Immune checkpoint inhibition in patients with *NRAS* mutated and *NRAS* wild type melanoma: a multicenter Dermatologic Cooperative Oncology Group study on 637 patients from the prospective skin cancer registry ADOREG

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1. Introduction

Melanoma often harbours somatic mutations affecting typically genes of the MAPKi and RAS pathway. They can be classified based on driver mutations as (I) *BRAF*-mutated (40–50%), (II) *RAS*-mutated (20–30%), (III) *NF1*-mutated (10–15%), or (IV) triple (*BRAF*, *NRAS*, and *NF1*) wild type (~10%) [1,2].

NRAS was the first oncogene to be recognised in melanoma [3] and mutations in the *NRAS* gene are

usually mutually exclusive of other mutations [2]. Melanoma harbouring *NRAS* mutations form a distinct subgroup of the disease and appear to have a poor prognosis [4–6]. These mutations primarily occur at position 61 and can involve amino acid changes from glutamine (Q) to arginine (R), lysine (K), or leucine (L), which locks the NRAS protein into a GTP-bound state thereby impairing its GTPase activity [7,8]. In about 20% of *NRAS* mutations, the mutation occurs at codons 12 or 13, resulting in an amino acid change from glycine (G) to aspartic acid (D), which prevents binding of GTPase activating proteins to NRAS [7].

Immune checkpoint inhibitors (ICI) have demonstrated high efficacy in many cancers, including melanoma. Anti-PD1 (e.g. nivolumab and pembrolizumab) and anti-CTLA4 (e.g. ipilimumab), either in combination or as a monotherapy, have improved clinical outcome in the adjuvant [9–11] and metastatic setting [12–14] and are currently tested as neoadjuvant strategies [15]. The impact of *NRAS* mutations on the outcome of ICI remains unclear. Some studies have found comparable progression-free survival (PFS) and overall survival (OS) for patients with *NRAS*-mutated (*NRAS*mut) and *NRAS*-wild type (*NRAS*wt) melanoma [16], while others have reported better responses [17] or less favourable survival [18] in patients with *NRAS*mut melanoma. However, although investigating large multicenter cohorts, these data were mainly retrospective [6,16–20]. Treatment outcomes for *NRAS*mut and *NRAS*wt patients were investigated in a prospective clinical trial for MEK inhibitors [21] and in two retrospective trials for MEK inhibitor and/or ICI [18,22] showing modest improvement. Few larger randomised trials included subgroup analysis of *NRAS* mutational status related to ICI outcome [23].

In a small sample, Johnson et al. showed that *NRAS*mut melanoma had higher programmed cell death ligand-1 (PD-L1) expression than *BRAF*-mutated and wild-type melanoma before ICI therapy [17]. To date, comprehensive data are lacking on the correlation between *NRAS* mutation status and PD-L1 expression in melanoma cells.

In this study, we investigated the potential effect of *NRAS* status (*NRAS*mut versus *NRAS*wt) on clinical characteristics and outcomes of anti-PD1-based therapy in patients with melanoma using a multicenter prospective database. We also investigated a complementary cohort of melanoma patients in whom PD-L1 staining was performed in tumour tissue.

2. Materials and methods

2.1. Study population and response assessment

Data were retrieved from the prospective multicenter skin cancer registry ADOREG of the German Dermatologic Cooperative Oncology Group (DeCOG) [24]. Patients were treated within 41 centres. Patients presenting at DeCOG academic cancer centres between 1 June 2014 and 31 May 2020 were identified according to the following inclusion criteria: histologically confirmed locally advanced or metastatic melanoma; first-line non-adjuvant ICI treatment with anti-PD1, anti-PD-L1, and anti-CTLA4 either as monotherapy or combination therapy; and known *NRAS* mutation status (*NRAS*mut or *NRAS*wt). Patients receiving adjuvant interferon were included. Exclusion criteria were (1) activating *BRAF* mutations, (2) prior ICI in an adjuvant therapy line, and (3) mucosal or ocular melanoma. Detailed information on patient history including prior treatments and follow-up information after the start of ICI therapy were extracted from local electronic patient files and captured within a central electronic data registry. Only first-line ICI therapy for advanced melanoma was considered in this analysis. Data-cut was July 2021.

2.2. Outcomes

The study end-points were overall response rate (ORR), PFS, and OS according to *NRAS* mutation status (*NRAS*mut or *NRAS*wt). The ORR was defined as the proportion of patients with partial or complete response to treatment. The disease control rate (DCR) additionally included patients with stable disease. Treatment response was evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 and was determined as best overall response from the start of ICI treatment until disease progression or death [25]. Best overall response (BOR) was defined as the best response recorded from the start of the treatment until disease progression/recurrence. During ICI therapy, tumours were staged every 3 months using computed tomography (CT), magnetic resonance imaging, or positron emission tomography-CT. The PFS was defined as the time from start of therapy until disease progression and OS was defined as the time from start of therapy until death. If disease progression or death did not occur, the date of the last patient contact was used as the end-point for assessing survival (censored PFS and OS). Median follow-up times were estimated as the median time in months among the event-free patients using reverse Kaplan-Meier approach (patients without recurrence or death).

2.3. Statistical analysis

Descriptive statistics are presented as percentages for categorical variables and as medians for continuous and ordinal variables. Baseline characteristics were compared using the Wilcoxon rank sum test for continuous variables and the Pearson's chi-square or Fisher's exact test for categorical variables. The ORR was measured as proportions in each group (*NRAS*mut and *NRAS*wt). Survival curves were estimated using the Kaplan–Meier

method and were censored according to the last patient contact. The PFS and OS were compared according to the *NRAS* mutation status and treatment using the log-rank test. The independent effect of *NRAS* mutation status and treatment type on PFS and OS was evaluated using univariate and multivariate Cox models. All variables were included in the initial multivariate model. The final multivariate models for PFS or OS included only those variables that were significant ($p < 0.05$) in the univariate analysis. To characterise prognostic baseline factors before start of treatment, information on site of metastasis categorised by American Joint Cancer Classification (AJCC)-v8, Eastern Cooperative Oncology Group performance status (ECOG), location of primary melanoma, number of involved organ sites and lactate dehydrogenase (LDH) were collected. Cox regression was used to calculate hazard ratios [HR] for OS and PFS. A p-value < 0.05 was considered statistically significant. All statistical analyses were done in R studio (version 4.1.1).

