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Author Contributions Statement

CMT, EC, SU, KG did the study design.

CMT, EC, JM, AZ, GL, PJ, LR, JK, IM, AS, RH, PT, JU, CP, JUl, PM, RG, FM, ED, MW, AP, EL, LZ, DS, EH, SU, KG contributed patient data.

All authors reviewed and edited the manuscript and confirmed the final version of the manuscript.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

C.M.T.: No relevant conflicts of interest.

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# NF1-mutated melanomas reveal distinct clinical characteristics depending on tumor origin and respond favorably to immune-checkpoint inhibitors

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## Abstract

**Background:** *NF1*-mutated tumors represent a small subset (10–15%) of melanomas, not sufficiently analyzed in large clinical cohorts. This study investigated the largest multicenter collection of *NF1*-mutated melanomas to date.

**Methods:** This study analyzed a multicenter tumor tissue sample cohort from 266 patients with *NF1*-mutated melanoma. Targeted next-generation sequencing of the *TERT* promoter and 29 relevant melanoma genes was performed. Survival was compared with *NF1*-wild-type cohorts from the TRIM project (n = 432).

**Results:** Most *NF1*-mutated melanoma arose in the head-and-neck region of patients > 60 years of age. *NF1* alterations were frequently inactivating, primarily non-sense, less frequently truncating mutations. Non-inactivating *NF1* mutations more frequently co-occurred with activating *BRAF* and *RAS* mutations. *NF1*-mutated tumors had higher numbers of gene mutations and UV-signature C>T and CC>TT transitions than *BRAF*, *RAS* and triple wild-type melanomas. *NF1*-mutated acral and mucosal melanomas harbored a different mutation signature and were frequent in females (69 and 83%, respectively), differing from non-acral cutaneous *NF1*-mutated melanomas (males 73%, females 27%). Overall survival in stage IV disease was comparable for patients with *NF1*-mutated or -wild-type melanoma. However, in patients receiving first-line immune checkpoint inhibitor treatment, better overall survival was observed for *NF1*-mutated than -wild-type tumors (mOS = not reached vs. mOS = 25.82, p = 0.0154, n = 80 and 432, respectively).

**Conclusions:** Cutaneous, acral, and mucosal *NF1*-mutated melanomas vary in clinical and genetic characteristics and demonstrate a favorable outcome upon immune checkpoint inhibition therapy.

### Keywords

*NF1*; *BRAF*; *NRAS*; melanoma; mutation profiling

## Introduction

Cutaneous melanoma is a highly malignant tumor with a potential for distant metastasis [1,2]. The prognosis for patients with metastatic disease remains poor despite significant recent improvements in therapeutic strategies [3].

The development of next-generation sequencing (NGS) technologies increasingly elucidated the genetic landscape of melanoma [4,5]. The Cancer Genome Atlas (TCGA) suggested to classify melanomas into four main genetic subtypes: *BRAF*-mutated, *NRAS*-mutated, *NF1*-

mutated or triple wild-type [6]. Alterations in the V600 codon of *BRAF* and the Q61, G12 or G13 codons of *RAS* genes all lead to MAP Kinase activation [6]. The *NF1* gene product is a GTPase-activating protein downregulating RAS activity. NF1 inactivation thus leads to MAPK activation [7].

Recently introduced therapeutic approaches have significantly improved overall survival of patients with advanced or unresectable melanoma [3,8,9]. These therapies can be classified into two groups, namely immune checkpoint inhibitors (ICI) targeting programmed death-1 and its ligand (PD-1/PD-L1) (nivolumab and pembrolizumab) or cytotoxic T lymphocyte antigen 4 (CTLA-4) (ipilimumab), and tyrosine kinase inhibitors (TKI) targeting the MAPK pathway, namely BRAF or MEK, which are applicable for patients with tumors harboring a *BRAF* V600 mutation. [10–13]. The most potent combination immunotherapy of anti-PD-1 (nivolumab) and anti-CTLA-4 (ipilimumab) antibodies has achieved a 5-year-survival rate of 52%, accompanied by a high rate of toxicity [14,15]. Besides these therapeutic regimens, treatment options for patients with advanced melanoma remain limited and targeted therapies for specific mutations in *NRAS* and *NF1* genes are not available.

Melanoma has a variable mutation frequency, largely based on varying exposure to UV radiation [5,16]. Melanomas arising in chronically sun-exposed skin harbor larger amounts of mutations including frequent mutations in the *NF1* gene [17]. A high mutational burden is associated with improved and more durable therapeutic responses to anti-CTLA-4 or anti-PD1 monotherapy in metastatic melanoma [18–20]. However, mutations in *NF1* also occur in tumors arising in anatomic sites with little or no UV exposure, such as acral and mucosal melanomas [17]. Patient age at diagnosis is also associated with the mutational pattern of melanoma - *BRAF* mutations are more common in younger, *NF1*-mutations in older patients [17,21]. *NF1* mutations occur particularly frequently in desmoplastic melanoma [22]. Loss-of-function mutations or deletions in *NF1* have been linked to a decreased sensitivity to BRAF-inhibitors in *BRAF*-mutated melanomas [23]. Enhanced sensitivity of *NF1*-mutated melanomas to MEK-inhibitors has been reported [7].

