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Original Research

Prognostic and predictive value of β-blockers in the EORTC 1325/KEYNOTE-054 phase III trial of pembrolizumab versus placebo in resected high-risk stage III melanoma

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Abstract ***Background:*** β-adrenergic receptors are upregulated in melanoma cells and contribute to an immunosuppressive, pro-tumorigenic microenvironment. This study investigated the prognostic and predictive value of β-adrenoreceptor blockade by β-blockers in the EORTC1325/KEYNOTE-054 randomised controlled trial.

Methods: Patients with resected stage IIIA, IIIB or IIIC melanoma and regional lymphadenectomy received 200 mg of adjuvant pembrolizumab (n = 514) or placebo (n = 505) every three weeks for one year or until recurrence or unacceptable toxicity. At a median follow-up of 3 years, pembrolizumab prolonged recurrence-free survival (RFS) compared to placebo (hazard ratio (HR) 0.56, 95% confidence interval (CI) 0.47–0.68). β-blocker use was defined as oral administration of any β-blocker within 30 days of randomisation. A multivariable Cox proportional hazard model was used to estimate the HR for the association between the use of β-blockers and RFS.

Results: Ninety-nine (10%) of 1019 randomised patients used β-blockers at baseline. β-blockers had no independent prognostic effect on RFS: HR 0.96 (95% CI 0.70–1.31). The HRs of RFS associated with β-blocker use were 0.67 (95% CI 0.38–1.19) in the pembrolizumab arm and 1.15 (95% CI 0.80–1.66) in the placebo arm. The HR of RFS associated with pembrolizumab compared to placebo was 0.34 (95% CI 0.18–0.65) among β-blocker users and 0.59 (95% CI 0.48–0.71) among those not using β-blockers.

Conclusions: This study suggests no prognostic effect of β-blockers in resected high-risk stage III melanoma. However, β-blockers may predict improved efficacy of adjuvant pembrolizumab treatment. The combination of immunotherapy with β-blockers merits further investigation.

This study is registered with ClinicalTrials.gov, NCT02362594, and EudraCT, 2014-004944-37.

1. Introduction

In vitro studies have demonstrated that expression of β-adrenoreceptors is up-regulated in a diverse range of cancers. β-adrenergic receptor activation contributes to a pro-tumorigenic microenvironment through its roles in apoptosis, inflammation, angiogenesis, DNA repair and cellular immunity [1,2]. Among the 29 most common human cancers, melanoma most strongly expresses β-adrenoreceptors, including the individual receptor subtypes β1, β2 and β3 [3,4]. All three subtypes are thought to play a role in melanoma development and progression through promoting the release of pro-tumorigenic cytokines and metalloproteases and reducing T-cell expansion and cytotoxicity [1,3].

β-blockers are a well-tolerated and frequently prescribed class of medication with a range of indications. They are subclassified as either β1-selective β-blockers or non-selective, which target β1, β2 and, in some cases, β3 receptors. Population-based studies have suggested that concomitant use of β-blockers has a beneficial effect on clinical outcomes in certain cancers.

The strongest evidence of a beneficial effect is in melanoma [5–7], though most studies have been conducted with poorly defined cases identified from cancer registers and of various stages and unspecified treatments.

A separate but related area of interest is the predictive effect of β-blockers use on immunotherapy efficacy. Immunotherapy has transformed the treatment of melanoma and is now a standard of care both in the metastatic setting and as adjuvant therapy for resected stage III disease [8–11]. Preclinical studies have demonstrated that programmed cell death protein 1 (PD-1) inhibitors are more effective in mice when combined with pharmacological β-blockade [12]. A retrospective clinical study in the United States involving 195 patients with metastatic melanoma treated with one or a combination of anti-PD-1, anti-CTLA-4 and IL-2 immunotherapy agents found improved overall survival (numerical estimates not reported) among patients using non-selective β-blockers [13].

This study aimed to further investigate the prognostic and predictive value of β-blockers in the European Organisation for Research and Treatment of Cancer (EORTC) 1325/KEYNOTE-054 phase III trial of pembrolizumab versus placebo in resected high-risk stage III melanoma [9–11].

