Genomic Profiling in Patients With Malignant Peripheral Nerve Sheath Tumors Reveals Multiple Pathways With Targetable Mutations

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Abstract

Background: The aim of this study was to determine the frequency of alterations in BRAF and other RAS/RAF genes, as well as other targetable pathways in malignant peripheral nerve sheath tumors (MPNSTs).

Patients and Methods: Pathology specimens were available for 2 cohorts: (1) patients with MPNST at Swedish Cancer Institute (n=17) from 2004 through 2016, and (2) patients with MPNST evaluated for >300 genomic alterations at Foundation Medicine from 2014 through 2016 (n=186; including 2 Swedish patients with BRAF-mutated MPNST).

Results: Of 201 MPNSTs, 13 (6.5%) demonstrated BRAF alterations. In the Foundation Medicine cohort, 10 of 84 tumors (11.9%) with no NF1 alterations had BRAF mutations (5 were V600E, 5 other), as did 3 of 102 (2.9%) tumors with NF1 alterations (1 V600E, 2 other).

In the Foundation Medicine cohort, 47% of patients had an alteration in at least one other gene in the RAS/RAF pathway (not including NF1 or BRAF); 46% had alterations in the PI3 pathway, with 70% having alterations in at least 1 of the 2 pathways; 57% had a CDKN2A alteration (80% in BRAF-mutated and 71% in NF1-altered patients); and 70% had an alteration in DNA repair genes. MPNST, both NF1 wild-type and NF1-mutated, often harbor alterations in the RAS/RAF pathway as well as changes related to DNA repair and CDKN2A/B. V600E and other mutations occur in BRAF, suggesting the need for second-generation activating BRAF inhibitors. The concurrence of BRAF and/or NF1 alterations with CDKN2A/B mutations, in particular, may be significant in the transformation of neurologic tumors from benign to malignant.

Conclusions: All MPNSTs would benefit from a comprehensive genomic analysis. Treatments targeted to RAS/RAF, DNA repair, and CDKN2A/B pathways should be used and/or developed to treat this uncommon tumor.

J Natl Compr Canc Netw 2018;16(8):967–974
doi: 10.6004/jnccn.2018.7033

Malignant peripheral nerve sheath tumors (MPNSTs) are rare tumors for which no established systemic treatment exists. MPNSTs occur in approximately 10% of patients with neurofibromatosis type 1, with malignancy usually present in the third or fourth decade of life. When MPNSTs occur sporadically in the absence of neurofibromatosis, they tend to present later in life, usually in the sixth or seventh decade, and often without an inactivating alteration in neurofibromin (NF1), which is seen in patients with neurofibromatosis type 1. Wild-type NF1 protein exhibits an inhibitory effect on the RAS/RAF pathway. Such tumors exhibit increased activity in the RAS/RAF pathway by the lack of negative feedback. Alterations in the PI3K/PTEN/AKT pathway can also affect RAS/RAF activity and can be stimulated by similar stimuli as the RAS/RAF pathway.

Previously, we reported on a patient with a sporadic MPNST, whose tumor featured a BRAF V600E mutation. This mutation results in activation of BRAF, leading to increased activity of the RAS/RAF pathway.

This patient had a dramatic response to vemurafenib, an
inhibitor of BRAF V600E. As a result, in the current study we analyzed the presence of BRAF alterations, other RAS/RAF alterations, and other genomic alterations in all MPNSTs studied at Swedish Cancer Institute and Foundation Medicine during the past 12 years.

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Results

A total of 201 patients with MPNSTs were included in this study, 17 of whom were identified at Swedish Cancer Institute; of these patients, 2 had BRAF alterations and were studied more extensively at Foundation Medicine.

Among the 186 MPNSTs profiled at Foundation Medicine (Table 1), 102 showed NF1 alterations (55%) and 84 were NF1 wild-type (45%). Of the patients with NF1 wild-type MPNST, 10 of 84 (12%) exhibited BRAF alterations. Of the 10 BRAF alterations, 9 were known to be pathogenic, including 5 BRAF V600E, 1 R389C, 1 I710fs*24, and 2 rearrangements (see supplemental eAppendix 1 for references, available with this article at JNCCN.org). One (P341I) was of unknown functionality. Among 102 patients with NF1 alterations, 2 exhibited likely pathogenic BRAF mutations, R389C and A762V, and one exhibited a functionally unknown G652E alteration.

