Annals of Case Reports
Hilts A and Samlowski W. Ann Case Rep: 7: 795.
www.doi.org/10.29011/2574-7754.100795
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Research Article

Cautious Addition of MEK Inhibitors to PD-1 Antibody Treatment in Patients with NRAS or NF1 Mutant Metastatic Melanoma Failing Initial Immunotherapy

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Citation: Hilts A, Samlowski W (2022) Cautious Addition of MEK Inhibitors to PD-1 Antibody Treatment in Patients with NRAS or NF1 Mutant Metastatic Melanoma Failing Initial Immunotherapy. Ann Case Report 7: 795. DOI: 10.29011/2574-7754.100795

Received Date: 06 March, 2022**; Accepted Date:** 11 March, 2022**; Published Date:** 14 March 2022

Abstract

Background: Improved treatment options are needed for patients with metastatic melanoma who lack activating BRAF mutations, and progress after initial checkpoint inhibitor therapy. Specifically, there is limited information to guide treatment for patients with NF1and NRAS mutations. MEK inhibitors have been tested in NRAS mutant melanomas, demonstrating minimal single-agent activity. We hypothesized that cautiously combining MEK targeted therapy with checkpoint inhibitor therapy would directly slow cancer progression, while potentiating anticancer immune responses. **Patients and Methods:** A retrospective analysis identified ten patients with initial progression on checkpoint inhibitor treatment, who continued PD-1 antibody treatment with the addition of low dose MEK inhibitors. Three patients had NF1 mutations, seven had NRAS mutations. **Results:** An overall complete response rate of 60% was observed. Median progression-free and overall survival in the entire group was not reached. Five of ten patients achieved durable complete responses. Four patients have been able to completely discontinue all treatment and remain in an ongoing remission. Toxicity was limited to CTCAE grade I-II during combined treatment in most patients. **Discussion:** Our results suggest a novel treatment option for patients with NF1- and NRAS-mutant melanomas who progress on initial immunotherapy.

Keywords: Cobimetinib; Trametinib; Binimetinib; Checkpoint inhibitors; Nivolumab; Pembrolizumab

Implications for Practice

Checkpoint inhibitors are usually employed as the initial therapy for melanoma patients lacking tumor mutations in BRAF V600E/K. If these patients progress, they have a poor outcome. Some of these patients have non-overlapping mutations in NRAS or NF1 by NextGen sequencing. MEK inhibitors have low single agent activity in this setting. We cautiously added low doses of MEK inhibitors to continued PD-1 therapy in patients with progressing metastatic melanoma who had NRAS or NF-1 mutations. This approach induced a significant response rate including durable remissions, and thus may represent an active treatment option for selected patients with immunotherapy-refractory melanoma.

Introduction

The incidence of invasive melanoma has increased steadily over many decades [1]. It is estimated that there were 106,110 new cases diagnosed in the United States in 2020, which resulted in 7,180 deaths [1]. Due to advances in cancer treatment, the median survival rate in metastatic melanoma has increased from

a 25% one-year survival rate in 2005 [2], to a five-year survival rate of over 50% in 2019 [3,4]. These advances in melanoma treatment outcome stem, in part, from a better understanding of anti-cancer immune responses and the mechanisms by which cancers selectively suppress immune recognition in order to avoid immune destruction [5]. Antibodies directed against T cell inhibitory checkpoints CTLA4 (ipilimumab) and PD-1 (nivolumab, pembrolizumab) have proven to be powerful agents in enhancing antitumor immunity and improving treatment outcome [6]. Clinical trials of checkpoint inhibitors (CKI) have shown a high clinical response rate, including durable complete remissions in metastatic melanoma [3,7]. Unfortunately, the majority of immunotherapy treated patients will eventually require additional therapy, as Progression Free Survival (PFS) at 5 years with combined ipilimumab plus nivolumab was only 37% [3]. Only 29% of patients treated with single agent nivolumab or and 8% of patients treated with ipilimumab monotherapy remained progression-free at 5 years, respectively [3]. Similarly, pembrolizumab monotherapy produced a 29% PFS at 5 years [7]. Thus, there is a great need for improved treatment options for patients with advanced melanoma who progress on initial CKI treatment. The discovery of a recurrent pattern of oncogene mutations in melanoma has allowed the development of "Targeted Therapy" (TT).