2.4. PD-L1 staining and quantification

Tumour PD-L1 expression was assessed in formalin-fixed paraffin embeded (FFPE) tissue samples. A rabbit antihuman PD-L1 monoclonal antibody (clone 28-8) and an analytically validated automated immunohistochemical assay (PD-L1 IHC 28-8 pharmDx for Autostainer Link 48; Dako, Glostrup, Denmark) was used, as previously described [26]. Per sample, a comparable tissue slide of the same preparation was stained with non-specific IgG and used as a negative control. PD-L1 expression in tumour tissue was quantified as the percentage of vital tumour cells that exhibited specific cell surface membrane staining of any intensity in a section containing at least 100 evaluable tumour cells, with ≥ 5% defined as positive staining, as previously described [26]. Quantification was performed by either pathologists or dermatologists, or both, with expertise in histopathology using conventional bright-field microscopy.

3. Results

3.1. Disease characteristics and first-line ICI of the total cohort

After excluding patients with an activating *BRAF* mutation and patients receiving ICI for adjuvant disease, 637 patients with metastatic melanoma stages III–IV were eligible for analysis (Fig. 1). The median age at melanoma diagnosis was 68 years (interquartile range [IQR] 55–76) and the majority of patients were male (n = 411, 65%). At the time of systemic treatment initiation, 58% of patients had ECOG 0, 36% had an elevated LDH, 84% were stage IV according to AJCC 8th edition and 28% had >= 3 organ sites involved (Table 1). Patients were treated with anti-PD1 monotherapy (n = 381, 60%), anti-PD1 plus anti-CTLA4 combination therapy (n = 192, 30%), or anti-CTLA4 monotherapy (n = 64, 10%) (Table 1). At a median follow-up of 35.9 months (IQR 18.0–54.6), the ORR to first-line ICI treatment was 190/637 (30%) for all patients (Table 1). For the total cohort, the median PFS was 10.3 months (95% confidence interval (CI), 7.9–13.2 months) and the median OS was 33.4 months (95% CI, 27.7–40.1) (Table 2). The 1- and 2-year PFS were 47% (95% CI, 42.3–51.0), and 38% (95% CI, 33.7–42.7), and the 1- and 2-year OS were 74% (95% CI, 70.6–77.8), and 56% (95% CI, 51.5–60.1) (Table 2, Fig. 2A,B).

3.2. Clinical and disease characteristics in NRAS mutant versus NRAS wild-type patients

From all 637 melanoma patients, 310 (49%) had *NRAS*mut melanoma and 327 (51%) had *NRAS*wt melanoma (Table 1). Nodular melanoma was significantly more common in *NRAS*mut patients compared with *NRAS*wt patients (39% versus 21% in *NRAS*wt, $p < 0.0001$). In *NRAS*wt patients, primary melanoma was most commonly localised on the lower extremities (22%), trunk (20%), and head and neck (20%), whereas in *NRAS*mut patients, primary melanoma were most frequently localised on the lower extremities (28%) and trunk (25%) (Table 1, $p = 0.001$). Prognostic factors were similar between the two groups (Table 1).

3.3. PFS and OS in first-line ICI in NRAS mutant versus NRAS wild-type patients

For the total cohort, the median PFS was 7.8 months (95% CI, 5.8–14.2) in *NRAS*mut patients and 11.4 months (95% CI, 9.1–16.7) in *NRAS*wt patients ($p = 0.3$) (Table 2, Fig. 2C). The median OS was 36.4 months (95% CI, 21.7–48.0) in *NRAS*mut patients and 32.9 months (95% CI, 25.0–45.1) in *NRAS*wt patients ($p = 0.5$) (Table 2, Fig. 2D). No significant differences in median PFS and OS were found in the anti-PD1 monotherapy cohort between *NRAS*mut patients (PFS: 9.0 months; 95% CI 5.9–16.6; OS: 27.9 months, 95% CI 20.1–48) and *NRAS*wt patients (PFS: 12.4, 95% CI 9.9–20.2, $p = 0.21$; OS: 29.4, 95% CI 22.8–37.3; $p = 0.97$) (Table 2, Fig. 3A,B). For the anti-CTLA4 combinational cohort, similar results were observed showing no differences in PFS and OS when comparing *NRAS*mut patients (PFS: 29.4; 95% CI 6.9-not reached; OS: 42.3; 95% CI 20.2-not reached) with *NRAS*wt patients (PFS: 24.2, 95% CI 16.6-NR; $p = 0.48$; OS: no reached, 95% CI 24.2-not reached; $p = 0.14$) (Table 2, Fig. 3C,D). In *NRAS*mut and

