BRAF Inhibitors in Melanoma Management: When Friends Become Foes

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The BRAF inhibitor (BRAFi) vemurafenib improves survival of patients with melanoma with \(BRAF^{V600E}\) mutations. However, effects of sustained BRAFis on BRAFi-resistant melanomas with dual mutations in \(BRAF\) and \(NRAS\) are not well characterized. Jandova and Wondrak (2021) report that vemurafenib selectively enhances expression of genes involved in the epithelial-to-mesenchymal transition in \(BRAF^{V600E}/NRAS^{Q61K}\) melanoma cells, paradoxically promoting tumor growth and metastasis in mice. This preclinical study provides compelling reasons to be cautious in the use of BRAFis in patients with NRAS-driven melanoma.


Melanoma is one of the most aggressive cutaneous malignancies. It is typically driven by somatic mutations in oncogenes, including \(BRAF\) and/or \(NRAS\), leading to uncontrolled proliferation caused by the activation of the MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK pathway (Adam et al., 2020). Although more than 50% of advanced (unresectable or metastatic) melanomas harbor driver mutations in the \(BRAF\) gene (\(BRAF^{V600E}\)) as the most common mutation, \(NRAS\) mutations (including \(NRAS^{Q61K}\)) are the second most common oncogenic alterations in melanoma (Echevarría-Vargas and Villanueva, 2017). Significant therapeutic progress has been achieved in the past few years targeting BRAF-driven melanomas. BRAF inhibitors (BRAFis), such as vemurafenib and dabrafenib, have been approved for the treatment of BRAF-mutation-bearing melanomas, after demonstration of significant improvement over cytotoxic chemotherapy. The approved combination of the BRAFi dabrafenib with the MEK inhibitor (MEKI) trametinib demonstrated improved progression-free survival as compared with monotherapy (Robert et al., 2015). Despite advances in targeting BRAF-driven melanomas, fewer treatment options exist for NRAS-driven melanomas, which are aggressive tumors with shorter patient survivals as compared with non-NRAS-mutant melanomas (Yin et al., 2019). Unfortunately, patients with NRAS mutations lack targetable BRAF mutations and feature a high degree of intrinsic and acquired resistance to MEK inhibition.

In previous studies, NRAS mutations \(Q61R, Q61K\) have been shown to cause resistance to BRAFis in melanomas with \(BRAF^{V600E}\) mutations. This represents the unique co-occurrence of \(BRAF\) and \(NRAS\) mutations that is facilitated and selected by BRAFi therapy (Johnson et al., 2015). Extensive research has been performed to determine the molecular mechanisms underlying acquired NRAS-driven BRAFi resistance. The most common mechanism is believed to be reactivation of the MAPK pathway that is stimulated by NRAS. However, there is a clear knowledge gap regarding the effects of BRAFi treatment on BRAFi-resistant tumors displaying dual BRAF and NRAS mutations. Valid research questions remain to be addressed: (i) Should BRAFi treatment be continued in patients harboring dual mutations of \(BRAF^{V600E}\) and \(NRAS^{Q61K}\) (ii) What would the BRAFi treatment response be in these patients with melanoma? Therefore, this is a very interesting and clinically relevant study in the context of BRAFi treatment efficacy, treatment failure, and acquired resistance in patients with melanoma.

In their new article in the Journal of Investigative Dermatology, Jandova and Wondrak (2021) analyzed the effects of a Food and Drug Administration-approved BRAFi (vemurafenib) on BRAF-sensitive A375-\(BRAF^{V600E}/NRAS^{Q61}\) and an accepted genetic model of BRAFi resistance, A375-BRAF\(^{V600E}/NRAS^{Q61K}\) melanoma cells, employing in vitro and in vivo approaches. This study provides novel information and suggests that BRAFi treatment selectively targets \(BRAF^{V600E}/NRAS^{Q61K}\) melanoma cells upregulating epithelial-to-mesenchymal transition (EMT) gene expression and paradoxically promoting invasiveness and metastasis. In this study, the authors conducted in vitro experiments using BRAFi-sensitive A375-BRAF\(^{V600E}/NRAS^{Q61}\) versus BRAF-resistant A375-BRAF\(^{V600E}/NRAS^{Q61K}\) melanoma cells followed by an in vivo validation in a severe combined immunodeficiency (SCID) mouse bioluminescent melanoma metastasis model. They determined that BRAFi treatment enhanced lung tumor burdens caused by mutant A375-BRAF\(^{V600E}/NRAS^{Q61K}\) cells while inhibiting the metastatic potential of A375-BRAF\(^{V600E}/NRAS^{Q61}\). Herein, we discuss the implications of these findings in the context of current research regarding the NRAS-driven BRAFi resistance in melanoma.

