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NF1 Mutated Metastatic Melanoma and Response to Immune Checkpoint Inhibitor Therapy: A Retrospective Analysis

De Backer C, Laenen A and Bechter O

[1]Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium

[2]Interuniversity Centre for Biostatistics and Statistical Bioinformatics, Leuven, Belgium

***Corresponding author:**

De Backer Cleo,

Department of General Medical Oncology, UZ Leuven,E-mail: cleo.debacker@uzleuven.be

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1. Abstract

1.1. Background: Based on molecular profiling, malignant melanoma is classified in four different groups. NF1-mutated tumors are a small subgroup occurring with a frequency of 13% of all malignant melanomas, usually harboring a high tumor mutational burden (TMB). Considering TMB as being a prerequisite for the effectiveness of immune checkpoint inhibitor therapy, we were wondering if this rare subtype is associated with a higher response rate to immunotherapy than it is known for the general melanoma population.

1.2. Methods: We analyzed a small cohort of 14 NF1 mutated metastatic melanoma patients and retrospectively assessed the response rate (RR) according to RECIST 1.1, Progression Free Survival (PFS) and Overall Survival (OS). We compared our results with outcome data reported in several clinical trials with immune checkpoint inhibitors.

1.3. Results: For our cohort, we noticed an objective response rate of 64%, which is higher than the response rate generally reported for anti-PD1 based therapy in a population of melanoma patients. The PFS rate at twelve months was 62%, next to an OS rate at twelve months of approximately 84%.

Additional mutations co-occur in NF1-mutated patients. Although we did not find an association between the number of additional mutations and response in general, we did notice a significant correlation between mutations in TERT promotor region and tumor response (p-value 0.027).

1.4. Conclusion: Despite the small patient group, we observed a higher response rate for NF1 mutated metastatic melanoma patients treated with immunotherapy. In addition, a significant correlation between response rate and the presence of hTERT promotor mutations was observed.

2. Introduction

Melanoma is a disease where driver mutations are known to be responsible for tumor proliferation for the vast majority of patients. Molecular profiling classifies melanoma in different subtypes. Generally, four different molecular subtypes of melanoma are identified: BRAF-mutated, NRAS-mutated, NF1-mutated and triple wild-type tumors. BRAF (V600E) mutations (present in 38,5% of cutaneous melanomas) and NRAS mutations (28,6% of melanomas) are the most prevalent alterations and are both mutually exclusive [1]. The group which is considered triple wildtype, is thought to have different mechanisms driving tumor growth, such as dependence on ERK pathway activation. The least prevalent group are melanomas with NF1 alterations. The frequency of somatic NF1 mutations overall is 12,2% (1): while this alteration has a low frequency in cutaneous melanoma (12-30%), it is vastly more present (45-90%) in a very rare subtype of melanoma, called desmoplastic melanomas which is known to respond with a high frequency to immunotherapy [2]. In a study published in Nature in 2018 by Eroglu et al, 60 patients with desmoplastic melanoma were included, and an objective response rate of 70% was observed. 45 % of these patients had a complete response, 55% a partial response [3].

Screening with NGS (next generation sequencing) to identify these mutations is standard of care since the identification of driv-

er mutations has direct implications for the treatment of patients at least when BRAF mutations are concerned. In 2011, FDA and EMA approved vemurafenib for metastatic melanoma carrying BRAFV600 mutations. Currently, for BRAF mutated patients, we have three approved combinations of BRAF/MEK inhibition as therapeutic options [4]. However other molecular alterations are not good candidates for targeted therapy thus far as it has been shown for NRAS and KIT mutated melanomas.

NF1 (neurofibromin 1) is a protein known as a tumor suppressor gene, as it downregulates RAS proteins [5], by facilitating the hydrolysis of GTP to GDP [6]. If NF1 is lost, RAS becomes refractory to negative feedback hence leading to consecutive RAS activation [6]. NF1 mutations can occur in germline, resulting in a hereditary disease known as neurofibromatosis type 1. This is an autosomal dominant disorder with an incidence of 1 in 3000 live births, characterized by café-au-lait-macules, benign neurofibromas and other tumors, mostly from the neural crest [2, 5]. As a somatic mutation, NF1 mutations are the third most frequent cause of melanoma [7]. These melanomas originate most typically on chronically sun-exposed skin in older male patients, and show in general a high mutational burden [5, 7]. The mechanism leading to a high tumor mutational burden as a possible result of NF1 mutation, still remains elusive.