The most common somatic mutation in melanoma involves the tyrosine kinase BRAF [8]. A BRAF V600E mutation is present in 36-52% of skin melanomas [9]. Treatment of patients with BRAF V600E mutant melanoma with BRAF±MEK inhibitors resulted in rapid onset of responses in a high percentage of patients, with improved progression-free and overall survival [10]. Unfortunately, patients who lack BRAF mutations currently do not have effective TT options. It should be noted that, in addition to BRAF mutations, the most common non-overlapping mutations in melanoma involve NRAS (~10-25% of metastatic melanomas) and NF1 (~5-10% of metastatic melanomas) genes [11,12]. Since these mutations seldom overlap with each other or BRAF mutations in melanomas, they are considered potential "driver mutations" for melanoma growth [11]. BRAF, NRAS and NF-1 mutations all appear to modulate the activity of the RAS/RAF/MEK/ERK signalling pathway in melanoma cells [13,14]. This pathway appears to be necessary for melanoma cell growth and survival [13].

NRAS mutations generally lead to constitutive activation of the RAF/MEK/ERK signalling pathway [15]. In contrast, NF1 acts as a GTP-ase to convert the active form of RAS (RAS-GTP) to the inactivated form (NRAS-GDP) [16]. Therefore, inactivating mutations in NF1 also function to increase NRAS activity [17]. Despite being downstream from activated NRAS, clinical trials of BRAF inhibition have not been successful in blocking the effects of mutant NRAS [18]. Inhibition of other components of the MAPK signalling pathway, such as MEK or ERK, have been considered as interventions in NRAS mutant melanoma [13]. Unfortunately, these trials have also not shown significant clinical activity. Basic science studies have shown that MEK-targeted agents enhance anticancer immune responses by counteracting inhibitory pathways in the tumour microenvironment and increasing natural killer cell number and function [19]. Since most melanoma patients with NRAS or NF1 mutations who progress after CKI therapy eventually die of their cancer, there is a need to develop additional effective treatments. Due to the potentially synergistic effects of TT and CKI, we have hypothesized combining the CKI treatment with currently approved MEK inhibitors would serve to arrest progressive cancer growth long enough to allow productive CKI-induced immune responses to develop. In our clinic, patients with NRAS and NF1 mutant melanoma who progressed on initial CKI therapy have been cautiously treated with the addition of low doses of MEK inhibitors to PD-1 maintenance therapy. The clinical outcome and toxicity of this approach are described.

Materials and Methods

Patients

Study patients were identified via a computer search of a HIPAA compliant password secured iKnowMed data base (McKesson, Houston, TX) installed on a secure network. The database was searched for patients who had received treatment with MEK inhibitors (binimetinib, trametinib, cobimetinib). We then identified patients from this group who had initially progressed on prior CKI therapy (pembrolizumab, nivolumab or ipilimumab plus nivolumab). For this study, we analyzed the patients who received ongoing CKI immunotherapy with cautious addition of low doses of MEK inhibitor. Eligible patients were treated between 2015–2020. The data collection period ended December 31, 2020. This retrospective data analysis study plan was reviewed by the Western International Review Board and deemed exempt from full IRB review.

Patient treatment

All patients underwent oncogene mutation testing [Foundation Medicine, Cambridge MA (NRAS and NF1), Genoptics (NRAS only), Carlsbad CA, Clearpoint Diagnostic Laboratory, Lewisville, TX (NRAS only)] and patients with NRAS and NF1 mutations were identified. All patients who progressed on initial CKI treatment were offered clinical trial participation if eligible. Patients with NRAS or NF1 mutations who were ineligible for trials continued ongoing PD-1 mAb therapy at standard doses with the cautious addition of an FDA-approved MEK inhibitor. Patients were started on a low dose of MEK inhibitor. After 1 week, if no toxicity was observed, there was an attempt to increase the dosage. The initial oral dose of trametinib was 1 mg daily, while the starting dose of binimetinib was 15 mg twice daily. The

initial cobimetinib dose was 20 mg/d. All patients were treated by one physician (WS) at a single institution. If the patient achieved a confirmed radiologic or pathologic complete remission, treatment was discontinued [20].

