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Immune checkpoint inhibition in metastatic or non-resectable melanoma after failure of adjuvant anti-PD-1 treatment. A EUMelaReg real-world evidence study

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ABSTRACT

Background: Adjuvant immune checkpoint inhibition (ICI) with anti-PD-1 antibodies in high-risk resected melanoma has been shown to improve recurrence-free survival. It is unclear whether prior adjuvant anti-PD-1 therapy is associated with altered response to subsequent ICI treatment in the metastatic setting.
Methods: Using data from the European Melanoma Registry (EUMelaReg), we analyzed the efficiency of first-line (1L) ICI in non-resectable or metastatic melanoma after failure from prior adjuvant anti-PD-1 treatment. Both single-agent anti-PD-1 and combined anti-PD-1/CTLA-4 (Ipi/Nivo) 1L regimes were included in the analysis. We identified 389 patients receiving 1L ICI with prior adjuvant anti-PD-1 treatment. The control population was selected from a pool of 3390 PD-1-naive cases by 1:1 matching for the type of 1L ICI and various prognostic factors. As outcome measure, overall remission rates (ORR) were calculated and progression-free survival (PFS) was evaluated by Kaplan-Meier and Cox regression analysis.
Results: Out of 389 patients, 303 (77.9 %) received Ipi/Nivo and 86 (22.1 %) anti-PD-1 in 1L. ORR was significantly lower in pre-treated patients (31.4 %) as compared to anti-PD-1 naive patients (48.8 %; $p < 0.0001$). Kaplan-Meier analysis showed significantly shorter median PFS for pre-treated patients. This applied to both anti-PD-1 and Ipi/Nivo treatment. Patients with early recurrence from adjuvant treatment (during or up to 12 weeks after end of treatment) showed lower ORR (28.5 %) and shorter PFS (3.1 months) than those who recurred later (37.7 % and 6.1 months, respectively).
Conclusions: Patients with metastatic melanoma, previously exposed to anti-PD-1 ICI in the adjuvant setting showed significantly lower ORR and shorter PFS to 1L ICI with either Ipi/Nivo or single-agent anti-PD-1 retreatment.

1. Introduction

Immune checkpoint inhibitors (ICI) have improved the outcome of patients with metastatic melanoma dramatically compared to former treatment standards [1,2]. Based on the results of clinical trials, the programmed death (PD) 1 inhibitors pembrolizumab and nivolumab were approved for treatment of non-resectable or metastatic melanoma [3–8]. Both drugs potentiate T-cell responses through blockade of PD-1 binding of PD-L1 and PD-L2, which are expressed by antigen presenting cells, but may also be expressed by tumor cells [9]. In metastatic melanoma, treatment with anti-PD-1 antibodies results in overall response rates (ORR) of about 40 % [5,10] and long-term overall survival (OS) rate at 5 years is also around 40 %. Furthermore, the development of combined ICI using nivolumab plus ipilimumab (anti-CTLA-4) with a 5-year OS rate of about 50 % constitutes a notable improvement as compared to the historically dismal prognosis of metastatic melanoma [11].

The success of ICI in metastatic melanoma has led to developments using these agents in the adjuvant setting for high-risk resected melanoma. Trials with single-agent anti-PD-1 antibodies showed highly significant prolongation of recurrence-free survival (RFS) when used in the adjuvant setting [12,13]. Based on these results, both pembrolizumab and nivolumab were approved for adjuvant treatment of resected stage III melanoma.

While adjuvant anti-PD-1 treatment improves RFS in patients with resected high-risk melanoma, still a proportion of these patients will develop a recurrence, many of them with non-resectable locoregional or metastatic disease. Overall in stage III eventually 50 % or more of patients might develop a recurrence despite adjuvant treatment according to long-term follow-up (FU) data [14–17]. A recurrence may occur while still on the 12 months of adjuvant treatment or later in the course of the disease, and is referred to as early and late ICI resistance, respectively [18].

In case of non-resectable failure or metastatic disease following adjuvant anti-PD-1 treatment it is unclear how the efficacy of a further ICI treatment is impacted by preceding ICI failure during or after adjuvant therapy.

2. Material and methods

2.1. Study design and participants

This retrospective study analyzed data on patients who were treated with first-line (1L) non-adjuvant ICI for non-resectable stage III or stage IV metastatic cutaneous melanoma (including melanoma of unknown primary; MUP) and stratified by history of adjuvant anti-PD-1 therapy. Both combined anti-PD-1/CTLA-4 (ipilimumab plus nivolumab; Ipi/Nivo) and single-agent anti-PD-1 antibodies (pembrolizumab or nivolumab) as 1L ICI treatments were included. Patients who had received adjuvant treatment with BRAF/MEK inhibitors (BRAF/MEKi) were excluded. Patients were selected from the European Melanoma Registry database (EUMelaReg; www.eumelareg.org) which collects and evaluates real-world treatment and outcome data of advanced melanoma patients across Europe. Data are submitted from collaborating centers on a voluntary basis, and they are subject to electronic and manual validation and monitoring measures to ensure sufficient quality for analyses.

2.2. Study objectives

This study was performed to analyze the efficiency of ICI treatment after failure of adjuvant anti-PD-1 therapy in patients with high-risk resected melanoma. Primary outcomes of interest were overall

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response rate (ORR) from 1L ICI treatment in the non-adjuvant setting and progression-free survival (PFS) from start of 1L ICI. Further outcomes of interest were rates of complete remissions (CR) and partial remissions (PR), and disease control rate (DCR). Demographic features, clinical tumor characteristics, and 1L ICI schedule (i.e. single agent anti-PD-1 vs. Ipi/Nivo) were used for correlational and multivariable analysis as well as criteria for the matched sample analysis, as described below. In the adjuvant pre-treatment group, the reason for discontinuation adjuvant anti-PD-1 and recurrence free interval were analyzed for correlations with 1L ICI efficiency. In accordance with the SITC (Society for Immunotherapy of Cancer) definitions [19] recurrences in the adjuvant setting were categorized as either early, occurring during or within 12 weeks after the end of treatment (EoT), or late recurrences, occurring $>$ 12 weeks after EoT.

2.3. Statistical analysis

Descriptive statistics were used to summarize baseline study cohort characteristics. Categorical variables are presented as the number of observations and the percentage, if appropriate also along with 95 % confidence intervals (CI). Continuous variables are presented as the mean, standard deviation, or median, minimum and maximum, as appropriate by distribution.