Very rarely, NF1 mutations are also present in patients harboring a BRAF V600 mutation, and response to BRAF/MEK inhibition has also been studied in these patients. Presumably, NF1 mutations could induce resistance to MAPK inhibition because of a sustained MAPK pathway activation [5]. Due to NF1 loss, the negative feedback on RAS activation is lacking, resulting in resistance to RAF inhibition. As a result, the pathway stays MEK dependent, and presumably susceptible to allosteric MEK inhibitors [6]. One explanation of the high mutational burden in these melanomas could be the chronically stimulated RAS pathway where the DNA damage repair machinery cannot keep up with the proliferative drive in these cells. The mechanism how mutational burden translates into a higher response to immunotherapy is not yet totally clear but likely mutations give rise to expression of more neo-antigens, and as a result the likelihood for immune recognition increases [8]. In line with this hypothesis, the rare subtype of desmoplastic melanomas (less than 4%), which significantly harbor NF1 mutations more frequently, are known to derive substantial benefit from anti PD-1 / anti PD-L1 therapy according to anecdotal reports, although this has not been systematically investigated so far [3]. However, a multitude of different markers exist which are presumably associated with response to anti-PD1 based therapy.

In melanoma, hTERT promotor mutations are reported to be the most frequently occurring mutation, and the prevalence is associated with increasing age, tumor site, and histological subtype. [10] Some authors describe that the presence of a mutation in the hTERT promotor corresponds with a worse prognosis and shorter survival, because of adverse characteristics like increased thickness, ulceration and mitotic rate, being more prevalent in hTERT mutated melanomas [11]. Until now, this worse prognosis is mostly described for hTERT promotor mutations and the simultaneous presence of BRAF and NRAS mutations. It is still unclear whether the same is true for NF1 mutated tumors [12, 13]. In this retrospective analysis, we were looking at a cohort of NF1 mutated metastatic melanoma patients, who were treated at our center with anti PD1 based therapy. In particular, we were interested in response, PFS and OS in patients treated with ICI, who suffer from this rare subtype of melanoma.

3. Methods

The patient population included in our analysis was from our melanoma database in UZ Leuven. All patients were diagnosed and treated at our site. Next generation sequencing is a standard of care diagnostic procedure and includes a panel of 96 genes, including the NF1 gene, relevant for diagnostic and therapeutic purposes in solid tumors. Only patients with available sequencing data were included. The melanoma database was extended to October 2018, because only since then NF1 was part of the Next Generation Sequencing (NGS) panel. A search for all NGS executed tests, irrespective of the type of malignant disease was done in September 2020, and 4077 NGS tests were identified. Melanoma was found to be the tumor of origin in 4.7 % of the cases, resulting in 194 NGS tests performed in malignant melanomas. 13 of these 194 NGS analyses did not yield a result because of numerous reasons, mostly due to poor DNA quality. 181 NGS tests were available for analysis, with 24 (13,3%) showing a NF1 alteration. 11 of these were classified as VUS, the other 13 were thought to be pathogenic or presumably pathogenic (Figure 1). When comparing our NF1 mutation positive population with other reports in the literature, a comparable mutation frequency of around 13-14% was found [14].

A database of 24 NF1 mutant tumors was established by extracting clinical and demographic data from the patients hospital charts. Of those 24 individual patients, 6 had undergone a curative resection of their melanoma and had no documented relapse until the last date of follow-up in January 2021. 4 out of 6 patients were treated in the adjuvant setting, one patient in this group relapsed during adjuvant treatment. 14 patients had upfront metastatic disease at the time of diagnosis. For the purpose of this analysis, we only focused on those patients with metastatic disease, hence 14 metastatic patients were then further analyzed. Characteristics as gender and age were extracted, but also stage of disease, first line therapy, number of metastasis sites, lactate dehydrogenase (LDH) level at time of diagnosis, type of melanoma (cutaneous, mucosal or unknown) and presence or absence of brain metastasis.

