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REVIEW

NRAS-mutant melanoma: current challenges and future prospect

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Abstract: Melanoma is one of the most common cutaneous cancers worldwide. Activating mutations in *RAS* oncogenes are found in a third of all human cancers and *NRAS* mutations are found in 15%–20% of melanomas. The *NRAS*-mutant subset of melanoma is more aggressive and associated with poorer outcomes, compared to non-*NRAS*-mutant melanoma. Although immune checkpoint inhibitors and targeted therapies for *BRAF*-mutant melanoma are transforming the treatment of metastatic melanoma, the ideal treatment for *NRAS*-mutant melanoma remains unknown. Despite promising preclinical data, current therapies for *NRAS*-mutant melanoma remain limited, showing a modest increase in progression-free survival but without any benefit in overall survival. Combining MEK inhibitors with agents inhibiting cell cycling and the PI3K–AKT pathway appears to provide additional benefit; in particular, a strategy of MEK inhibition and CDK4/6 inhibition is likely to be a viable treatment option in the future. Patients whose tumors had *NRAS* mutations had better response to immunotherapy and better outcomes than patients whose tumors had other genetic subtypes, suggesting that immune therapies – especially immune checkpoint inhibitors – may be particularly effective as treatment options for *NRAS*-mutant melanoma. Improved understanding of *NRAS*-mutant melanoma will be essential to develop new treatment strategies for this subset of patients with melanoma.

Keywords: metastatic melanoma, *NRAS* mutation, MEK inhibitor, immunotherapy, trametinib, binimetinib

Introduction

Throughout the recent 5 years, there has been a significant shift in the therapeutic management of disseminated melanoma. The clinical success of *BRAF*-targeted therapy and immunotherapy with high responders and long survivors suggests that short- and long-term disease control can be a reality for the unclearly defined subgroups of patients with melanoma. *BRAF* and *NRAS* co-mutations are not mutually exclusive; however, the sole finding of double-mutated cells in a resistant tumor is insufficient to determine follow-up therapy, and combinational therapy targeting different pathways will be necessary.[1–4]

Some specific *driver mutations*, each with well-known unique clinical and genetic features, have been described; these *driver mutations* occur in multiple oncogenes including *BRAF*, *NRAS*, and *CKIT*, which are the most commonly described, and may serve as potential therapeutic targets. Despite this progress, few advances have been made in developing targeted therapeutic strategies for the 50% of patients whose melanomas are *BRAF* wild-type (WT). The best-characterized subgroup of *BRAF*-WT tumors comprises 15%–20% of all melanomas that harbor activating *NRAS* mutations. The small GTPase, *NRAS*, was the first oncogene identified in melanoma and other mutational subtypes of melanoma; patients with mutant *NRAS* tumors tend to be older

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and have a history of chronic ultraviolet (UV) exposure.[5–7] Histologically, mutant *NRAS* tumors are more aggressive than other subtypes and have thicker lesions, elevated mitotic activity, and higher rates of lymph node metastasis.[8,9] Given the more aggressive disease seen with mutant *NRAS* patients who have not received any specific therapy for the disease, it is not surprising that *NRAS* mutation status is a predictor of poorer outcomes in patients with melanoma who harbor this mutation, with lower median overall survival (OS) compared to non-*NRAS*-mutant melanoma.[6–8] Moreover, and in contrast with *BRAF*-mutant melanoma, little progress has been made in developing targeted therapeutic strategies for *NRAS*-mutant melanoma; no effective small-molecule inhibitors have been approved that specifically target NRAS, although MEK inhibitors have demonstrated modest clinical activity in a phase II trial, with clinical benefit in progression-free survival (PFS), but without a clear benefit in OS.[3] A better understanding of the biological and signaling characteristics of the *NRAS*-mutant melanoma will outline some effective therapeutic strategies for its therapeutic management that are now urgently needed.