For PFS, time-to-event analyses were conducted using the Kaplan-Meier method to generate plots and to estimate median intervals in months with 95 % CI, and events rates with 95 % CI at 1 year. PFS was defined as time from start of non-adjuvant immunotherapy to date of first progression according to physiciańs assessment or death due to any cause. If no event occurred before a switch to another line of treatment, patients were censored at the start of next treatment, otherwise with the date of last contact. FU time was calculated by Kaplan Meier analysis from start of first non-adjuvant ICI treatment to the date of last contact with censoring for death events.

Statistical comparisons for categorical rates were done by chi^2 or Fisher's test, for numerical variables by *t*-test or Mann-Whitney rank test, as appropriate by the data. Survival times were compared using log-rank test, associated hazard ratios (HR) are displayed with their 95 % CI's from Cox regression analysis.

Matching was performed with an optimal matching algorithm using mahalanobis distance as distance metric. Samples were matched for Eastern Cooperative Oncology Group (ECOG), American Joint Committee on Cancer (AJCC) stage, Lactate dehydrogenase (LDH), number of metastatic sites, sex, BRAF status, age and Charlson comorbidity score. Additionally, an exact matching on the type of immunotherapy was performed. All statistical analyses were performed using the statistical software package R (version 4.3.2 including packages matchIt, survival and survminer).

3. Results

3.1. Patient characteristics

After applying the selection criteria, a total of 389 eligible patients with non-resectable recurrence after adjuvant anti-PD-1 pre-treatment and 1L ICI treatment in the advanced/metastatic setting were included (Fig. 1). Demographic characteristics of patients treated with 1L ICI after

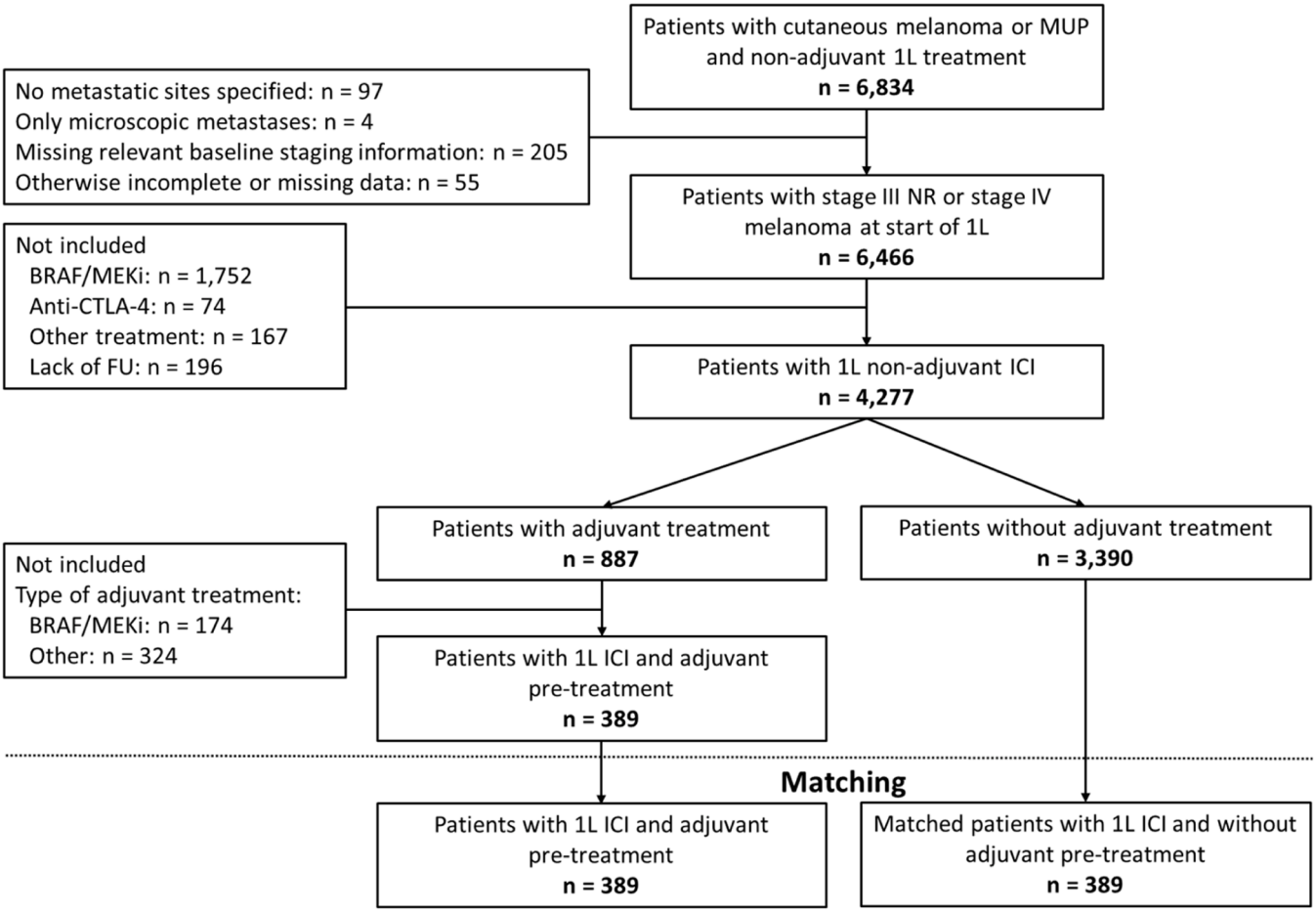

Fig. 1. STROBE flow chart of the study population. N: number of patients; FU: follow-up; ICI; Immune checkpoint inhibitors; MUP: melanoma of unknown primary; NR: non-resectable; 1L: first-line.

disease recurrence on adjuvant anti-PD-1 therapy (pre-treated cohort) compared to patients without adjuvant anti-PD-1 therapy (PD-1 naive cohort) are shown in Table 1. The majority of patients were male (62.5 %), 93.1 % had cutaneous melanoma, and 6.9 % had MUP. A $BRAF^{V600}$ mutation was reported in 25.4 % of the patients. The baseline characteristics of the pre-treated cohort were significantly different from the PD-1 naive unmatched control cohort at 1L ICI. These patients had a significantly lower age, more often ECOG performance status (PS) 0, and less often a MUP (Table 1). Multivariable cox regression analysis confirmed the significance of common prognostic factors in the given population (Fig. S1) and several of these variables showed significant differences in favor of the pre-treated group, including substages, number of metastatic sites, and rate of elevated serum LDH.