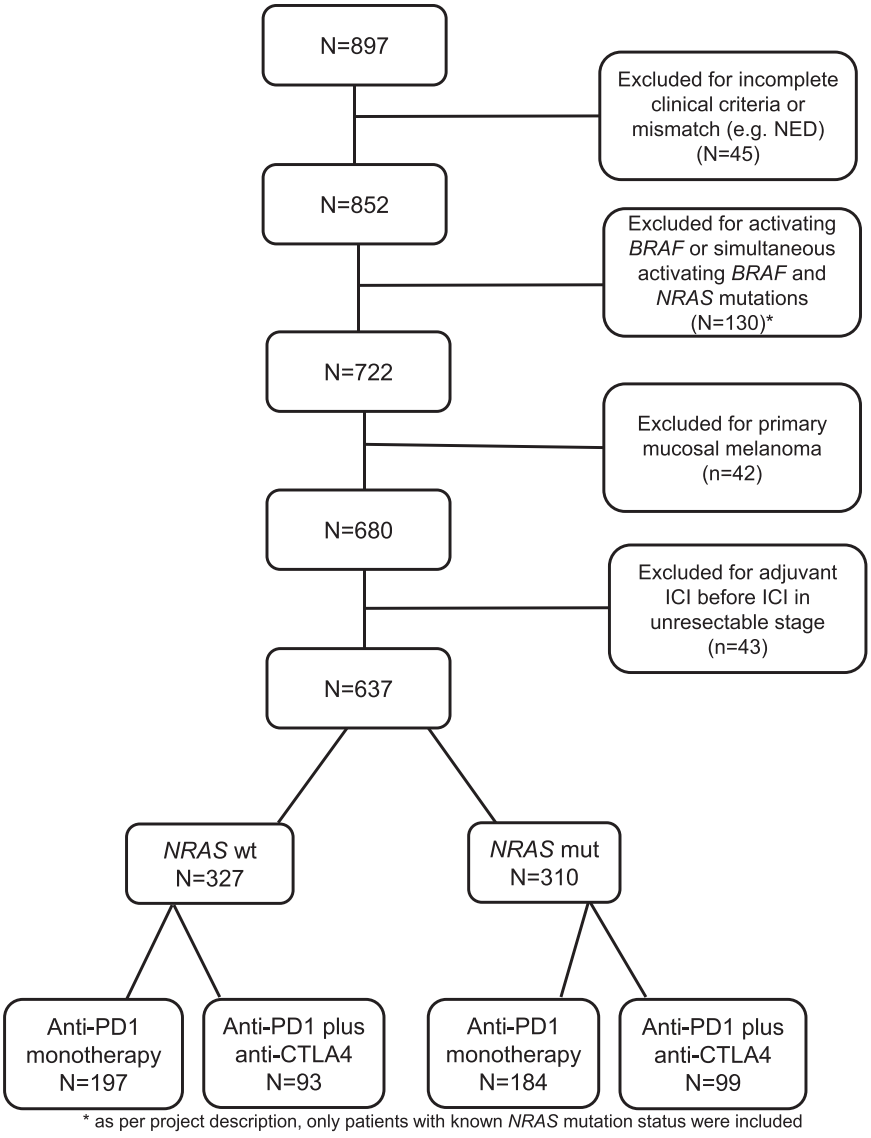

Fig. 1. CONSORT chart.

*NRAS*wt patients the 2-year PFS was higher in patients who received first-line combination therapy (54% in *NRAS*mut patients and 53% in *NRAS*wt patients) than in patients who received anti-PD1 monotherapy (39% in *NRAS*mut patients and 41% in *NRAS*wt patients) (Table 2, Supplementary Fig. 1A,C). Despite worse clinical prognostic factors, *NRAS*wt patients who received combination therapy had a significantly longer OS (median OS NR; 95% CI, 24.2–NR) than patients who received anti-PD1 monotherapy (median OS 29.4 months; 95% CI, 22.8–37.3) ($p = 0.03$) (Supplementary Table 2, Supplementary Fig. 1B,D). In *NRAS*mut patients multivariate Cox regression analysis confirmed that ECOG >= 1 (PFS: HR 1.5, $p = 0.02$; OS: HR 2.8, $p < 0.0001$), and elevated LDH (PFS: HR 1.5 $p = 0.04$; OS: HR 2.2, $p = 0.0002$) were associated with shorter PFS and OS. In *NRAS*wt patients multivariate Cox regression analysis showed that ECOG >= 1 (HR 1.5; $p = 0.04$) and elevated LDH (HR 1.5; $p = 0.02$) were associated with shorter PFS and primary melanoma in the head/neck (HR 0.6, $p = 0.02$), and treatment group (anti-PD1 HR 0.55, $p = 0.007$; and anti-PD1 plus anti-CTLA-4 HR 0.37, $p = 0.0001$) were still significantly associated with longer OS (Supplementary Tables 3–6).

3.4. Univariate and multivariate analysis for all patients receiving anti-PD1 monotherapy or combination therapy

Univariate Cox regression analysis showed that the following variables were associated with shorter PFS: elevated LDH levels (HR 1.35, $p = 0.01$), and ECOG performance status (PS) ≥ 1 (HR 1.43, $p = 0.005$), while primary melanoma in the head and neck (HR 0.7, $p = 0.04$), and treatment group (anti-PD1 versus anti-

Table 1
Patient and disease characteristics for the overall population divided by *NRAS* mutation status (*NRAS*mut versus *NRAS*wt).