Ras proteins as an oncogene

Despite the attention focused upon *BRAF*-mutant melanoma, *NRAS* was the first melanoma oncogene to be identified. It is known that approximately one-third of all human cancers have oncogenic mutations in the small GTPase *RAS* family.[10] The *RAS* family of GTPases consists of *KRAS*, *HRAS*, and *NRAS*. Although *NRAS*, *KRAS*, and *HRAS* share structural and functional similarities, mutations in *KRAS* are the most frequent *RAS* mutations in human malignant disease; moreover, in melanoma, the most commonly mutated isoform of *RAS* mutation typically occur at codons 12, 61, or, less frequently, 13, with 15% of cases harboring point mutations.[11] Whereas mutant *NRAS(Q61)* disrupts the GTPase activity of *RAS*, locking it in its active conformation, *NRAS(G12)* and *NRAS(G13)* mutations affect the Walker A-motif (p-loop) of the protein, thus decreasing its sensitivity to GTPase-accelerating proteins.[12,13] Mutations in G12/13 and Q61 can all be described as activating; yet, they affect the NRAS protein in a distinct way as they favor the formation of GTP-bound, active RAS proteins.

Genetic evidence in experimental systems provides strong evidence that the RAF–MEK–ERK pathway is critical to the ability of RAS to induce cell proliferation, migration, and survival – highlighting the functional and biochemical relationship between RAS and this pathway in cancer.[14] Whereas *KRAS* mutations are frequent in colorectal, lung, and pancreatic cancers, *NRAS* mutations are, by far, the predominant alteration among *RAS* isoforms in melanoma.

Mutations in *NRAS* constitutively activate intracellular signaling through a variety of pathways – most notably, the RAS–RAF–MAPK and PI3K–AKT pathways. These mutations activate MAPK signaling to a similar degree as *BRAF* mutations and rarely co-occur with mutations in the PI3K–AKT pathways, suggesting that mutant *NRAS* drives this pathway as well. These activated signaling pathways induce cell-cycle dysregulation, pro-survival pathways, and cellular proliferation.[15]

NRAS-mutant melanoma

NRAS was the first melanoma oncogene to be identified in 1984 in a screen of melanoma cell lines for genes that possessed transforming properties and were identified as activating mutations in *NRAS* in 4/30 samples.[16] Currently, mutations of *NRAS*, *KRAS*, or *HRAS* are known to be present in 20%, 2%, and 1% of all melanomas tested, respectively.[17] The most common oncogenic change present in >80% of all *NRAS* mutations is a point mutation leading to the substitution of glutamine to leucine at position 61, with mutations at positions 12 and 13 occurring with less frequency.[18]

NRAS mutations occur at a fairly consistent rate of 15%–20% at all non-uveal sites of melanoma, including sun-exposed and sun-unexposed skin, mucosal, and acral sites of origin. This distribution contrasts with *BRAF* mutations, which are more common in intermittently sun-exposed skin, and with *KIT* mutations, which are present predominantly in mucosal and acral melanomas. Furthermore, in contrast to *BRAF*, *NRAS* mutations are rarely present in benign melanocytic nevi – with the exception of congenital nevi.[9] The presence of *NRAS* mutations in melanoma has prognostic significance.[12,19] Typical patients harboring *NRAS* mutations tend to be older (>55 years) than patients with *BRAF* mutations, with a chronic pattern of UV exposure and lesions are usually located at the extremities and have greater levels of mitosis than *BRAF*-mutant melanomas. Moreover, *NRAS* mutations are associated with lower rates of ulceration and thicker primary tumors, with the presence of NRAS mutations an adverse prognostic factor leading to shorter MSS. Several studies examining the effect of NRAS mutations on OS have found different results; when OS was measured from the time of primary disease, *NRAS* mutations were found to have no impact on OS.[20,21] However, in two other studies where OS was measured from the time of biopsy of advanced disease, *NRAS* mutations were associated with

improved OS when compared to tumors with *BRAF* mutations or those WT for both.[22,23]

Current management strategies

Improved understanding of genetic and molecular basis of melanoma has revolutionized treatment options for this disease. Genetic profiling of melanomas varies widely between institutions and practices and, in spite of these advances, the treatment of melanoma remains challenging in terms of therapy selection. Because no therapeutic agents have been approved specifically for *NRAS*-mutant melanoma, due to the fact that several different strategies of directly targeting *RAS* have not resulted in effective therapeutics, mutational profiling of *NRAS* is not performed routinely by many clinicians although identifying *NRAS* mutations may have prognostic implications and facilitate clinical trial enrolment.