After applying a stepwise matching procedure, to address anticipated baseline imbalances and eliminate selection bias, sufficient balance for all these variables could be reached. A 1:1 matching for the type of 1L ICI could be applied with only limited deviances on the other variables, as measured by standardized mean differences (Table 1, Fig. 2). In the next step, we separated the analysis into 1L treatment with Ipi/Nivo and anti-PD-1 and matched them to the corresponding PD-1 naive patients on the same variables. Of the 389 patients, 77.9 % (n = 303) were treated with Ipi/Nivo and 22.1 % (n = 86) were treated with anti-PD-1 in 1L (Table 2, S4). Demographic characteristics of these populations are shown in Table 2.

3.2. Treatment response and outcome

The treatment outcome of matched samples of the pre-treated and PD-1 naive cohort at 1L ICI is shown in Table 3. ORR of ICI after adjuvant anti-PD-1 treatment failure was 31.4 % with CR in 60 (15.4 %) and PR in 62 (15.9 %) of cases. This was significantly lower as compared to the PD-1 naive matched cases with an ORR of 48.8 %, including 76 (19.5 %) CR and 114 (29.3 %) PR, respectively ($p < 0.0001$) (Table 3).

Considering only patients who were treated with Ipi/Nivo in 1L, a similar statistical difference in regard to ORR between pre-treated (29.0 %) and PD-1 naive (48.5 %) cohort can be observed ($p < 0.0001$). This effect was also found in patients receiving anti-PD-1 in 1L, but did not reach statistical significance between the treated and naive cohorts ($p = 0.13$, Table 4).

Kaplan-Meier estimates show a shorter PFS for patients pre-treated with anti-PD-1 as compared to the PD-1 naive patients ($p < 0.0001$). The median PFS was 3.8 [3.1–4–6] months and 12.5 [8.4–20.4] months, respectively (Table 3, Fig. 3A). The resulting 12 months PFS rates (95 % CI) were accordingly lower for the pre-treated cases (27.8 % [23.5–32.9] vs. 50.6 % [45.7–56.1]). This applied similarly to both anti-PD-1 pre-treatment and Ipi/Nivo (Fig. 4).

Patients with adjuvant pre-treatment showed a significantly shorter FU compared to the naive group ($p < 0.0001$), which could not be accounted for by matching (Table 3).

The multivariable analysis showed impaired ICI treatment efficiency

Table 1
Patient demographics and disease characteristics at 1L ICI treatment.

	PD-1 Naive+1L ICI (N = 3390)	P-value	Pre-treated+1L ICI (N = 389)	P-value	Matched controls PD-1 Naive+1L ICI (N = 389)
Sex					
Female	1331 (39.3 %)	0.54	146 (37.5 %)	0.77	141 (36.2 %)
Male	2059 (60.7 %)		243 (62.5 %)		248 (63.8 %)
Age (years)					
Mean (SD)	67.1 (13.8)	< 0.001	62.0 (14.5)	0.61	62.5 (13.0)
Melanoma type					
Cutaneous	2833 (83.6 %)	< 0.001	362 (93.1 %)	0.68	358 (92.0 %)
MUP	557 (16.4 %)		27 (6.9 %)		31 (8.0 %)
BRAF					
Wildtype	2105 (62.1 %)	< 0.001	259 (66.6 %)	0.85	252 (64.8 %)
Mutated	1124 (33.2 %)		99 (25.4 %)		106 (27.2 %)
Unknown	161 (4.7 %)		31 (8.0 %)		31 (8.0 %)
ECOG					
0	1810 (53.4 %)	< 0.001	286 (73.5 %)	0.99	283 (72.8 %)
1	797 (23.5 %)		62 (15.9 %)		65 (16.7 %)
≥ 2	239 (7.1 %)		12 (3.1 %)		11 (2.8 %)
Missing/Unknown	544 (16.0 %)		29 (7.5 %)		30 (7.7 %)
CCS					
6	2225 (65.6 %)	< 0.001	242 (62.2 %)	0.75	256 (65.8 %)
7	327 (9.6 %)		61 (15.7 %)		53 (13.6 %)
≥ 8	122 (3.6 %)		31 (8.0 %)		30 (7.7 %)
Missing/Unknown	716 (21.1 %)		55 (14.1 %)		50 (12.9 %)
AJCC stage					
Stage III, NR	253 (7.5 %)	0.05	45 (11.6 %)	0.99	42 (10.8 %)
Stage IV M1a	604 (17.8 %)		58 (14.9 %)		54 (13.9 %)
Stage IV M1b	636 (18.8 %)		71 (18.3 %)		73 (18.8 %)
Stage IV M1c	1280 (37.8 %)		149 (38.3 %)		152 (39.1 %)
Stage IV M1d	617 (18.2 %)		66 (17.0 %)		68 (17.5 %)
Serum LDH					
Normal	1949 (57.5 %)	< 0.001	268 (68.9 %)	0.87	261 (67.1 %)
Elevated	1008 (29.7 %)		90 (23.1 %)		95 (24.4 %)
Missing	433 (12.8 %)		31 (8.0 %)		33 (8.5 %)
N° of metastatic sites					
1	1037 (30.6 %)	< 0.001	162 (41.6 %)	0.58	152 (39.1 %)
2	1022 (30.1 %)		117 (30.1 %)		114 (29.3 %)
≥ 3	1331 (39.3 %)		110 (28.3 %)		123 (31.6 %)
Type of 1 L therapy					
Anti-PD−1	2459 (72.5 %)	< 0.001	86 (22.1 %)	1	86 (22.1 %)
Ipi/Nivo	931 (27.5 %)		303 (77.9 %)		303 (77.9 %)

Patient demographics and disease characteristics for the unmatched and matched samples after disease recurrence on adjuvant anti-PD-1 therapy (pre-treated cohort) and without adjuvant pre-treatment (PD-1 naive cohort) at first-line (1L) ICI treatment. N: number of patients; SD: standard deviation; MUP: melanoma of unknown primary; BRAF: BRAF V600 mutation status; ECOG: Eastern Cooperative Oncology Group; CCS: Charlson comorbidity score; AJCC stage: American Joint Committee on Cancer 8th edition; NR: non-resectable; LDH: serum lactate dehydrogenase; Ipi/Nivo: ipilimumab/nivolumab.