Data acquisition

Patients were analysed for gender, age, and their specific NRAS or NF1 mutation. The initial CKI regimen used was recorded, as was total dose of CKI, number of CKI doses, TT agent(s) added, TT total dose and number of months of TT, the start and termination date of all drugs, time to progression, best response using RECIST 1.1 criteria [21,22]. Data was de-identified after extraction. We analyzed the duration of the initial response following CKI treatment (R1) and the duration of response to combined CKI and TT (R2) separately. R1 was calculated from the start of CKI to the start of the targeted therapy. R2 was computed from the start of the targeted therapy to the date of eventual progression or death. If patients were alive, the date of the last clinic visit was used in the analysis. Cause of death was also identified. Any treatment-related toxicity was recorded using CTCAE 4.0 criteria [23]. Overall survival was calculated from the start of CKI treatment.

Data analysis

Descriptive statistics were calculated via an Excel spreadsheet (Microsoft). Survival and progression-free survival were evaluated via Kaplan-Meier analysis [24].

Results

Patient Characteristics

This retrospective chart review study identified ten patients with metastatic melanoma with either a NRAS or NF1 mutation who had progressed after initial CKI therapy. In our clinic, 38.5% of all NRAS patients (n=13) and 45.8% of all NF1 patients (n=24) achieved a durable complete response with their initial CKI therapy. No statistical comparison of these groups was performed due to the small sample size. Following progression, patients were considered for available clinical trials. If ineligible, patients were subsequently treated with concurrent PD-1 antibody treatment with cautious addition of low dose MEK inhibitor. Patient characteristics are shown (Table 1). The median age at diagnosis was 65.8 years old, with the minimum age 41 and maximum age 77 years old, 80% of the patients were male and 20% were female. There were 3 patients with NF1 mutations and 7 with NRAS mutations. The specific NRAS and NF1 mutations are shown (Table 1). Codon 61 mutations were present in 5 of 7 NRAS mutant patients. Each NF1 patient had a distinct mutation. In addition, all patients with a NF1 mutation had a concomitant telomerase (TERT) promoter mutation.

Pt	Sex	Age	Primary site	NRAS/NF1 mutation	IO agent(s)	R1 (mo)	Targeted agent	R2 (mo)	Best response	Current status	Toxicity (TT+CKI)
1	M	63	back	NF1-T2575FS*48 & C1367*	Ipi/Nivo	0.7	T	2.7	PD	DOD	Dry Skin
2	M	65	shoulder	NF1-G1756fs*6, SS 2002-1G>A	Nivo	3.9	T	22.2	CR	CR-off Rx	None
3	M	77	preauricular	NF1-S168*	Nivo	5.5	T	29.0	CR	CR-off Rx	Rash
4	M	62	trunk	NRAS-Q61K	Nivo	2.1	T, B, C	39.7	CR	CR-off Rx	T, B: fatigue, malaise, Vitiligo, Endocrinopathy, rash, puritus C: none
5	M	76	shoulder	NRAS-Q61L	Pembro	6.4	T	7.2	CR	D-CAD	None
6	F	41	arm	NRAS-Q61R	Pembro	2.1	T	12.8	PD	DOD	None
7	M	72	unknown	NRAS-G60E; E62A	Pembro	2.9	T	13.1	PR	D-cardiac	Rash, puritus
8	M	65	back	NRAS-Q61R	Pembro	6.8	T	15.9	PD	DOD	Diarrhea, gas, fatigue, malaise, sweats
9	M	68	chest	NRAS-Q61L	Pembro	4.0	T	33.0	PD	DOD	nausea, weight loss
10	F	69	vulva	NRAS-A59D	Pembro	3.1	T	45.5	CR	CR-off Rx	Rash, hypophysitis

Table 1: Patient characteristics.

Footnote:

Ipi: ipilimumab; nivo, nivolumab; pembro, pembrolizumab; R1, response to immunotherapy alone; R2, response to immunotherapy plus MEK inhibitor; CR, complete response; PR, partial response; PD, progressive disease; T, trametinib; B, binimetinib; C, cobimetinib; CR-On Rx, Complete response currently on treatment; A-PD, alive with progressive disease; CR-off Rx, complete remission therapy discontinued; DOD, died of disease, D-cardiac, died of intercurrent cardiac disease.