2. Patients and methods

2.1. Study population

A retrospective analysis of patients enrolled in the EORTC 1325 trial was conducted, which compared adjuvant pembrolizumab to placebo among patients aged 18 years or older, with biopsy confirmed high-risk stage IIIA (minimum sentinel node tumour deposit >1 mm), IIIB or IIIC melanoma and complete regional lymphadenectomy within 13 weeks prior to starting treatment. Recruitment was between August 2015 and November 2016 across 123 centres in 23 countries. Patients were excluded if they had an Eastern Cooperative Oncology Group performance status of greater than 1, in-transit metastases, an autoimmune disease, uncontrolled infection, used systemic glucocorticoids or received previous systemic treatment for melanoma. Assignment was by 1:1 randomisation to groups receiving either 200 mg of adjuvant pembrolizumab (N = 514) or placebo (N = 505) every three weeks for one year or until recurrence, unacceptable toxicity, protocol violation or withdrawal. Follow-up was by clinical review with computed tomography and/or magnetic resonance imaging every 12 weeks for the initial 2 years, then every 6 months until the end of year 5 and annually thereafter. For the present study, clinical cut-off date was 30th September 2019.

This study obtained ethical approval from the EORTC protocol-review committee and was conducted in compliance with the Declaration of Helsinki. All patients in the 1325 trial provided written informed consent.

2.2. β-blocker use

The use of β-blockers was defined as oral administration of any β-blocker between the date of randomization and 30 days thereafter. Both β1-selective agents (e.g. acebutolol, atenolol, betaxolol, bisoprolol, celiprolol, esmolol, metoprolol and nebivolol) and non-selective agents (e.g. carteolol, carvedilol, labetatol, levobunolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, practolol, propranolol, sotalol and timolol) were included. The use of topical β-blockers at baseline was defined using the same time window as for oral β-blockers. Patients who received them were not included in the main analyses as described subsequently.

2.3. Outcomes

As previously published [9, 10], the primary end-point was recurrence-free survival (RFS). RFS was defined as the time between the date of randomization and the date of recurrence (local, regional, distant metastasis) or death (whatever the cause), whichever occurred first. The date of recurrence was assumed to be the date of clinical assessment when recurrence was first detected. For patients who remained alive and without recurrence, RFS was censored at the most recent date of clinical assessment.

2.4. Statistical analysis

RFS was estimated using the Kaplan–Meier estimator and confidence intervals using the normal approximation of the distribution of log(-log(survival)) and the Greenwood variance formula. Univariable and multivariable Cox models were used to estimate hazard ratios (HRs) and 95% confidence interval (95% CI) for the associations between baseline oral β-blocker use and RFS. The multivariable Cox model included adjustments for treatment arm, disease stage (stage IIIA versus stage IIIB versus stage IIIC with 1–3 positive nodes versus stage IIIC with >3 positive nodes), age (<50 vs 50-<65 vs ≥ 65 years), sex and BMI (<25 vs 25-<30 vs ≥ 30 kg/m^2). The predictive value of oral β-blocker use for RFS was estimated by adding a further

term to the aforementioned Cox model for the interaction between β-blockers and treatment arm.

In order to avoid potential bias due to topically administered β-blockers, a sensitivity analysis was performed by excluding patients that were using topical β-blockers from the group not using oral β-blockers. Finally, RFS according to use of β1-selective and non-selective β-blockers separately was estimated using the Kaplan–Meier estimator, as described previously.

For all estimated parameters, point estimates and 95% confidence intervals were used. The proportional hazards assumption was verified using the approach by Lin, Wei and Ying [14] based on martingale residuals. All tests were performed at a two-sided significance level of 0.05. SAS software (SAS Institute) was used for all analyses.

3. Results

There were 1019 patients randomised to either pembrolizumab (n = 514) or placebo (n = 505) treatment arms. Baseline clinical characteristics are presented in Table 1. The majority had higher stage disease - stage IIIA (n = 152), IIIB (n = 472) or IIIC (n = 395). Ninety-nine (10%) patients were using oral β-blockers at baseline. Among the 920 patients not using oral β-blockers, eight patients (<1%) used topical β-blockers. The median follow-up was 37 months, and the interquartile range was 35–40 months.

Patients using oral β-blockers were older (41.4% versus 22.8% aged ≥65 years), had more advanced melanoma (46.4% versus 37.9% were stage IIIc) and had more comorbidities, including obesity (34.3% versus

Table 1
Baseline characteristics of patients according to oral β-blocker use at baseline.