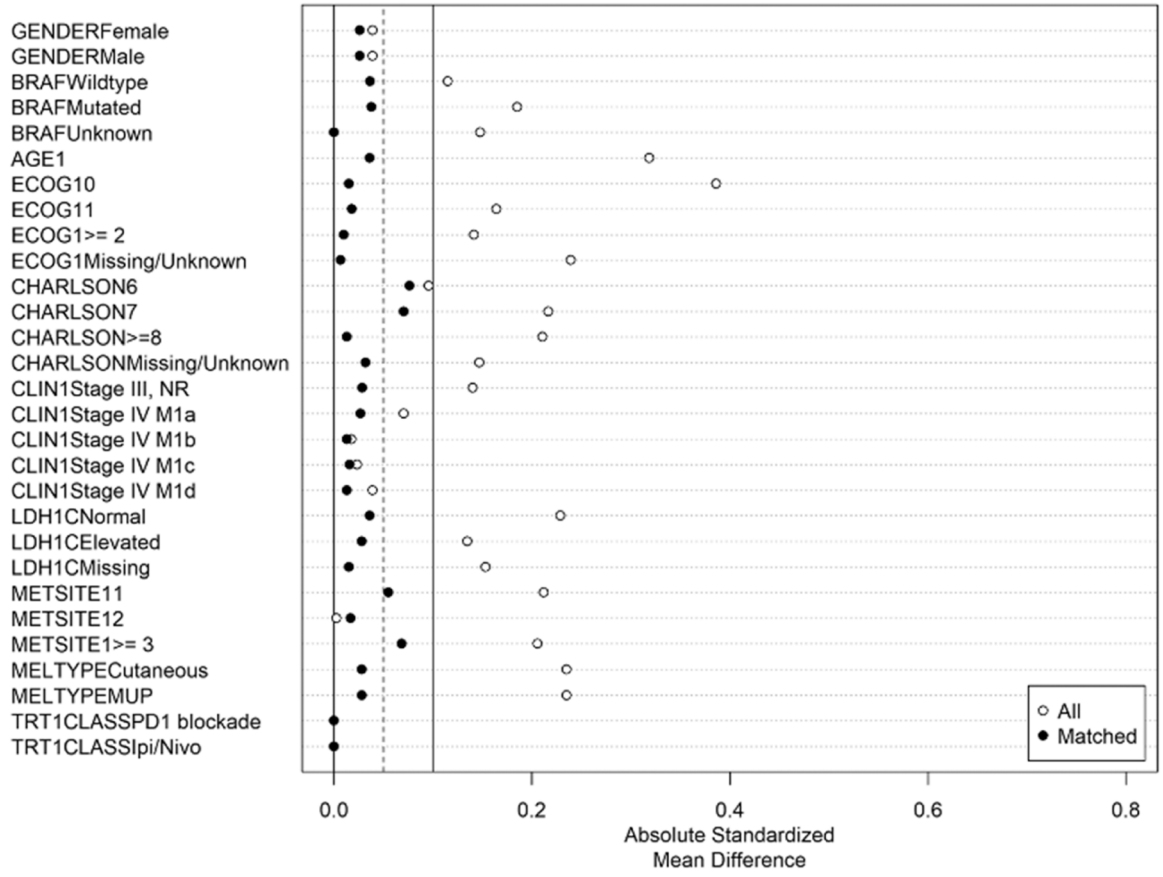

Fig. 2. Love plot illustrating the quality of the matching procedure. AGE1: age at first-line treatment (1L); BRAF: $BRAF^{V600}$ mutation status; ECOG 10/11/1 > =2: Eastern Cooperative Oncology Group score 0/1/> =2 at 1L; NR: non-resectable; CLIN1: clinical stage based on American Joint Committee on Cancer at 1 L; LDH1: lactate dehydrogenase at 1L; METSITE 11/12/1 > =3: number (1, 2 or >=3) of metastatic sites at 1L; MELTYPE: melanoma type; TRT1: treatment at 1L.

with a HR of 0.51 (95 % CI 0.42–0.61) for pre-treated patients and a homogeneous presence in all subgroups, with a comparatively strong effect for patients with MUP and patients with an impaired PS (Fig. 4).

To explore whether the time of recurrence had an influence on ICI retreatment efficiency, we analyzed subgroups with recurrence before or up to 12 weeks after EoT (early recurrence) as compared to later recurrences (>12 weeks after EoT). Early disease recurrence was reported in 267 (68.6 %) patients, while late recurrence occurred in 122 (31.4 %) patients (Table S1). The median time (95 %CI) to retreatment from the timepoint of recurrence on adjuvant treatment was 1.35 (0.03–26.5) months for anti-PD-1 and 1.15 (0.03–37.9) months for Ipi/Nivo (Table S3). Out of 86 patients receiving anti-PD-1 single agent retreatment 40 (46.5 %) patients had an early recurrence from their adjuvant pre-treatment. 17 (19.7 %) of them had a delayed retreatment, mostly due to other treatment modalities after recurrence, i.e. surgery and/or radiotherapy. Patients receiving immediate retreatment with anti-PD-1 were mostly ineligible for receiving Ipi/Nivo due to side effects or availability of the combination.

The ORR of 1L ICI was higher in late recurrences (37.7 %) as compared to early recurrences (28.5 %), without reaching statistical significance ($p = 0.08$) (Table 5), and median PFS (95 % CI) from 1L was significantly longer in late compared to early recurrences regardless of the type of 1L agent treatment (Table 5, Fig. 3 Panel B, D, E).

We further explored whether this was correlated with different baseline parameters at 1L retreatment, which were compared by stratification for time of recurrence. There was a small difference for baseline LDH elevation in favor of late recurrences (19.7 % vs. 24.7 % in early recurrence; $p = 0.023$) (Table S1). In multivariable Cox regression analysis late recurrence was a significant factor with a HR of 0.70 (95 % CI 0.52–0.92), generally well distributed over various subgroups (Fig. S2).

Early termination of adjuvant anti-PD-1 was most frequently due to early disease recurrence (56.8 %, n = 221) during. Another 34 (8.7 %) and 31 (8.0 %) patients stopped adjuvant treatment for side effects or due to other reasons, respectively (Table S2 and S3). Although patients who stopped treatment for side effects or other reasons received combined Ipi/Nivo less often (Table S2), the ORR to 1L ICI for these patients was higher (41.2 % and 41.9 %, respectively) as compared to 34.0 % in patients who regularly completed adjuvant treatment, although not statistically significant ($p = 0.14$). Accordingly, median PFS of 1L ICI retreatment was highest in patients who terminated adjuvant treatment for side effects (Table S2).

