologically staged IIB or IIC melanoma.[4 5] The results from the first interim analysis of KEYNOTE-716 showed that pembrolizumab significantly improved recurrence-free survival (RFS) compared with placebo in patients with resected stage IIB or IIC melanoma (HR, 0.65 (95% CI, 0.46 to 0.92); p=0.0066), a result which was sustained at the second interim analysis.[4] At the third interim analysis, adjuvant pembrolizumab was also shown to significantly improve distant metastasis-free survival (DMFS) compared with placebo (HR, 0.64; 95% CI, 0.47 to 0.88; p=0.0029) and continued to show an improvement in RFS (HR, 0.64; 95% CI, 0.50 to 0.84).[6] Pembrolizumab also had a manageable safety profile that was consistent with prior reports for pembrolizumab monotherapy and was not associated with a decline in health-related quality of life.[4 6 7]

While these results support the use of adjuvant pembrolizumab for patients with stage IIB or IIC melanoma, outcomes can vary depending on the histopathologic features of an individual's disease. Some of these features, such as tumor thickness and ulceration, are reflected in the American Joint Committee on Cancer (AJCC) 8th edition melanoma staging system.[8] However, there are other histopathologic parameters that are associated with poor outcome, including high mitotic rate, an absence of tumor-infiltrating lymphocytes (TILs), and having a more aggressive subtype such as nodular melanoma.[9 10] To further characterize the efficacy of adjuvant pembrolizumab in stage IIB and IIC melanoma, we present a post hoc subgroup analysis of efficacy among patients in KEYNOTE-716 by histopathologic features of interest, including tumor thickness, presence of ulceration, mitotic rate, melanoma subtype, and presence of TILs.

Table 1 Baseline characteristics

	Pembrolizumab n=487	Placebo n=489
Age, median (IQR), years	60 (51–68)*	61 (53–69)†
Sex		
Male	300 (61.6)	289 (59.1)
Female	187 (38.4)	200 (40.9)
Cancer stage‡		
IIA	1 (0.2)	0
IIB	309 (63.4)	316 (64.6)
IIC	171 (35.1)	169 (34.6)
IIIC	4 (0.8)	1 (0.2)
IV	0	2 (0.4)
Missing	2 (0.4)	1 (0.2)
Melanoma subtype		
Nodular	232 (47.6)	241 (49.3)
Superficial spreading	103 (21.1)	94 (19.2)
Acral	23 (4.7)	28 (5.7)
Lentiginous	25 (5.1)	18 (3.7)
Desmoplastic	20 (4.1)	21 (4.3)
Unknown	21 (4.3)	21 (4.3)
Other	63 (12.9)	66 (11.2)
Tumor thickness		
≤4 mm	202 (41.5)	201 (41.1)
>4 mm	285 (58.5)	288 (58.9)
Ulceration		
Yes	373 (76.6)	373 (76.3)
No	114 (23.4)	116 (23.7)
Mitotic rate, median (range), per mm^2	5.0 (0–67)§	5.0 (0–38)§
<5 per mm^2	186 (38.2)	198 (40.5)
≥5 per mm^2	245 (50.3)	223 (45.6)
Missing/unknown	56 (11.5)	68 (13.9)
Tumor-infiltrating lymphocytes		
Absent	97 (19.9)	118 (24.1)
Present	220 (45.2)	203 (41.5)
Brisk	58 (11.9)	53 (10.8)
Non-brisk	162 (33.3)	150 (30.7)
Unknown	170 (34.9)	168 (34.4)

Data are n (%) unless otherwise specified.
*Age ranged from 16 to 84 years. One (0.2%) patient was aged <18 years.
†Age ranged from 17 to 87 years. One (0.2%) patient was aged <18 years.
‡Enrollment of patients with IIA, IIIC, and IV disease was recorded as a protocol deviation.
§Mitotic rate of "0/mm^2" indicates that mitosis was not detected.