	Oral β-blockers at baseline		
	No (N = 920)	Yes (N = 99)	Total (N = 1019)
	N (%)	N (%)	N (%)
Treatment arm			
Pembrolizumab	466 (50.7)	48 (48.5)	514 (50.4)
Placebo	454 (49.3)	51 (51.5)	505 (49.6)
Age (years)			
<50	368 (40.0)	11 (11.1)	379 (37.2)
50-64	342 (37.2)	47 (47.5)	389 (38.2)
65+	210 (22.8)	41 (41.4)	251 (24.6)
Sex			
Male	566 (61.5)	62 (62.6)	628 (61.6)
Female	354 (38.5)	37 (37.4)	391 (38.4)
ECOG PS			
0	869 (94.5)	91 (91.9)	960 (94.2)
1	51 (5.5)	8 (8.1)	59 (5.8)
AJCC-7 stage at baseline			
III A	137 (14.9)	15 (15.2)	152 (14.9)
III B	434 (47.2)	38 (38.4)	472 (46.3)
III C (1–3 LN+)	161 (17.5)	22 (22.2)	183 (18.0)
III C (>3 LN+)	188 (20.4)	24 (24.2)	212 (20.8)
BMI			
<25	321 (34.9)	18 (18.2)	339 (33.3)
25-<30	372 (40.4)	46 (46.5)	418 (41.0)
≥30	212 (23.0)	34 (34.3)	246 (24.1)
Missing	15 (1.6)	1 (1.0)	16 (1.6)
β-blocker use prior to baseline			
No reported	918 (99.8)	0 (0.0)	918 (90.1)
Less than 1 year	1 (0.1)	22 (22.2)	23 (2.3)
1-<2 years	0 (0.0)	9 (9.1)	9 (0.9)
2+ years	0 (0.0)	57 (57.6)	57 (5.6)
Reported, duration unknown	1 (0.1)	11 (11.1)	12 (1.2)
Oral selective β-blockers at baseline			
No	920 (100.0)	19 (19.2)	939 (92.1)
Yes	0 (0.0)	80 (80.8)	80 (7.9)
Oral non-selective β-blockers at baseline			
No	920 (100.0)	80 (80.8)	1000 (98.1)
Yes	0 (0.0)	19 (19.2)	19 (1.9)
β-blockers eye drops at baseline			
No	912 (99.1)	93 (93.9)	1005 (98.6)
Yes	8 (0.9)	6 (6.1)	14 (1.4)

Abbreviations: N (number), ECOG (Eastern Cooperative Oncology Group), PS (performance status), AJCC-7 (American Joint Committee on Cancer), BMI (body mass index).

