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Treatment of *NRAS*-Mutant Melanoma

Douglas B. Johnson, M.D. and **Igor Puzanov, M.D.**

Department of Medicine, Division of Hematology/Oncology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 777 Preston Research Building, 2220 Pierce Avenue, Nashville, TN 37232, USA

Douglas B. Johnson: douglas.b.johnson@vanderbilt.edu

Opinion statement

NRAS mutations in codons 12, 13, and 61 arise in 15–20 % of all melanomas. These alterations have been associated with aggressive clinical behavior and a poor prognosis. Until recently, there has been a paucity of promising genetically targeted therapy approaches for *NRAS*-mutant melanoma (and *RAS*-mutant malignancies in general). MEK inhibitors, particularly binimetinib, have shown activity in this cohort. Based on pre-clinical and early clinical studies, combining MEK inhibitors with agents inhibiting the 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, and is the most promising genetically targeted treatment strategy for *NRAS*-mutant melanoma developed to date. In addition, immune-based therapies have shown increasing activity in advanced melanoma and may be particularly effective in those with *NRAS* mutations. Combination strategies of immune and targeted therapies may also play a role in the future although clinical trials testing these approaches are in early stages.

Keywords

Melanoma; *NRAS*; Mutation; MEK inhibitor; CDK4; MAPK; Immunotherapy; Trametinib; Binimetinib

Introduction

Mutations in RAS occur in a large percentage of prevalent and deadly malignancies including melanoma, lung adenocarcinoma, colon cancer, pancreatic cancer, acute leukemias, and others [1]. Targeting RAS, therefore, has remained a critical priority for cancer therapy but an effective, approved treatment option has remained elusive in any cancer to date. *NRAS* is recurrently altered in 15–20 % of melanomas at codons 12, 13, or 61, and is the second most common oncogenic “driver” mutation in this disease. In parallel with melanoma therapy in general, there have been few treatment options for this genetic

Correspondence to: Douglas B. Johnson, douglas.b.johnson@vanderbilt.edu.

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cohort in the past. More recently, the development of genetically targeted agents and immune-based therapies has yielded numerous available and potential therapeutic strategies for treating *NRAS*-mutant melanomas. These treatment approaches, if effective for patients with *NRAS*-mutant melanoma, may also have implications extending to other RAS-mutant malignancies. In this review, we will cover the clinical and pathologic characteristics of *NRAS*-mutant melanoma and ongoing treatment options for patients in this genetically defined cohort of melanoma (Table 1).

Epidemiology

NRAS mutations occur at a fairly consistent rate of 15– 20 % at all non-uveal sites of melanoma, including sun exposed and non-sun exposed skin, mucosal, and acral sites of origin [10, 11]. This distribution contrasts with *BRAF* mutations, which are more common in intermittently sun-exposed skin, and *KIT* mutations, which are present predominantly in mucosal and acral melanomas [12]. Also, in contrast to *BRAF, NRAS* mutations are rarely present in benign melanocytic nevi, with the exception of congenital nevi [13, 14]. *NRAS* mutations are associated with thicker primary tumors, increased mitotic rate, and lower incidence of ulceration [15]. More importantly, *NRAS* mutations have been generally linked to a poorer overall survival, although this has varied across studies [15, 16•, 17].

Pathogenesis

Mutations in *NRAS* constitutively activate intracellular signaling through a variety of pathways, most notably the RAS-RAF-MAPK and PI3K-AKT pathways. *NRAS* 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 also [18•]. These activated signaling pathways induce cell cycle dysregulation, pro-survival pathways, and cellular proliferation. *NRAS* point mutations occur in codon 61 in >80 % of cases with the remaining mutations occurring in codons 12 and 13 [16•, 19]. This distribution contrasts with *KRAS* mutations in lung cancer or colon cancer, which occur largely in codons 12 and 13 [20, 21]. Mutations at any of these codons produce similar effects by locking *NRAS* into its active conformation [22]. It remains unclear whether different *NRAS* mutations induce distinct biologic or clinical features in melanoma.