In the present study, we gathered the largest multi-center cohort of *NF1*-mutated melanomas investigated to date in order to further understand the *NF1*-mutated melanoma subtype and its implications on clinical course and possible therapeutic approaches in the respective patients.

## Materials and Methods

### Patients and Samples:

Screening 3837 NGS reports of melanoma patients, we identified 266 patients with *NF1*-mutated melanoma diagnosed between 2013 and 2020. Related data and tumor samples were obtained from the Westdeutsche Biobank Essen (WBE/SCABIO), University Hospital Essen (11-4715-BO, n=157) and from the multicenter prospective translational study “tissue registry in melanoma” (TRIM; 15-6566-BO, n=109). Tumors were classified according to the American Joint Committee on Cancer (AJCC 8th) staging system [24,25]. *NF1*-wild-type cohort data was obtained from the TRIM cohort. This study was performed in accordance with the Declaration of Helsinki, was approved by the Ethics Committee of the Medical

Faculty of the University of Duisburg-Essen (ethics approval no. 20-9606-BO) and followed the guidelines for good clinical practice (GCP).

A customized amplicon-based sequencing panel covering the *NF1* gene as well as 29 additional genes known to harbor oncogenic mutations relevant for melanoma was used (genes list in Supplemental Table 4).

### Targeted sequencing:

DNA was isolated from Formalin-fixed paraffin-embedded (FFPE) tumor tissue according to standard procedures as previously described[26]. A custom amplicon-based sequencing panel covering 29 genes (listed in Supplemental Table 4) known to be recurrently mutated in cutaneous and uveal melanoma was designed and prepared applying the GeneRead Library Prep Kit from QIAGEN® according to the manufacturer's instructions. For adapter ligation and barcoding of individual samples, the NEBNext Ultra DNA Library Prep Mastermix Set and NEBNext Multiplex Oligos for Illumina from New England Biolabs were applied. Twelve samples were sequenced in parallel on an Illumina MiSeq next generation sequencer.

Sequencing analysis was performed applying the CLC Cancer Research Workbench from QIAGEN® (currently version 20.0.4). In brief, the following steps were applied. The workflow in CLC included adapter trimming and read pair merging before mapping to the human reference genome (hg19). InDels and Structural Variants were assessed allowing 3 maximum mismatches (unaligned end breakpoints). Single nucleotide variant detection, local realignment and primer trimming followed. Additional information was then obtained regarding potential mutation type, known single nucleotide polymorphisms and conservation scores by cross-referencing varying databases (COSMIC, ClinVar, dbSNP, 1000 Genomes Project, HAPMAP and PhastCons-Conservation_scores_hg19). After the CLC Cancer Research Workbench processing, resulting csv files were analyzed manually. Mutations affecting the protein coding portion of the gene were considered if predicted to result in non-synonymous amino acid changes. The functional consequences of mutations were predicted by later by performing an analysis on the server based SIFT[27], PROVEAN[28] and PolyPhen-2[27] assays. A detailed list of all mutations and database references is included in Supplemental Table 4. To eliminate questionable low frequency background mutation calls, mutations were reported only if ≥ 10 reads reported the mutated variant, coverage of the mutation site was ≥ 30 reads and the mutation frequency was ≥ 10%. The average read coverage of the targeted area achieved was 2607x. (A detailed listing of the individual settings applied in CLC cancer research workbench are listed in the Supplemental Material and Methods)

### Statistical analysis:

Associations of tumor origin with clinical parameters were investigated using chi-squared tests or Fisher's exact tests as indicated. Continuous variables are presented as means with standard deviation or as median with interquartile range, as appropriate. Categorical variables are presented as counts and percentages. Survival curves were drawn using the Kaplan-Meier method and the log-rank test was used for comparisons. OS was calculated from first date of stage IV diagnosis or start of ICI therapy until death or last patient contact

(censored observation), respectively. Statistical analyses were performed using Microsoft Excel, GraphPad Prism (version 6), SPSS 27.0 (IBM Corp., Armonk NY, USA), R (R version 4.0.3 (2020-10-10)) and RStudio [29,30]. A p-value < 0.05 was considered significant.

## Results

### Patient characteristics

Two hundred sixty-six patients (95 females and 171 males) diagnosed with *NF1*-mutated melanoma were included in this study. The clinical characteristics are shown in Table 1 (additional data for all patients are shown in Supplemental Table 1). Tumors of the head and neck region were the most common (44% of tumors with documented primary tumor localization) (Figure 1, B middle). Mucosal melanomas consisted of 8 vaginal, 3 anal, 2 nasal, 2 esophageal and 1 urethral mucosal cases. In two cases the localization was not documented. Of all 16 acral melanomas, 7 were located on the lower extremities, whereas 2 were on the upper extremities with information missing in 7 cases. An overview of the clinical characteristics of stage IV *NF1*-mutated melanomas in relation to available date from the *NF1*-wild-type cohort used for survival and treatment comparisons is shown in Supplemental Table 2.