	*NRAS*MUT (N = 310)	*NRAS*WT (N = 327)	*p*-value	Total (N = 637)
Age at diagnosis (years), median [range]	69.2 [13.8, 92.0]	66.4 [15.9, 92.6]	0.15	67.7 [13.8, 92.6]
Gender			0.6806	
Male	203 (65.5%)	208 (63.6%)		411 (64.5%)
Histological subtype			1.034819e-06	
NMM	121 (39.0%)	70 (21.4%)		191 (30.0%)
SSM	54 (17.4%)	52 (15.9%)		106 (16.6%)
ALM	22 (7.1%)	40 (12.2%)		62 (9.7%)
LMM	2 (0.6%)	17 (5.2%)		19 (3.0%)
Unknown primary	46 (14.8%)	51 (15.6%)		97 (15.2%)
Cutaneous, other subtype	37 (11.9%)	64 (19.6%)		101 (15.9%)
Unclassified	28 (9.0%)	33 (10.1%)		61 (9.6%)
Ulceration of the primary tumour			0.231	
Yes	130 (41.9%)	124 (37.9%)		254 (39.9%)
Unknown	77 (24.8%)	101 (30.9%)		178 (27.9%)
Localisation primary tumour			0.001017	
Head/neck	31 (10.0%)	66 (20.2%)		97 (15.2%)
Upper extremity	62 (20.0%)	50 (15.3%)		112 (17.6%)
Torso	78 (25.2%)	65 (19.9%)		143 (22.4%)
Lower extremity	86 (27.7%)	73 (22.3%)		159 (25.0%)
Unknown/skin	10 (3.2%)	21 (6.4%)		31 (4.9%)
Unknown	43 (13.9%)	52 (15.9%)		95 (14.9%)
Prior adjuvant therapy[a]			0.07789	
Yes	51 (16.5%)	37 (11.3%)		88 (13.8%)
No	259 (83.5%)	290 (88.7%)		549 (86.2%)
Age at therapy start (years, median) [range]	72.2 [19.1, 92.4]	70.1 [23.4, 93.2]	0.13	71.0 [19.1, 93.2]
First-line systemic therapy			0.2	
Ipilimumab + Nivolumab	99 (31.9%)	93 (28.4%)		192 (30.1%)
Nivolumab	84 (27.1%)	73 (22.3%)		157 (24.6%)
Pembrolizumab	100 (32.3%)	124 (37.9%)		224 (35.2%)
Ipilimumab	27 (8.7%)	37 (11.3%)		64 (10.0%)
Stage at therapy start			0.3533	
IIIB	12 (3.9%)	14 (4.3%)		26 (4.1%)
IIIC	37 (11.9%)	36 (11.0%)		73 (11.5%)
IIID	2 (0.6%)	3 (0.9%)		5 (0.8%)
IV	259 (83.5%)	274 (83.8%)		533 (83.7%)
Organ sites at therapy start			0.9418	
< 3	221 (71.3%)	235 (71.9%)		456 (71.6%)
≥ 3	89 (28.7%)	92 (28.1%)		181 (28.4%)
Brain metastases			1	
Yes	54 (17.4%)	56 (17.1%)		110 (17.3%)
ECOG status at therapy start			0.4428	
0	177 (57.1%)	193 (59.0%)		370 (58.1%)
≥ 1	74 (23.9%)	68 (20.8%)		142 (22.3%)
Unknown	59 (19.0%)	66 (20.2%)		125 (19.6%)
Best overall response			0.2377	
CR	30 (9.7%)	38 (11.6%)		68 (10.7%)
PR	55 (17.7%)	67 (20.5%)		122 (19.2%)
SD	48 (15.5%)	59 (18.0%)		107 (16.8%)
PD	147 (47.4%)	125 (38.2%)		272 (42.7%)
ND	30 (9.7%)	38 (11.6%)		68 (10.7%)
LDH			0.1193	
> ULN	121 (39.0%)	106 (32.4%)		227 (35.6%)
Unknown	57 (18.4%)	66 (20.2%)		123 (19.3%)
S100			0.4034	
> ULN	142 (45.8%)	142 (43.4%)		284 (44.6%)
Unknown	124 (40.0%)	130 (39.8%)		254 (39.9%)
PFS (months), median (95% CI)	7.8 (5.8–14.2)	11.4 (9.1–16.7)	0.26	10.3 (7.9–13.2)
OS (months), median (95% CI)	36.4 (21.7–48.0)	32.9 (25.0–45.1)	0.52	33.4 (27.7–40.1)

ALM, acrolentiginous melanoma; CR, complete response; LMM, lentigo maligna melanoma; MUT, mutated; ND, not determined/unknown; NMM, nodular malignant melanoma; PD, progressive disease; PR, partial response; SD, stable disease; SSM, superficial spreading melanoma; ULN, upper limit of normal; wt, wild type; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PFS, progression-free survival; OS, overall survival.

[a] Adjuvant interferon therapy.

Table 2
PFS and OS by first-line systemic treatment in *NRAS*mutant versus *NRAS*wt melanoma patients.

		All (n = 637)		First-line treatment					
		All		Anti-PD1		Anti-PD1 plus anti-CTLA4		Anti-CTLA4	
		*NRAS*mut (N = 310)	*NRAS*wt (N = 327)	*NRAS*mut (N = 184)	*NRAS*wt (N = 197)	*NRAS*mut (N = 99)	*NRAS*wt (N = 93)	*NRAS*mut (N = 27)	*NRAS*wt (N = 37)
PFS	Median, months	10.3	11.4	9.0	12.4	29.4	24.2	3.7	2.6
	(95% CI)	(7.9–13.2)	(9.1–16.7)	(5.9–16.6)	(9.9–20.2)	(6.9-NR)	(16.6-NR)	(2.3-NR)	(2.3–20.7)
	1-year rate	46.5%	47.7%	45.6%	50.5%	53.6%	63.4%	45.6%	25.1%
	(95% CI)	(42.3–51.0)	(42.0–54.2)	(40.0–52.5%)	(44.4–57.4%)	(43.4–66.2%)	(53.1–75.9%)	(29.7–69.9%)	(12.4–50.6%)
	2-year rate	37.9%	37.3%	38.5%	39.4%	41.1%	53.6%	52.5%	9.1%	16.7%
	(95% CI)	(33.7–42.7)	(31.5–44.2)	(32.7–45.4)	(33.3–46.6%)	(34.9–48.4%)	(43.4–66.2%)	(41.1–67.1%)	(1.5–55.4%)	(5.8–48.5%)
OS	Median, months	33.4	36.4	32.9	27.9	29.4	42.3	NR	37.6	37.6
	(95% CI)	(27.7–40.1)	(21.7–48.0)	(25.0–45.1)	(20.1–48.0)	(22.8–37.3)	(20.2-NR)	(24.2-NR)	(20.6-NR)	(19.6-NR)
	1-year rate	74.1%	69.5%	78.4%	70.8%	75.9%	62.6%	83.4%	84.9%	80.7%
	(95% CI)	(70.6–77.8)	(64.3–75.2)	(73.8–83.3)	(64.2–78.1)	(69.9–82.5)	(53.2–73.7%)	(75.5–92.1%)	(72.3–99.7%)	(68.8–94.6%)
	2-year rate	55.6%	54.7%	56.6%	51.5%	54.9%	58.4%	62.0%	64.9%	55.0%
	(95% CI)	(51.5–60.1)	(48.9–61.1)	(50.9–63.0)	(44.1–60.2)	(47.7–63.2)	(48.8–70.0%)	(51.2–75.1%)	(48.7–86.4%)	(40.8–74.1%)