METHODS

Study design and patients

The design of the randomized, double-blind, phase 3 KEYNOTE-716 study has been reported previously.[4 6] The study was conducted at 160 centers in 16 countries. Eligible patients were aged 12 years or older and had newly diagnosed and completely resected stage IIB (T3b or T4a) or IIC (T4b) cutaneous melanoma per AJCC 8th edition criteria.[8] A negative sentinel lymph node biopsy was required, with no evidence of regional (N0) or distant metastatic disease (M0). Patients could not have received prior treatment for melanoma beyond complete resection. Full eligibility criteria are available in the protocol.[4]

The study was conducted in accordance with principles of Good Clinical Practice and was approved by the appropriate institutional review boards and regulatory

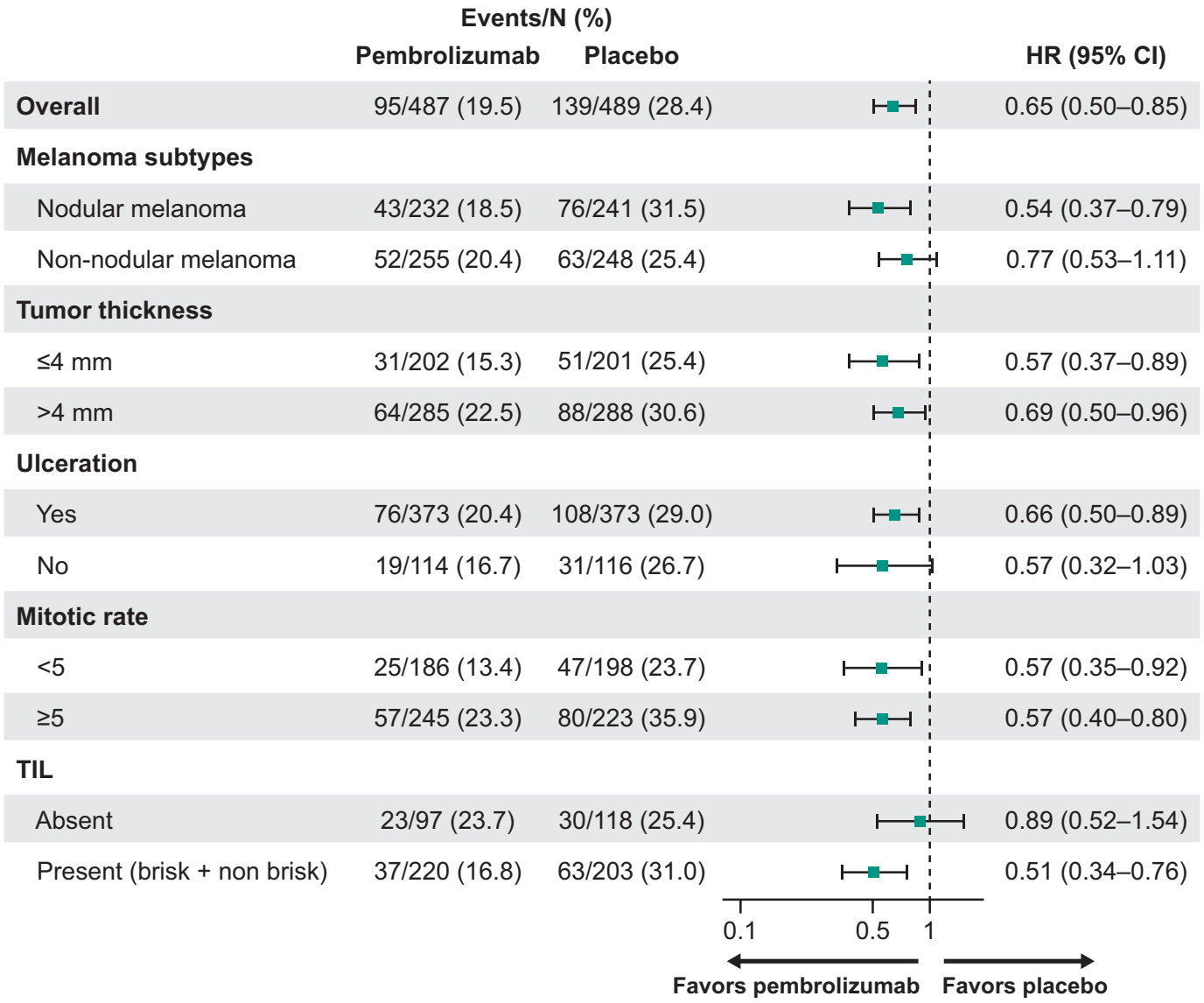

Figure 1 Forest plot of recurrence-free survival by subgroup factors. HRs (95% CI) calculated using a Cox regression model with the Efron method of tie handling, with treatment as a covariate. TIL, tumor-infiltrating lymphocytes.

agencies. All patients or their legally acceptable representatives provided written informed consent.

KEYNOTE-716 is a two-part study, comprising a double-blind phase (part 1) followed by an unblinded rechallenge or crossover phase (part 2). In part 1, patients were randomly assigned (1:1) to receive adjuvant pembrolizumab 200 mg (2 mg/kg up to a maximum of 200 mg for pediatric patients) or saline placebo intravenously every 3 weeks. Randomization was stratified by T stage for adult patients, with a separate stratum for patients aged 12–17 years. Treatment continued for up to 17 cycles (approximately 1 year) or until disease recurrence, unacceptable toxicity, patient or investigator decision to withdraw, or other discontinuation criteria were met.

In part 2, patients with confirmed disease recurrence were unmasked and allowed to cross over from placebo to pembrolizumab or to receive pembrolizumab rechallenge. Patients with resectable disease received up to 17 cycles of pembrolizumab after resection, and patients with unresectable disease received up to 35 cycles of pembrolizumab. Patients who completed 17 cycles of placebo or pembrolizumab in part 1 and who did not experience recurrence within 6 months of completing treatment or did not stop pembrolizumab because of recurrence or intolerability were also eligible to receive pembrolizumab in part 2.