Of the Foundation Medicine cohort, 46% of patients had alterations in the PI3 pathway (only pathogenic mutations were counted), 57% had CDKN2A alterations, and 70% had an alteration in DNA repair genes. The cohorts of 102 NF1-mutated patients and 84 NF1 wild-type patients analyzed at Foundation Medicine were compared, with separate breakout of the 10 BRAF-altered cases. Differences in alteration rates were evident (Figures 1 and 2). Of note, CDKN2A was altered at a significantly higher rate in NF1-altered and BRAF-altered tumors versus non-NF1/BRAF (71%, 80%, and 34%, respectively). Rates of TP53 (32% of NF1, and only 14% of

Table 1. Relationship Between BRAF Mutation and NF1 Status (N=186)*

<table>
<thead>
<tr>
<th>NF1 Status</th>
<th>BRAF Alterations</th>
<th>Functional Status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n=84)</td>
<td>V600E, n=4</td>
<td>Pathogenic</td>
</tr>
<tr>
<td></td>
<td>V600E and exon duplication, n=1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P341I, n=1</td>
<td>Unknown significance</td>
</tr>
<tr>
<td></td>
<td>Rearrangement, n=2</td>
<td>Pathogenic</td>
</tr>
<tr>
<td></td>
<td>I710fs*24, n=1</td>
<td>Pathogenic</td>
</tr>
<tr>
<td></td>
<td>R389C, n=1</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td>NF1 alteration, somatic or germline (n=102)</td>
<td>R389C, n=1</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td></td>
<td>A762V, n=1</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td></td>
<td>G652E, n=1</td>
<td>Unknown significance</td>
</tr>
</tbody>
</table>

*includes only patients whose tissue was submitted for comprehensive genomic profiling at Foundation Medicine (2 from the Swedish cohort and 184 others).

*Functional status identified through several databases, including COSMIC, 1000 Genomes Project, dbSNP, and ExAC (to filter out common germline variants). Published literature and conference proceedings were also used to assess functional significance of variants, adjusting default rules as needed to reflect whether a variant is likely pathogenic.
non-NF1 and 13% of BRAF), SUZ12 (20% of NF1-altered, 13% of BRAF-altered, 9% of non–NF1/non–BRAF-altered), and EED (8% of NF1-altered, 13% of BRAF-altered, 3% of non–NF1/non–BRAF-altered) also varied with subtype.

Across the entire Foundation Medicine MPNST cohort, 47% of tumors had an alteration in at least one other gene in the RAS/RAF pathway (ERBB2, ERBB3, ERBB4, KRAS, MET, HRAS, MAP2K1, MAP2K2, NRAS), not including NF1 or BRAF. By group, 52% of NF1-altered, 50% of BRAF-altered, and 38% of non–NF1/BRAF-altered had at least 1 RAS/RAF alteration (Figure 2). Most of the alterations in the RAS/RAF pathway are known alterations, for which targeted therapies have been associated with positive responses in other cancers. Among tumors with alterations in the PI3 pathway (AKT1, AKT2, FBXW7, INPP4B, MTOR, PIK3C2B, PIK3C2G, PIK3C3, PIK3CA, PIK3CB, PIK3CG, PIK3R1, PIK3R2, Pten, Rictor, STK11, Tsc1, or Tsc2), those with NF1 mutations had almost 2.5 times as many alterations in this pathway as those with non–NF1-altered; 70% of patients’ tumors had alterations in at least 1 of the 2 pathways. Alterations in DNA repair genes (ATM, BARD1, BRCA1/2, FANCx, PBRM1, CHEK2, MSH2, MSH3, MSH6, NBN, PBRM1, POLE, RAD51, RAD51C) were found equally in 70% of tumors across subgroups.

