Long-Term Response to Intermittent Binimetinib in Patients with *NRAS*-Mutant Melanoma

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Disclosures of potential conflicts of interest may be found at the end of this article.

ABSTRACT

Melanoma can be classified based on the detection of relevant oncogenic driver mutations. These mutations partially determine a patient's treatment options. MEK inhibitors have demonstrated little efficacy in patients with *NRAS*-mutated melanoma owing to primary and secondary resistance. We report two patients with *NRAS*-mutant metastatic melanoma with long-term response to intermittent MEK-inhibitor binimetinib therapy. Intermittent dosing schedules could play a key role in preventing resistance to targeted therapy. This article highlights the efficacy of an intermittent dosing schedule, toxicities associated with binimetinib, and possible mechanisms preventing resistance in targeted therapy. Intermittent MEK-inhibitor therapy may be considered in patients with *NRAS*-mutated melanoma that have failed all standard therapies. *The Oncologist* 2020;25:e1593–e1597

KEY POINTS

- Melanomas harbor *NRAS* mutations in 10%–30% of the cases. These mutations promote hyperactivation of the MAPK pathway, leading to proliferation and prolonged survival of tumor cells.
- Currently, drugs directly targeting *NRAS* are not available. Downstream inhibition of the MAPK pathway can be considered as a therapeutic option after immunotherapeutic failure.
- Intermittent administration of kinase inhibitors might be the way to partially overcome the development of drug resistance by (a) inducing a fitness deficit for drug-resistant cells on treatment break, (b) increasing the immunogenicity, and (c) inducing apoptosis and cell cycle arrest. It also enhances expression of numerous immunomodulating molecules, and reduction of immunosuppressive factors, which suggests better access of the immune system to the tumor.

PATIENT STORIES

Patient 1

A 71-year-old female presented with multiple subcutaneous in-transit metastases on the shin after resection of a melanoma on her right lower leg (Breslow 1.1 mm). Her sentinel lymph nodes were negative. Several surgical procedures were performed because of local relapses. The mutational analysis of an in-transit metastasis by polymerase chain reaction (PCR) and sequencing analysis detected a *NRAS* mutation (p.Q61R in Exon 3). Despite treatment with anti–cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) antibodies (ipilimumab 200 mg intravenously, four infusions), the disease progressed (locoregional lymph node involvement and in-transit soft tissue metastasis up to 4.5 cm in size). As anti–programmed cell death protein 1 (PD-1) blockade was not available at the time, the patient was enrolled in a clinical trial investigating binimetinib (45-mg tablets p.o. b.i.d.; NCT01763164) in March 2015. Binimetinib was stopped after 6 weeks of treatment owing to myalgia and severe creatine phosphokinase (CPK) elevation grade (G) 3. The patient also developed acneiform dermatitis G2, bilateral retinopathy G1, and eye pressure elevation G2. All adverse events (AEs) resolved when binimetinib was
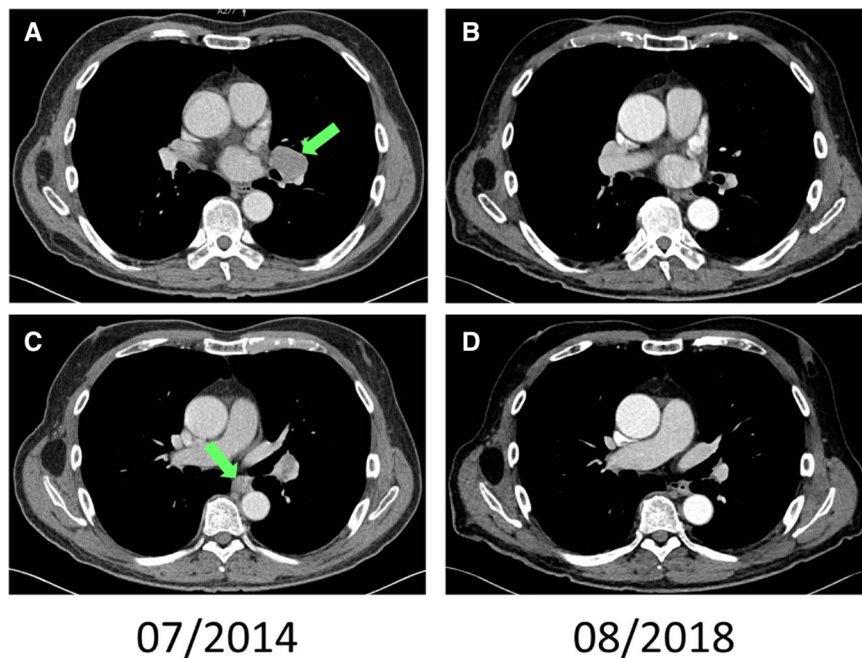
Figure 1. Representative transverse chest computed tomography sections of Patient 2. Left hilar metastasis measuring 3.4 × 2.6 cm at baseline **(A)** showing complete remission under intermittent therapy with binimetinib **(B)**. Mediastinal metastasis of 1.5 × 1 cm adjacent to the esophagus **(C)** was not detectable at the end of therapy in August 2018 **(D)**.