### Cutaneous melanomas harbor more mutations than mucosal melanomas

A subgroup analysis of the total 231 patients with known primary tumor localization for different melanoma subtypes revealed 197 (85.3%), 16 (6.9%) and 18 (7.8%) patients with cutaneous non-acral, acral and mucosal melanomas, respectively (Table 1). No age difference was noted (medians of 67, 68.5 and 67 years, respectively). A highly significant ($p<0.0001$) difference in sex distribution was noticed. In non-acral cutaneous melanomas, the majority of patients was male (72%), whereas both mucosal and acral melanomas arose more often in female patients (83% and 69%, respectively). *BRAF*, *NRAS* and *TERT* promoter mutations were more frequent in patients with non-acral cutaneous melanoma (Figure 1, A; Table 1). Mucosal melanomas had significantly fewer mutations than both acral and non-acral cutaneous melanoma (Figure 1, B right).

### Distribution of UV-induced mutations amongst melanoma subtypes

Analysis of mutational patterns within the *NF1*-mutated tumors revealed a UV-signature. Single nucleotide variants were grouped into six mutation types, as previously described [31]. We compared mutational patterns of single nucleotide variants of *NF1*-mutated melanomas to *BRAF*, *NRAS* and triple-wild-type melanomas. *NF1*-mutated melanomas revealed the highest number of UV-induced signature C>T substitutions and CC>TT substitutions (Figure 1, C and Supplemental Figure 1, B). Upon normalization per sample, mucosal melanomas showed a considerably lower number of UV-induced signature C>T transitions compared to other *NF1*-mutated melanomas (Supplemental Figure 1, A).

### *NF1*-mutated melanoma exhibits the highest mutation number

Comparing the number of mutations identified in our sequencing panel (Supplemental Table 4, 0.88 megabase coverage) *NF1*-mutated melanomas were found to exhibit higher numbers

of mutations per tumor (median 6) compared to *BRAF*-mutated (median 3), *NRAS*-mutated (median 4) and triple-WT (median 2) melanomas. (Figure 1, C).

### *NF1*-mutated melanomas show a better response to ICI

Comparison of overall survival after first diagnosis of distant metastatic disease (AJCC stage IV) independently of treatment revealed no difference between patients with *NF1*-mutated (n = 128) or -wild-type (n = 387) melanomas (Supplemental Table 2) (mOS = 47 vs. 37 months, respectively [p = 0.35]) (Figure 2, A). However, a trend towards longer overall survival was noted in *NF1*-mutated melanoma patients receiving first line immune-checkpoint inhibitors (anti-PD1-monotherapy, or anti-PD1 + anti-CTLA-4 combination therapy) for stage IV disease compared to other first-line treatments (p = 0.32) (other treatments included targeted therapies, chemotherapy and tyrosine kinase inhibitors) (Figure 2, B). Further analysis of survival upon first-line immune checkpoint inhibitor treatment in advanced or metastatic disease, revealed a prolonged overall survival of *NF1*-mutated melanoma patients (n = 80) compared to a cohort of patients with *NF1* wild-type melanoma (n = 432, TRIM cohort) (mOS = not reached vs. 25.15, respectively [p = 0.01]) (HR (95% CI) = 0.60 (0.40 to 0.90); p = 0.01). Combination nivolumab and ipilimumab, nivolumab or pembrolizumab treatment was given in 28, 31 and 21 cases, respectively in the *NF1*-mutated group and 132, 153 and 147 cases, respectively in the *NF1* wild-type group (p = 0.39) (Figure 2, C).

### Targeted next generation sequencing

Within 266 study samples, 538 *NF1* mutations were identified, with many harboring more than one mutation. *BRAF* mutations were frequent (n = 99, 38%) (Figure 3, Supplemental Table 3), with 45 V600E, 11 V600K, and 1 V600D activating mutation. *NRAS* mutations were found in 77 samples (29%), 56 of which were activating Q61/G12/G13 mutations. Q61 mutations included 4 Q61H, 9 Q61K, 16 Q61L and 17 Q61R (Supplemental Table 3). *KRAS* and *HRAS* mutations were less frequent, in 7% and 8% of samples, respectively (Figure 3). Activating *TERT*-promoter mutations were present in 166 samples (62%) (Supplemental Table 3). Other frequently mutated genes included *TP53* (33%), *ARID1A* (38%), *ARID2* (36%) and *KIT* (20%). Less frequent mutations were reported in *RAC1, CDKN2A, GNAQ, GNA11, PTEN, CDK4, SMARCA4, MAP2K1, MAP2K2, CTNNB1, PIK3CA, EZH2, IDH1, FBXW7, WT1, BAP1, SF3B1, PIK3R1, MITF, and TERT.*

### *NF1* mutations are distributed throughout the neurofibromin protein, not sparing the GTPase-activating protein-related domain

*NF1* mutations in the 2818 amino acid protein neurofibromin occured in a pattern typical of loss-of-function gene alterations, being evenly distributed across the entire protein with an enrichment of inactivating truncating mutations. (Figure 4). The well characterized Ras-GTPase-activating protein (GAP)-related domain (GRD), which down-regulates the Ras signaling pathway, harbored both missense and inactivating mutations.