Assessments and outcomes

CT or MRI was performed 6 months from the date of randomization, then every 6 months from years 2 to 4 from randomization, and then once in year 5, or until disease recurrence. During follow-up, survival was assessed every 12 weeks. Analysis of melanoma subtype, tumor thickness, presence or absence of ulceration, mitotic rate, and presence or absence of TILs was based on local pathology reports.

The primary end point was RFS per investigator review. DMFS per investigator review was a secondary end point.

Table 2 Adjusted association of covariates with recurrence-free survival estimated using a multivariable Cox model

Factor	Comparison	HR (95% CI)	Nominal p value
Treatment arm	Placebo	Reference	**0.0001**
	Pembrolizumab	0.56 (0.43 to 0.75)	
Melanoma subtypes	Nodular melanoma	Reference	0.631
	Non-nodular melanoma	0.94 (0.71 to 1.23)	
Tumor thickness	≤4 mm	Reference	**0.0246**
	>4 mm	1.43 (1.05 to 1.95)	
Ulceration	No	Reference	0.131
	Yes	1.32 (0.92 to 1.90)	
Mitotic rate	<5 per mm^2	Reference	**0.0004**
	≥5 per mm^2	1.65 (1.25 to 2.18)	
Tumor-infiltrating lymphocytes	Present	Reference	
	Absent	1.06 (0.74 to 1.50)	0.762
	Unknown	1.09 (0.80 to 1.50)	0.584

p values in bold are nominally significant (p<0.025).

Statistical analysis

In this post hoc subgroup analysis, RFS and DMFS outcomes were assessed using a multivariate Cox model with the following covariates: melanoma subtype (nodular melanoma vs non-nodular melanoma), tumor thickness (≤4 mm vs >4 mm), presence of ulceration (yes vs no), mitotic rate (less than median (<5 per mm^2 vs equal to or greater than median (≥5 per mm^2), and presence of TILs (absent vs present (brisk and non-brisk)). All patients randomly assigned to treatment, including patients with a recorded protocol deviation, were included in the efficacy analysis. Nominal p values are reported; significance was prespecified as p<0.025.