withheld. On resumption of binimetinib, the same AEs recurred after 3–6 weeks. Consequently, a dose reduction (30 mg b.i.d.) and intermittent treatment (3 weeks on and 10 days off) schedule was trialed. On this regimen, CPK elevation fluctuated between G1 and G3. The acneiform dermatitis peaked to G2 at the end of each treatment phase but settled between dosing. Both retinopathy and elevated eye pressure were never symptomatic but present on ophthalmic review. A combination of a beta-blocker and a carbonic anhydrase inhibitor (cosopt eye drops) led to improvement of the eye pressure. The patient did not experience myalgia.

Patient 2

A 75-year-old male presented with a melanoma (Breslow 0.95 mm) on the trunk. He developed right axillary lymph node metastasis developed right lymph node metastasis axillary in December 2013. Mutational analysis by PCR and sequencing revealed a *NRAS* mutation (p.Q61K in Exon 3). Within 6 months, the patient developed hilar lymph node metastasis of 3.4 cm, lung and soft tissue metastases (stage IV disease) (Figure 1). In July 2014, binimetinib (45-mg tablets b.i.d.) was started (NCT01763164). Immunotherapy was kept as salvage therapy. Four weeks into therapy, treatment had to be suspended because of CPK elevation G4. The patient experienced facial acneiform dermatitis G2 and retinopathy G1, but no myalgias. A 2-weeks-on and 1-week-off schedule with reduced dose (30 mg b.i.d.) was commenced. With this regimen, CPK elevation was between G1 and G3, acneiform dermatitis between G1 and G2, and the retinopathy between G0 and G1. Within the first year of treatment, the tumor burden continuously decreased with overall partial response. By April 2017, all lesions had disappeared or shrunk to a maximum of 5 mm and remained stable. In

August 2018, binimetinib was ceased owing to right central retinal vein thrombosis. The visual disturbance improved and eventually returned to normal with two intravitreal anti–vascular endothelial growth factor injections and eye pressure–reducing drops (dorzolamide and timolol).

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Melanoma and Tumor Biology

Development of melanoma can be triggered by activating oncogenes or inactivating tumor-suppressor genes through specific mutations. Some of the most important signaling pathways involved in the pathogenesis of melanoma include MAPK, PI3K/PTEN/AKT, and MITF [1].

Cutaneous melanomas can be divided into four genomic subtypes: BRAF, RAS, NF1, and triple-WT melanomas [2].

In The Cancer Genome Atlas (TCGA), *NRAS* somatic mutations were present in 28% of the analyzed samples [2]. *NRAS* and *BRAF* mutations are thought to be often mutually exclusive. However, the presence of both mutations in one cell line was proved [3]. Either of them is sufficient to promote hyperactivation of the MAPK pathway, leading to proliferation and prolonged survival of tumor cells.

Genotyping Results and Interpretation of the Molecular Results

In the presented cases, *NRAS* mutations (p.Q61K in Exon 3 and p.Q61R in Exon 3) were detected by both PCR and sequencing. BRAF mutation was not present.

The most common *NRAS* mutation in melanoma (occurring in more than 80% of *NRAS*-mutated samples) is a substitution of glutamine with arginine or lysine at position p.61 (NRASQ61R/K/L) as a result of the c.181C > A

transversion (38%) of the c.182A > G transition (34%) or of the c.182A > T transversion (10%) in exon 3 of the gene, respectively [4]. Codon 61 mutations are associated with locking of the RAS protein into its activating conformation and impaired GTPase activity [5], leading to activated RAS signaling (MAPK pathway) with consecutive cell growth, motility, and survival, thereby enhancing tumor growth [6]. *NRAS* mutation not only activates the MAPK pathway, but can also trigger activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway and other survival signaling pathways [7].