Diagnosis

Genetic profiling of melanomas varies widely between institutions and practices. Testing platforms range from single-point mutation assays (for $BRAF^{V600E}$), PCR-based tests evaluating a limited number of recurrently mutated “hotspots ” in several genes, to targeted next generation sequencing assays which may assess hundreds of genes [11, 23, 24]. Testing for $BRAF^{V600E}$ mutations is nearly universal in metastatic disease since three clinically active small molecule inhibitors have now been approved for $BRAF^{V600}$-mutant melanoma (vemurafenib, dabrafenib, and trametinib) [25–27]. Since no therapeutic agents have been approved specifically for *NRAS*-mutant melanoma, mutational profiling of *NRAS* is not performed routinely by many clinicians. Identifying *NRAS* mutations may have prognostic implications and facilitate clinical trial enrollment. Most institutions that routinely perform more extensive multiplexed PCR platforms or targeted next generation sequencing assays

will include at least the frequently mutated “hotspot ” codons (12, 13, 61) or exons (1 and 2) in their respective panels.

Treatment

Identifying an effective treatment option for mutant RAS is a long sought, elusive goal in cancer therapeutics since this gene drives many of the most aggressive malignancies. Previous strategies have focused on posttranslational modification of *NRAS* (farnesyltransferase inhibitors) whereas current approaches are largely concentrated on downstream signaling pathways. Directly targeting RAS with small molecules or small interfering RNAs (siRNAs) may also play a role in the future. Possible therapeutic approaches are outlined in (Fig. 1).

Farnesyltransferase inhibitors (FTIs)

Farnesylation of a cysteine residue is a critical posttranslational modification of the RAS oncoprotein which occurs prior to its insertion into the cell membrane [28]. Although inhibition of this step of RAS activation showed a promising pre-clinical activity, the experience in clinical trials has generally been disappointing [29–31]. In melanoma, an FTI (R115777) was evaluated in 14 patients in a phase II clinical trial. No patients experienced a clinical response, although the *NRAS* mutation status of these patients was not reported [32]. Tipifarnib demonstrated efficacy in AML in occasional patients with complete remissions observed in three of 54 treated patients; the *NRAS* mutation status in this population is likewise unknown [33]. Further clinical trials of FTIs in melanoma are not ongoing.

MEK inhibitors

Mutated *NRAS* triggers the MAPK signaling cascade through activation of RAF, which in turn activates MEK, triggering ERK phosphorylation and cellular proliferation. Blocking a downstream signaling partner, therefore, is an attractive therapeutic strategy. Inhibition of RAF in this setting is a challenge since both CRAF and BRAF have been implicated as critical downstream signaling partners for mutant *NRAS*. Inhibitors of BRAF, in fact, appear to paradoxically activate signaling in RAS-mutated malignancies and likely promote neoplastic proliferation. Efforts to inhibit MAPK signaling, therefore, have largely focused on MEK.

Early MEK inhibitors

- CI-1040 was among the first MEK inhibitors evaluated in clinical trials. Although its toxicity profile was tolerable, objective responses were rare across malignancies (1 of 144 in phase I/II trials) [34, 35]. Of note, baseline phospho-ERK expression correlated with stable disease, suggesting that this class of therapies may be effective in tumors with MAPK activation.
- PD-03225901 is a second-generation MEK inhibitor with higher potency and improved MAPK-blocking activity [36]. Forty-eight patients with advanced melanoma were enrolled with much greater activity; three patients experienced partial responses and ten had temporary stable disease [37]. High incidence of

toxicity, particularly ocular and neurologic, limited further development of PD-03225901 [37, 38].

- Selumetinib (AZD-6244; ARRY-142886) is another second-generation inhibitor of MEK1/2 with potent inhibition of cell lines and xenografts in both RAS and RAF-mutant cancers [39]. In a phase I clinical trial, 11 melanoma patients had evaluable responses, of which 1 had a partial response (with an *NRAS* mutation) and seven had stable disease [40]. Two subsequent melanoma trials did not show a benefit with selumetinib, however. A phase II trial in unselected melanoma patients compared selumetinib with temozolomide and reported equivalent or inferior response rates for selumetinib (5.8 vs. 9.7 %) and PFS (hazard ratio 1.07) [41]. This agent was subsequently evaluated only in $BRAF^{V600}$-mutant melanoma in combination with dacarbazine 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) [42]. A phase II trial in uveal melanoma identified a PFS advantage compared to chemotherapy as well [43]. No trials specifically for *NRAS*-mutant melanoma have been performed; development is ongoing for *KRAS*-mutant lung cancer, uveal melanoma, and other malignancies.