RFS and DMFS were estimated using the non-parametric Kaplan-Meier method. HRs and 95% CIs were estimated using a Cox regression model with the Efron method of tie handling, with treatment as a covariate. A multivariate Cox model was used to estimate the association of covariates with RFS and DMFS.

The data cut-off for the prespecified third interim analysis was January 4, 2022.

RESULTS

Between September 23, 2018, and November 4, 2020, 976 patients were enrolled and assigned to treatment (pembrolizumab, n=487; placebo, n=489). The most common melanoma subtype was nodular melanoma (n=473 (48.5%)) and most patients had tumor thickness >4 mm (n=573 (58.7%)) and ulceration (n=746 (76.4%)) (table 1). Subgroup sizes were balanced between treatment arms. The median mitotic rate was 5 per mm^2. The median time from randomization to the data cut-off in the overall population was 27.4 months (range, 14.0–39.4).

In the overall population, with 95 events having occurred in the pembrolizumab group and 139 in the placebo group, the HR for RFS was 0.64 (95% CI, 0.50 to 0.84).[6] Subgroup analysis of RFS is presented as a forest plot in figure 1. The HR for RFS by melanoma subtype was 0.54 (95% CI, 0.37 to 0.79) in patients with nodular melanoma and 0.77 (95% CI, 0.53 to 1.11) in patients with non-nodular melanoma (Kaplan-Meier curve presented in online supplemental figure S1); 0.57 (95% CI, 0.37 to 0.89) in patients with tumor thickness ≤4 mm and 0.69 (95% CI, 0.50 to 0.96) in patients with tumor thickness >4 mm (Kaplan-Meier curve presented in online supplemental figure S2); 0.66 (95% CI, 0.50 to 0.89) in patients with ulceration and 0.57 (95% CI, 0.32 to 1.03) in patients with no ulceration (Kaplan-Meier curve presented in online supplemental figure S3); 0.57 (95% CI, 0.35 to 0.92) in patients with a mitotic rate <5 per mm^2 and 0.57 (95% CI, 0.40 to 0.80) in patients with a mitotic rate ≥5 per mm^2 (Kaplan-Meier curve presented in online supplemental figure S4); and 0.89 (95% CI, 0.52 to 1.54) in patients with TILs absent and 0.51 (95% CI, 0.34 to 0.76) in patients with TILs present (brisk and non-brisk) (Kaplan-Meier curve presented in online supplemental figure S5). In the Cox multivariate analysis, treatment arm (pembrolizumab vs placebo: HR, 0.56 (95% CI, 0.43 to 0.75); p=0.0001), tumor thickness (>4 mm vs ≤4 mm: HR, 1.43 (95% CI, 1.05 to 1.95); p=0.0246), and mitotic rate (≥5 per mm^2 vs <5 per mm^2: HR, 1.65 (95% CI, 1.25 to 2.18); p=0.0004) were all significant independent factors for RFS (table 2).

In the overall population, with 63 events having occurred in the pembrolizumab group and 95 in the placebo group, the HR for DMFS was 0.64 (95% CI, 0.47 to 0.88; p=0.0029).[6] Subgroup analysis of DMFS is presented as a forest plot in figure 2. The HR for DMFS by melanoma subtype was 0.52 (95% CI, 0.33 to 0.83) in patients with nodular melanoma and 0.78 (95% CI, 0.50 to 1.22)