Farnesyl transferase inhibitors

Farnesyl transferase inhibitors are a class of drugs designed to prevent the posttranslational modification of Ras and its insertion into the plasma membrane. Although this mechanism of inhibition showed promising preclinical activity, the clinical experience in different clinical trials with these inhibitors has been very disappointing – with several serious side effects and very few responses observed.[24–26] In melanoma, farnesyl transferase inhibitors were evaluated in a small phase II trial with 14 patients whose NRAS mutation status was unknown; in this trial, none of the patients experienced a clinical response.[27] The lack of success observed with these drugs is attributable to the fact that many critical cellular proteins are farnesylated, in addition to Ras. Currently, there are no further ongoing clinical trials with farnesyl transferase inhibitors.

MEK inhibitors

After the lack of success in directly targeting *NRAS* was objectivized, focus developed toward targeting the critical signal-transduction pathways of the MAPK pathway, using MEK inhibitors. First-generation MEK inhibitors (PD 098059, U0126) showed promising inhibition in preclinical models of melanoma, but they did not progress to clinical trials.[28] CI-1040 (PD 184352) and its derivate PD-0325901 (PD-901) were the first MEK inhibitors tested in a clinical trial. CI-1040 was identified as a drug with a favorable safety profile, but with low oral bioavailability and high metabolism that led to plasma drug levels insufficient for antitumor activity.[29] PD-901 is a second-generation MEK inhibitor with some clinical response, as demonstrated in a clinical trial with 48 patients with melanoma (three patients experienced partial responses and 10 had temporary stable disease); however, the high incidence of adverse events – particularly ocular and neurologic – were observed, and limited further development of this drug.[30] Overall, the results of early clinical trials with early-generation MEK inhibitors were disappointing (10% objective response rate) and retrospective genotyping for the *NRAS*/*BRAF* mutations did predict for clinical benefit.

Newer MEK inhibitors have been developed with a better safety profile and antitumor activity. The first of these newer generation MEK inhibitors to be developed was selumetinib (AZD-6244; ARRY-142886) – another second-generation inhibitor of MEK1/2 with potent inhibition of cell lines in both RAS- and RAF-mutant cancers.[31] Initially, it was tested in a phase I trial with 11 patients with melanoma, where activity was observed with one partial response (in a *NRAS*-mutant patient) and seven patients had stable disease; two other clinical trials were performed, but they did not show clear benefit with selumetinib.[32] In a phase II trial study with *BRAF*-WT and *NRAS*-unselected patients with melanoma, selumetinib was compared with temozolomide; the study reported equivalent/inferior response rates for selumetinib (5.8% vs 9.7%) and no difference in PFS between the two groups [hazard ratio (HR) 1.07].[33] In another phase II trial of docetaxel with or without selumetinib in patients with *BRAF*-WT advanced melanoma, no difference in OS was noted between the two groups.[34] This agent was subsequently evaluated only in *BRAF* V600-mutant melanoma in combination with dacarbazine as compared to dacarbazine alone; although PFS was extended (median 5.6 vs 3.0 months), no improvement in OS was identified (13.9 vs 10.5 months).[35] Selumetinib has, moreover, been tested for uveal melanoma in two phase II trials with a PFS advantage, as compared to chemotherapy.[36,37] To date, no trials have been specifically conducted for *NRAS*-mutant melanoma.