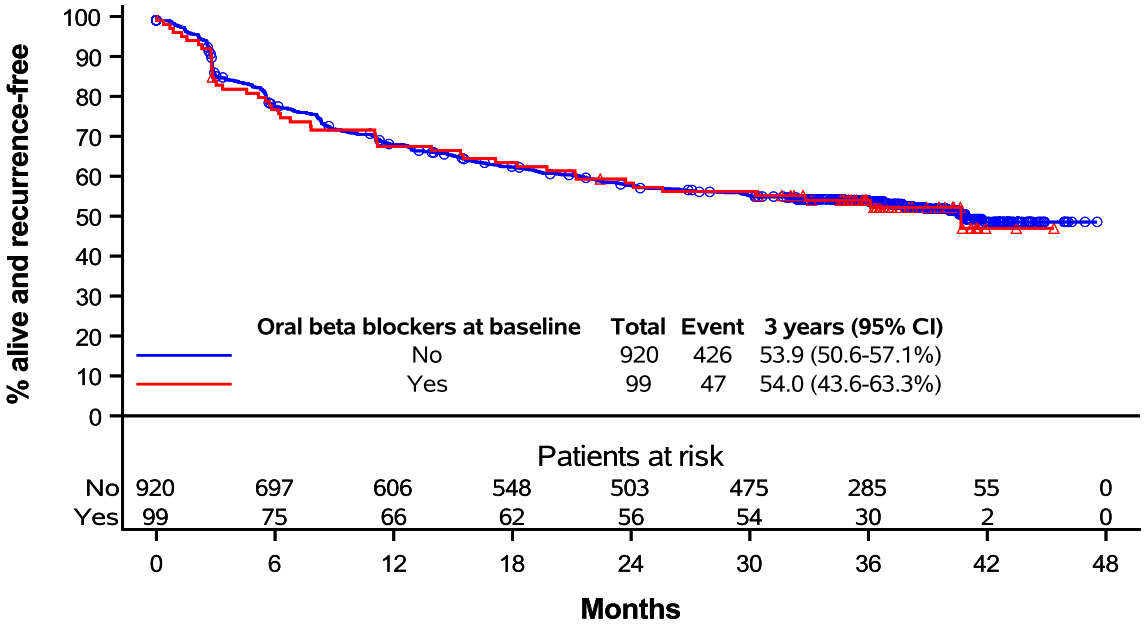

Fig. 1. Recurrence-free survival by the use of oral β-blockers at baseline.

23.0% BMI ≥30 kg/m^2), diabetes mellitus (24.2% versus 5.8%) and hypertension (85.9% versus 27.7%). Supplementary Table 1 shows the distribution of comorbidities according to oral β-blocker use. Supplementary Tables 2 and 3 show the baseline characteristics of patients according to both oral β-blocker use and treatment arm.

As previously published, there were 473 RFS events during follow-up, including newly diagnosed distant metastases (n = 307), locoregional recurrence only (n = 160), death (n = 5) or unknown type (n = 1). Pembrolizumab (n = 190 RFS events) compared with placebo (n = 283 RFS events) resulted in prolonged RFS: 3-year RFS rate, 63.7% versus 44.1% for pembrolizumab versus placebo, respectively; HR, 0.56; 95% CI, 0.47 to 0.68) [10].

Patients using oral β-blockers (in both arms combined) had a similar RFS compared to those not using β-blockers: the 3-year RFS rates were 54.0% (95% CI 43.6–63.3%) and 53.9% (95% CI: 50.6–57.1%), respectively, and the HR was 1.02 (95% CI 0.75–1.38) (Fig. 1). In addition, Cox multivariable analysis indicated that the use of β-blockers had no prognostic importance (RFS HR 0.96, 95% CI 0.70–1.31) when adjusting for treatment arm and possible confounding factors (Table 2). Pembrolizumab, a lower disease stage and a BMI between 25 and 30 kg/m^2 were significantly associated with a longer RFS whereas age and sex were not.

Fig. 2 shows estimated RFS by treatment arm and β-blocker use. In a Cox multivariable analysis, the HR for the association between baseline oral β-blocker use and RFS was 0.67 (95% CI 0.38–1.19) in the pembrolizumab arm and 1.15 (95% CI 0.80–1.66) in the placebo arm (Table 3). The p-value for the interaction between treatment arms and β-blockers was 0.12. Conversely, the HR for pembrolizumab compared to placebo comparison regarding RFS was 0.34 (95% CI 0.18–0.65) among patients using β-blockers and 0.59 (95% CI 0.48–0.71) among patients not using β-blockers. The difference between these two estimates was not statistically significant.

Excluding eight patients not using oral β-blockers at baseline that were using topical β-blockers made little difference to the association between oral β-blocker use and RFS among all patients (data not shown).

Among 99 patients using oral β-blockers at baseline, 80 were using β1-selective β-blockers, while 19 only were using non-selective β-blockers. A Kaplan–Meier plot of RFS according to oral β-blocker subclass is shown in Fig. 3. The 3-year RFS rates were 50.7% (95% CI: 39.3%–61.1%) and 66.9% (95% CI: 40.5–83.6%) for patients using β1-selective and nonselective β-blockers, respectively. Fig. 4 shows the Kaplan–Meier plot of RFS according to both oral β-blocker classes and treatment arm. In the pembrolizumab group, the 3-year RFS rates were 76.1% (95% CI: 59.0%–86.8%) and 65.6% (95% CI: 26.0%–87.6%) for patients using β1-selective and non-selective β-blockers, respectively, and 28.6% (95% CI: 16.0%–42.5%) and 66.7% (95% CI: 28.2%–87.8%), respectively, in the placebo group.