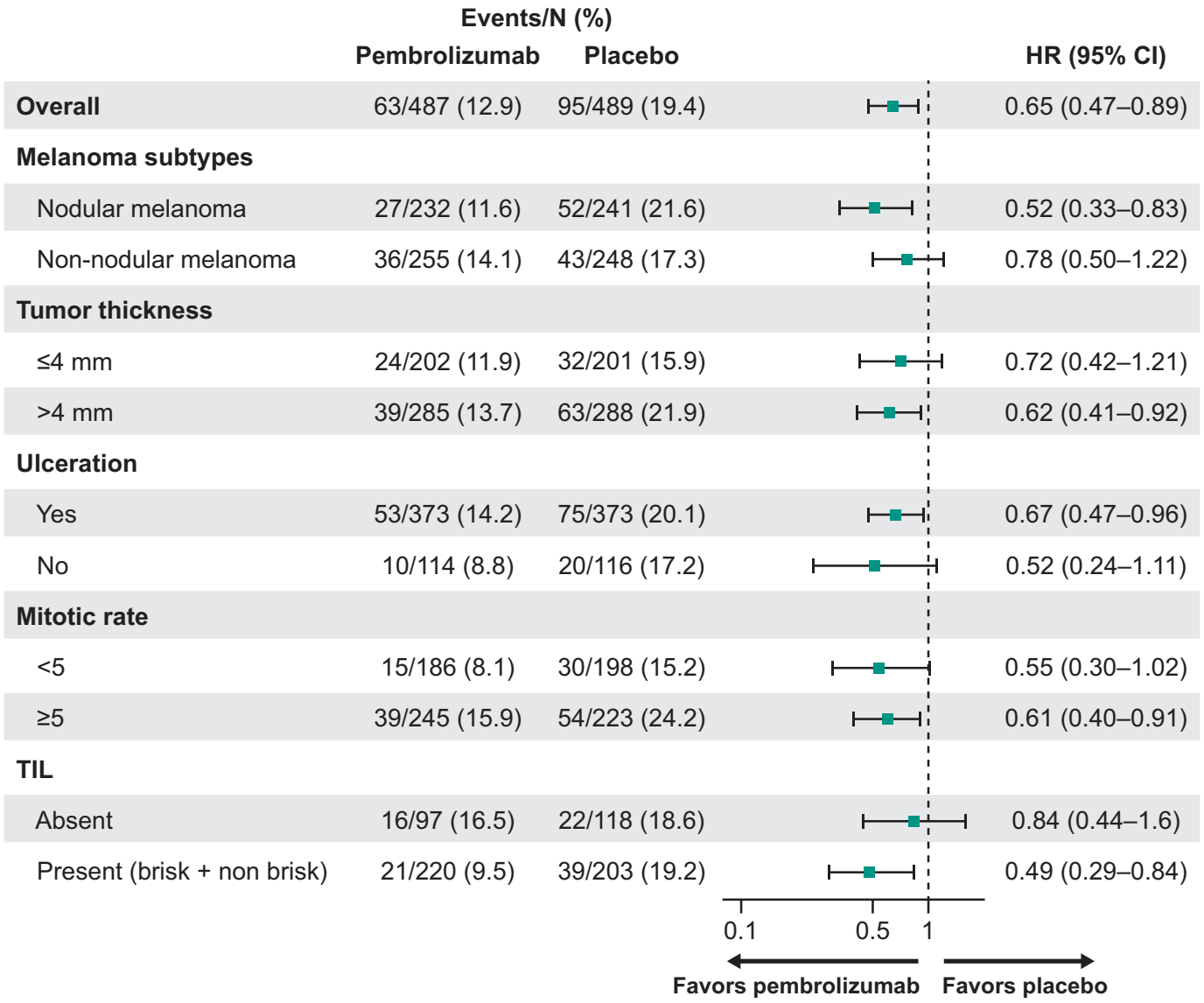

Figure 2 Forest plot of distant metastasis-free survival by subgroup factors. HRs (95% CI) calculated using a Cox regression model with the Efron method of tie handling, with treatment as a covariate. TIL, tumor-infiltrating lymphocyte.