## Discussion

To our knowledge, this study reports the largest cohort of *NF1*-mutated melanomas investigated to date. Analysis revealed a striking difference in the sex distribution pattern of *NF1*-mutated melanomas in favor of male patients - this distribution varied among different subgroups of *NF1*-mutated melanoma: 72% of patients with non-acral cutaneous melanoma were male, whereas 69% and 83% of patients with acral and mucosal melanoma were female, respectively. Mucosal melanoma being more frequent amongst female patients was documented previously [32,33]. The reason for the strong male predominance in non-acral cutaneous *NF1*-mutated melanoma in our cohort is not apparent. The median age at diagnosis of *NF1*-mutated patients in our cohort (67 years), is significantly older compared to patients of other large melanoma cohort studies [34,35].

A large proportion of *NF1*-mutated cutaneous melanomas arose in the head and neck region. This observation is consistent with previous findings that *NF1* mutations preferentially occur in sun-exposed areas as UV-radiation is the main driver for mutagenesis and represents a clustering specific for this subgroup [22,36].

The frequency of concurrent $BRAF^{V600}$ mutations (17%) and $NRAS^{Q61}$ mutations (17%) was higher compared to previous studies of *NF1*-mutated melanomas [6,22,36]. This discrepancy could be due to smaller numbers of *NF1*-mutated melanoma assessed in previous studies and differences in terms of tumor origin. Activating *BRAF* and *NRAS* mutations were less frequent in tumors harboring inactivating *NF1* mutations (Table 2). This fits with missense or truncating *NF1* mutations leading to a stronger activation of the MAPK pathway. Both mucosal and acral melanomas harbored comparatively fewer *BRAF* and *NRAS* mutations, fitting previous studies [33,37–40].

Patients with mucosal melanoma showed significantly fewer tumor mutations (Figure 1, B), a consequence of the lack of UV exposure, the main driver of mutations in cutaneous melanoma [41]. Mucosal melanomas revealed, as expected, a significantly lower number of UV-signature C>T substitutions (Supplemental Figure 1).

Mutations in the *TERT* promoter region, usually exhibiting C>T transitions, may be UV-induced [42] but are also frequent in non-UV-induced tumors such as gliomas [43]. *TERT* promoter mutations were rare in mucosal melanoma in our study, but were present in >30% of acral melanomas and >70% of non-acral cutaneous melanomas. We found that *NF1*-mutated melanoma exhibits more UV-induced C>T and CC>TT substitutions compared to other genetic subtypes. Further, *NF1*-mutated melanomas in our cohort harbor significantly more mutations compared to other melanoma subtypes, supporting previously published data [17].

Both missense and inactivating mutations in *NF1* were distributed throughout the neurofibromin protein, typical of loss-of-function mutations [44]. Whereas activating, gain-of-function, mutations have to occur at specific sites, inactivating alterations, in particular frame-shift and truncating mutations can generally occur throughout the gene. An enrichment of truncating mutations was identified in our cohort (Figure 4). We detected 96 truncating and 23 frameshift mutations. This is in line with findings from previous

studies, in which an enrichment of truncating, but not frameshift mutations was noted [36]. A limitation of our study is that our assay could not reliably detect losses of larger indels of the *NF1* gene, meaning that likely some patients with bona fide *NF1* alterations were not recognized[45]. Another generally difficult issue, is that *NF1* is a very large gene (cDNA of circa 9000bp, depending on splice variant) and mutations can occur throughout the gene. Considering melanoma generally has a very high number of passenger mutations, these certainly do also occur in the *NF1* gene. As demonstrated in other studies[36,45,46], one can assume *NF1* mutations are functionally more relevant when inactivating and occurring in tumors not harboring other known activating mutations (i.e. *BRAF, NRAS*, etc.). The predicted functional relevance of the individual *NF1* mutations as determined by different algorithms (PROVEAN, SIFT and PolyPhen-2) is listed in Supplemental Table 6.

Survival analysis of stage IV melanoma patients showed no significant difference between the overall survival in *NF1*-mutated to -wild-type melanoma. The clinical data (Supplemental Table 1) showed a significant age difference with *NF1*-mutated melanomas occuring in older individuals. The prolonged OS observed in *NF1*-mutant tumor patients receiving first-line immunotherapy compared to patients with *NF1* wild-type melanoma may be associated with tumor mutational burden. The size of our sequencing panel did not allow a valid estimation of tumor mutational burden (generally requiring sequencing of 1 megabase of DNA), however in our panel, we did observe a higher average number of mutations per sample in *NF1*-mutated melanoma than other subtypes (Figure 1). This supports existing data from patients with a high mutational burden compared to patients with a low mutational burden [18–20]. The trend of *NF1*-mutated tumors for better OS of patients receiving ICI compared to other therapies including targeted therapies, tyrosine kinase inhibitors, and chemotherapies (Figure 2, B) did not reach statistical significance, probably due to the limited available patient numbers (n = 80 ICI vs. n = 19 other). Within the group of *NF1*-mutated ICI therapy treated patients, the only parameter determined to be significantly different in non-responders (SD/PD) versus responders (PR/CR) was the pre-treatment tumor PD-L1 status, which was more often positive (>= 5% of tumor cells stained) in the group of treatment responders (Supplemental Table 5). The prognosis of all *NF1*-mutated melanoma patients has been reported to be significantly worse than patients with other mutation patterns [17]. This was not observed in our cohort, but this may be due to many of the patients having received immunotherapy. If validated in larger studies, the improved response to immune checkpoint inhibition therapy we observed, supports treating patients with *NF1*-mutated non-acral cutaneous melanoma with immunotherapy.