Trametinib

Trametinib is an allosteric inhibitor of MEK1/2 that has received FDA approval for the treatment of $BRAF^{V600}$-mutant melanoma as a single agent and in combination with dabrafenib. This approval was based on improved overall survival in a phase III trial for trametinib compared with dacarbazine in *BRAF*-mutant melanoma [26]. The trametinib/dabrafenib combination approval was based on a randomized phase II trial which demonstrated improved PFS and response rates for the dabrafenib and trametinib compared to single-agent dabrafenib [44]. The phase I study of trametinib included a limited number of patients with *NRAS*-mutant melanoma. Of seven treated patients, two experienced stable disease (29 %) including one patient who remained on treatment for approximately 8 months [45•]. Given the more robust data in the *BRAF*-mutant population, no additional studies were performed for the *NRAS*-mutant cohort. Potential combination strategies with trametinib may play a role in the future (see combination therapy below).

Binimetinib (MEK162)

Binimetinib, an allosteric MEK1/2 inhibitor, has been the first agent to show robust activity specifically in *NRAS*-mutant melanoma. An open-label phase II trial enrolled 71 patients with advanced melanoma including 30 with *NRAS* mutations [2••]. Of these 30 patients, six demonstrated a partial response (20 %) and another 13 (43 %) had temporary stable disease. Median PFS for this cohort was 3.7 months (95 % CI 2.5–5.4 months). Of note, the PFS and response rate appeared to be very similar for the *NRAS* and *BRAF* cohorts in this study. Based on these results, a randomized phase III trial is ongoing comparing binimetinib to chemotherapy in *NRAS*-mutant melanoma (NCT01763164). Combination strategies are also being explored with binimetinib.

Other MEK inhibitors

- RO4987655 (CH4987655) is a MEK inhibitor recently assessed in phase I trials. Eight patients with *NRAS*-mutant melanoma were treated with one PR (13 %) and two additional cases of stable disease [46].
- Cobimetinib (GDC-0973) has not been evaluated for the *NRAS* cohort. In combination with vemurafenib, however, promising activity was observed in $BRAF^{V600}$-mutant melanoma with an objective response rate of 87 % and a median PFS of 13.7 months for patients not previously treated with a BRAF inhibitor [47].
- Other MEK inhibitors in development: An intriguing structural and functional study suggested that experimental MEK inhibitors with superior pre-clinical activity in *KRAS*-mutant cancers (GCD-0623 and G-573) form a hydrogen bond with MEK to prevent feedback phosphorylation by wild-type BRAF [48]. By contrast, cobimetinib, which is more effective in *BRAF*-mutant pre-clinical models, inhibits activated MEK. This suggests that through distinct modes of action, RAS and RAF-specific MEK inhibitors may be utilized in the future.

MEK inhibitor-based combinations

Although the clinical activity of single-agent binimetinib is unprecedented for a RAS-targeted therapy, the relatively suboptimal response rate and PFS has led to interest in MEK inhibitor-based combination strategies. Mutant *NRAS* activates multiple cell signaling pathways, likely necessitating such an approach. Two particular pathways of interest are CDK4/Rb and PI3K/AKT.

- MEK + CDK4 inhibition: An inducible mouse model of *NRAS*-mutant melanoma demonstrated that MEK inhibition was sufficient to cause apoptosis but not cell cyle arrest. In contrast, complete *NRAS* extinction not only induced apoptosis but also drove arrest of the cell cycle [49•]. Network modeling demonstrated that CDK4 was a critical driver of these differential results; subsequent combination of MEK inhibition (trametinib) with CDK4/6 inhibition (palbociclib) caused tumor regression. In addition, alterations in genes causing cell cycle dysregulation (*CDKN2A, CDK4, CCND1*, others) occur with high frequency in melanoma [18•, 50]. Pre-clinical cell line data suggests that alterations in these genes predict for sensitivity to CDK4 inhibition [51, 52]. Based on these observations, two phase I/II trials particularly focusing on *NRAS*-mutant melanomas are ongoing (binimetinib + LEE011; NCT01781572 and trametinib + palbociclib; NCT01781572). Early results from the binimetinib + LEE011 study were presented in July 2014 with promising activity [3]. Among the first 21 patients treated, seven experienced a partial response (33 %) and another 11 (52 %) experiencing stable disease. Only three patients (14 %) had primary progressive disease. Several clinically significant toxicities have occurred (including high-grade creatine phosphokinase (CPK) elevations and a single fatal case of cardiomyopathy) and dose finding is ongoing.
- MEK + PI3K/AKT inhibition: Combined targeting of MAPK and PI3K/AKT signaling has a significant rationale as dual inhibition was required to restrain tumor growth in *NRAS*-mutant models [53, 54]. Several early-phase studies for

advanced cancers are underway co-targeting MEK and PI3K/AKT. These include the combination of trametinib and an AKT inhibitor (uprosertib; GSK2141795) in *BRAF* wild-type melanoma (NCT01941927) and in AML (NCT01907815). Binimetinib and various PI3K/AKT pathway inhibitors are also being assessed in early-stage trials (NCT01363232, NCT01337765, NCT01449058) [55].