Treatment

All patients were initially treated with a CKI (usually a PD-1 antibody alone or in combination with CTLA4 antibody), as shown (Table 1). The median time from the start of the initial CKI to the start of TT (R1) was 3.8 ± 2.0 months (± SD). The median number of CKI doses from the start of initial treatment until progression, prior to addition of MEK inhibitor was 5.9 ± 2.9 doses.

Analysis of progression-free and overall survival

The initial objective response rate (CR+PR) after addition of MEK inhibitors to ongoing PD-1 therapy was 60% (Table 1). Median progression free survival from the start of CKI plus MEK inhibitor therapy in the combined cohort of patients was not reached (Figure 1). Overall progression-free survival has remained at 52% at over 24 months median follow-up. Median progression-free survival for NF1 mutant subset was also not reached. Median progression-free survival in the NRAS mutant subset was reached at 15.9 months. Two of three (66.7%) of patients with NF1 mutations achieved a complete response with second line TT+ PD-1 treatment. In NRAS mutant patients, 3/7 (45%) achieved an unmaintained complete response lasting over 3 years. No responding patient has progressed after 15 months. Median overall survival was also not reached for the overall group or in patients with NF-1 mutations. The median survival in patients with an NRAS mutations was 24 months (Figure 2). There appeared to be a survival plateau after 24 months for patients with either NRAS or NF1 mutations. Survival of responding patients is also shown, demonstrating the durable nature of clinical responses in these patients (Figure 3). Two NRAS-mutant patients died of intercurrent illness without disease progression. One patient with a partial response had sudden death at home after 13 months of treatment for unknown reasons (without prolonged QT syndrome). The other was an elderly man who died in complete remission of melanoma, 6 months after treatment discontinuation, from pre-existing coronary artery disease. The relative contribution of initial immunotherapy and TT+PD1 therapy is shown in a "swim-lane" plot (Figure 4). In all patients, R2 (combined TT and PD-1 therapy) exceeded R1 (initial CKI therapy). This plot also shows the timing of treatment discontinuation in 4/6 responding patients. To date none of these 4 responding patients have relapsed after elective treatment discontinuation.

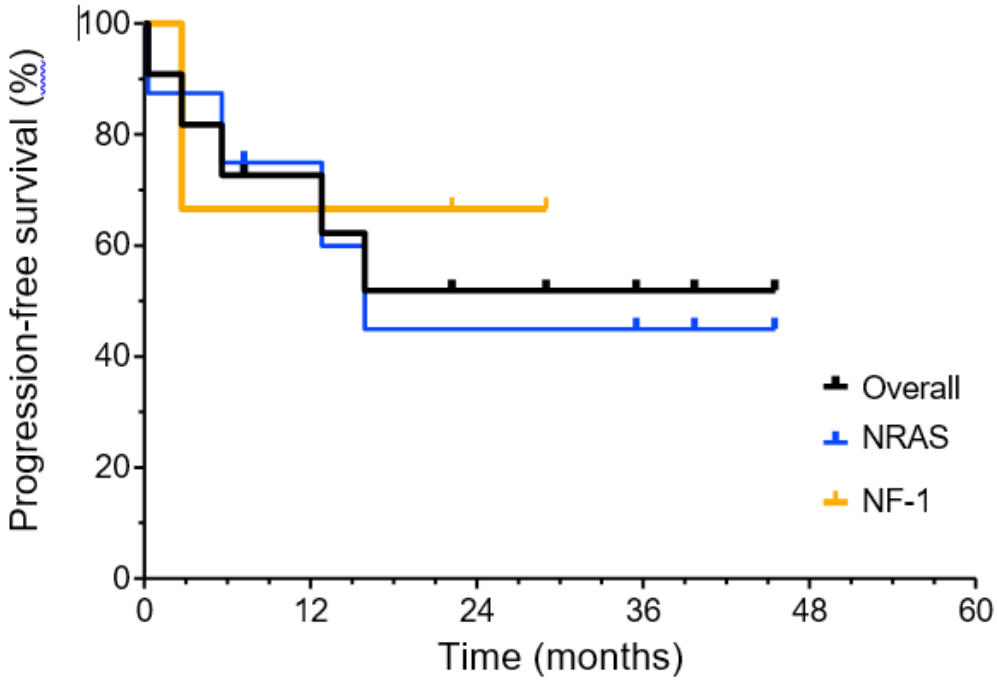

Figure 1: Progression free survival of patients overall and by specific mutation. Censored patients are indicated by hash marks.