in patients with non-nodular melanoma (Kaplan-Meier curve presented in online supplemental figure S1); 0.72 (95% CI, 0.42 to 1.21) in patients with tumor thickness ≤4 mm and 0.62 (95% CI, 0.41 to 0.92) in patients with tumor thickness >4 mm (Kaplan-Meier curve presented in online supplemental figure S2); 0.67 (95% CI, 0.47 to 0.96) in patients with ulceration and 0.52 (95% CI, 0.24 to 1.11) in patients with no ulceration (Kaplan-Meier curve presented in online supplemental figure S3); 0.55 (95% CI, 0.30 to 1.02) in patients with a mitotic rate <5 per mm^2 and 0.61 (95% CI, 0.40 to 0.91) in patients with a mitotic rate ≥5 per mm^2 (Kaplan-Meier curve presented in online supplemental figure S4); and 0.84 (95% CI, 0.44 to 1.60) in patients with TILs absent and 0.49 (95% CI, 0.29 to 0.84) in patients with TILs present (brisk and non-brisk) (Kaplan-Meier curve presented in online supplemental figure S5). In the Cox multivariate analysis, treatment arm (pembrolizumab vs placebo: HR, 0.58 (95% CI, 0.41 to 0.82); p=0.0019) and mitotic rate (≥5 per mm^2 vs <5 per mm^2: HR, 1.67 (95% CI, 1.19 to 2.36); p=0.0034) were significant independent factors for DMFS (table 3).

DISCUSSION

The results of this post hoc analysis showed that the benefit of adjuvant pembrolizumab in terms of RFS and DMFS across patient subgroups defined by various histopathologic characteristics was largely consistent with that observed for the overall population of patients with resected stage IIB or IIC melanoma. This included patients with prognostic features generally considered to be associated with poor outcome, including having nodular melanoma, tumor thickness >4 mm, presence of ulceration, and a mitotic rate of ≥5 per mm^2. The assessment of TILs in the current study showed that the HRs for RFS and DMFS were numerically lower in the subgroup with TILs present compared with the subgroup with TILs absent,

Table 3 Adjusted association of covariates with distant metastasis-free survival estimated using a multivariable Cox model

Factor	Comparison	HR (95% CI)	Nominal p value
Treatment arm	Placebo	Reference	**0.0019**
	Pembrolizumab	0.58 (0.41 to 0.82)	
Melanoma subtypes	Nodular melanoma	Reference	0.822
	Non-nodular melanoma	1.04 (0.74 to 1.45)	
Tumor thickness	≤4 mm	Reference	0.0946
	>4 mm	1.38 (0.95 to 2.01)	
Ulceration	No	Reference	0.0769
	Yes	1.51 (0.96 to 2.39)	
Mitotic rate	<5 per mm^2	Reference	**0.0034**
	≥5 per mm^2	1.67 (1.19 to 2.36)	
Tumor-infiltrating lymphocytes	Present	Reference	
	Absent	1.35 (0.89 to 2.06)	0.160
	Unknown	1.34 (0.90 to 1.97)	0.147

p values in bold are nominally significant (p<0.025).

suggesting greater benefit of pembrolizumab in those with TILs present; however, the small sample size led to wide CIs. In a multivariate analysis, treatment arm, tumor thickness, and mitotic rate were identified as significant independent factors for RFS, and treatment arm and mitotic rate were significant independent factors for DMFS.

The principal histopathologic parameter affecting the prognosis of localized melanoma is the Breslow thickness, with tumors that penetrate more deeply associated with poorer outcomes.[9] The presence of ulceration is also an adverse prognostic factor and, together with tumor thickness, is reflected in the T category of the AJCC 8th edition melanoma staging system.[8] Mitotic rate was also previously considered in the T category, and, while it has been removed from the AJCC 8th edition criteria, it is still considered a strong independent predictor of outcome.[9] The subtype of melanoma is also important, with nodular melanoma associated with poorer survival than other subtypes, such as superficial spreading melanoma or lentigo maligna melanoma.[10] The presence of TILs in the tumor is considered a favorable prognostic marker, as it signals that the immune system has recognized and responded to the malignancy.[9 11 12]