PFS, progression-free survival; OS, overall survival; *NRAS*mut, NRAS-mutated; *NRAS*wt, NRAS-wild type.

CTLA-4, HR 0.6, $p = 0.002$; and anti-PD1 plus anti-CTLA4 versus anti-CTLA4, HR 0.42, $p < 0.0001$) were associated with improved PFS (Supplementary Table 7). Multivariate analyses confirmed that primary melanoma in the head/neck (HR 0.6, $p = 0.01$), ECOG PS ≥ 1 (HR 1.44, $p = 0.008$), and treatment group (anti-PD1 HR 0.52, $p = 0.002$; and anti-PD1 plus anti-CTLA-4 HR 0.38, $p < 0.0001$) were still significantly associated with PFS (Supplementary Table 7).

Univariate analysis showed the following factors to be associated with shorter OS: ≥ three affected organ sites (HR 1.33, $p = 0.02$), brain metastases (HR 1.9, $p < 0.0001$), ECOG PS ≥ 1 (HR 2.54, $p < 0.0001$), elevated LDH (HR 1.76, $p < 0.0001$), while primary melanoma in the head/neck (HR 0.67, $p = 0.02$), and prior adjuvant therapy (HR 0.66, $p = 0.02$) (Supplementary Table 8) were associated with improved OS. Multivariate analyses confirmed that elevated LDH (HR 1.47, $p = 0.004$), ECOG PS ≥ 1 (HR 2.37, $p < 0.0001$), primary melanoma in the head/neck (HR 0.6, $p = 0.01$), and brain metastases (HR 1.55, $p = 0.007$) were associated with OS (Supplementary Table 8).

3.5. ORR in first-line ICI in NRAS mutant versus NRAS wild-type patients

The ORR was not significant different in *NRAS*wt patients (69/194, 35%) than in *NRAS*mut patients (48/184, 26%, $p = 0.2$). In addition, there were no significant differences in DCR between *NRAS*mut patients (78/184, 42%) and *NRAS*wt patients (106/194, 54%) (Table 3). *NRAS*mut patients and *NRAS*wt patients also showed similar response rates to first-line treatment with anti-PD1 plus anti-CTLA4 combination therapy (ORR: 32% in *NRAS*mut versus 34% in *NRAS*wt) (Table 3). *NRAS*mut patients treated with combinational therapy showed significant better ORR compared to patients who received anti-PD1 monotherapy ($p = 0.05$, Table 3, Supplementary Table 1).

3.6. Clinical characteristics and therapy response in different NRAS genotypes

NRAS mutation subtyping revealed > 11 different genotypes, with the most frequent *NRAS* mutations being Q61R (n = 128, 41%), Q61K (n = 100, 32%), and Q61L (n = 32, 10%) (Supplementary Table 9). Clinical characteristics at first-line ICI were similar between *NRAS* Q61R and *NRAS* Q61K patients (Supplementary Table 10). No significant differences in median PFS and OS were found in the anti-PD1 monotherapy cohort and in the combinational cohort between patients with *NRAS* Q61K-mutated and *NRAS* Q61R-mutated melanoma (Supplementary Fig. 2A–D). The ORR and DCR to anti-PD1 therapy were higher in patients with *NRAS* Q61R mutations than in patients with *NRAS* Q61K mutations (ORR 36%, DCR 52% in *NRAS* Q61R