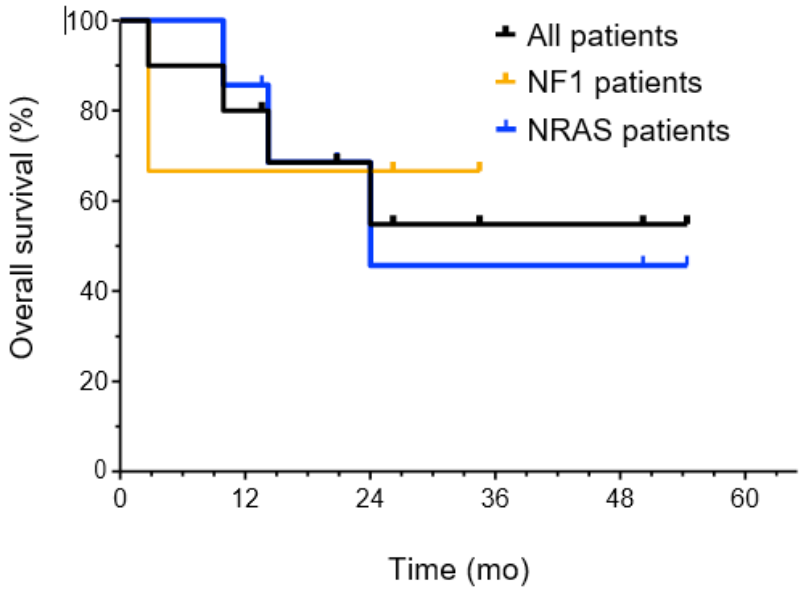

Figure 2: Overall survival of patients overall and by specific mutation.

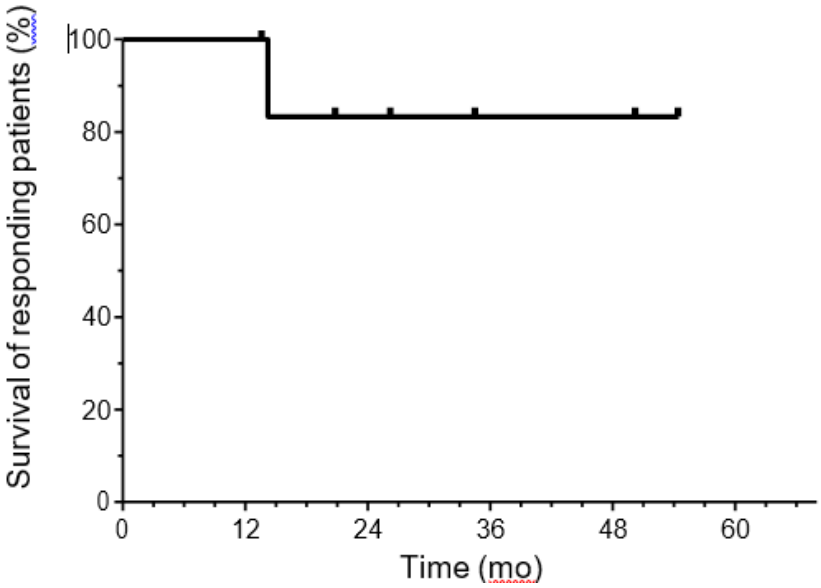

Figure 3: Disease-specific survival of responding patients.