Other altered genes identified that might inform targeted therapy included KDR, Kit, PDGFR, and FGFR1 (7% each) and NTRK1 and FGFR2 (5% each) (see supplemental eAppendix 2 for references).

The specific NF1 alterations in the Foundation Medicine cohort were further evaluated for frequency of specific alterations (Table 2). Notably, 2 cases harbored 4 NF1 alterations each; 14 tumors had >1 NF1 alteration.

Case Reports of Patients From the Swedish Cohort

Patient 1 was a 51-year-old woman who presented with an MPNST. Her initial presentation and treat-
ment were previously reported, including a detailed pathologic description and clinical photographs documenting response. The present study provides details of further follow-up. She presented with a large, high right axillary mass. The tumor was intimately associated with the brachial plexus. She was treated with surgical removal and postoperative radiation therapy. Nine months later she developed a recurrence on the contralateral shoulder, which was also removed and radiated. This tumor was found to have a BRAF V600E mutation. No clinical or genomic evidence of neurofibromatosis was seen. Shortly thereafter the patient developed widespread bulky metastatic disease. She had a transient modest response to sorafenib but a dramatic almost complete response to vemurafenib that lasted 4 months. At that time, she developed progressive disease. A second biopsy demonstrated a BRAF kinase domain (BRAF-KD) duplication. Therapy was discontinued for 1 month, followed by re-treatment with vemurafenib, this time with the addition of trametinib. She had a very good partial response, which lasted for 3 months. On disease progression, she was treated with vemurafenib plus everolimus without response. She experienced a 4-month partial response to carboplatin (her tumor was also BRCA2-mutated), but then developed progressive disease and died from her MPNST.

Patient 2 was a 58-year-old woman with a history of neurofibromatosis with multiple subcutaneous tumors. She developed a large tumor in the subcutaneous tissues of the occipital region. An 8-cm MPNST was surgically removed. Multiple nodules were present in the resection, which represented the benign plexiform neurofibroma characteristic of neurofibromatosis. Superimposed were varying degrees of malignant involvement by MPNST spreading within the confines of the perineurium of the plexiform neurofibroma. At its maximal involvement, the sarcoma was composed of a highly cellular population of monomorphous cells, which stained positively for S100. Sox 10 and p16 showed diffuse positive staining. BRAF V600E was detected in the malignant tumor. Analysis at Foundation Medicine revealed another BRAF alteration, which was identified at a very low frequency in a homopolymer repeat region of the gene, and could not be excluded as an artifact. Loss of CDKN2A/B was also detected. Focal areas of necrosis were seen. The Ki-67 labeling index was moderately elevated to 21%, in contrast to nearly 0% in the benign neurofibromatosis regions. Tissue obtained from the neurofibroma at that time did not harbor either mutation. The sarcomatous component did not show extension to the soft tissue margins and was rather confined to the scaffolding of the plexiform neurofibroma. The peripheral nerve margins, however, showed, in addition to benign neurofibroma, rare pleomorphic and Ki-67 positive nuclei, consistent with extension of the sarcoma to the nerve margins. Because of the positive surgical

### Table 2. Frequency of Specific NF1 Alterations in the Foundation Medicine Cohort

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Protein Effect</th>
<th>Known Pathogenic?</th>
<th>Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Deletion</td>
<td>Known</td>
<td>CN</td>
</tr>
<tr>
<td>8</td>
<td>Truncation</td>
<td>Likely</td>
<td>RE</td>
</tr>
<tr>
<td>4</td>
<td>Rearrangement</td>
<td>Unknown</td>
<td>RE</td>
</tr>
<tr>
<td>3</td>
<td>q514fs*43</td>
<td>Known</td>
<td>SV</td>
</tr>
<tr>
<td>3</td>
<td>r1534*</td>
<td>Known</td>
<td>SV</td>
</tr>
<tr>
<td>3</td>
<td>r1276*</td>
<td>Known</td>
<td>SV</td>
</tr>
<tr>
<td>2</td>
<td>q400*</td>
<td>Known</td>
<td>SV</td>
</tr>
<tr>
<td>2</td>
<td>r2258*</td>
<td>Likely</td>
<td>SV</td>
</tr>
<tr>
<td>2</td>
<td>Splice site 7190-1g&gt;a</td>
<td>Likely</td>
<td>SV</td>
</tr>
<tr>
<td>2</td>
<td>y80*</td>
<td>Likely</td>
<td>SV</td>
</tr>
<tr>
<td>2</td>
<td>q519*</td>
<td>Likely</td>
<td>SV</td>
</tr>
<tr>
<td>104</td>
<td>All different sites, spanning length of protein</td>
<td>15 known, 68 likely, 21 unknown</td>
<td>103 SV, splice site, or frame shift 1 amplification</td>
</tr>
</tbody>
</table>