The outcome of anti-PD-1 vs. Ipi/Nivo as 1L cannot directly be compared, since the bias by significantly different baseline characteristics could not be adjusted given the limited sample size. Overall, the usage of Ipi/Nivo was dominant with 78 % of all cases as compared to anti-PD-1 alone. Importantly, this was much more prevalent in early recurrences, where Ipi/Nivo dominated with 85 % of all 1L treatments as compared to 62 % in late recurrences. Likely confounders as evidenced by multivariable Cox regression for PFS (Fig. S2) were not evenly distributed at 1L metastatic setting between both treatment schemes. Poor prognostic markers such as higher AJCC substage, including presence of brain metastasis (M1d), and number of metastatic sites were more frequently observed in patients receiving Ipi/Nivo, while ECOG PS and LDH were not significantly different (Table S3). In the multivariable analysis assessing the impact of adjuvant pre-treatment, stratification for the type of 1L treatment showed a significant impairment of 1L ICI efficiency for both single-agent and combination ICI, with a HR of 0.48 (95 %CI 0.39–0.59) for Ipi/Nivo and HR of 0.61 (95 %CI 0.42–0.90) for anti-PD-1 (Fig. 4).

Table 2
Patient demographics and disease characteristics at 1L of matched samples separated to Ipi/Nivo and anti-PD-1 treatment.

	Pre-treated+ 1L Ipi/Nivo (N = 303)	PD-1 Naive+ 1L Ipi/Nivo (N = 303)	P-value	Pre-treated+ 1L Anti-PD-1 (N = 86)	PD-1 Naive+ 1L Anti-PD-1 (N = 86)	P-value
Sex						
Female	111 (36.6 %)	106 (35.0 %)	0.75	35 (40.7 %)	32 (37.2 %)	0.75
Male	192 (63.4 %)	197 (65.0 %)		51 (59.3 %)	54 (62.8 %)	
Age (years)						
Mean (SD)	60.3 (13.8)	60.8 (12.4)	0.65	68.0 (15.6)	69.7 (11.9)	0.41
Melanoma type						
Cutaneous	286 (94.4 %)	281 (92.7 %)	0.51	76 (88.4 %)	77 (89.5 %)	1
MUP	17 (5.6 %)	22 (7.3 %)		10 (11.6 %)	9 (10.5 %)	
BRAF						
Wildtype	192 (63.4 %)	182 (60.1 %)	0.62	67 (77.9 %)	66 (76.7 %)	0.98
Mutated	87 (28.7 %)	98 (32.3 %)		12 (14.0 %)	13 (15.1 %)	
Unknown	24 (7.9 %)	23 (7.6 %)		7 (8.1 %)	7 (8.1 %)	
ECOG						
0	228 (75.2 %)	224 (73.9 %)	0.95	58 (67.4 %)	60 (69.8 %)	0.98
1	43 (14.2 %)	48 (15.8 %)		19 (22.1 %)	18 (20.9 %)	
> = 2	8 (2.6 %)	8 (2.6 %)		4 (4.7 %)	4 (4.7 %)	
Missing/ Unknown	24 (7.9 %)	23 (7.6 %)		5 (5.8 %)	4 (4.7 %)	
CCS						
6	187 (61.7 %)	201 (66.3 %)	0.70	55 (64.0 %)	57 (66.3 %)	0.99
7	49 (16.2 %)	42 (13.9 %)		12 (14.0 %)	11 (12.8 %)	
> =8	21 (6.9 %)	19 (6.3 %)		10 (11.6 %)	10 (11.6 %)	
Missing/ Unknown	46 (15.2 %)	41 (13.5 %)		9 (10.5 %)	8 (9.3 %)	
AJCC stage						
Stage III, NR	31 (10.2 %)	28 (9.2 %)	0.96	14 (16.3 %)	14 (16.3 %)	1
Stage IV M1a	35 (11.6 %)	31 (10.2 %)		23 (26.7 %)	23 (26.7 %)	
Stage IV M1b	53 (17.5 %)	55 (18.2 %)		18 (20.9 %)	18 (20.9 %)	
Stage IV M1c	122 (40.3 %)	122 (40.3 %)		27 (31.4 %)	27 (31.4 %)	
Stage IV M1d	62 (20.5 %)	67 (22.1 %)		4 (4.7 %)	4 (4.7 %)	
Serum LDH						
Normal	206 (68.0 %)	199 (65.7 %)	0.69	62 (72.1 %)	64 (74.4 %)	0.82
Elevated	73 (24.1 %)	82 (27.1 %)		17 (19.8 %)	14 (16.3 %)	
Missing	24 (7.9 %)	22 (7.3 %)		7 (8.1 %)	8 (9.3 %)	
N° of metastatic sites						
1	115 (38.0 %)	101 (33.3 %)	0.41	47 (54.7 %)	47 (54.7 %)	1
2	96 (31.7 %)	97 (32.0 %)		21 (24.4 %)	21 (24.4 %)	
> = 3	92 (30.4 %)	105 (34.7 %)		18 (20.9 %)	18 (20.9 %)	

Patient demographics and disease characteristics for the matched samples after disease recurrence on adjuvant anti-PD-1 therapy (pre-treated cohort) and without adjuvant pre-treatment (PD-1 naive cohort) at first-line (1L) Ipi/Nivo and anti-PD-1 treatment. N: number of patients; SD: standard deviation; MUP: melanoma of unknown primary; BRAF: BRAF V600 mutation status; ECOG: Eastern Cooperative Oncology Group; CCS: Charlson comorbidity score; AJCC stage: American Joint Committee on Cancer 8th edition; NR: non-resectable; LDH: serum lactate dehydrogenase; Ipi/Nivo: ipilimumab/nivolumab.

Table 3
Therapy outcome in matched samples after 1L ICI treatment.