Clinical development of third-generation MEK inhibitors led to focused attention in targeting MEK in patients with NRAS-mutant melanoma. Both trametinib (GSK1120212) and MEK 162 (ARRY-438162) are potent inhibitors of MEK1/2, with sustained MAPK pathway inhibition at clinically achievable doses.[38,39] Trametinib is an allosteric inhibitor of MEK1/2 that was first tested in a phase I trial among patients with advanced melanoma; efficacy was demonstrated in *BRAF*-mutant patients, but no responses were seen in *NRAS*-mutant patients.[40] Trametinib has received FDA approval for use in the treatment of *BRAF* V600-mutant melanoma as a single agent and in combination with dabrafenib. This approval was based on improved OS in a

phase III trial for trametinib compared with dacarbazine in *BRAF*-mutant melanoma,[38] and the strong activity demonstrated in another phase III trial among patients with *BRAF*-mutant melanoma treated in the METRIC study; an overall response rate of 24% and a median PFS of 4.8 months, both significantly better than with standard chemotherapy, were observed.[38] Additionally, a phase III trial analyzing the efficacy of *BRAF* inhibition with dabrafenib alone compared to a combination of dabrafenib and trametinib demonstrated clear benefit with the combination treatment in terms of response rate, PFS, and OS.[41] Potential combination strategies of trametinib combined with other agents may play a role in the future of *NRAS*-mutant patients. Binimetinib (MEK 162) is the MEK1/2 inhibitor that was the first to show significant activity in *NRAS*-mutant melanoma. In a phase I trial with patients who had advanced solid tumors, MEK 162 showed promising signs of clinical activity.[42] In a phase II study of binimetinib in patients with advanced *NRAS*-mutant melanoma, 20% showed partial responses and 43.3% stable disease, with a median PFS of 3.7 months.[43] Based on these promising results, a randomized phase III trial, the NEMO trial was performed. In this trial, binimetinib improved PFS compared with dacarbazine (2.8 months vs 1.5 months, HR 0.62 [95% confidence interval 0.47–0.80]) and was tolerable. Grade 3–4 adverse events seen in at least 5% of patients in either group were increased creatine phosphokinase (19% vs 0%), hypertension (7% vs 2%), anemia (2% vs 5%), and neutropenia (1% vs 9%) in binimetinib group versus dacarbazine group, respectively; serious adverse events (all grades) occurred in 34% patients in the binimetinib group and in 22% patients in the dacarbazine group. Binimetinib might represent a new treatment option for patients with *NRAS*-mutant melanoma after failed immunotherapy.[44]

Other MEK inhibitors such as pimasertib (AS703026), cobimetinib (GDC-0973), TAK-733, and RO4987655 have been tested for efficacy in different clinical trials with patients with melanoma. Pimasertib has been evaluated in a phase I trial with 17 *NRAS*-mutant patients, with two partial and two complete responses.[45] Cobimetinib has not been evaluated specifically for the *NRAS* cohort but, in combination with vemurafenib, has demonstrated a promising activity for patients with *BRAF*-mutant melanoma[46] (Table 1).

Table 1 Phase I/II/III trials of MEK inhibitors in *NRAS*-mutant melanoma

	ORR	PFS	OS
Trametinib[40]	22%	4.8 months	81% at 6 months
Binimetinib[44]	15%	2.8 months	11 months
Selumetinib[33]	5.8%	2.2 months	8 months

Abbreviations: ORR, overall response rate; PFS, progression-free survival; OS, overall survival.

MEK inhibitor combinations

Although the next generation of MEK inhibitors are showing promising clinical efficacy, the relatively suboptimal response rate and PFS has led to interest in different MEK inhibitor-based combinations. The combination of MEK inhibition with RAF, EGFR–PI3K–AKT, and CDK4/6, which are the two particular pathways of interest, are currently being evaluated in clinical trials.

MEK + CDK4-6 inhibitors, which are regulators of the G1/S cell-cycle checkpoint inhibiting cancer cell growth, are being tested in phase I/II trials. Early results for the combination of ribociclib (LEE001) with binimetinib in patients with NRAS-mutant melanoma have shown a partial response in 33% and stable disease in 52% of patients.[47] Another phase I/II trial with a combination of trametinib and palbociclib in patients with solid tumors and with a specific cohort for NRAS-mutant melanoma is ongoing.

The combination of MEK + PI3K/AKT inhibitors has been shown to synergistically inhibit the growth of *NRAS*-mutant melanoma cell lines. In melanoma cell lines where BRAF inhibitor resistance is mediated through an acquired *NRAS* mutation, the combination of a MEK inhibitor plus a PI3K/mTOR inhibitor was noted to overcome drug resistance and inhibit cell survival.[48] Several early-phase studies in solid tumors have been performed, but it is not yet clear what the optimal combination of signal transduction inhibitors will be for *NRAS*-mutant melanoma. There are multiple phase I/II studies examining these combinations in patients with melanoma.