4. Discussion

This study estimated the prognostic and predictive value of oral β-blockers on RFS in the EORTC 1325 clinical trial, which compared pembrolizumab to placebo among 1019 patients age 18 years or older, with biopsy confirmed high-risk stage IIIA (minimum sentinel node tumour deposit >1 mm), IIIB or IIIC melanoma and complete regional lymphadenectomy within 13 weeks

Table 2
Adjusted association of oral β-blocker use and baseline covariates with recurrence-free survival, estimated using a multivariable Cox model.

Parameter	Levels	Hazard Ratio (95% CI)	P-value
Oral β-blockers at baseline	No	1.00	0.793
	Yes	0.96 (0.70, 1.31)	
Treatment arm	Placebo	1.00	<0.001
	Pembrolizumab	0.56 (0.46, 0.67)	
AJCC-7 stage at baseline	III A	1.00	<0.001
	III B	1.90 (1.36, 2.65)	
	III C (1–3 LN+)	2.39 (1.66, 3.44)	
	III C (>3 LN+)	3.17 (2.24, 4.51)	
Sex	Male	1.00	0.069
	Female	0.84 (0.69, 1.01)	
Age (years)	<50	1.00	0.552
	50–64	1.04 (0.84, 1.29)	
	65+	1.14 (0.90, 1.45)	
BMI	<25	1.00	0.019
	25-<30	0.75 (0.60, 0.94)	
	≥30	0.98 (0.77, 1.24)	

Abbreviations: AJCC-7 (American Joint Committee on Cancer), BMI (body mass index). There was no evidence of non-proportional hazards for any of the covariates ($p > 0.05$).

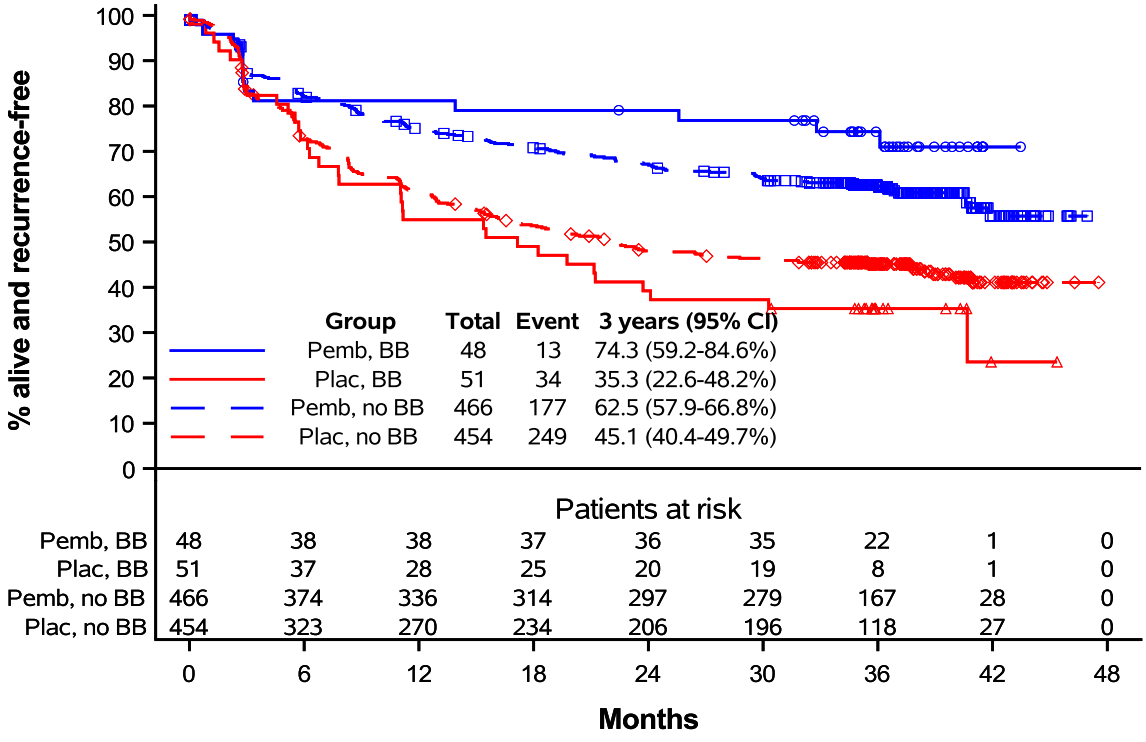

Fig. 2. Recurrence-free survival by the use of oral β-blockers at baseline and treatment arm. Abbreviations: Pemb (pembrolizumab), Plac (placebo), BB (β-blockers).

prior to starting treatment. To our knowledge, this is the first study to estimate the prognostic and predictive value of β-blockers within a large, randomised controlled trial for adjuvant immunotherapy in melanoma.

