BRAF L597K mutation: an opportunity to treat

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Abstract

The outcomes of patients with metastatic melanoma (MM) have significantly improved after the introduction of BRAF-specific inhibitors. Herein is reported a patient with MM and non-V600-*BRAF* mutation who responded to iBRAF/iMEK therapy. In July 2014, a 63-year-old man presented with a 4.1mm-thick V600E-*BRAF* wild type melanoma on the back. Metastases were identified in one sentinel node and two of 11 subsequently excised lymph nodes, with no signs of distant metastatic disease. In September 2017, lung metastasis was observed and pembrolizumab was started. Progressive disease was apparent at cycle 10 and therapy was switched to ipilimumab. After four cycles, an asymmetric response was observed. In November 2017, next generation sequencing genomic profiling disclosed a rare L597K-*BRAF* mutation and vemurafenib plus cobimetinib therapy was initiated in January 2018. Seven days after treatment start, a remarkable clinical improvement was observed. In April 2018, the patient achieved partial response, which was sustained until October 2018. Cases of patients with non-V600-*BRAF* mutations responding to iBRAF/iMEK therapy have been reported over the last years. To the best of our knowledge, this is the first case reporting response to combined iBRAF/iMEK therapy in a patient with metastatic melanoma harboring L597K mutation.

Keywords: metastatic melanoma, non-V600 BRAF mutation, target therapy

Introduction

The outcomes of patients with metastatic melanoma (MM) have significantly improved after the discovery of oncogenic BRAF signaling and introduction of BRAF-specific inhibitors (BRAFi), [1]. Activating mutations in the *BRAF* gene are present in nearly 50% of advanced melanomas [2]. To date, more than 80 somatic mutations have been identified in exon 15, 80–90% of which are at the V600 locus [3,4]. BRAF is a serine/threonine protein kinase encoded on chromosome 7q and is part of the mitogen-activated protein kinase (MAPK) pathway [5,6]. BRAF has been implicated in senescence and apoptosis evasion, unchecked replicative potential, angiogenesis, tissue invasion and metastasis development, and immune response evasion [5]. Owing to widespread availability of detection tools [4], such as next-generation sequencing (NGS), an increasing number of rare BRAF mutations have been reported. Herein we report a patient with MM and a non-V600-BRAF mutation, who responded to combined BRAFi/MEK inhibitor (MEKi) treatment.

Case Synopsis

A 63-year-old man presented with a 4.1mm thick, non-ulcerated, superficial extension melanoma on the back that was surgically removed in July 2014 (Clark level four and 7 mitosis/mm^2). After wide local excision and sentinel lymph node (LN) biopsy showing one MM metastasis, locoregional lymphadenectomy was performed, revealing

metastases in two of the 11 excised LNs. Whole-body PET-CT performed in December 2014 showed no signs of metastatic disease.

In July 2015, the patient relapsed with multiple skin metastases in the axillary region but no evidence of distant recurrence. After four sessions of electrochemotherapy (a combination of electroporation and chemotherapy) partial response was achieved. In this treatment modality, an electric field is applied to cells, eliciting changes in tissue geometrical and material properties that cause local deficiencies in cell membrane and make it permeable to chemotherapy. Real-time PCR showed no V600E *BRAF* (exon 15) or *NRAS* (codons 12, 13, and 61) mutations. Eastern Cooperative Oncology Group (ECOG) performance status was zero and lactate dehydrogenase (LDH) level was 181UI/L (normal range 100–250 UI/L).

In September 2017, lung metastases were identified, and pembrolizumab (2 mg/kg every three weeks) was started. During the first three cycles, no new lesions were observed but older lesions increased. By cycle 5, asymmetric skin response was observed (with some lesions significantly decreasing and others increasing); lung disease did not improve but remained stable. Pembrolizumab was maintained, but at cycle 9 skin metastases became hemorrhagic and unacceptably painful and palliative radiotherapy was performed. Progressive disease was apparent at cycle 10, with development of new lesions and increase in size of older ones. At that time therapy was switched to ipilimumab (3mg/kg every three weeks). After cycle 4, an asymmetric response was again observed.