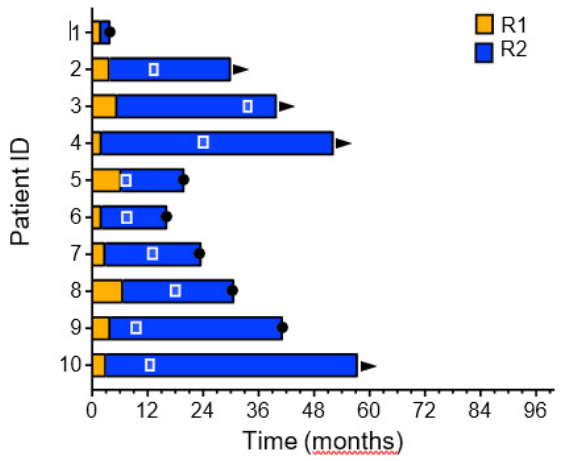

Figure 4: Swim-lane plot of the response duration to CKI treatment (R1) and subsequent response duration to treatment with MEK inhibitor + PD-1 antibody therapy (R2). Arrows indicate ongoing response. Blunt ended bars indicate patient death. White boxes indicate the timing of treatment discontinuation.

Toxicity

Eight of the eleven patients (70%) experienced adverse events while on initial CKI therapy. Some patients experienced multiple side effects while 30% of patients experienced no CKI toxicity. The most common CKI-related immune toxicity was a rash or colitis (3/10 patients each), and neurologic toxicity (2/10 patients). When patients were started on combined therapy, 70% of patients experienced at least one adverse event (Table 1). The most common side effect continued to be rash (4/10 patients) although this was generally more typical of MEK-inhibitor induced skin changes. One patient developed a significant rash with both trametinib and binimetinib and was finally able to tolerate cobimetinib. Two patients developed clinically significant fatigue. In one patient this was associated with endocrinopathy and in the other it was associated with colitis. Most toxicity related to combined therapy was grade II and was controllable with low dose steroids or antipruritic agents. Patients with endocrinopathy had appropriate thyroid and glucocorticoid replacement. Two patients were hospitalized during initial CKI therapy. One patient required transient hospitalization for toxicity during combined CKI+TT therapy for symptomatic hypopituitarism.

Discussion

Progressive growth of melanoma cells is often driven via the mitogen activated protein kinase (MAPK) pathway [16]. The most common somatic mutations in the MAPK pathway in patients with melanoma involve the BRAF (in 36-52%), NRAS (10-25%) and NF1 (5-10%) genes [11,12]. Since these mutations are non-overlapping in most untreated patients, they are felt to be "driver" mutations involved in melanoma development [25]. The successful development of kinase inhibitors targeting the most common mutation (V600E) in BRAF has proven to be an important milestone in improving melanoma therapy [26]. BRAF targeted agents induce a rapid and deep anti-cancer response in most BRAF-mutant melanomas [11,12]. Unfortunately, the eventual development of adaptive resistance has been frequent [4]. Currently there is minimal evidence for the effectiveness of targeted therapy for patients with other MAPK pathway "driver" mutations, such as in NRAS or NF-1. There have been previous attempts to target NRAS in metastatic melanoma [13]. NRAS signalling appears to be inhibited by MEK inhibitors, but not BRAF inhibitors [27]. Unfortunately, monotherapy using MEK inhibitors alone has not shown significant clinical activity in melanoma [28-30].

In patients without an activating BRAF mutation (e.g., NF-1, NRAS mutant or "triple negative"), CKI treatment represent the most effective option. Unfortunately, eventual disease progression remains a significant clinical challenge. Additional treatment options for patients who lack BRAF mutations are still badly needed. More recently, several clinical trials in metastatic melanoma patients who lacked BRAF mutations have been performed using combinations of MEK inhibitors plus PD-(L)1 antibodies. These trials included patients with NRAS and NF-1 mutations, but also included triple-negative patients (no BRAF, NRAS or NF1 mutations). These studies have shown mixed results. Hellman et al. reported a phase I/IB study of PD-L1/PD-1-naive patients with solid tumors treated with cobimetinib plus the PD-1L MAb atezolizumab [31]. A total of 152 patients with various tumor types were enrolled, including 22 patients with melanoma. Confirmed responses were observed in 9 of 22 patients (41%) with melanoma. There was a high treatment discontinuation rate due to toxicity of the combination regimen [31]. Gogas et al. subsequently reported a larger trial of combination of MEK inhibitor plus PD-1L Mab in previously untreated BRAF wild-type metastatic melanoma patients [32]. Patients were randomized to receive either cobimetinib plus atezolizumab (222 patients) or pembrolizumab (224 patients). Median PFS was 5.5 months with cobimetinib plus atezolizumab versus 5.7 months with pembrolizumab monotherapy (p=0.30). Hazard ratios for PFS were similar across all prespecified subgroups including patients with NRAS (18% of patients) and NF-1 mutations (23% of patients). However, it should be noted that NRAS and NF1 testing results were not available in 62/222 patients (28%) in the combination arm [32].