	Pre-treated+ 1L ICI (N = 389)	PD-1 Naive+ 1L ICI (N = 389)	P-value*
Best response			
CR	60 (15.4 %)	76 (19.5 %)	< 0.0001
PR	62 (15.9 %)	114 (29.3 %)	
SD	56 (14.4 %)	52 (13.4 %)	
PD	182 (46.8 %)	105 (27.0 %)	
Not assessable	29 (7.5 %)	42 (10.8 %)	
ORR	122 (31.4 %)	190 (48.8 %)	< 0.0001
DCR	178 (45.8 %)	242 (62.2 %)	< 0.0001
Survival (95 % CI)			
Median PFS	3.8 (3.1–4.6)	12.5 (8.4–20.4)	< 0.0001
Median FU	20.0 (17.7–21.0)	36.8 (33.0–40.5)	< 0.0001

Treatment outcome in matched samples of patients after disease recurrence on adjuvant anti-PD-1 therapy (pre-treated cohort) compared to adjuvant anti-PD-1 naive (PD-1 naive cohort) patients at first-line (1L) ICI. N: number of patients; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; ORR: overall response rate; DCR: disease control rate; PFS: progression-free survival; FU; follow-up; CI: confidence interval. *p-values for survival differences between groups were calculated using the log-rank test.

4. Discussion

This study provides evidence that an adjuvant pre-treatment with anti-PD-1 in melanoma is associated with impaired efficiency of a subsequent ICI treatment for metastatic disease using either combined anti-PD-1/CTLA-4 therapy, or anti-PD-1 single agent rechallenge. This is reflected by significantly lower response rates and significantly shorter PFS as compared to treatment naive matched controls.

In principle, the question under study can be referred to as the impact of ICI resistance on further treatment options. ICI resistance may occur in the adjuvant and in the metastatic setting, and it is unclear so far whether the consequences are similar in both settings. In the adjuvant setting there is only sparse data available. In the KEYNOTE-054 trial on adjuvant treatment with pembrolizumab, patients in the verum arm were allowed a rechallenge with pembrolizumab after recurrence, if occurring more than 6 months after the end of adjuvant treatment and if no brain metastases were present. Among 9 patients with evaluable response in the metastatic setting, one reached CR, 3 patients were considered SD and 5 as PD [20]. Owen et al. reported on 147 cases of systemic treatment after adjuvant anti-PD-1 treatment failure, either while receiving adjuvant anti-PD-1 or within 1 month or greater than 1 month after last dose of adjuvant anti-PD-1. The treatments included ipilimumab (± anti-PD-1), anti-PD-1 monotherapy, BRAF/MEKi, or anti-PD(L)-1 plus novel agents. 1 L anti-PD-1 was only

Table 4
Therapy outcome in matched samples after 1 L separated to Ipi/Nivo and anti-PD-1 treatment.

	Pre-treated+ 1L Ipi/Nivo (N = 303)	PD-1 Naive+ 1L Ipi/Nivo (N = 303)	P-value*	Pre-treated+ 1L Anti-PD- (N = 86)	PD-1 Naive+ 1L Anti-PD-1 (N = 86)	P-value*
Best response						
CR	40 (13.2 %)	52 (17.2 %)	< 0.0001	20 (23.3 %)	26 (30.2 %)	0.25
PR	48 (15.8 %)	95 (31.4 %)		14 (16.3 %)	19 (22.1 %)	
SD	40 (13.2 %)	34 (11.2 %)		16 (18.6 %)	19 (22.1 %)	
PD	149 (49.2 %)	84 (27.7 %)		33 (38.4 %)	21 (24.4 %)	
Unknown	26 (8.6 %)	38 (12.5 %)		3 (3.5 %)	1 (1.2 %)	
ORR	88 (29.0 %)	147 (48.5 %)	< 0.0001	34 (39.5 %)	45 (52.3 %)	0.13
DCR	128 (42.2 %)	181 (59.7 %)	< 0.0001	50 (58.1 %)	64 (74.4 %)	0.04
Survival (95 % CI)						
Median PFS	3.2 (2.8–4.4)	12.7 (8.0–23.2)	< 0.0001	5.9 (3.8–10.1)	15.7 (8.0–27.5)	0.02
Median FU	18.6 (16.2–20.3)	37.3 (33.0–40.3)	< 0.0001	21.1 (18.0–24.4)	38.8 (27.9–46.2)	< 0.0001

Treatment outcome in matched samples of patients after disease recurrence on adjuvant anti-PD-1 therapy (pre-treated cohort) compared to adjuvant anti-PD-1 naive (PD-1 naive cohort) patients at first-line (1L) Ipi/Nivo and 1L anti-PD-1. N: number of patients; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; ORR: overall response rate; DCR: disease control rate; PFS: progression-free survival; FU; follow-up; CI: confidence interval. *p-values for survival differences between groups were calculated using the log-rank test.

effective in those recurring after end of adjuvant treatment (2 PR out of n = 5), and not in patients recurring while on treatment (0 out of 9). Ipilimumab or Ipi/Nivo achieved 24 % ORR (8/38) in patients recurring while on treatment, and 40 % ORR (2/6) when recurring off treatment [21]. This is well in accordance with the data presented here, showing response rates of 29.0 % for Ipi/Nivo in the pre-treated cohort and that a delayed time of recurrence was associated with a higher response rate of 37.7 %.

In general, the pre-treated population investigated in our study had more favorable prognostic factors than in typical metastatic cohorts, particularly evidenced by highly significant imbalances compared to the unmatched PD-1 naive population in the EUMelaReg database. This likely explains why we observed high response rates for the pre-treated and naive cohort, in particular for CR. Lower AJCC substages, number of brain metastases and LDH elevations for this group of patients were also found for those re-treated with anit-PD-1 alone compared to those treated with Ipi/Nivo. In addition, due to the matching process also the PD-1 naive cohort treated with 1L anti-PD-1 displays higher response rates and therefore cannot be used for comparative purposes for general metastatic melanoma patients or for patients in clinical trials. In the metastatic setting, studies on rechallenge of single-agent anti-PD-1 in patients with advanced melanoma that relapsed from initial remission showed anti-tumor activity with ORR's ranging from 15 % to 63 % [22–25]. Thus, anti-PD-1 therapy may induce reactivation of tumor immunity in patients who lost it while off-treatment. In our study, patients who recurred late after the end of adjuvant treatment may have a similar situation, explaining a meaningful response rate after retreatment with anti-PD-1, although still less than in anti-PD-1 naive cases.

For patients recurring while on adjuvant treatment, the situation seems more similar to anti-PD-1 failure in the metastatic setting. Since the addition of anti-CTLA-4 antibodies to anti-PD-1 results in somewhat higher response rates and PFS as compared to anti-PD-1 in metastatic melanoma [26], combined ICI with Ipi/Nivo is frequently being used in anti-PD-1 refractory patients resulting in secondary response rates ranging from 20 % to 31 % [24,27,28]. Also, there is some evidence of superiority of Ipi/Nivo vs ipilimumab alone in a phase II randomized trial with a response rate of 28 % for Ipi/Nivo and a HR of 0.63 over ipilimumab for PFS [29]. These results are in line with what we found in early recurrences from anti-PD-1 adjuvant treatment in the current study. A minority of patients with early recurrence were retreated with single-agent anti-PD-1, which compares to the second-line response rate of pembrolizumab in the KEYNOTE-002 trial. Here, pembrolizumab was given in second-line for metastatic melanoma after failure from 1L ICI with ipilimumab and the ORR was 21 %-26 % with different schedules of pembrolizumab [4].