Response was assessed in all patients with CT scan or PET-CT scan according to RECIST v1.1, and response assessment was done every twelve weeks. In the event of progressive disease, response assessment was complemented with criteria used in iRE-

CIST unless patients showed unequivocal disease progression. Progression Free Survival (PFS) was defined as time from initiation of anti-PD1 based therapy until disease progression per RECIST or death from any cause. Overall Survival (OS) was defined as time from treatment initiation until death from any cause.

Statistical analysis used the Kaplan-Meier method for estimating PFS and OS. The cumulative incidence function was used for estimating time to best response; death without response was treated as a competing event. The Mann-Whitney U test was used to compare groups on ordinal variables. The Fisher exact test was used for group comparisons on binary variables. A two-sided 5% significance level was adopted for all tests. Analyses have been performed using SAS software (version 9.4 of the SAS system for Windows).

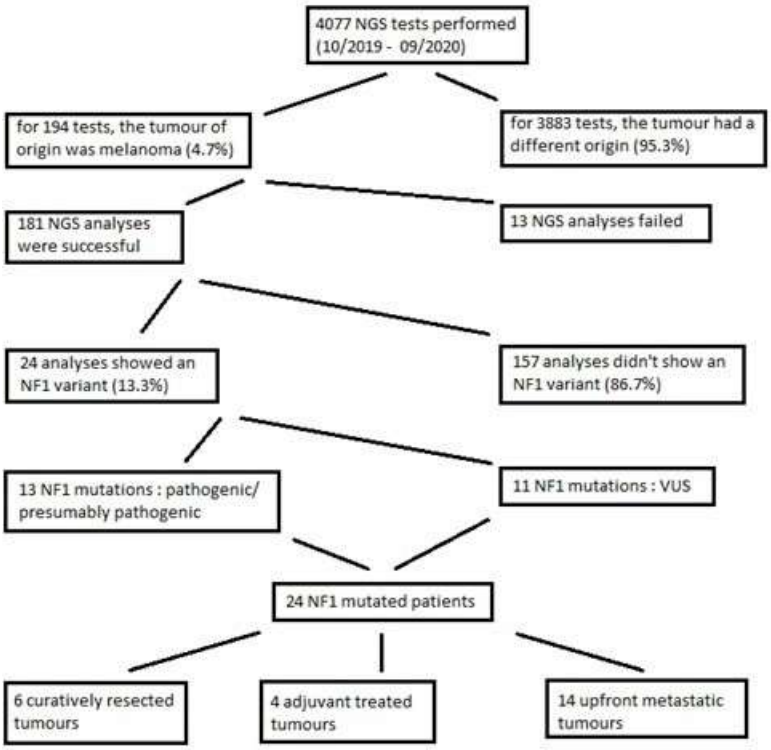

Figure 1: Selection methods

4. Results and Discussion

14 patients with metastatic, irresectable malignant melanoma were included in this retrospective analysis, all of them harboring a NF1 mutation, in addition to a multitude of secondary mutations associated with this rare melanoma subtype. All these patients had upfront metastatic disease at the time of their diagnosis. Median age was 73 years, 9 patients were male, 5 patients were female. hTERT promoter mutation was the second most frequent mutation in our cohort. At the time of initiation of therapy, 29% had elevated LDH. 7 out of 14 (50%) included patients had a primary cutaneous melanoma, while 3 (21%) were diagnosed with a mucosal melanoma. For the remaining 4, the primary tumor was not known. 5 patients (36%) had brain metastasis at the time of diagnosis. Almost half of the included patients (43%) had more than three metastatic sites (Table 1).

More than half of the NF1 mutations were classified as VUS (57%). 11 out of 14 patients harbored an additional TERT promotor mutation, next to the NF1 mutation. Three patients (21%) didn't harbor a mutation in the TERT promotor gene. Most of the included patients harbored more mutations than only one NF1 mutation. Besides the most prevalent hTERT promotor mutations (79%), additional BRAF mutations (3 patients (21%): two mutations in exon 15, one in exon 1), and additional NRAS mutation (4 patients (29%); three mutations in exon 3, one in exon 2) were detected. A variety of other mutations were also found although less frequently (CDKN2A (36%), TP53 (21%), KIT (21%), MET (14%), SMARCA4 (14%), GNAQ (7%), PDGFRA (7%), BAP1 (7%), MAP2K1 (7%), GNA11 (7%), PIK3CA (7%)) (Table 7).