- Other MEK inhibitor combinations have pre-clinical support as well. Adding an ERK inhibitor to MEK inhibition increased apoptosis, suppressed *CCND1* signaling, and delayed resistance in *NRAS*-mutant cell lines [56]. Augmenting MEK inhibitor-induced apoptosis with WNT signaling stimulation is another potential strategy with pre-clinical support [57].

Other MAPK-directed therapies

While MEK inhibitors are the most developed, additional strategies targeting the MAPK pathway are being evaluated in various stages of development. These include RAF inhibitors (alone and in combination), ERK inhibitors, and other more experimental strategies.

- Sorafenib, a multitargeted kinase inhibitor, and tivantinib, a MET inhibitor, were assessed in eight patients with *NRAS*-mutant melanoma [58]. Two patients experienced a complete or partial response and another two had stable disease as best response.
- RAF-265, an inhibitor of BRAF and VEGFR2, demonstrated pre-clinical efficacy in *NRAS*-mutant and *NRAS/BRAF* wild-type patient-derived xenografts [59]. In 71 patients, the objective response rate was 16 %for *BRAF* mutant melanoma patients and 13 % for BRAF wild-type (*NRAS* status not reported) [60].
- Inhibitors of ERK, the final step of canonical MAPK signaling, have also generated interest in both *NRAS* and *BRAF*-mutant melanoma. SCH772984 has demonstrated efficacy in cell lines with *NRAS, KRAS,* and *BRAF* mutations as well as in models of BRAF inhibitor-resistant melanoma [61•]. Additionally, ERK inhibition in combination with PI3K/AKT inhibitors appeared effective against BRAF inhibitor-resistant cell lines, including those with secondary *NRAS* mutations [62].
- An intriguing recent study identified a scaffolding protein, IQGAP1, that appears to be essential for MAPK signaling in both RAS and RAF driven tumors [63]. A peptide disrupting IQGAP1-ERK interactions was effective in cell lines and mouse models of RAS-mutant malignancies.

Other targeted strategies

- Direct targeting of *NRAS* by RNA interference (RNAi) to block protein translation is an effective technique in laboratory models, particularly cell lines, but has not yet been implemented in clinical practice [64]. Challenges include intravascular degradation, intracellular trafficking, and potential off target effects [65]. An early-stage clinical trial demonstrated intratumoral presence of small interfering RNAs (siRNAs) with a nanoparticle delivery system and decreased expression of the target (RRM2) [66].

- Heat shock protein-90 (HSP90) inhibitors may have a role in single agent or combination therapy through inhibition of multiple down-stream targets of RAS. The HSP90 inhibitor XL888 had promising activity in *NRAS*-mutant melanoma cell lines [67].
- Combination inhibition of NF-KB and AKT by a small molecule inhibitor BI-69-A11 also was effective in *NRAS*-mutant mouse models [68].

Immune-based therapies

Immune-based therapies are the other cornerstone of melanoma therapeutics and are used regardless of tumor genotype. Immune therapies are generally used as first-line therapy, particularly for patients with $BRAF^{V600}$ wild-type melanoma (including *NRAS* mutant).

- High-dose interleukin-2 (IL-2) has been used for many years and causes durable responses in 5–8 % of treated patients [4, 69]. Acute toxicities, however, are severe and limit therapy to young and otherwise healthy patients [70]. Despite the drawbacks, IL-2 is still a potential therapeutic strategy for patients who qualify and adds another treatment option.
- Ipilimumab is a monoclonal antibody to cytotoxic T lymphocyte antigen-4 (CTLA4) which activates an antitumor immune response by removing a key negative T cell regulator. Ipilimumab improved overall survival in pre-treated melanoma patients compared to a peptide vaccine, and in combination with dacarbazine as first-line therapy when compared to chemotherapy alone [5•, 71]. This agent is associated with durable benefit, with approximately 20 % of patients surviving 3–5 years [72, 73]. Toxicities are related to aberrant immune activation and include colitis, hepatitis, endocrinopathies, and skin rash.
- Agents targeting the programmed cell death-1 receptor and its ligand (PD-1/PD-L1) appear still more promising. Nivolumab (BMS-936558) and pembrolizumab (MK-3475) are associated with objective response rates in the 25–40 % range with many appearing durable [6, 7•, 74•, 75]. Furthermore, immune-related adverse events occur much less commonly than with ipilimumab. Pembrolizumab recently received FDA approval for treatment of patients who previously progressed on ipilimumab and BRAF inhibitors (if applicable). Clinical trials comparing these agents with ipilimumab as well as numerous combination therapy trials are ongoing.
- Although these therapies are used irrespective of genotype, some retrospective data suggests that patients with *NRAS*-mutant melanoma may have higher response rates to immune-based therapies. Joseph and colleagues observed nearly a 50 % response rate to IL-2 among a small *NRAS*-mutated cohort [76•]. Our group saw a similar effect among patients treated with newer immune checkpoint inhibitors, particularly for those treated with anti-PD-1/PD-L1 [77]. The underlying mechanism for this finding is not clear and prospective validation is needed.
- Combined immune and targeted therapy strategies may also hold promise. Several clinical trials are ongoing for the *BRAF*-mutant population combining immune