Abbreviations: CN, copy number change; RE, rearrangement; SV, single base variation.
Discussion

MPNSTs are uncommon, occurring in approximately 1 in 10 million patients per year.\(^9,10\) They can sometimes be treated with local therapy, including surgery and radiation. However, these tumors can metastasize and be very aggressive, with an average long-term survival of only 20% to 50%. The SARC006 trial reported that systemic chemotheraphy with drugs used for sarcoma, such as doxorubicin, ifosfamide, and etoposide, produced approximately a 25% objective response rate, but these responses have not been durable.\(^11\) Trials with a variety of targeted agents are underway. Some responses have been reported with imatinib, an inhibitor of cKIT, but no clearly dramatic activity has been reported thus far.\(^10\) Therefore, new systemic treatments are sorely needed.

Approximately 50% of MPNSTs are considered to be derived from neurofibromas and possess an inactivating mutation in NF1, which would likely lead to increased RAS/RAF activity. Because alterations in the PI3K/PTEN/AKT pathway can also affect RAS/RAF activity, alterations in these pathways were also evaluated. In this series, PI3K alterations were significantly more common in NF1-altered versus NF1 wild-type tumors. During review, alterations in the DNA repair pathways were also identified frequently, suggesting that inability to correctly repair DNA damage caused an additive effect in malignancy. CDKN2A/B mutations were also extremely frequent, particularly in BRAF-mutated tumors.

Four other studies have reported activating BRAF V600E mutations in MPNST (Table 3).\(^12–15\) Combining these studies with our own (including the entire Swedish cohort), 20 of 351 patients (5.7%) with MPNST demonstrated BRAF alterations, of which 12 of 351 (3.4%) were the classic V600E mutation. In our study of the patients more fully analyzed at Foundation Medicine, 11.9% of NF1 wild-type MPNSTs harbored BRAF alterations, 5 of which were classic V600E activations (6.0%), 4 were known or likely to be pathogenic, and 1 of unknown pathogenicity. Among NF1-mutated tumors, 2.9% harbored a BRAF mutation, 2 of which were likely pathogenic and 1 was of unknown pathogenicity. This study, in agreement with that of Hirbe et al.,\(^12\) suggests that BRAF alterations may be more common in patients with NF1 wild-type versus NF1-mutated tumors who develop MPNSTs. It is unclear whether methods used in the prior studies listed here would have detected BRAF mutations other than V600E. Our results demonstrate that, although uncommon, BRAF mutations are common enough that they should be searched for in all patients with MPNST.

In the one patient treated with a BRAF V600E inhibitor in this series, a remarkable response was seen. However, it lasted only approximately 4 months. After a month off treatment, she was rechallenged with the same drug plus an MEK inhibitor and briefly responded again. It is possible that the level of BRAF-mutated cells had decreased in her tumor to preresistant levels rendering the tumor again susceptible to vemurafenib, as has been reported and reviewed in mutated melanoma studies in both animals and humans.\(^16–18\) It is also possible that the addition of an MEK inhibitor was able to partially overcome tumor resistance by blocking the RAS/RAF pathway further downstream.\(^19\) The combination of a BRAF V600E blocker and an MEK inhibitor has been shown to be superior to single-agent therapy in melanoma.\(^20,21\) Subsequent treatment of this patient with vemurafenib and everolimus, an inhibitor of the mTOR/AKT pathway, was not effective, despite the demonstration of compensatory amplification of this alternate pathway as another mechanism of resistance to activated BRAF inhibitors.\(^4\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients, N</th>
<th>All BRAF Mutations, n</th>
<th>V600E Mutations, n</th>
<th>V600E Mutations, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schindler et al (^13)</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Botillo et al (^15)</td>
<td>47</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Je et al (^14)</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hirbe et al (^12)</td>
<td>61</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Kaplan</td>
<td>201</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>351</td>
<td>20</td>
<td>12</td>
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</tr>
</tbody>
</table>