13 out of 14 patients received immunotherapy in first line. Only one patient received pembrolizumab in second line. 4 out of 13 patients started their treatment with combination immunotherapy ipilimumab – nivolumab, whereas 3 patients received nivolumab in monotherapy. 7 patients received pembrolizumab mono.

With regard to response, we observed an objective response rate of 64% in our total patient population. 36 % (n=5) of the patients showed either stable disease (n=3; 21%) or progressive disease (n=2; 14%) as their best response. A total of 28% of the patients achieved a complete response, whereas 36% had a partial response (Table 2). When subdividing by type of treatment, patients treated with combination immunotherapy showed a response in 50%

of the cases (2/4), whereas patients treated with anti-PD1 monotherapy, responded in 70% of the cases (7/10). As shown in the swimmer plot analysis, responses usually occurred within the first 4 months of treatment (Figure 2). The median time of follow up in our study was 11.7 months.

Considering progression free survival, in our cohort 6/14 patients had a progression event during the time of follow-up compared to 8/14 who had no progression (Table 3). The PFS rate was 86% (CI 54-96%) at three months, and the estimated PFS at six, twelve and twenty-four months was approximately of 62% (CI 32-82%) (Table 4). The Kaplan Meier curve for progression free survival shows a plateau which is expected and typical for ICI treated patients (Figure 3).

Overall survival estimates at three months are 100%, 92% (CI 57-99%) at six months, 84 % (CI 49-96%) at twelve months and 72 % (CI 34-90%) at twenty-four months (Table 6). The number of deaths in this small cohort was 29% (n=4) (Table 5). Figure 4 shows the overall survival curve as estimated by the Kaplan-Meier method.

Table 1: Patient characteristics

NF1 mutated metastatic melanoma	ALL (14)
Median age, years (range)	73 (54-92)
Male / Female	
Male	9 (64%)
Female	5 (36%)
LDH	
Normal	10 (71%)
Elevated	4 (29%)
Immuno in first / second line	
First	13 (93%)
Second	1 (7%)
Type of melanoma	
Cutaneous	7 (50%)
Mucosal	3 (21%)
Unknown primary	4 (29%)
Metastatic sites	
3 or less	8 (57%)
more than 3	6 (43%)
Number of mutations (except for NF1)	
0, 1 or 2	6 (43%)
3 or 4	6 (43%)
5 or 6	2 (14%)
Brain mets	
yes	5 (36%)
no	9 (64%)
Type of immunotherapy	
ipilimumab - nivolumab	4 (29%)
nivolumab	3 (21%)
pembrolizumab	7 (50%)

Table 2: Response rate

Variable	**Statistic**	**All**
Response		
No	n/N (%)	5/14 (35.71%)
Yes	n/N (%)	9/14 (64.29%)
Best response		
CR	n/N (%)	4/14 (28.57%)
PR	n/N (%)	5/14 (35.71%)
SD	n/N (%)	3/14 (21.43%)
PD	n/N (%)	2/14 (14.29%)

Table 3: Frequency of progression

Variable	**Statistic**	**All**
Progression		
No	n/N (%)	8/14 (57.14%)
Yes	n/N (%)	6/14 (42.86%)