In November 2017, the patient presented normal LDH levels but ECOG performance status of two, and difficulty controlling pain (opioid therapy was ongoing). Since no other effective therapies were available, the patient agreed to provide a tumor sample for for pathological analyses (**Figure 1**) and NGS genomic profiling (FoundationOne protocol).

Genomic analysis revealed a high tumor mutational burden (30 mutations/Mb) and stable microsatellite status. *ARID1A* Q557 and *DAXX* Q425 mutations and *CDKN2A/B* loss were identified. Also, a rare L597K *BRAF* mutation was detected. Considering the absence of other active treatment options and rapid disease progression, BRAFi/MEKi therapy with vemurafenib plus cobimetinib was started in January 2018 after tumor board discussion (**Figure 2**). After seven days, remarkable clinical improvement was observed, with rapid reduction of all lesions enabling the opioid dose to be down-titrated and subsequently discontinued.

Two months after starting BRAFi/MEKi therapy, grade 3 retinal detachment prompted cobimetinib dose reduction (March 2018) and subsequent suspension with clinical improvement and almost complete resolution.

In April 2018, skin lesion volume reduction and significant metabolic activity decline of skin (**Figure 3**) and lung lesions was observed in PET-CT. Partial response was sustained until October 2018, when a new subcutaneous nodule became evident. By this time, cobimetinib 20mg was resumed, but after 14 days it was again suspended owing to increased ocular toxicity. In December 2018, treatment was switched to dabrafenib plus trametinib (full dose), with asymmetric response and no ocular toxicity. However, in February 2019 progression was again evident, with development of new skin lesions and increase in older ones. Dacarbazine was started, but the patient died in May 2019.

Case Discussion

Among *BRAF* mutations associated with human cancer, the high-activity V600E *BRAF* mutation is by far the most common, accounting for >90% of cases [7,8]. However, several other mutations are detected at lower frequency [7]. Novel BRAF-specific target therapy kinase inhibitors (verumafenib, dabrafenib, and encorafenib) have shown high rates of objective response and important overall survival benefit compared to chemotherapy [6]. However, roughly 50% of all BRAFi-treated patients experience disease progression within 6–7 months [6], which has been attributed to reactivation of the MAPK pathway, either by upstream activating mutations or downstream alterations [6]. BRAFi-elicited squamous cell carcinoma and keratoacanthoma led to the