Oral β-blockers did not confer a prognostic effect on RFS in either the unadjusted (HR 1.02) or adjusted analysis (HR 0.96). Interestingly, in a Cox multivariable model, which considered the β-blocker–treatment interaction, patients using oral β-blockers had a larger improvement in RFS in the pembrolizumab arm (HR 0.67) than the placebo arm (HR 1.15). However, the differential prognostic importance of β-blockers in the two treatment arms did not reach statistical significance ($P = 0.12$). Conversely, patients using β-blockers had a larger treatment benefit from pembrolizumab

Table 3
Adjusted associations between β-blockers at baseline and recurrence-free survival by treatment arm, estimated using a multivariable Cox model.[a]

Group	HR (95% CI)	P-value for the interaction
All patients	0.96 (0.70–1.31)	0.112
Treatment arm: Pembrolizumab	0.67 (0.38–1.19)	
Treatment arm: Placebo	1.15 (0.80–1.66)	

Abbreviations: HR (hazard ratio).
[a] Cf model indicated in Table 3, where an interaction term, treatment group x β-blockers use, was added.

(HR = 0.34) than patients not using β-blockers (HR = 0.59), although the difference was not significant.

The observed benefit of β-blockers in patients receiving pembrolizumab supports the hypothesis of a role of β-adrenergic signalling in the inhibition of T cell activity, which in part mediates the action of pembrolizumab [15, 16]. β2 signalling is thought to play the most important role (i.e. compared to β1 and β3) [13].

A number of other studies have investigated associations between β-blocker use and outcomes in melanoma. However, unlike the present work, most previous studies have been population-based studies (i.e. outside of a clinical trial) involving melanoma identified from health care records of various stages and unspecified treatments. In one such study involving 4,179 cases of stages I-IV melanoma in a Danish cancer register, Lemeshow *et al.* [17] found that use of β-blockers within 90 days of diagnosis was associated with prolonged overall survival (HR 0.81, 95% CI 0.67–0.97) and melanoma-specific survival (HR 0.87, 95% CI 0.64–1.20). In a similar study, involving 121 patients with Breslow thickness >1 mm melanoma, De Giorgi *et al.* [6] found that β-blockers were also significantly associated with overall survival (HR 0.62, 95% CI 43–90). Other population-based studies have only reported weak associations between β-blocker use and survival [18–20]. In addition, a small off-label study has investigated the effect of 80 mg of propranolol daily (taken from the time of diagnosis) on adverse outcomes among 19 of 53 patients with histologically confirmed stage IB to IIIA cutaneous melanoma and no evidence of metastasis [21]. In that study, patients receiving propranolol had markedly reduced risk of recurrence compared to those not receiving propranolol (adjusted HR 0.18, 95% CI, 0.04–0.89).

Only one published clinical study has investigated the effect of β-blockers on the efficacy of immunotherapy treatment in metastatic melanoma. Kokolus *et al.* [13] reported the results of a retrospective study involving a cohort of 195 patients with advanced melanoma who received different immunotherapy agents: IL-2, anti-CTLA-4 and/or anti-PD-1. They found that patients (N = 17) who used non-selective β-blockers had longer overall survival (numerical estimates were not reported) than those who received selective β-blockers (N = 45) or no β-blockers (N = 133). Therefore, there was consistency with our findings regarding non-selective β-blockers but not selective β-blockers. There is an ongoing early phase Ib/II trial investigating the effects of propranolol hydrochloride, a nonselective β-blocker, when given with pembrolizumab to patients with stage IIIC-IV melanoma that is not surgically resectable (NCT03384836).