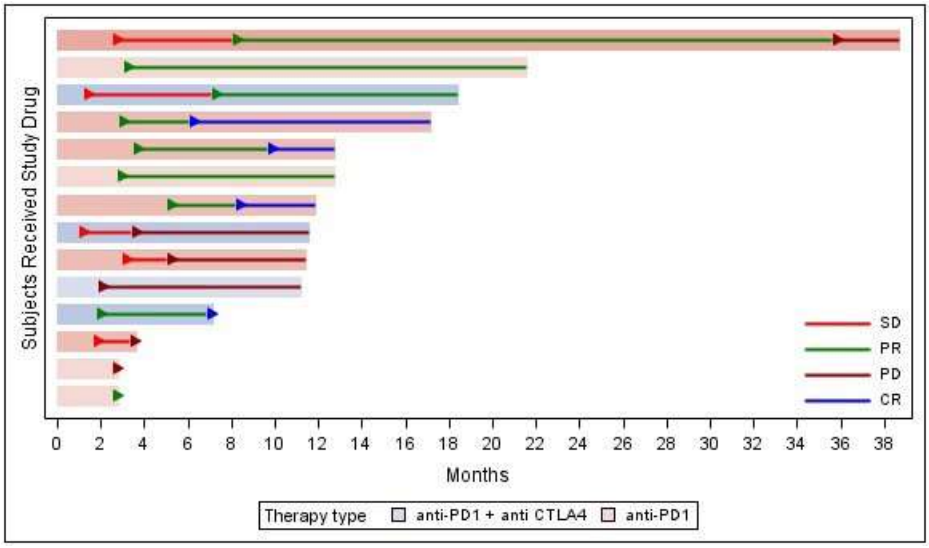

Figure 2: Swimmers plot

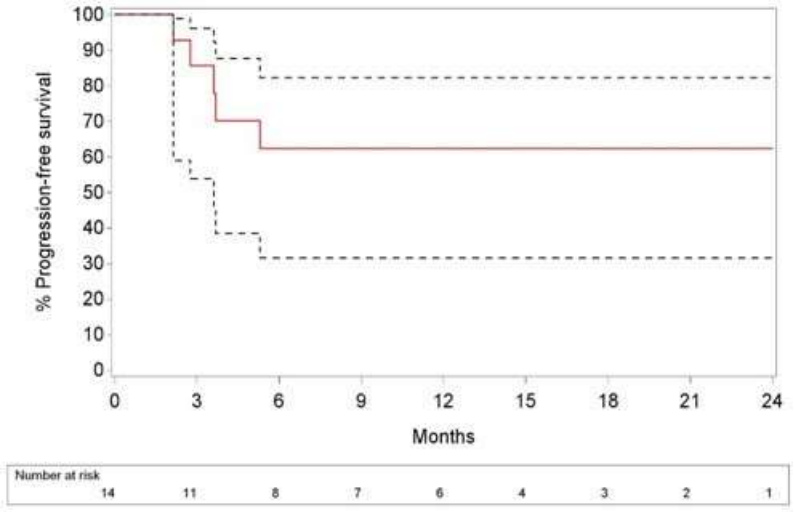

Figure 3: Progression-free survival curve (+95% CI)

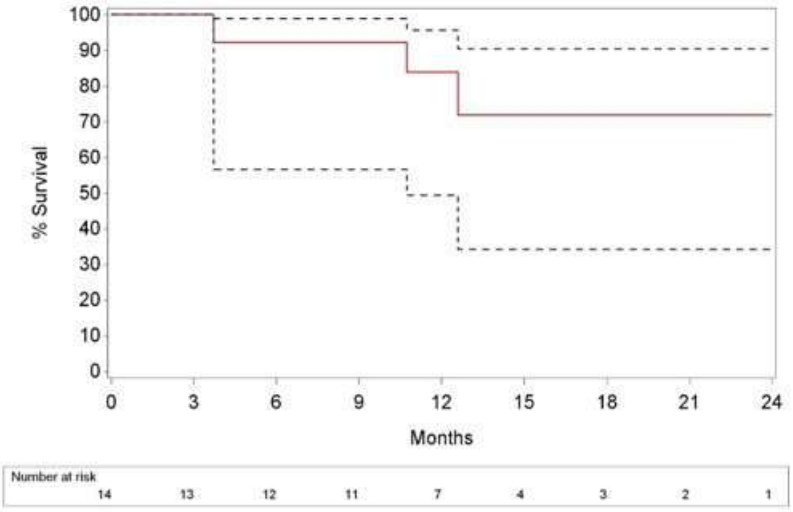

Figure 4: Overall survival curve (95% CI)

Table 4: Progression free survival estimates

Months	% PFS (95% CI)
3	85.71 (53.94;96.22)
6	62.34 (31.66;82.35)
12	62.34 (31.66;82.35)
24	62.34 (31.66;82.35)

Table 5: Frequency of death

Variable	Statistic	All
Death		
No	n/N (%)	10/14 (71.43%)
Yes	n/N (%)	4/14 (28.57%)