Other combinations for *NRAS*-mutant melanoma

Other targets such as polo-like kinase 1 (PLK1), TANK-binding kinase 1 (TBK1), and ROCK 1/2 are overexpressed in *NRAS*-mutant melanoma and the combination of MEK inhibitors plus specific inhibitors in these targets may show promising results in the different phase I/II trials that are ongoing.[49–51]

Immune-based therapies

Immune therapies are playing an increasing role in the treatment of patients with metastatic melanoma, regardless of its *BRAF* or *NRAS* mutation status, particularly when there is no specific targeted therapy available.[52]

Despite the current lack of effective and specifically approved targeted therapies for *NRAS*-mutant melanoma, there is some evidence that *NRAS* mutational status may predict for response to other therapies. Immune-based therapies are the standard of care in melanoma therapeutics, mainly used as first-line therapy particularly in patients with *BRAF*-WT melanoma, and are used regardless of tumor genotype. Some retrospective data suggest that patients with *NRAS*-mutant melanoma may have higher response rates to immunotherapies.

A retrospective analysis of patients treated with high-dose interleukin-2 demonstrated that the majority of the responders were patients with *NRAS*-mutant melanoma, and that those patients with either *BRAF*-mutant or *BRAF*/*NRAS*-WT melanoma were less likely to respond.[53] It is not yet clear whether *BRAF* or *NRAS* mutational status predicts for better responses in patients with melanoma receiving the antiCTLA-4 antibody ipilimumab or anti-PD-1/PD-L1 antibodies; however, some publications suggest that patients with *NRAS* mutations respond better to these agents.[54,55] In the only published analysis to date, the disease control rate of patients with melanoma on ipilimumab therapy was noted to be 30% and 33% for those with and without *BRAF* mutations, respectively[55] (Table 2).

Future perspectives

Although the current target and immunotherapeutic agents may offer some hope to patients with *NRAS*-mutant melanoma, none of these therapies are mutation specific and have shown modest response rates and carry risk of significant toxicities. In contrast with *BRAF*-mutant melanoma, to date, no effective molecularly targeted therapeutic strategies have been approved for *NRAS*-mutant or -WT melanoma.

Many targeted strategies are now being evaluated for *NRAS*-mutant melanoma, although these tumors appear to be more heterogeneous than those with *BRAF* mutations. The most promising data from clinical investigations are with regard to MEK inhibition; however, the relatively short PFS indicates that either combination strategies or other targeted approaches will be necessary to achieve more clinically important disease responses. Both combinations for pathway interference (MEK + PI3K/mTOR and MEK + CDK 4,6) as well as combination of targeted therapy with immunotherapy are, to date, the most promising strategy to interfere with current targets refractory to chemotherapy, such as NRAS-mutant melanoma.

Table 2 Response rate and clinical benefit in patients with *NRAS*-mutant melanoma receiving immunotherapy

	NRAS-mutant	WT
Anti-PD1-PD-L1		
Objective response	64%	35%
Clinical benefit	73%	43%
Ipilimumab		
Objective response	19%	11%
Clinical benefit	42%	20%
IL-2		
Objective response	33%	26%
Clinical benefit	33%	37%

Abbreviations: WT, wild-type; IL-2, interleukin-2.

Conclusion

NRAS-mutant melanoma is a relatively common subtype of this disease (15%–20% of patients harbor this mutation) with a known poor prognosis. Although, currently, there are no targeted therapies that directly target NRAS, a high number of newer targeted therapeutic strategies, particularly mono- and combination therapy with MEK inhibitors, hold promise of being effective treatment strategies in the near future in the several clinical trials that are being conducted. Immune-based therapies are not genotype-specific but appear to be at least as or even more effective in the *NRAS*-mutant population compared to other melanoma subtypes.

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Author contributions

All authors contributed toward data analysis, drafting and revising of the paper, and agree to be accountable for all aspects of the work.

Disclosure

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