There are limited data available regarding the impact of these histopathologic features on outcomes for patients treated with PD-1 inhibitors in the adjuvant setting. A subgroup analysis of the phase 3 KEYNOTE-054 study, which was conducted to investigate the efficacy and safety of adjuvant pembrolizumab versus placebo in patients with resected, high-risk stage III melanoma, indicated an RFS benefit with pembrolizumab regardless of the presence or absence of ulceration, but the analysis did not include any of the other subgroups investigated in the current report.[13] Similar results were observed in the phase 3 CheckMate 238 study, which was conducted to investigate the efficacy of nivolumab versus ipilimumab for the treatment of resected stage IIIB, IIIC, or IV melanoma, with an RFS benefit observed with nivolumab irrespective of ulceration status.[14] The CheckMate 238 study also included an analysis by melanoma subtype, including mucosal, cutaneous, acral, and other, but did not differentiate between cutaneous melanoma subtypes.[14] To our understanding, the current analysis includes the only data currently available reporting the efficacy of adjuvant PD-1 inhibitors in stage II melanoma analyzed by various histopathologic characteristics. Although some pathologic variables studied here have a prognostic impact, and some may be considered predictive for immune reaction, the consistency of the adjuvant effect of pembrolizumab suggests that none of these variables defines a subgroup of stage II melanoma with a different natural history and immune reactivity.

This study is primarily limited by its post hoc nature. Further, the patient numbers in some subgroups were small. Consequently, any numeric differences apparent between subgroups should be interpreted with caution. The study also did not mandate *BRAF* status reporting at baseline, because BRAF testing is not considered part of the standard of care for stage IIB/IIC melanoma.

The results of this post hoc subgroup analysis show that pembrolizumab provides a consistent benefit as adjuvant therapy for resected stage IIB or IIC melanoma regardless of histopathologic features, including melanoma subtype, tumor thickness, presence of ulceration, mitotic rate, and presence of TILs. These data add to the body of evidence supporting the use of adjuvant pembrolizumab in patients with resected stage IIB or IIC melanoma.

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Competing interests DS reports being an invited speaker for BMS, Merck Serono, MSD, Novartis, Roche, and Sanofi; having advisory board roles with BMS, Immunocore, MSD, Neracare, Novartis, Pfizer, Philogen, Pierre Fabre, and Sanofi/Regeneron; research grants from BMS and MSD; being a steering committee member for BMS, MSD, and Novartis; being a coordinating PI for BMS, MSD, Novartis, and Pierre Fabre; being a local PI for Philogen and Sanofi; and being member of the board of directors for EORTC-MG (non-financial). 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Ethics approval University of Iowa Human Subjects Office - IRB00000099; Western Institutional Review Board - IRB00000533; Memorial Sloan Kettering Cancer Center IRB - IRB00000533; University of Chicago IRB - IRB00000967; Mayo Clinic – IRB - IRB00000020; IRB00003294; IRB00003295; IRB00005256; OHSU Institutional Review Board - IRB00000471; Icahn School of Medicine at Mount Sinai Program for the Protection of Human Subjects - IRB00000533; Johns Hopkins Medicine Institutional Review Boards - IRB00001555; Horizon Health Network Research Ethics Board - RS 2018-2655; OCREB - Ontario Cancer Research Ethics Board - 1571; Dana Farber Cancer Institute Institutional Review Board - IRB00000052; IRB00000753; IRB00001186; IRB00003340; IRB00005504; IRB00006224; IRB00007493; UCLA Office of Human Research Protection Program - IRB00000173; Advarra IRB - IRB00000971; UCSD Human Research Protection Program IRB - IRB00000354; University of Texas MD Anderson Cancer - IRB00000121; Northwestern University IRB - IRB00000418; NYU Institutional Review Board - IRB00001015. Participants gave informed consent to participate in the study before taking part.

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