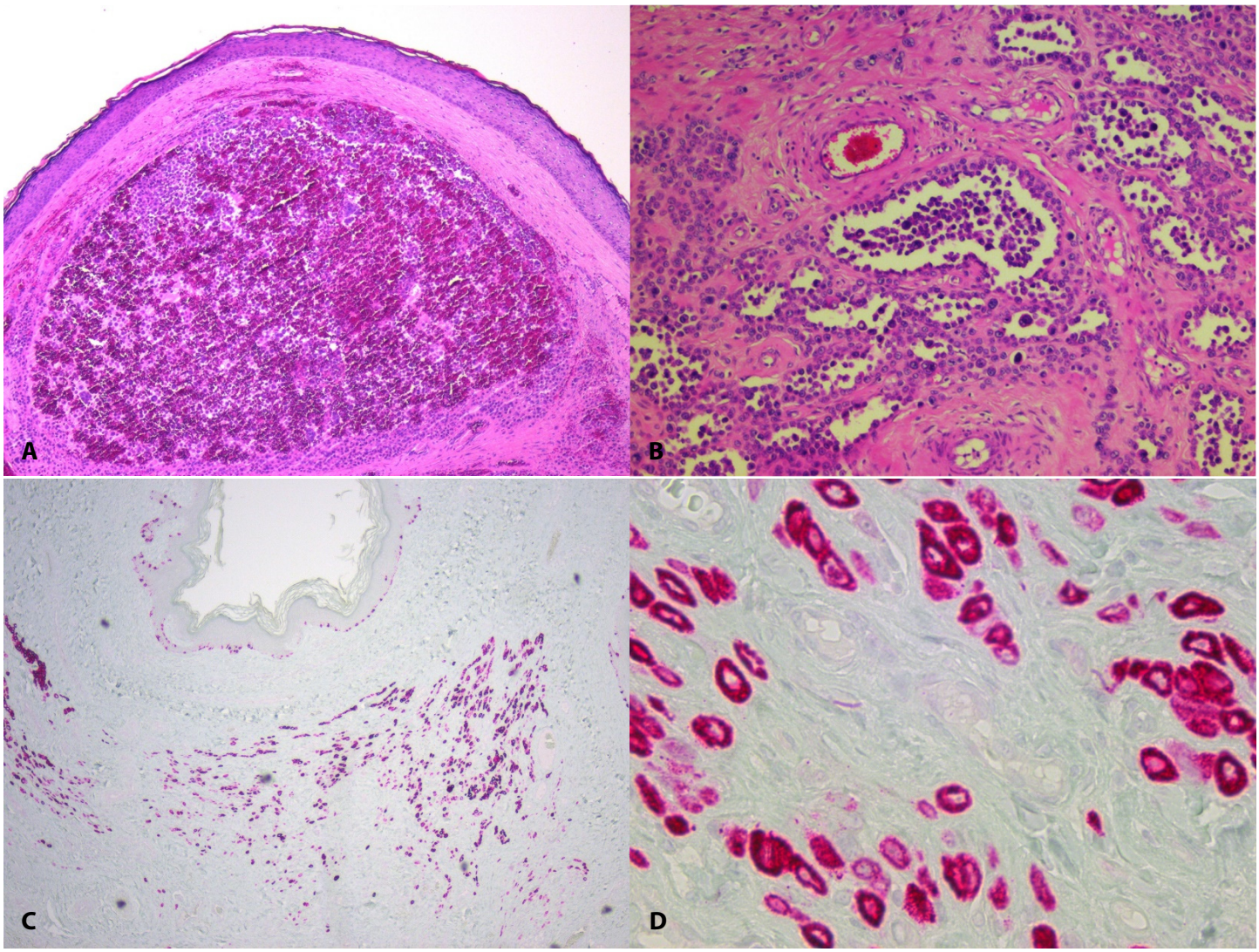

Figure 1. *Pathology images of metastases: H&E, **A)** 40×, **B)** 100×; Melan-A immunohistochemistry, **C)** 40×, **D)** 400×.*

discovery of BRAFi-induced paradoxical hyperactivation of MAPK downstream pathway [6]. To counteract these side effects, BRAFi and MEKi (cobimetinib, trametinib, and binimetinib) combination therapy was instituted to maximize MAPK pathway inhibition and prevent resistance. The efficacy of this combination was confirmed by numerous clinical trials, as described in **Table 1**.

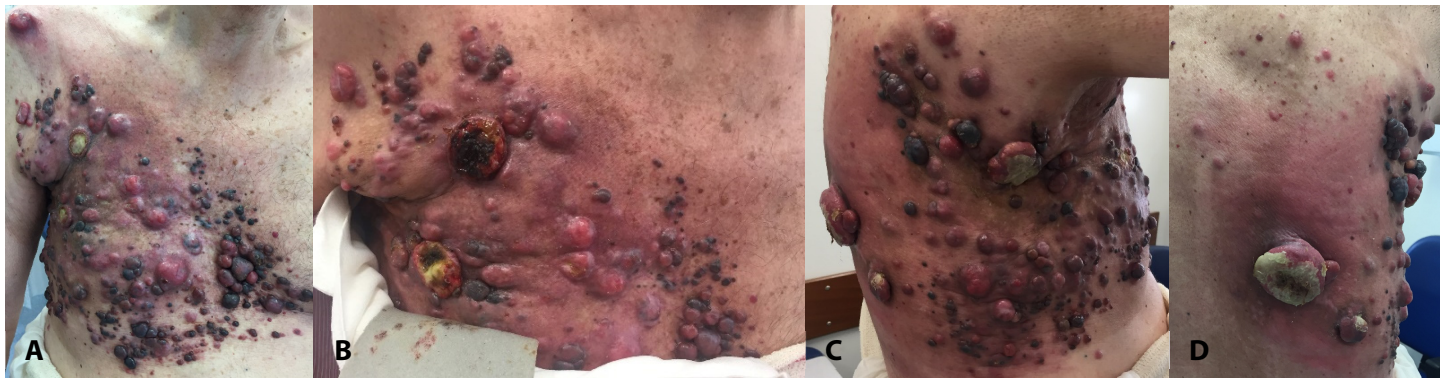

Figure 2. *Skin metastases before treatment: **A)** thorax; **B)** right breast and axillary region; **C)** right mid axillary chest wall; **D)** right scapula.*