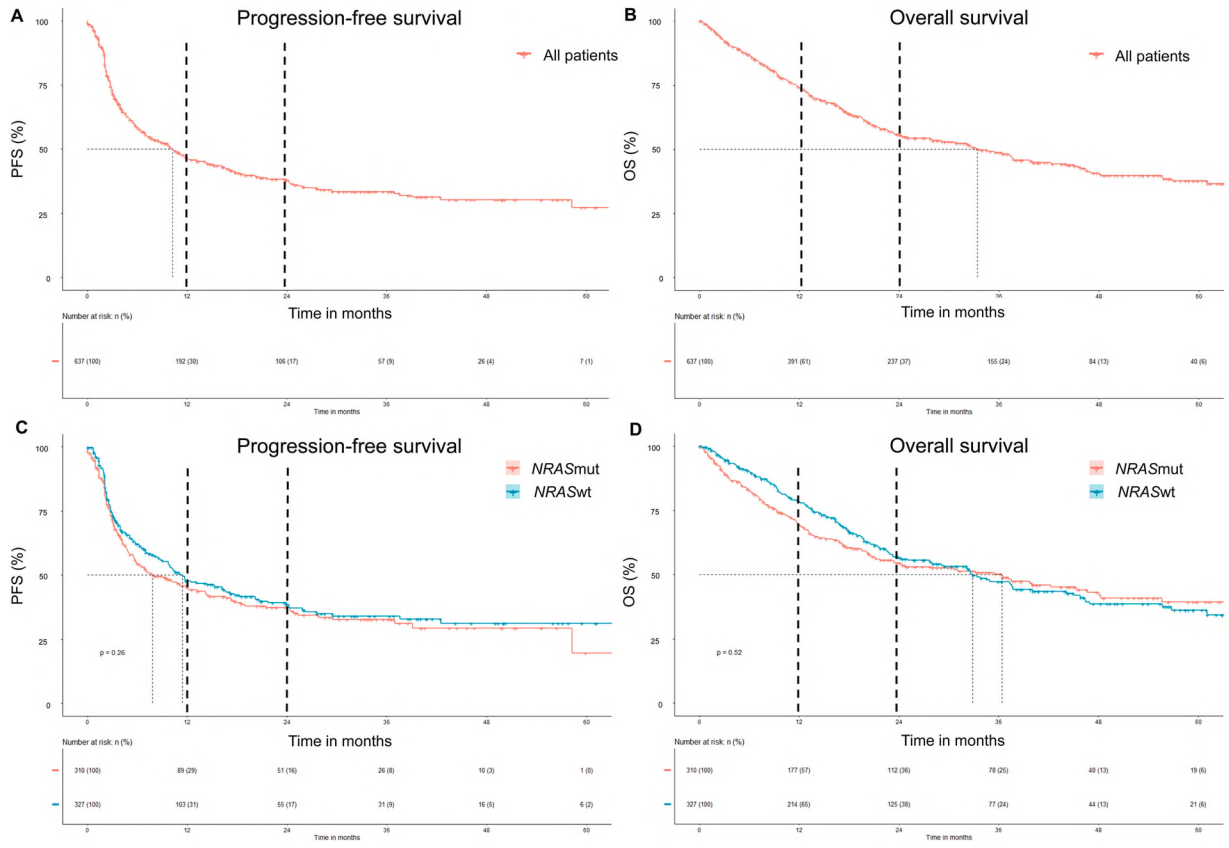

Fig. 2. PFS and OS in all NRASmut and NRASwt patients; (A) Progression-free survival (PFS) in the full cohort including *NRAS*-mutated (*NRAS*mut) and *NRAS*-wild type (*NRAS*wt) patients (n = 637). (B) Overall survival (OS) in the full cohort including *NRAS*mut and *NRAS*wt patients (n = 637). (C) PFS in *NRAS*mut (n = 310) and *NRAS*wt (n = 327) melanoma patients. (D) OS in *NRAS*mut (n = 310) and *NRAS*wt (n = 327) melanoma patients.

versus ORR 24%, DCR 39% in *NRAS* Q61K) but was comparable when anti-CTLA4 therapy was given in combination (ORR 30%, DCR 50% in *NRAS* Q61R versus ORR 36%, DCR 46% in *NRAS* Q61K, Supplementary Table 11).

3.7. Higher PD-L1 expression is related to improved OS in NRAS mutant and NRAS wild-type melanoma patients

We investigated whether PD-L1 expression was affected by *NRAS* mutation status in 125 patients with advanced melanoma (55 *NRAS*mut patients and 70 *NRAS*/*BRAF*wt patients). PD-L1 expression data were available for 82 of these patients (36 *NRAS*mut patients and 46 *NRAS*wt patients). In most cases, patients were naive to systemic therapy and had not yet received checkpoint inhibitor therapy at the time of PD-L1 analysis (see Supplementary Tables 12 and 13 for clinical characteristics). Using a 5% cutoff, 49% (n = 18) of *NRAS*mut and 51% (n = 19) of *NRAS*wt samples stained positive for PD-L1 ($p = 0.6$, Supplementary Table 13). The PFS was slightly improved and OS was significantly improved in patients with a high PD-L1 expression of > 5% (Supplementary Fig. 3A,B). Clinical and tumour characteristics were similar between patients with high and low PD-L1 expression (Supplementary Table 13). Positive PD-L1 expression improved OS in *NRAS*mut and *NRAS*wt patients but did not affect the PFS (Supplementary Fig. 3 A–G).

4. Discussion

This multicenter prospective analysis shows that patients with advanced *NRAS*mut and *NRAS*wt melanoma respond similar to combined anti-PD1 and anti-CTLA4 therapy and to anti-PD1 monotherapy. Although the characteristics of primary *NRAS*mut and *NRAS*wt melanoma were significantly different, the disease-specific prognostic factors were similar and significantly affected survival regardless of *NRAS* mutation status. To our knowledge, this is one of the largest prospective studies on the survival and treatment response of patients with *NRAS*mut and *NRAS*/*BRAF*wt melanoma treated with first-line anti-PD1-based therapy in a real-world setting.

*NRAS*mut melanoma was most frequently located in the lower extremities and trunk. This is in line with previous findings [16,17,27–29] and suggests that localisation of origin has an impact on the type of driver mutation. The histological subtype of the primary