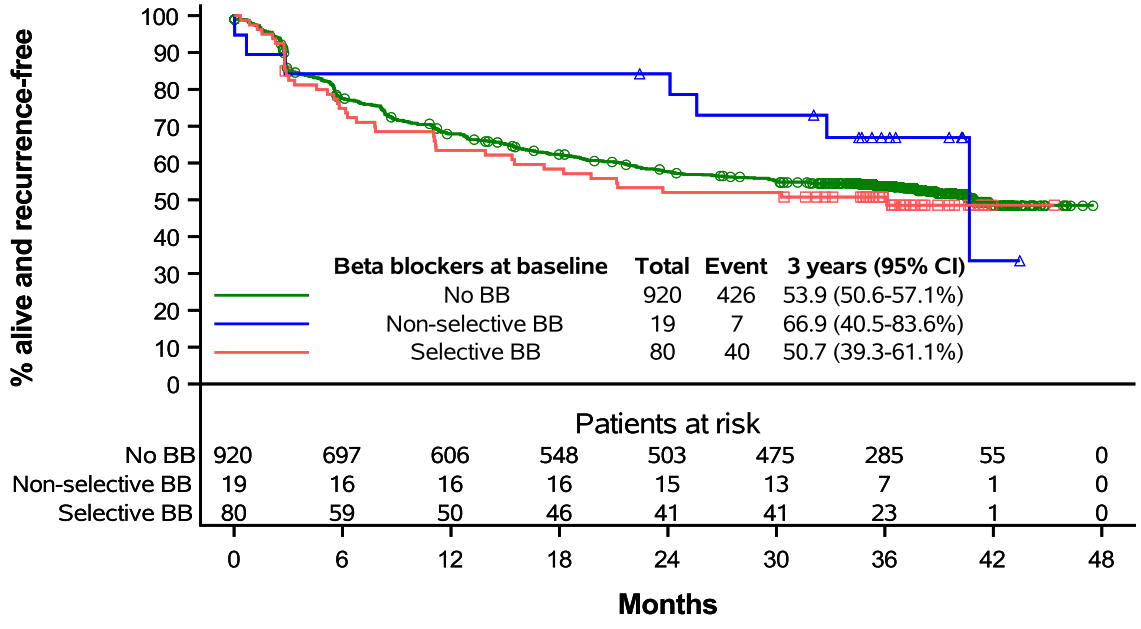

Fig. 3. Recurrence-free survival by oral β1-selective and non-selective β-blocker use at baseline. Abbreviations: BB (β-blockers).