Table 6: Overall survival estimates

Months	% OS (95% CI)
3	100.00 (.;.)
6	92.31 (56.63;98.88)
12	83.92 (49.40;95.73)
24	71.93 (34.24;90.37)

Table 7: Mutational analysis

	NF1	TERT	CDKN2A	NRAS	TP53	KIT	BRAF	MET	SMARCA4	GNAQ	PDGFRA	BAP1	MAP2K1	GNA11	PIK3CA
1	exon13	promotor													
2	exon30	promotor								exon 3					
3	exon18				Intron 5+8										
4	exon14			exon 3			exon 1				exon 12				
5	exon40 +17	promotor			exon 7			exon 21				exon 13			
6	exon57	promotor	exon 2	exon 3									exon 3		
7	exon21	promotor	exon 2	exon 2	exon 10+9	exon 11			exon 6						
8	exon54	promotor					exon 15								
9	exon19 +45	promotor +exon2	exon 2					exon 2+11	exon 20					exon 7	
10	eon21	promotor				exon 13+17									
11	exon18	promotor	exon 2												exon 5
12	intron 43														
13	exon8	promotor	exon 2	exon 3											
14	exon50	promotor				exon 15	exon 15								

Knowledge about response and outcome of anti-PD1 based therapy in rare melanoma subtypes is sparse. To the best of our knowledge there are no reports describing the outcome of NF1 mutated metastatic melanoma and ICI therapy. Several molecular studies have shown that NF1 mutated melanomas harbor a higher mutational burden (8, 15). Several lines of evidence exist, showing that tumor mutational burden is an important prerequisite for immune checkpoint inhibitors efficacy. Although this is not considered to be the only predictive factor, studies have shown that mutational burden is associated with clinical outcome in a variety of different malignant diseases [16].

Anti PD1 based therapy has emerged as a standard of care in the treatment of patients with irresectable or metastatic melanoma.

In the Keynote-006 study where pembrolizumab was compared to ipilimumab for advanced melanoma, an objective response rate (partial response plus complete response) of 36% was noted with 13% of the patients achieving a complete response. 67 % of these patients were treated in first line, 33% were treated in second line. Median PFS was 4.1 months for pembrolizumab given every three weeks. PFS at two years of follow-up was 28 months [17].

In the Checkmate-066 study, previously untreated patients without BRAF mutation received either nivolumab or placebo. The median progression free survival was 5.1 months in the nivolumab group. The objective response rate was 40 %. 7.6 % of the patients had a complete response [18].

In the Checkmate-067 study, the combination therapy of ipilimumab plus nivolumab was compared to ipilimumab and nivolumab monotherapy. An unprecedented objective response rate of 58% was noted for the combination therapy group, compared to 45% in the nivolumab group and 19% in the ipilimumab group. The rate of complete response was 22%, 19% and 6%, respectively. The median progression free survival was 11.5 months in the combination group, 6.9 months in the nivolumab group and 2.9 months in the ipilimumab group [19].

We observe a higher response rate (64%) in our small observational cohort, than what was observed in pivotal phase III studies for anti PD1 therapy in melanoma.

Although due to the small sample size, no firm conclusion can be made, but the fact that the objective response rate in our cohort is as high as 64% suggests that NF1 mutated melanoma patients might at least not have an inferior response to anti-PD1 based therapy. Although the higher mutational burden associated with NF1 mutated melanoma would suggest a more favorable response rate we did not find a significant correlation between number of additional mutations and outcome [16]. The fact that we did not actually measure TMB might explain this lack of correlation. The number of additional mutations might thus not be a good surrogate marker for a high TMB. What we did find is a significant correlation between the presence of an additional hTERT promotor mutation and a favorable response (Table 8). A causal relationship between NF1 and hTERT promotor mutations remains elusive at this point.