In the mucosal melanoma group, five patients received treatment with PD-1 agents. One patient exhibited a complete response, three progressive disease and one stable disease were noted. In acral melanoma group, of six patients received treatment with PD-1 agents, 5 patients exhibited progressive disease and one stable disease. This data suggests patients with *NF1*-mutated acral or mucosal melanoma may differ from cutaneous non-acral melanoma and a potential benefit from checkpoint-blockade therapy will need to be evaluated in larger studies.

Our data suggests that patients with *NF1*-mutated melanoma treated with immunotherapy exhibit better overall survival than those with *NF1*-wild-type melanoma. If validated in

future studies, *NF1* mutation status may be a biomarker to consider when selecting which melanoma patients may benefit from immunotherapy. However, given the retrospective nature of this study, survival analysis should be adjusted for factors such as LDH in future analyses. Unfortunately, we were unable to perform this analysis because of missing information in some cases of ICI-treated melanoma. Acral and mucosal *NF1*-mutated melanomas should be considered distinct subtypes of *NF1*-mutated melanomas with unique clinical characteristics and mutational patterns meriting further exploration in larger studies.

## Conclusion:

- Non-acral *NF1*-mutated melanoma occurs frequently in male patients and is clinically distinct from *NF1*-mutated acral and mucosal melanoma
- *NF1*-mutated melanoma had a similar overall survival to *NF1* wild-type melanoma when not stratifying for therapy received
- *NF1*-mutated melanoma harbored more UV signature mutations than *BRAF*-mutated, *NRAS*-mutated or Triple-wild-type melanomas
- *NF1*-mutated melanoma patients receiving immunotherapy had improved overall survival compared to *NF1* wild-type melanoma patients

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Highlights

1. *NF1*-mutated melanoma patients respond favorably to PD-1 based immunotherapy
2. *NF1*-mutated metastatatic melanoma had a similar overall survival to *NF1* wild-type
3. Non-acral, acral and mucosal *NF1*-mutated melanoma are clinically distinct
4. *NF1*-mutated melanoma exhibit a large amount of UV signature mutations