This illustrates that the case of ICI "resistance" in the adjuvant situation as compared to the metastatic stages needs to be carefully differentiated. In particular, the situation of recurrence while on adjuvant treatment seems to be more similar to ICI failure in the metastatic state, reflected by the response rate of 25.3 % in patients who had recurred while on adjuvant treatment.

Impaired outcome of subsequent ICI may also be due to worse prognostic factors in the recurrent situation. But since the patients recurring from adjuvant treatment had a more favourable pattern of prognostic factors compared to the unmatched controls, such bias would rather be in favour of the pre-treated group. The response rates in the matched control group are in accordance with those from other real-world studies on anti-PD-1 based treatments [30–34].

Patients in our analysis recurring early had slightly worse prognostic factors, reflected by rates of elevated serum LDH level, limited stage M1a/b, and number of metastatic sites compared to those with late recurrence. In accordance with the literature these all are prognostic factors for poorer outcome in patients with metastatic melanoma [35]. However, the differences were limited and not likely to explain the overall effect on the subsequent outcome.

Limitations of our study include the fact that response evaluation in clinical practice not always follows strict criteria like RECIST or immune-related response criteria, including no pre-specified target lesion list available in the registry. While this might be comparable with clinical trial data, selective confounding for the strata analyzed in our study seems less likely. Our study has some further limitations inherent in observational data analysis including the possibility of selection bias and limitations in representativity, but the given effect size and robust statistics warrant to consider our findings both robust and relevant.

5. Conclusion

In conclusion, this real-world data analysis demonstrates that re-treatment with ICI in melanoma patients who have failed from adjuvant anti-PD-1 therapy is associated with significantly reduced efficiency as compared to treatment naive patients. The impact on real-world treatment decisions warrants further research, as does the question on which sequence of adjuvant and potential non-adjuvant treatments might be preferable for patients with BRAF mutated melanoma.

Study/ethical approval and consent to participate

This study is a retrospective database analysis. Ethical approval was not required. Patient informed consent was obtained by patients in writing before documentation or transfer of any data occurred into a national country or multi-country registry. Since the national informed consent covered data transfers from a country or multi-country registry

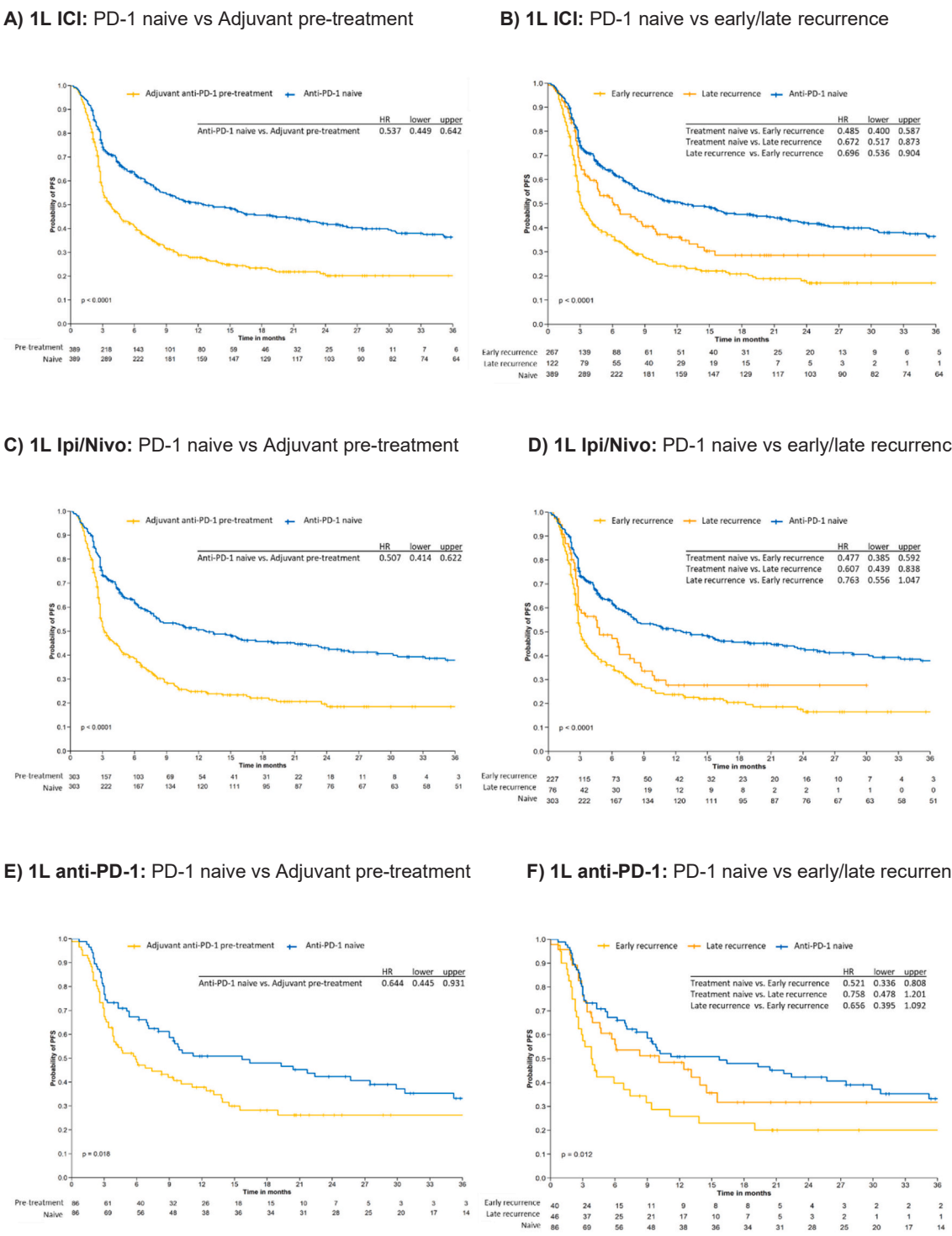

Fig. 3. Kaplan-Meier curves of progression-free survival (PFS) stratified by adjuvant pre-treatment (left side) and by timing of recurrence (right side) for matched cohorts. (A, C, E) Estimates are shown for patients treated with 1L ICI, Ipi/Nivo, and anti-PD-1 and pre-treatment with adjuvant anti-PD-1 (yellow line) or anti-PD-1 naive (blue line). (B, D, F) Estimates are stratified by timing of recurrence to adjuvant anti-PD-1 pre-treatment: early recurrence (yellow line, defined as on adjuvant anti-PD-1 or within 3 months after end of anti-PD-1), late recurrence (blue line; defined as more than 3 months after end of adjuvant treatment), and anti-PD1 naive (grey line). HR: hazard ratio.

to other international scientific research organizations such as EUMelaReg, no further consent was necessary. For the anonymous analysis of EUMelaReg patient data within this study, no separate patient information was needed.