EMT and EMT to metastasis pathways in response to NRAS-driven BRAFi resistance in melanoma

EMT is a process by which malignant melanocytic cells lose their epithelial...
characteristics and acquire a mesenchymal phenotype leading to an increase in migration, invasiveness, and metastatic potential (Pearlman et al., 2017). The results obtained by Jandova and Wondrak (2021) regarding the increased expression of genes associated with EMT and EMT to metastasis pathways in BRAFi-resistant NRASQ61K melanoma cells are consistent with prior research showing increased metastatic potential of NRAS-driven melanomas (Eskandarpour et al., 2009). An important aspect of this study is that the authors performed a comprehensive high throughput NanoString (NanoString Technologies, Seattle, WA)-based transcriptomic profiling and identified the differential upregulation of EMT- and metastasis-related gene expression networks. They identified modulation of 10 genes encoding EMT- and metastasis-related transcription factors (TWIST1, extracellular matrix components (CLDN4, COL6A2, FN1, ITGA1, ITGA7, LAMA5), and components of inflammatory and GF signaling cascades (AREG, CXCL8, PDGFRB).

The authors further validated the differential expression of EMT-related genes as a function of NRASQ61K genotype and BRAFi treatment employing a more specific EMT-focused RT2-Profiler PCR array. They observed that BRAFi paradoxically enhanced EMT-related gene expression and transcription factors ZEB1 and ZEB2 in double mutant BRAFV600E/NRASQ61K melanoma cells in comparison to the BRAFV600E/NRASQ61 melanoma cells that harbored only BRAF mutations. A review of this literature shows that EMT has a well-established role in melanoma progression and that EMT-related proteins are considered to be valid therapeutic targets for melanoma metastasis (reviewed in [Pedri et al., 2021]). Thus, the findings demonstrating that BRAFi treatment selectively targets BRAFV600E/NRASQ61K melanoma cells by promoting EMT-related gene expression (Figure 1) are relevant toward the development of future treatment strategies to overcome drug resistance in patients with melanoma.

Enhanced migratory and invasive potential of BRAFV600E/NRASQ61K melanoma cells in response to BRAFi treatment
As melanoma progresses and penetrates the basal layer of the epidermis, tumor cells gain access to blood and lymph vessels, leading to an increase in

![Figure 1. Opposing effects of the BRAFi vemurafinib on melanoma cells harboring BRAFV600E and BRAFV600E/NRASQ61K. Vemurafinib treatment selectively targets BRAFV600E/NRASQ61K melanoma cells upregulating expression of EMT genes, paradoxically promoting invasiveness and metastasis. BRAFi, BRAF inhibitor; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal–regulated kinase; MEK, MAPK/extracellular signal–regulated kinase kinase; p-Akt, phosphorylated protein kinase B; TF, transcription factor.](image)
metastatic potential. This is often accompanied by phenotypic changes in melanoma cells with enhanced cell motility, migration, and invasiveness. Together, migration and invasion potential of tumor cells are important parameters to assess metastatic potential. Using in vitro experiments, Jandova and Wondrak (2021) first determined the effect of vemurafenib on cellular viability, proliferation, and colony formation, followed by migration and invasion potential, employing isogenic melanoma cell lines harboring BRAF mutations and BRAF/NRAS double mutations. The authors demonstrated that vemurafenib treatment affected cellular proliferation in opposing ways as a function of genotype. However, the viability of melanoma cells remained unaffected by BRAFi irrespective of genotype. The cellular proliferation and colony formation data showed that BRAFi treatment inhibited BRAFi-sensitive A375-BRAF^{V600E/NRAS^{Q61K}} melanoma cells. A375 melanoma cells with BRAF^{V600E/NRAS^{Q61K}} displayed the opposite phenotype, showing that BRAFi treatment caused an increase in proliferation, considered a genotype-specific effect. Most importantly, Jandova and Wondrak (2021) observed a significant increase in migration and invasiveness of A375-BRAF^{V600E/NRAS^{Q61K}} melanoma cells after BRAFi treatment. As a possible mechanism for these genotype-specific effects of BRAFi treatment, the authors observed loss of BRAFi-induced blockade of phosphorylated ERK1/2 and an increase in protein kinase B phosphorylation (Ser473) in BRAF^{V600E/NRAS^{Q61K}} melanoma cells compared with BRAFi-treated BRAF^{V600E/NRAS^{Q61K}} cells. However, a thorough evaluation and cause-and-effect validation of these mechanisms will require additional investigation.