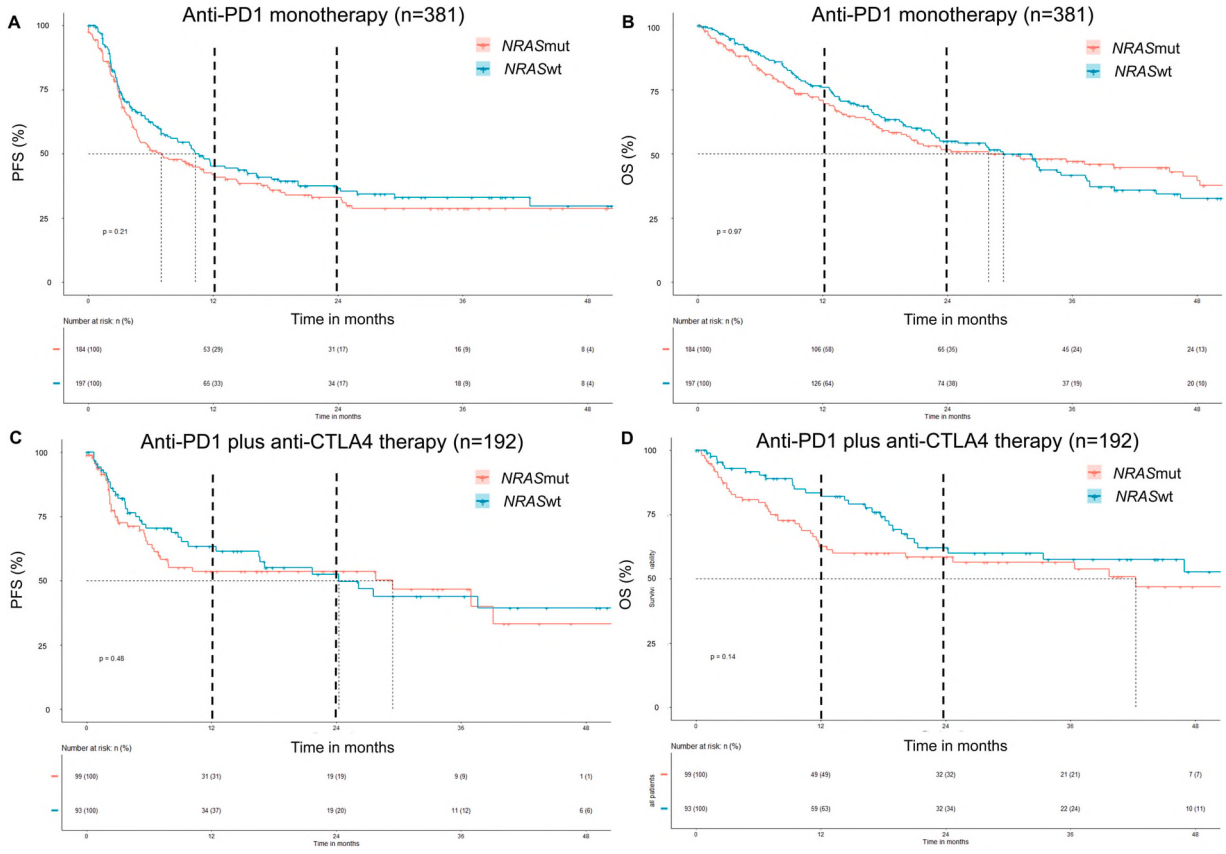

Fig. 3. PFS and OS to immune checkpoint inhibitor therapy in NRASmut and NRASwt patients; (A) Progression-free survival (PFS) in *NRAS*-mutated (*NRAS*mut) (n = 184) and *NRAS*-wild type (*NRAS*wt) (n = 197) patients treated with anti-PD1 monotherapy. (B) Overall survival (OS) in *NRAS*mut (n = 184) and *NRAS*wt (n = 197) patients treated with anti-PD1 monotherapy. (C) PFS in *NRAS*mut (n = 99) and *NRAS*wt (n = 93) patients treated with anti-PD1 plus anti-CTLA4 combination therapy. (D) OS in *NRAS*mut (n = 99) and *NRAS*wt (n = 93) patients treated with anti-PD1 plus anti-CTLA4 combination therapy.

tumour was also different between *NRAS*mut and *NRAS*wt melanoma; 39% of *NRAS*mut melanoma were nodular compared with 21% of *NRAS*wt melanoma. This is consistent with data presented by others [5,28,29] and indicates an association between *NRAS* mutations and nodular melanoma. Favourable prognosis for head/neck region might be explained as melanomas arising in the head/neck region presumably have a UV damage signature and therefore show a higher response rate to immunotherapy. In addition, we included nearly 50% of *BRAF*/*NRAS*wt melanomas, which are known to have a higher mutational burden [30]. Taken together, these findings show that data on the subtype and localisation of *NRAS*mut primary melanoma are consistent.

The effect of *NRAS* mutations on melanoma responsiveness to ICI and patient survival remains controversial. Retrospective studies have reported significantly shorter OS [18] but a trend towards a

Table 3
Overall response in all patients stratified by first-line ICI therapy according to *NRAS* status.

	All ICI treatments	Anti-PD1		Anti-PD1 + anti-CTLA4		Anti-CTLA4	
		*NRAS*mut (N = 184)	*NRAS*wt (N = 194)	*NRAS*mut (n = 99)	*NRAS*wt (N = 93)	*NRAS*mut (n = 27)	*NRAS*wt (n = 37)
ORR (CR + PR)	190 (29.9%)	48 (26.1)	69 (35.0)	32 (32.4)	32 (34.4)	5 (18.5)	4 (10.8)
DCR (CR + PR + SD)	297 (46.7%)	78 (42.4)	106 (53.8)	47 (47.6)	50 (53.8)	8 (29.6)	8 (21.6)
CR	68 (10.7%)	23 (12.5)	24 (12.2)	6 (6.1)	13 (14.0)	1 (3.7)	1 (2.7)
PR	122 (19.2%)	25 (13.6)	45 (22.8)	26 (26.3)	19 (20.4)	4 (14.8)	3 (8.1)
SD	107 (16.8%)	30 (16.3)	37 (18.8)	15 (15.2)	18 (19.4)	3 (11.1)	4 (10.8)
PD	272 (42.7%)	88 (47.8)	70 (35.5)	40 (40.4)	28 (30.1)	19 (70.4)	27 (73.0)
ND - Missing	68 (10.7%)	18 (9.8)	21 (10.7)	12 (12.1)	15 (16.1)	0	2 (5.4)

CR, complete response; DCR, disease control rate; ND, not defined; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; ICI, immune checkpoint inhibitors.