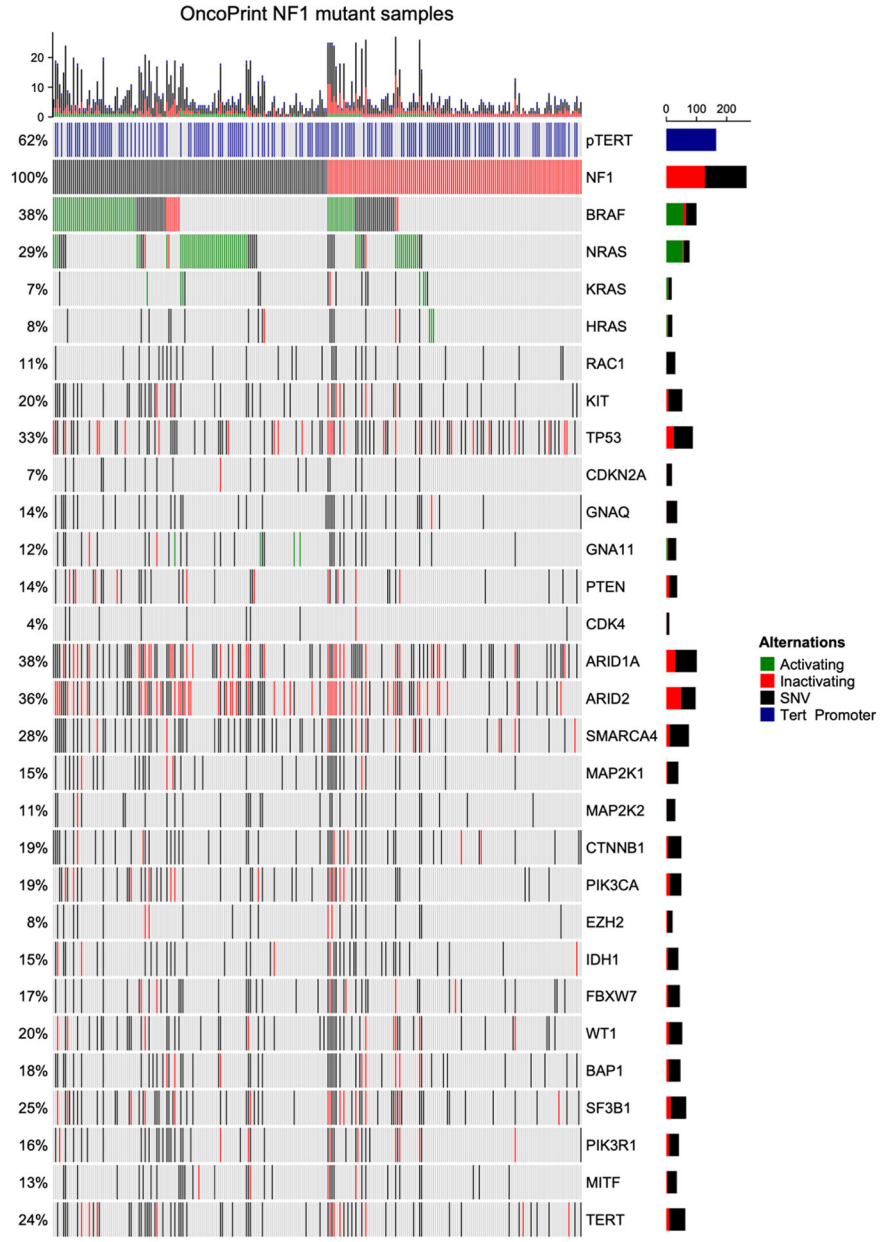


**Figure 1 –. Characteristics of NF1-mutated melanomas**

Non-acral cutaneous *NF1*-mutated melanoma harbored more frequent concurrent *BRAF* and *NRAS* mutations compared to both acral and mucosal melanoma (**A**). Left: Non-acral cutaneous melanoma shows a trend towards higher Breslow tumor thickness. Middle: Most non-acral cutaneous melanomas were located within the head and neck region. Right: Acral and non-acral cutaneous melanoma harbor significantly more mutations compared with mucosal melanoma (**B**). Left: *NF1* mutated melanomas harbor more C>T substitutions compared to *BRAF*, *NRAS* or triple-wt melanomas. Right: *NF1* mutated melanomas harbor the highest mutational burden among melanoma genetic subtypes. (**C**). Statistical tests performed are Mann-Whitney U test and Wilcoxon test. Data is shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

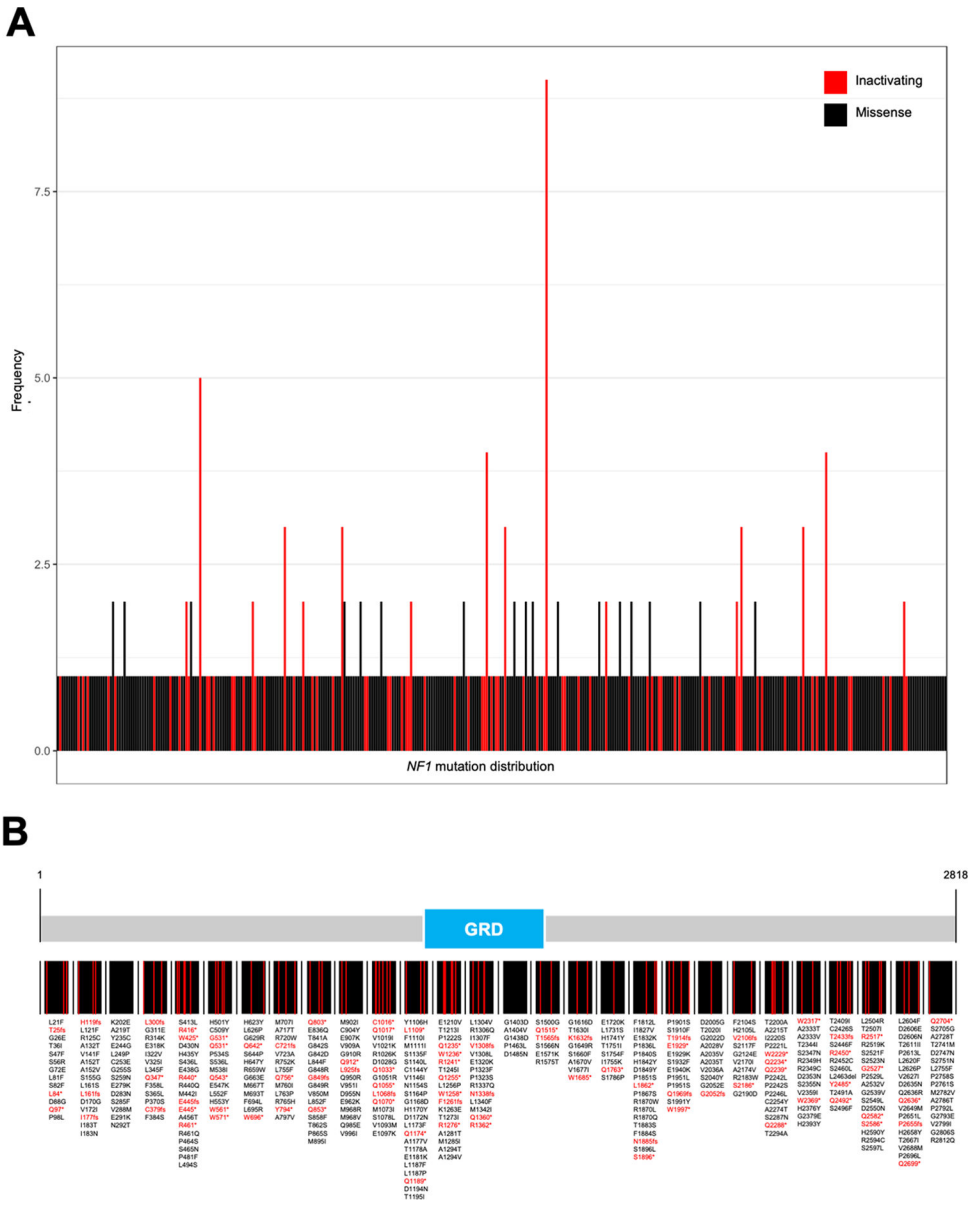


**Figure 2 –. NF1-mutated melanomas exhibit favorable ICI therapy response**

Patients with stage IV *NF1*-mutated melanoma (n = 128) do not show a difference in overall survival compared to patients with stage IV *NF1*-wild-type melanoma (n = 387) (**A**). Within the group of *NF1* mutated melanoma, patients undergoing therapy with immune-checkpoint inhibitors exhibit a trend towards better overall survival compared to other therapies (**B**). Patients treated with first-line ICI therapies with *NF1* mutated melanomas show a prolonged mOS compared to *NF1*-wild-type melanomas (**C**).