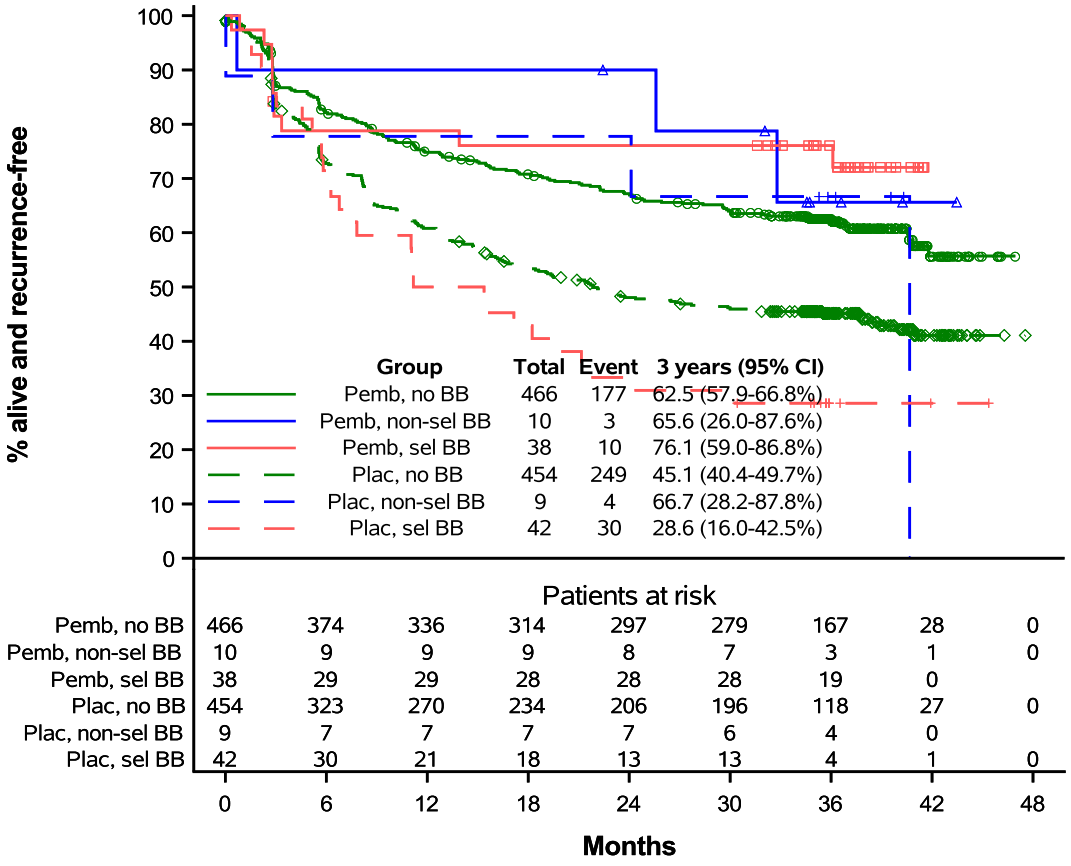

Fig. 4. Recurrence-free survival by oral β1-selective and non-selective β-blocker use at baseline and treatment arm. Abbreviations: Pemb (pembrolizumab), Plac (placebo), BB (β-blockers), sel (selective).

The present study has a number of strengths, including a well-characterised cohort of patients with radiologically and biopsy proven resected stage III melanoma, a large sample size, prospective design and rigorous standards of data collection and follow-up of patients in this registration study. It is the first study, to our knowledge, to report quantitative estimates for the effects of β-blockers in melanoma treated with pembrolizumab. This makes the present study more relevant to current practice than the previous studies mentioned above.

There were also some limitations. First, the study's observational design did not allow one to infer causation of the associations between β-blocker use and clinical outcomes. Second, there were some important differences in the distribution of comorbidities at baseline. Patients using β-blockers were more exposed to chronic health conditions, which are associated with increased mortality. This imbalance may have reduced the chance of observing a statistically significant association between β-blocker use and RFS, although death only accounted for 0.5% of RFS events. While the analyses adjusted for sex, age, BMI and stage, baseline comorbidities were not adjusted for due to a strong collinearity with β-blockers. Moderately increased BMI in this study was associated with increased survival similar to a previous report [22]. However, our analyses did not explore possible interactions between BMI, sex and pembrolizumab treatment. A number of previous studies suggest that the pro-inflammatory phenotype of high BMI, particularly in males, influences immunotherapy efficacy [23, 24], although other studies have found no effect [25]. Third, there may have been misclassification of exposure to β-blockers if patients started to use β-blockers after the 30-day window used to define baseline exposure. For example, β-blockers may have been used for hyperthyroidism, which is a complication of pembrolizumab treatment. Conversely, other patients may have started to use β-blockers during the window but stopped shortly thereafter. The misclassification of β-blocker use would have reduced the chance of observing a statistically significant association between β-blockers and RFS. Finally, the numbers of patients were too small to better explore (e.g. uni- and multi-variable statistical analysis) the effects on RFS of different β-blocker subclasses (i.e. β1-selective and nonselective) overall and in each treatment group. For the same reasons, the effect of β-blockers on different types of recurrences (e.g. diagnosed distant metastases, locoregional recurrence and so on) was not assessed.

In conclusion, this study investigated the prognostic and predictive value of oral β-blockers in a cohort of

patients with resected high-risk stage III melanoma treated with pembrolizumab or placebo. β-blockers did not appear to confer a prognostic effect on RFS. However, the analysis of the predictive value raised the possibility of increased efficacy of pembrolizumab among patients receiving β-blockers. The potential benefits of β-blockers when combined with immunotherapy in melanoma merit further investigation, including the study of the influence of β-blocker selectivity, other commonly used immunotherapy agents and the potential impact in the metastatic setting.

Author contributions

Oliver John Kennedy: Conceptualization, Methodology, Writing-original draft preparation, Writing-review and editing.

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Michal Kicinski: Conceptualization, Methodology, Data Curation, Formal analysis, Visualization, Writing-original draft preparation, Writing-review and editing.

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Alexander MM Eggermont: Supervision, Writing-review and editing.

Paul Lorigan: Methodology, Resources (patients), Investigation, Supervision, Writing-original draft preparation, Writing-review and editing.

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Data sharing statement

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (MSD) is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

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Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.01.017.

Appendix

List A1
The list additional investigators participated in the EORTC 1325/KEYNOTE 054 trial, but their names were not included in the author list of this article.

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(*continued on next page*)

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