Table 8: Association mutations with response

Variable	Statistic	All
NF1		
exon 13	n/N (%)	1/14 (7.14%)
exon 14	n/N (%)	1/14 (7.14%)
exon 18	n/N (%)	2/14 (14.29%)
exon 19 + exon 45	n/N (%)	1/14 (7.14%)
exon 21	n/N (%)	2/14 (14.29%)
exon 30	n/N (%)	1/14 (7.14%)
exon 40 + exon 17	n/N (%)	1/14 (7.14%)
exon 50	n/N (%)	1/14 (7.14%)
exon 54	n/N (%)	1/14 (7.14%)
exon 57	n/N (%)	1/14 (7.14%)
exon 8	n/N (%)	1/14 (7.14%)
intron 43	n/N (%)	1/14 (7.14%)
Number of mutations		
0	n/N (%)	1/14 (7.14%)
1	n/N (%)	2/14 (14.29%)
2	n/N (%)	3/14 (21.43%)
3	n/N (%)	4/14 (28.57%)
4	n/N (%)	2/14 (14.29%)
5	n/N (%)	1/14 (7.14%)
6	n/N (%)	1/14 (7.14%)
TERT		
No	n/N (%)	3/14 (21.43%)
Yes	n/N (%)	11/14 (78.57%)
CDKN2A		
No	n/N (%)	8/13 (61.54%)
Yes	n/N (%)	5/13 (38.46%)
NRAS		
No	n/N (%)	9/13 (69.23%)
Yes	n/N (%)	4/13 (30.77%)
TP53		
No	n/N (%)	11/14 (78.57%)
Yes	n/N (%)	3/14 (21.43%)
KIT		
No	n/N (%)	11/14 (78.57%)
Yes	n/N (%)	3/14 (21.43%)
BRAF		
No	n/N (%)	11/14 (78.57%)
Yes	n/N (%)	3/14 (21.43%)
MET		
No	n/N (%)	12/14 (85.71%)
Yes	n/N (%)	2/14 (14.29%)
SMARCA4		
No	n/N (%)	12/14 (85.71%)
Yes	n/N (%)	2/14 (14.29%)
GNAQ		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
PDGFRA		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
BAP1		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
MAP2K1		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
GNA11		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
PIK3CA		
No	n/N (%)	13/14 (92.86%)
Yes	n/N (%)	1/14 (7.14%)
CTNNB1		
No	n/N (%)	14/14 (100.00%)

A potential working hypothesis could be that NF1 mutations lead to a dysfunctional tumor suppressor gene and promote proliferation, hence these cells are also more prone to acquire more mutations, leading to a higher tumor mutational burden [6]. However, a direct link between NF1 mutations and their effect on DNA repair or DNA damage response signaling is not yet been described. Dysregulation of telomerase likely resulting from hTERT promotor mutations would add an additional proliferative stimulus thereby increasing the likelihood of acquired mutations. In 83 % of the NF1 mutated melanomas, a co-occurrence of hTERT promotor mutation is described in the literature (20). This is similar to what we have found in our cohort (79%). Possibly this suggests a link between MAPK activation and TERT expression. When MAPK pathway is activated, it promotes phosphorylation and activation of the ETS1 transcription factor by ERK. Interestingly, the hTERT promotor harbors an ETS binding site [20].

In summary, we did find a higher response rate in this small cohort of NF1 mutated patients, compared to the response rate expected and reported for anti PD1 therapy in phase III melanoma trials. Although NF1 mutated melanomas are associated with a worse prognosis in untreated melanoma, our results suggest that when patients receive anti PD1 based therapy, their outcome might not differ substantially from other melanoma patients. Clearly, much more work is needed to elucidate the impact of NF1 mutations on tumor mutational burden or other molecular features associated with response to ICI therapy. In addition, more robust clinical data are needed to clarify whether this rare subtype of melanoma is associated with a better clinical outcome when treated with immunotherapy.

5. Conclusion

We want to emphasize that this small descriptive study had a very limited patient number and interpretation of the results should therefore be done with caution. However, we saw a high response rate in this small number of NF1 mutated patients treated with immunotherapy, which presumably is higher compared to reported efficacy data generated in large phase III studies. We didn't find a clear correlation between the total number of additional mutations and response rate. This is likely due to the fact we did not measure TMB, and the number of additional mutations might not be a perfect surrogate for this purpose.

We also found a significant correlation between response rate and the presence of a mutation in the promotor of TERT. Reports in the literature show a negative prognostic impact of the presence of this mutation especially if simultaneously present with NF1 mutation.

In summary, our data suggest that the outcome of anti PD1 based ICI therapy in NF1 mutated melanoma is at least comparable with the outcome seen in other melanoma subtypes and should therefore also be considered in patients with this rare genomic alteration.

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