* BRAF mutations other than V600E from the current series are noted in Table 1.
Patient 1 was also found to harbor a BRAF-KD duplication in addition to the V600E mutation when she was rebiopsied at the time of tumor progression. This mutation was sought in her original biopsy and was not present at that time. We have recently reported the occurrence of this mutation in a variety of tumor types, including patient 1. It is possible that this mutation became as, or more, important to tumor survival than the V600E activation, and that this second mutation was partially overcome by the addition of an MEK inhibitor. Regorafenib has produced a dramatic response in one patient with this BRAF-KD mutation in a salivary acinic cell malignancy. The patient whose tumor harbored the fusion alteration treated with regorafenib in that study did not have the V600E mutation, suggesting that regorafenib may well be active against the kinase duplication mutation. Hutchinson et al also reported the development of 2 different BRAF fusion mutations in patients with melanoma. In that study, therapy with an MEK inhibitor in vitro was active in inhibiting tumor growth. Further, Kulkarni et al described BRAF V600E–mutated melanoma in a patient who developed an AGAP3-BRAF fusion mutation that was resistant to vemurafenib but responded to vemurafenib plus an MEK inhibitor. After treatment was discontinued, the fusion mutation was no longer detectable, suggesting that such mutations may be clonally treatment-selected by exposure to BRAF inhibitors alone.

In our study, we identified a number of BRAF mutations in addition to BRAF V600E and the fusion mutation described earlier, many of which have been shown to be pathogenic (supplemental eAppendix 1). Chakraborty et al showed in Langerhans cell histiocytosis that an additional BRAF fusion mutation and other small in-frame BRAF deletions can lead to BRAF activation. These tumors are resistant to traditional BRAF V600E inhibitors but sensitive to MEK inhibitors, as well as the second-generation BRAF inhibitor PLX8394. Similarly, Diamond et al recently reported in histiocytosis that first-generation BRAF inhibitors are clinically effective in BRAF V600–mutant disease, whereas MEK inhibition was very successful against such patients harboring a variety of BRAF mutations, as well as altered MAP2K1, MAP2K2, KRAS, CRAF, and ARAF. Yao et al have studied the mechanism of BRAF activation in various BRAF mutants. Activating BRAF V600 mutants are activated monomers when RAS activity is low and are inhibited by first-generation BRAF V600 blockers, whereas other activating BRAF mutants noted in their study were constitutive RAS-independent dimers. First-generation BRAF blockers are effective against mutant monomers but not dimers. Their binding to one site in the dimer significantly reduces their affinity for the second, rendering those mutants resistant. Alternatively, the second-generation BRAF inhibitor PLX8394 binds to both sites of mutant BRAF dimers and is active against both monomer and dimer BRAF mutants. Although none of the non–V600 BRAF mutations reported in the current study were tested by Yao et al, it will be important to explore use of this drug in such patients. A clinical trial in patients exhibiting these types of mutations is currently underway (ClinicalTrials.gov identifier: NCT02428712).

Use of MEK inhibitors is particularly interesting in patients with loss of neurofibromin activity, which results in unrestrained activity of the RAS/RAF pathway. Multiple studies have suggested loss of neurofibromin activity as a driver of growth in melanomas that can be blunted by MEK inhibitors. The clinical utility of MEK inhibition was confirmed in the study by Dombi et al, in which 71% of children with neurofibromatosis type 1 had clinically significant durable shrinkage of benign inoperable plexiform neurofibromas with selumetinib, a known MEK inhibitor. Whether inhibition of MEK will yield clinically significant responses in patients with somatic NF1 mutations in MPNSTs remains to be explored. A number of compounds are in clinical development in an attempt to block this pathway further downstream at the level of ERK. Morris et al reported activity of one such compound even in tumors with BRAF V600E and MEK1/2L overexpression and acquired resistance to both BRAF and MEK inhibitors, whereas Sullivan et al reported clinical activity of BVD-523 in MAPK-mutant tumors, including in patients with BRAF V600E and other BRAF-activating mutations.