**Increased tumor growth and lung metastasis with BRAFi treatment in BRAF^{V600E/NRAS^{Q61K}} melanomas**

Another important aspect of this study is that the authors used an animal model to validate their in vitro findings. They determined the effects of BRAFi treatment in a bioluminescent SCID melanoma xenograft mouse model and compared tumor growth and metastatic potential of BRAF^{V600E/NRAS^{Q61K}} and BRAF^{V600E/NRAS^{Q61K}} melanoma cells. Corresponding to the in vitro phenotypic changes, the authors found significant enhancements of tumor growth and metastasis as a response to BRAFi treatment of BRAF^{V600E/NRAS^{Q61K}} recipient mice. Immunohistochemical (IHC) analysis of tumor tissues demonstrated enhanced proliferation marker proliferating cell nuclear antigen—positive cells only in BRAF^{V600E/NRAS^{Q61K}} recipient mice in response to BRAFi treatment when compared with BRAF^{V600E/NRAS^{Q61K}} recipient mice. It is important to note that one contradictory result was observed in IHC staining of tumor tissue sections regarding the expression of matrix metalloproteinase 9 (MMP9), an indicator of invasiveness in melanoma. In vitro data using RT-qPCR and immunoblot analysis on BRAFi-induced MMP9 upregulation in A375-BRAF^{V600E/NRAS^{Q61K}} cells contrasted with the in vivo situation showing no increase in MMP9 expression in IHC staining of tumors from A375-BRAF^{V600E/NRAS^{Q61K}} recipient mice after BRAFi treatment. In a recent study, MMP9 has been shown as a candidate marker of responses to BRAFis in patients with melanoma harboring BRAF^{V600E} mutation (Salemi et al., 2018). However, studies correlating MMP9 expression with NRAS mutations in melanoma are lacking.

**Innovation and Limitations**

This study is innovative in several respects. For the first time, the effects of BRAFi treatment have been determined using a stringent genetic model with dual BRAF/NRAS-mutated BRAFi-resistant melanomas, employing a genetic model of NRAS^{Q61K}—driven BRAFi resistance in isogenic BRAF^{V600E}—driven melanoma cell lines. Further, NanoString nCounter (NanoString Technologies) profiling of isogenic malignant melanoma cells was used to determine gene expression changes in BRAF^{V600E}/NRAS^{Q61K} cells relative to BRAF^{V600E}/NRAS^{Q61K} cells. The genes involved in melanoma EMT have been observed to be responsive to BRAFi treatment as a function of NRAS genotype. Moreover, to our knowledge, this study is the first to show invasiveness and lung metastasis imposed by BRAF^{V600E}/NRAS^{Q61K} cells (while inactivating the BRAF^{V600E}/NRAS^{Q61K} genotype), a preclinical finding that has the potential to inform clinical decisions in the future.

As in any research study, there are limitations that have been acknowledged by the authors. First, this study is focused on only one NRAS mutation (NRAS^{Q61K}) in combination with BRAF^{V600E}. Further detailed investigations are required to show if these results are representative of other NRAS mutations that also confer BRAFi resistance in malignant melanoma. Similarly, the findings are based on one specific BRAFi (vemurafenib), and the effects observed in this study should be validated with other clinically relevant BRAFi therapeutics. In addition, the findings in this study should be expanded to BRAFi and MEKi combination therapies, because this is the current standard of care for melanoma in the first-line setting. Moreover, in this study, in vivo findings are restricted to one melanoma xenograft model. Future clinical validations of the findings of this study are needed using patient-derived xenograft models and clinical BRAFi resistance patient tumors.

**Conclusion**

Successes of currently used targeted therapies for melanoma are limited owing to the occurrence of drug resistance and paradoxical oncogenesis. Therefore, understanding the mechanisms of resistance and effects of currently approved drugs on resistant tumors fills a knowledge gap and promotes development of new strategies to overcome drug resistance during melanoma management. The paper by Jandova and Wondrak (2021) provides substantial evidence regarding the opposing effects of the BRAFi vemurafenib on BRAFi-sensitive (BRAF^{V600E}/NRAS^{Q61K}) and BRAFi-resistant (BRAF^{V600E}/NRAS^{Q61K}) isogenic melanoma cells in vitro as well as in vivo. Although this is a preclinical study, the findings could help identify novel avenues of melanoma management for patients with melanoma with NRAS-driven BRAFi-resistant tumors who are receiving BRAFi treatment. In light of the observation by Jandova and Wondrak (2021) that the BRAFi vemurafenib increases proliferation, tumor growth, and metastasis of BRAFi-resistant (BRAF^{V600E/NRAS^{Q61K}}) melanoma cells (Figure 1), it is important...
that clinical decisions in the treatment of BRAF/NRAS double mutated patients with melanoma with vemurafenib, which could lead to enhanced invasiveness and metastasis, should be made deliberately. Because current research is increasingly moving toward personalized medicine that is based on specific markers and mutations in individual patients for effective cancer management, the work published by Jandova and Wondrak (2021) could be a valuable contribution related to treatment of melanoma, a highly heterogeneous malignancy.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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REFERENCES