Functional and Clinical Significance of *NRAS* Mutation in Melanoma

NRAS mutation is considered the second most common oncogenic driver mutation in melanoma. Compared with *BRAF*-mutated or WT *NRAS* melanoma, there are conflicting data regarding the importance of *NRAS* mutations in outcomes to new therapies, particularly checkpoint inhibitors.

Potential Strategies to Target the Pathway and Implications for Clinical Practice

Currently, there are no drugs that target *NRAS*. Downstream inhibition of the MAPK pathway can be considered as a therapeutic option after immunotherapeutic failure.

Binimetinib, a selective inhibitor of mitogen-activated protein kinase (MEK1 and 2), demonstrated some benefit in patients with *NRAS*-mutated melanoma (median progression-free survival [PFS] 3.7 months, 95% confidence interval [CI] 2.5–5.4) [8]. In the pivotal trial comparing dacarbazine and binimetinib, median PFS for patients with MEK inhibitor–treated *NRAS*-mutant melanoma was 2.8 months (95% CI 2.8–3.6) versus 1.5 months (95% CI 1.5–1.7) in the dacarbazine group [9]. Unfortunately, most patients develop resistance within the first year [10].

Common AEs of MEK inhibitors include diarrhea (G1–2 39%, G3–4 1%), acneiform dermatitis (G1–2 33%, G3–4 3%), increased CPK (G1–2 23%, G3–4 19%), and various ocular events such as retinal pigment epithelial detachment (retinopathy; G1–2 32%, G3–4 1%) and retinal vein occlusion (G1–4 2%) [9].

In view of the modest outcomes and the AEs, MEK inhibitors are not routinely used for *NRAS*-mutant melanomas.

Despite low clinical efficacy, we report two cases of long-term response to binimetinib treated with an intermittent dosing schedule. Intermittent dosing was well tolerated and toxicity was manageable. Yaeger et al. also reported long-term response to intermittent MEK-inhibitor treatment in a patient with melanoma with RAF1 mutation [11].

Intermittent MEK Inhibition as a Possible Mechanism Preventing Resistance

Most tyrosine kinase–resistant tumor cells remain dependent on MAPK pathway signaling and rely on ERK (extracellular signal-regulated kinase) reactivation [12, 13]. Although reliant on oncogenes (MAPK pathway and ERK signaling), resistant tumor cells experience a fitness deficit in the absence of the drug. They can induce apoptosis and cell cycle arrest [13]. This is observed with both inhibition and hyperactivation of the MAPK pathway leading to an

excessive MEK–ERK signaling [14]. It seems that melanoma cells require a specific level of activated ERK for optimal tumor growth. Drug-resistant tumor cells have a selective disadvantage in the absence of the drug, leading to regrowth of drug-sensitive tumor cells during drug holiday [15]. Moreover, MITF (microphthalmia-associated transcription factor), an important regulator of melanoma cell proliferation and survival, is strongly linked to the MAPK pathway. In MEK inhibitor–resistant melanoma cells, MITF expression can be highly upregulated, whereas strong activation of MAPK signaling will reduce MITF protein levels through degradation [16].

We propose that the dosing interval during intermittent therapy targets drug-sensitive tumor cells and the off interval drives drug-resistant tumor cells into cell cycle arrest and apoptosis.

Different data support the strategy of intermittent dosing for MEK-inhibitor therapy. First, MEK inhibitors lead to an initial increase of HLA-1 and HLA-2, enhanced expression of numerous immunomodulating molecules, and reduction of immunosuppressive factors such as IL1A, IL8, programmed death-ligand 1 (PD-L1), and others [17]. Higher immunogenicity at the beginning of treatment would suggest more efficient antitumor response. Second, after a longer exposure to MEK inhibitor, melanoma cells can switch phenotype into more invasive cell behavior. Zipser et al. demonstrated a phenotype switch in MITF-expressing tumor cells with an activated MAPK pathway after 2 weeks of treatment. This was characterized by a change in morphology, increased invasiveness, and a decline in expression of melanocytic differentiation antigens. [18] Third, Deken et al. reported a high influx of T cells within the first week and lesser presence of T cells later on in a mouse model treated with kinase inhibitors [19]. An enrichment of tumor-infiltrating, antigen-specific CD8^{+} effector T cells in the MEK inhibitor–treated mice has also been demonstrated [20]. Consequently, this implies a more effective access of the immune system to the tumor at the beginning of the treatment.