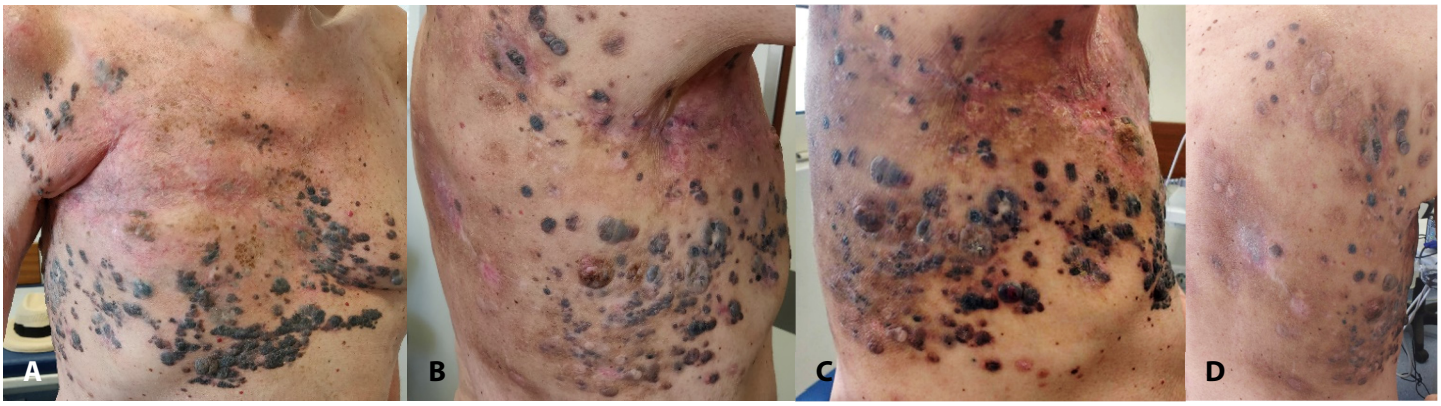

Figure 3. *Skin metastases after 5 cycles of treatment:* ***A)*** *Thorax;* ***B)*** *Right mid axillary chest wall;* ***C)*** *Right axillary chest wall;* ***D)*** *Right scapula.*

The FoundationOne study reported other alterations than the non-V600-*BRAF* mutations, such as *ARID1A*, *DAXX*, and *CDKN2A/B*. *ARID1A* encodes the Baf250a protein, a member of the SWI/SNF chromatin remodeling complex and this gene is considered to be a tumor suppressor [9,10]. *DAXX* encodes a multifunctional protein that participates as a cytoplasmic signaling component in FAS-mediated apoptosis [11,12]. *CDKN2A/B* encodes proteins that bind and inhibit CDK4 and 6, maintaining the tumor suppressor activity of *Rb* [13,14]. The first two mutations had no potentially valuable target therapy and CDKN2A/B was preclinically associated to potential sensitivity to CDK4/6 inhibitors, including abemaciclib, palbociclib, and ribociclib [15–17]. Considering previous immunotherapy use and the fact that no target treatment options were available, as well as the rapidly progressive disease with quality-of-life impact, the patient was started on BRAFi/MEKi.

In 2011, one study reported that tumor samples negative for V600 *BRAF* mutations could harbor other 'non-V600 ' *BRAF* exon 15 mutations in more than 8% of cases [1]. L597 *BRAF* is located in the activation segment of the kinase domain and adjacent to V600 [1], with the L597Q variant displaying high kinase activity [4]. Other than this, no further evidence regarding L597K mutation was found.

Several reports of non-V600 *BRAF* MM cases treated with BRAFi/MEKi have been published, including in vitro reports and a phase I study also reporting PR, with a 60% tumor reduction and sustained response for over two years [1,3,8,22,23]. Bowyer et al. investigated trametinib activity in five patients with K601E or L597Q *BRAF*-positive MM. The authors reported trametinib antitumor activity in both first and second-line settings, with objective response in three of five (60%) patients, including the one with a L597Q *BRAF* mutation [3].