The high percentage of patients who harbored at least one alteration in the RAS/RAF pathway suggests that therapy options might exist for a large subgroup of patients with MPNST, including BRAF-altered tumors for which we have shown response. As previously discussed, a variety of resistance mechanisms have been demonstrated either in animals or...
Genomic Profiling of MPNST

in vitro, suggesting that activation of other metabolic pathways can bypass the RAS/RAF pathway.\(^{4,5}\) Combining blockade of multiple interacting pathways such as RAS/RAF and PI3K/PTEN/AKT may also prove useful in the clinical setting. The other studies noted in Table 3 performed varying degrees of extensiveness of genomic testing. In particular, we have found additional BRAF alterations other than V600E that are pathogenic. It will be important for future studies to perform genomic evaluation of all the relevant pathways discussed.

These considerations may prove useful in multiple types of tumors. As noted, activating BRAF mutations and BRAF fusions and alterations in other pathways that impact RAS/RAF activity have been reported in many malignancies.\(^{8,34}\) In addition, multiple kinases and transcription factors are known to stimulate this pathway both under normal and malignant conditions. Some of these, such as EGFR, have already been shown to be important in multiple tumor types, and inhibitors of these are clinically readily available. Such inhibitors could be evaluated both alone as well as in combination with the compounds discussed.\(^{35}\)

Analysis of other pathways and driver genes identified alterations in DNA repair pathways, CDKN2A/B, the PI3 kinase pathway,\(^{46}\) KIT, KDR, PDGFR, FGFR1/2/3, and NTRK1. The striking occurrence of CDKN2A/B alterations in patients with BRAF and NF1 alterations may be of particular clinical significance. Studies have suggested that these 2 mutations taken together may be important in progression of pediatric low-grade glioma to secondary high-grade glioma,\(^{37–39}\) another neurologic malignancy developing from a less aggressive lesion, whereas Schiffman et al\(^{40}\) noted similar findings in pediatric malignant astrocytoma. Of note, patient 2 with BRAF-mutated MPNST also demonstrated loss of CDKN2A/B. She had a nearby benign neurofibroma that harbored neither mutation. The incidence of BRAF-activating mutations in benign neurofibromatosis tumors is not known. However, it is known that BRAF mutations are often seen in benign melanocytic nevi,\(^{41}\) wherein CDKN2A alteration has been shown to be a late step in the development of malignancy.\(^{42}\) Thus, it is likely that other events besides BRAF activation or NF1 deactivation, such as CDKN2A/B mutation, may be needed to confer malignant behavior. Indeed, Sohier et al\(^{43}\) hypothesized this very evolution in MPNST.

SUZ12 and EED mutations have been previously reported in MPNSTs. These are components of the polycomb repressive complex 2 (PRC2), and loss-of-function mutations have been reported in high frequencies in these tumors, particularly in association with NF1 and CDKN2A alterations.\(^{44}\) Further, loss-of-function PRC2 mutations have been associated with amplification of RAS-driven transcription and vulnerability to MEK and BRD4 inhibitors.\(^{45}\) Our finding of an association between CDKN2A/B, BRAF, PRC2, and NF1 mutations is consistent with these studies, raising the question of a critical role in tumorigenesis.\(^{43}\) Trials are currently underway testing the utility of clinically available CDK4/6 inhibitors against this mutation.\(^{46}\)

**Conclusions**

Comprehensive genomic evaluation of MPNSTs is warranted to identify not only BRAF alterations but also alterations in pathways related to RAS/RAF. The findings of frequent mutations in DNA repair genes and CDKN2A/B suggest additional targets for therapeutic intervention in this rare malignancy.\(^{47–50}\)

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**References**

29. Kaplan et al