Ribas et al. evaluated durvalumab and trametinib given concomitantly (n=20) or sequentially (n=22) in patients with BRAF-wild type melanoma [33]. It should be noted that the patients were not selected for NRAS or NF1 mutations. Prior therapy was allowed in this trial. Objective responses were observed in 20.0%

and 31.8% of these patients, respectively. Several patients achieved long-term remissions. There was evidence of improved tumor immune infiltration and durable responses in a subset of patients with available biopsy samples. Adverse events and treatment discontinuation rates were more common than previously reported for these agents given as monotherapy [33]. The explanation for the variable results of prior trials is not clear. NextGen sequencing based oncogene mutation screening can identify potentially significant genetic mutations beyond BRAF V600E that may be clinically actionable. NRAS mutations generally appear to involve codons 12, 13 and 61 [15]. In particular, the most common NRAS mutations in codon 61 appears to function by locking the NRAS protein into a constitutively active conformation, leading to downstream MAPK pathway activation [34]. Activating NRAS mutations result in increased melanoma cell growth, motility and survival [35].

NRAS mutations also appear to correlate clinically with aggressive melanoma growth, including an increased risk of visceral metastases and brain metastases [36]. NF1 encodes the protein neurofibromin, which negatively regulates the RAS/MAPK pathway by converting active NRAS-GTP to inactive NRAS-GDP [37]. NF1 inactivation is, therefore, considered a 'RAS-opathy' as the growth of these tumors results from increased NRAS signalling and MEK dependence [37,38]. Somatic deletions and inactivating mutations in NF1 have been described in 5-10% of patients with cutaneous melanoma and are associated with increased growth-dependent RAS-RAF-MEK signalling [17,38]. NF1 mutant melanomas are typically found on heavily sun-exposed skin and exhibit a high DNA mutation burden [39]. It is surprising that no trials of MEK inhibitors have been reported in NF1mutant melanoma. Due the apparent MEK dependence of patients with NRAS and NF1mutation, combining MEK inhibitors with CKI appeared highly attractive. This was particularly of interest, since both NRAS and NF1 mutant melanomas are often characterized by a high mutation burden that may sensitize to immunotherapy [40]. Unfortunately, previously attempted combinations of CKI and TT have led to increased levels of toxicity [41-43]. This added toxicity appeared due to the unanticipated immunologic activity of both BRAF and MEK inhibitors. While BRAF inhibitors strongly potentiate immune responsiveness, less is known about the effect of MEK inhibition on CKI antitumor immune responses [44]. Based on preclinical experiments in isolated T cells, there were initially theoretical concerns that MEK 1/2 inhibitors might suppress immunologic responsiveness due to direct inhibitory effects on lymphocytes [45-51].

In vivo murine studies have suggested that MEK inhibitors increase antitumor T cell responses and synergize with immunotherapy [45,52,53]. MEK inhibitors augment immune responses in vivo by inducing permissive alterations in the tumor microenvironment. These mechanisms include inhibition of myeloid suppressor cells, induction of tumor associated macrophage activation, and decreases of the inhibitory mediators, Cox 2, and arginase [54]. We have approached the problem of immunotherapy-resistant patients differently than many ongoing trials. In a community setting, there are frequent delays in identifying NRAS or NF1 mutant patients, as this requires Next-Gen sequencing panels to be sent to a reference laboratory [55]. Thus, initiating treatment with CKI avoids lengthy treatment delays while awaiting molecular test results. There is developing data in BRAF mutant patients that initial CKI therapy followed by BRAF inhibition at relapse may have a higher progression free survival than the reverse sequence [56-59]. Whether this also holds true in NRAS or NF1 mutant melanoma is not yet known.