longer median PFS in melanoma patients with *NRAS* mutations [17]. In contrast, two studies by Guida et al. and van Not et al. found comparable PFS and OS between patients with and without *NRAS* mutations [16,19]. In our prospective study, clinical factors relevant to disease prognosis were similar between the *NRAS*mut and *NRAS*wt subgroups and our results showed no significant differences in PFS and OS following anti-PD1-based therapy between these subgroups. However, comparing results between studies is difficult because of major differences in cohort design. For example, some studies have included patients with *BRAF* mutations in the *NRAS*wt group; these patients may have received targeted therapy that significantly affected their survival [18]. Prognostic factors (number of liver metastases and LDH levels) and patient characteristics (e.g. ECOG PS, breslow thickness) were also significantly different between *NRAS* mutant and *BRAF* mutant patients in another large register study [19]. The Imspire170 trial has provided prospective data on *RAS*mut and *RAS*wt/*NF*wt patients with previously untreated *BRAF*V600wt advanced melanoma who received therapy with cobimetinib plus atezolizumab versus pembrolizumab monotherapy. Similar to our results subgroup analysis between *RAS*mut (195 patients, 111 in the pembrolizumab arm) and *RAS*wt/*NF*wt (97 patients, 32 in the pembrolizumab arm) patients showed comparable PFS in the pembrolizumab arm (HR, 0.75; 95% CI, 0.44–1.30) and the cobimetinib plus atezolizumab arm (HR, 0.90; 95% CI, 0.57–0.140) [23].

Johnson et al. retrospectively analysed 229 melanoma patients treated with first-line immunotherapies. They reported a global response rate of 28% in *NRAS*mut melanoma versus 16% in *NRAS*wt melanoma (excluding *BRAF*mut) ($p = 0.04$), with a clinical benefit of 50% in *NRAS*mut patients versus 31% in *NRAS*wt patients ($p < 0.01$). The benefit was higher in the *NRAS*mut cohort (73%) treated with anti-PD1/PD-L1 than in the *NRAS*wt cohort (35%) [17]. Guida et al. performed a retrospective analysis of 331 patients and found no substantial differences between ORR (42% in *NRAS*mut/*BRAF*wt and 37% in *NRAS*wt/*BRAF*wt). No significant differences in BOR were found between *NRAS*mut and *NRAS*wt patients in our study, while ORR was slightly improved for anti-PD1 monotherapy in *NRAS*wt patients. This again underlines inconsistent findings possibly related to divergent clinical characteristics of patients included.

The effect of PD-L1 tumour expression on *NRAS* mutation status has not been well described. In a small cohort study, Johnson et al. showed higher PD-L1 expression in *NRAS*mut melanoma than in *BRAF*mut and wild-type melanoma prior to ICI therapy, which partially explained the improved therapy response they observed to ICI [17]. However, the sample sizes were low in this study (a maximum of 15 patients per group). In our analysis, PD-L1 expression was similar between *NRAS*mut and *NRAS*wt melanoma. OS was higher in patients with melanoma expressing PD-L1, regardless of *NRAS* mutation status, PFS was not affected, arguing against an influence of *NRAS* mutation status on PD-L1 expression of tumour cells. Our data suggest that PD-L1 expression of 5% or higher is an independent positive prognostic factor for OS in melanoma patients regardless of mutation status.

Our data were collected prospectively from a multicenter skin cancer registry and comprise a large number of melanoma patients. Prognostic factors were well balanced in relation to *NRAS* mutation status, however, varied in subgroup analyses for mono or combination therapy. The study size was too small in some analyses and caused imprecise effect estimates (wide CIs) leading to greater statistical uncertainty in these results. The reader should also be aware that *p*-values must be interpreted in the context of multiple testing. Patients harbouring activating *BRAF* mutations were not included because they have additional targeted therapy options. Therefore, a comparison between *NRAS*mut and *BRAF*mut patients is not meaningful. Furthermore, PD-L1 expression in melanoma tissue was only available for 13% of total patients, resulting in limited evaluability.

In summary, we found significant differences in localisation of the primary tumour and histological subtype between *NRAS*mut and *NRAS*wt melanoma patients. Survival was independent of *NRAS* mutation status, and was only related to clinical prognostic factors. The ORR was not significant different in *NRAS*wt patients than in *NRAS*mut patients. In addition, PD-L1 expression in melanoma tissue correlated positively with OS but not with *NRAS* mutation status in patients with melanoma.

Ethics approval and consent to participate

The ADOREG registry was approved by the ethics committee of the University Duisburg-Essen (14-5921-BO), and provides real-world data from skin cancer patients in the DeCOG. Molecular testing was performed with the informed consent of patients.

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CRediT authorship contribution statement

AZ, LZ: Conceptualisation. AZ, RG, FriM, CP, MW, PT, AF, JaU, JoU, JW, MK, CG, RH, AS, ED, MS, FraM, LH, SH, CW, HL, SK, KG, EL, DS, SU, LZ:

Data curation. AZ, LZ: Formal analysis. DS: Funding acquisition. AZ, LZ: Investigation. AZ, LZ: Methodology. AZ, LZ: Supervision. AZ, LZ: Visualisation. AZ, LZ: Writing – original draft. All other authors: Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A.Z. received travel support from Novartis, Sanofi Genzyme, and Bristol-Myers Squibb, outside the submitted work. Fri.M. has received travel support or/and speaker's fees or/and advisor's honoraria by Novartis, Roche, BMS, MSD, Pierre Fabre and Sanofi and research funding from Novartis and Roche. CP received honoraria (speaker honoraria or honoraria as a consultant) and travel support from: Novartis, BMS, MSD, Merck Serono, MSD, Celgene, AbbVie, Sunpharma, Pierre Fabre, UCB, Nutricia Milupa, Janssen and LEO outside the submitted work. P.T. declares speakers and advisory board honoraria from Almirall, Bristol-Myers Squibb, Novartis, Merck Sharp & Dohme, Pierre-Fabre, CureVac, Merck Serono, Sanofi, Roche, Kyowa Kirin, Biofrontera and 4SC; travel support from Bristol-Myers Squibb and Pierre-Fabre. 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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.2023.04.008.

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