These alterations give reasonable justification to assume that immunogenicity could be elevated at the beginning of MAPK pathway inhibition and reduced after long-term exposure. Intermittent administration of kinase inhibitors may overcome this problem [21]. Consistent with our hypothesis, Choi et al. recently demonstrated that pulsatile, rather than continuous, treatment with MEK inhibitors in murine models can maintain T-cell activity better and prolong survival in KRAS mutant cancer. This effect is further enhanced when combined with immunotherapy [22].

Continuous MEK-inhibitor administration combined with anti–PD-L1 inhibitor atezolizumab failed in a recent clinical study in humans [23]. Based on our experience, this study protocol should be repeated with an intermittent MEK-inhibitor dosing.

Another strategy to optimize tumor apoptosis is a combination of MEK inhibitors and CDK4/6 inhibitors. Teh et al. demonstrated in an in vivo study a more effective tumor inhibition with less toxicity on an intermittent dosing

Patient Update

Patient 1

The patient's tumor burden steadily decreased in the first 6 months of treatment and remained stable for more than 4 years. Side effects were well tolerated with the intermittent dosing schedule. In April 2019, two progressive nodules were detected on the right shin leading to end of treatment because of progressive disease. A next-generation sequencing–based test (FoundationOne CDx, Foundation Medicine Inc. Cambridge, MA, USA) of the excised metastasis confirmed *NRAS* Q61R mutation as well as CDKN2A/B loss, EED R441 alteration, MTAP loss, and TERT promoter alteration 1146 C > T. The tumor mutational burden was intermediate with 11 mutations per megabase. *NRAS* mutations with CDKN2A/B loss and TERT promoter alterations are likely present at baseline based on their high prevalence in primary samples [2]. Whereas the role of MTPA loss in melanoma is not clearly characterized, the EED R441 alteration might favor resistance by EZH2-mediated epigenetic changes [25]. But as no serial biopsies were performed, we can only speculate on the resistance mechanisms.

Immunotherapy with anti–PD1-inhibitor nivolumab (six infusions of 240 mg intravenously every 2 weeks) was started in June 2019. Despite this, the patient's condition progressed. Combined intermittent binimetinib (Mektovi Pfizer, New York) with nivolumab initiated in September 2019 finally resulted in complete metabolic response by June 2020.

Patient 2

No further therapy was required as regular imaging by positron emission tomography–computed tomography demonstrated complete metabolic response. This prolonged response is unusual in the landscape of targeted therapy. Supportive factors might be the small tumor burden and normal LDH at therapy start, as well as favorable organ involvement (lymph node, lung) [9].

Conclusion

NRAS-mutated melanomas have fewer treatment options compared with *BRAF*-mutated melanomas. We report two patients with *NRAS*-mutant melanoma with long-term response on an intermittent MEK-inhibitor treatment with reduced dosing. Both patients did not develop resistance for more than 3 years, and the regimen was well tolerated with manageable side effects. Intermittent therapy may be key to achieving better responses, reducing side effects, and delaying drug resistance. Larger-cohort studies are required to investigate these findings.

Author Contributions

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Glossary of Genomic Terms and Nomenclature

AKT: protein kinase B
BRAF: v-raf murine sarcoma viral oncogene homolog B
CDKN2A/B: cyclin-dependent kinase inhibitor 2A/B
EED: embryonic ectoderm development
ERK: extracellular signal-regulated kinase
HLA: human leukocyte antigens, also called major histocompatibility complex (MHC)
KRAS: Kirsten rat sarcoma viral oncogene homolog
MAPK: mitogen-activated protein kinases
MEK: mitogen-activated protein kinase kinase
MITF: microphthalmia transcription factor
MTAP: methylthioadenosine phosphorylase
NRAS: neuroblastoma RAS viral oncogene homolog
NF1: neurofibromin 1
RAS: rat sarcoma
PI3K: phosphatidylinositol 3-kinase/ protein kinase B
PTEN: phosphatase and tensin homologue
TCGA: The Cancer Genome Atlas
TERT: telomerase reverse transcriptase
WT: wildtype

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