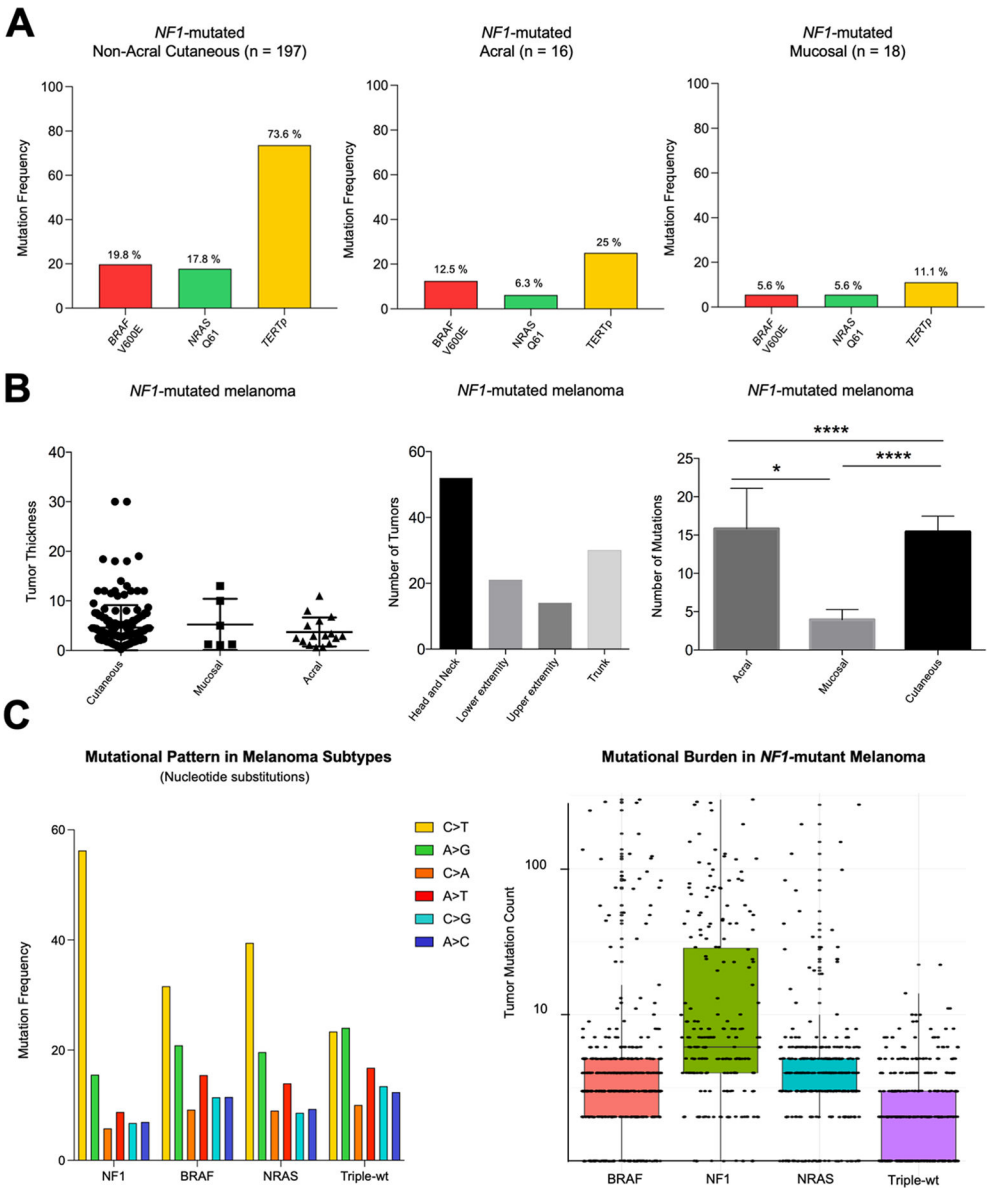


**Figure 3 –. Mutation distribution in NF1-mutated melanomas**

Green: mutations known or assumed to be activating. Red: loss of function mutations. Blue: mutations in the *TERT* promoter region.