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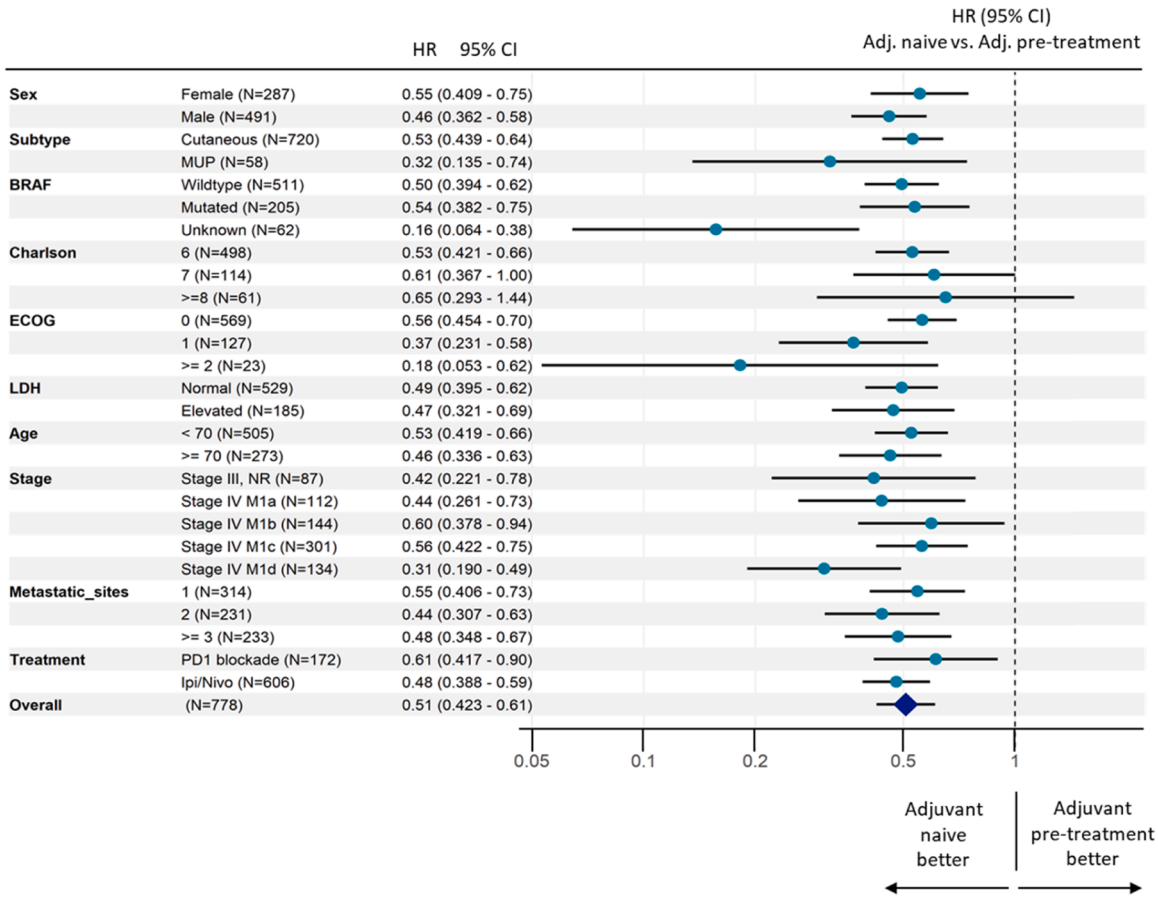

Fig. 4. Hazard ratios for progression of 1L ICI in matched samples with or without adjuvant anti-PD-1 pre-treatment. HRs and 95 % CI from a multivariate cox regression model are reported for 1L ICI for PFS comparing adjuvant anti-PD-1 naive vs adjuvant anti-PD-1 pre-treated patients. Stratified analyses for the given subgroups are adjusted for all other variables in the model. N: number of patients; MUP: melanoma of unknown primary; BRAF: $BRAF^{V600}$ mutation status; Charlson: Charlson comorbidity score; ECOG: Eastern Cooperative Oncology Group performance score; LDH: serum lactate dehydrogenase; Stage: American Joint Committee on Cancer 8th edition; treatment: non-adjuvant treatment in 1L advanced setting; ICI: immune checkpoint inhibitors; PFS: progression-free survival; HR: hazard ratio; CI: confidence interval.

Table 5
Therapy outcome by time of recurrence.

	Early recurrence (N = 278)	Late recurrence (N = 111)	P-value*
Best response			
CR	35 (13.1 %)	25 (20.5 %)	0.28
PR	41 (15.4 %)	21 (17.2 %)	
SD	39 (14.6 %)	17 (13.9 %)	
PD	133 (49.8 %)	49 (40.2 %)	
Not assessable	19 (7.1 %)	10 (8.2 %)	
ORR	76 (28.5 %)	46 (37.7 %)	0.08
DCR	115 (43.1 %)	63 (51.6 %)	0.13
Median PFS (95 % CI)	3.1 (2.8–3.8)	6.1 (4.5–8.8)	0.006

First-line treatment outcome by time of recurrence from adjuvant anti-PD-1. Early recurrence is defined as while on adjuvant anti-PD-1 or within 3 months after end of adjuvant anti-PD-1. Late recurrence is defined as more than 3 months after end of adjuvant anti-PD-1 treatment. N: number of patients; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; ORR: overall response rate; DCR: disease control rate; PFS: progression-free survival; CI: confidence interval. *p-values for survival differences between groups were calculated using the log-rank test.

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Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.115339.

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