We also recognized that a substantial percentage of patients (20-40%) treated with initial immunotherapy achieve complete and durable remissions without any additional therapy [3,7]. This may especially apply to NF1 mutant patients, who appeared to have a lower progression rate after initial CKI therapy. Inclusion of patients in clinical trials who would have achieved remissions with CKI therapy alone may have confounded the previously described clinical trials employing combination CKI and TT treatment. We chose to treat only patients with NRAS and NF1 mutations who had clearly progressed on immunotherapy with cautious addition of TT to ongoing PD-1 directed treatment. We also discarded the chemotherapy concept of "maximum tolerated dose". It is not clear that this applies to either CKI or TT therapy [60-62]. We sought to find a minimum effective dose of TT with CKI. Our hypothesis was that this might arrest tumour progression while enhancing potentially synergistic immune activation and decrease the risk of additive toxicity. Our results support the clinical activity of this sequential treatment approach. We were able to cautiously escalate MEK inhibitors with PD-1 therapy. Our approach was able to produce a 60% objective response rate in NRAS and NF1 patients after initial CKI failure. Median progression-free survival (with >36-month follow-up from the start of second line therapy) was not reached in the overall group of patients or in patients with NF1mutation. Median progression-free survival was 24 months in patients with NRAS mutations. Median overall survival in our patients was not reached with 24-month median follow-up from the start of initial CKI therapy, either in the overall group or in the NF1 group. The NRAS mutant patients had a median survival of 24 months. There appeared to be a survival plateau after 15 months. Of 5 complete responders, 4 have been able to discontinue therapy without subsequent progression. Two responding patients have died of unrelated conditions.

In our series, treatment-related toxicity was manageable and reached Grade 1-2 intensity in 4 of 10 patients. Only one patient required hospitalization during combined therapy, due to dizziness and dehydration related to development of hypopituitarism, which was subsequently controlled with medications. This allowed the

patient to continue treatment and achieve a durable complete response. Toxicity was generally managed with temporary treatment interruption and steroid administration if toxicity was typical for CKI immunologic toxicity. MEK inhibitors were interrupted and restarted with a dose reduction if toxicity was believed to be TT related (e.g., a typical non-pruritic MEK inhibitor rash). Treatment was resumed once toxicity reached grade 1 or less. Due to the recurrence of TT toxicity after re-challenge, two patients were converted to alternate MEK inhibitors. One patient required a trial of 3 separate MEK inhibitors to achieve acceptable levels of rash. All patients were able to remain on therapy after toxicity was treated. Unfortunately, 4/10 patients (40 %) failed to respond at all to the addition of TT to PD-1 treatment. Further work will be needed to understand the mechanism(s) of resistance and to identify more active treatment options in this subset of patients. The dichotomous response pattern in our patients is curious: most responding patients eventually reached a complete response and virtually all initially progressing patients died. The basis for this observation remains to be elucidated.

Summary

Our data suggests that 60% of patients with NRAS/or NF1 mutations who progress on initial CKI therapy respond to the addition of low doses of MEK inhibitor to ongoing PD-1 treatment. A significant number of these patients achieved durable complete remissions and were able to eventually discontinue all therapy. Our current report is intended to be hypothesis-generating, with a goal of providing preliminary data to support development of further clinical trials in patients who express NRAS or NF1 mutations. Potential limitations of this study include that it represents a retrospective review of patient outcomes over 5 years involving a relatively small number of patients. Patients were treated with two different PD-1 antibodies and different MEK inhibitors based on availability over this time interval. It is possible that adding additional agents to overcome CKI or TT resistance mechanisms may increase responses. We are hopeful that our results will stimulate further interest in trials for metastatic melanoma patients whose tumors express NRAS and NF1 mutations.

Acknowledgements

We thank the patients, families, clinic staff, and referring physicians who made this case series possible. The helpful editorial suggestions of Suzanne Samlowski, M. Arch., are also appreciated.

Author Contributions

Conception and design: Alexis Hilts and Wolfram Samlowski

Provision of study materials and patients: Wolfram Samlowski

Collection and assembly of data: Alexis Hilts and Wolfram Samlowski

Manuscript writing: Wolfram Alexis Hilts and Wolfram Samlowski

Final approval of manuscript: Alexis Hilts and Wolfram Samlowski

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