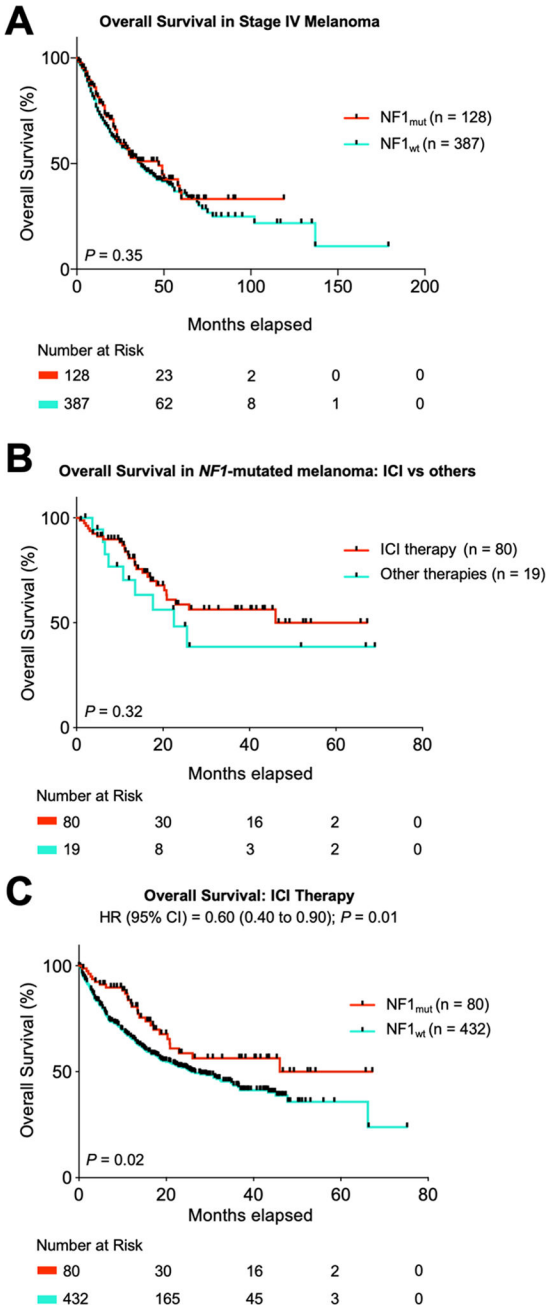


**Figure 4 –. Distribution of NF1-mutations within neurofibromin**

Analysis of mutations in *NF1* mutated melanoma reveals no clustering or hotspot of mutations and no sparing of the GRD region on neurofibromin (**A**, **B**).

**Table 1 –**

Clinical characteristics of non-acral, acral and mucosal melanomas

| Variable, n (%) | Non acral cutaneous (n = 197) | Mucosal (n = 18) | Acral (n = 16) | p-Value |
|---|---|---|---|---|
| **Age at first diagnosis** | | | | *.86* |
| Median (range) | 67 (16 – 94) | 67 (38 – 85) | 68,5 (41 – 80) | |
| ≤60 years | 67 (34.0) | 6 (33.3) | 7 (43.8) | |
| >60 years | 130 (66.0) | 12 (66.7) | 9 (56.3) | |
| **Sex** | | | | ***< .0001*** |
| Female | 55 (27.9) | 15 (83.3) | 11 (68.8) | |
| Male | 142 (72.1) | 3 (16.7) | 5 (31.3) | |
| **Mutated oncogene** | | | | *.39* |
| *BRAF* V600E | 39 (19.8) | 1 (5.6) | 2 (12.5) | |
| *NRAS* Q61 | 35 (17.8) | 1 (5.6) | 1 (6.3) | |
| *NF1* | 197 (100) | 18 (100) | 16 (100) | |
| **Invasion depth of primary** | | | | *.69* |
| Mean ± SD | 4,61 ± 4,54 | 5,26 ± 4,29 | 3,73 ± 2,82 | |
| < 1 mm | 11 (5.6) | 0 (0) | 2 (12.5) | |
| 1 – 2 mm | 37 (18.8) | 3 (16.7) | 3 (18.8) | |
| 2 – 4 mm | 53 (26.9) | 0 (0) | 6 (37.5) | |
| > 4 mm | 72 (36.6) | 3 (16.7) | 5 (31.3) | |
| Unknown | 24 (12.2) | 12 (66.7) | 0 (0) | |
| **Ulceration of primary** | | | | *.26* |
| Present | 80 (40.6) | 2 (11.1) | 11 (68.8) | |
| Absent | 93 (47.2) | 3 (16.7) | 5 (31.3) | |
| Unknown | 24 (12.2) | 13 (72.2) | 0 (0) | |
| **TERT promoter** | | | | |
| *TERTp228* | 106 (49.8) | 0 (0) | 4 (25.0) | |
| *TERTp242* | 15 (7.0) | 0 (0) | 1 (6.3) | |
| *TERTp250* | 60 (28.2) | 2 (11.1) | 1 (6.3) | |
| **PD-L1** | | | | *.33* |
| Positive | 72 (36.6) | 2 (11.1) | 5 (31.3) | |
| Negative | 93 (47.2) | 8 (44.4) | 6 (37.5) | |
| Not performed | 32 (16.2) | 8 (44.4) | 5 (31.3) | |

**Table 2 –**

Distribution of BRAF and NRAS mutations among NF1 mutated melanoma

| Variable, n (%) | Truncating / Frameshift (n = 115) | Other Mutations (n = 151) | p-Value |
|---|---|---|---|
| **Mutation** | | | **p < 0.0001** |
| *BRAF* V600E | 10 (8.7) | 34 (22.5) | |
| *NRAS* Q61 | 9 (7.8) | 36 (23.8) | |