checkpoint inhibitors and BRAF and/or MEK inhibitors. Early phase studies are also combining MEK inhibitors with anti-PD-1 or anti-PD-L1 across a variety of solid tumors. Concerns to such an approach include aberrant T cell activation (toxicity) or dampening the immune response (lack of efficacy), highlighting the need for rational and carefully conducted clinical trials [78, 79].

Conclusions

NRAS-mutant melanoma is a common subtype of this disease with a poor prognosis. No approved therapies specifically targeting *NRAS* have been approved. However, newer targeted therapy strategies, particularly MEK inhibitor monotherapy and combinations, will likely provide effective treatment strategies in the near future. These therapies may also have an impact in other RAS-mutant malignancies. Immune-based therapies, while not genotype-specific, are also incredibly promising and appear at least as effective in the *NRAS*-mutant cohort compared to other melanoma populations.

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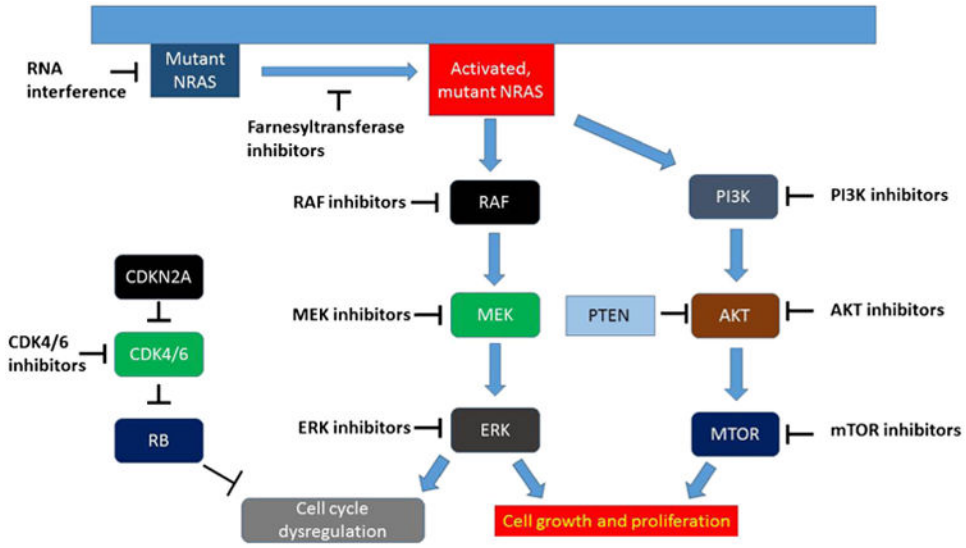

Fig. 1.
NRAS-driven cellular signalling and possible targeted therapeutic strategies.

Table 1

Selected experimental and approved treatment options for advanced NRAS-mutant melanoma

Agent (reference)	NRAS specific?	Response rate	OS (median)	FDA approved?
Binimetinib [2••]	Yes	20 % (6 of 30)	*	No
Binimetinib + LEE011 [3]	Yes	33 % (7 of 21)	*	No
IL-2 [4]	No	16 %	11.4 months	Yes
Ipilimumab [5•]	No	10.9 %	10.1 months[a] (2nd line +)	Yes
Pembrolizumab [6, 7•]	No	38 % (ipi naïve) 25 % (ipi pre-tx)	*	Yes[b]
Nivolumab [8, 9]	No	31 % (ipi naïve) 25 % (ipi pre-tx)	16.8 months	No

ipi ipilimumab, pre-tx pre-treated

*Not reported

[a]Associated with a ∼20 % 5-year survival