Although BRAF and MEK inhibitors are not approved treatments for non-V600-mutated MM, based on previously reported evidence and absence of other active options, BRAFi/MEKi combination therapy was administered in an off-label setting in the present case.

Melanoma is highly heterogeneous and has the highest mutational rate of all cancers (average of 16.8 mutations per Mb), [24]. This refers to both inter- and intratumor heterogeneity. The first refers to differences between tumors from different patients whereas the latter describes different tumor cell populations within the same tumor [25]. Intertumor heterogeneity is the result of primary neoplasm intratumor heterogeneity owing to secondary lesions arising from different subpopulations within the primary tumor [25]. It can be induced by microenvironmental factors and result from constant tumor evolution within a patient and from treatment sequences [25]. It has been shown that most targets with approved therapy options are not homogeneously present in tumor tissue [26]. This means that the same patient can have two or more molecularly distinct metastatic lesions. For this

Table 1. *Registration trials for BRAFi plus MEKi combinations in melanoma.*

	Vemurafenib + Cobimetinib	Dabrafenib + Trametinib		Encorafenib+ Binimetinib
Indication	Advanced melanoma 1st line setting	Advanced melanoma 1st line setting	Adjuvant melanoma	Advanced melanoma 1st line setting
FDA approval	2015*	2014#	2018§	2018*
Trial	coBRIM [18]	COMBI-d/v [19]	COMBI-AD [20]	COLUMBUS (part 1) [21]
mFollow-up, mo	21.2	22	44	48.8
ORR, n (%)	172 (70)	383 (68)	–	(64)
CR (%)	52 (21)	109 (19)	–	(13)
PR (%)	120 (49)	274 (49)	–	(51)
SD (%)	45 (18)	130 (23)	–	(29)
mPFS, mo (95% CI)	12.6 (9.5–14.8)	11.1 (9.5–12.8)	–	14.9 (11.0–20.2)
mRFS, mo (95% CI)	–	–	NE (46.9–NE)	–
mOS, mo (95% CI)	22.5 (20.3–28.8)	25.9 (22.6–31.5)	–	33.6 (24.4–39.2)
Grade ≥3 AEs, %	78	59	41	68

*–, not applicable/not available; AE, adverse event; CI, confidence interval; CR, complete response; m, median; mo, months; NE, not estimable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; RFS, relapse-free survival; SD, stable disease. FDA approval for: *BRAFV600+,#BRAF V600E, §BRAFV600E/K+.*

reason, it was decided to biopsy a large, new thoracic skin metastatic nodule for NGS testing in the present patient, so that the most recent genomic alterations and evolution could be captured. Tumor heterogeneity is a major hurdle for clinical efficacy of anticancer therapies and it is associated with poor prognosis and outcome [26,27]. Targeting one pathway may select another clone harboring different mutations, eliciting tumor progression and drug resistance [25]. Furthermore, heterogeneity contributes to asymmetric treatment response within a patient [25]. In this study, the observed early response followed by progressive disease is similar to what is described in V600 *BRAF* mutated patients. Target blocking and initial tumor regression allow non-*BRAF*-mutant clones to emerge, leading to resistance and disease progression [26].

By the time BRAFi/MEKi therapy was started, the patient had an ECOG performance status of 2/3, uncontrolled pain (even with opioid therapy escalation), and was in severe depression. He had received two lines of therapy, with disease progression to both. There was no evidence for immunotherapy rechallenge and it was believed that the patient would have died shortly after February 2018 had he not started the BRAFi/MEKi therapy. Instead, BRAFi/MEKi treatment conferred one year of clinical benefit with improved quality of life (similarly to the median progression-free survival reported in registration clinical trials with targeted therapies in this setting).

Conclusion

Cases of patients with non-V600-BRAF mutations responding to BRAFi/MEKi therapy have been reported in the last years. To the best of our knowledge, this is the first case reporting response to a BRAFi/MEKi combination treatment in a patient with MM harboring the very rare L597K mutation.

Potential conflicts of interest

The authors declare no conflicts of interests.

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