NRAS mutant melanoma: an overview for the clinician for melanoma management

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Practice points

- Aberrant signaling through the RAS-RAF pathway occurs in up to 75% of melanomas.
- NRAS mutations are present in 15–30% of metastatic melanoma.
- Despite the development of targeted therapies and immunomodulatory monoclonal antibodies, additional therapeutic approaches are needed for melanoma.
- MEK inhibitors show modest activity in NRAS mutant melanoma.
- Trials with MEK inhibitors in combination with other therapies are underway.
- Efficacy of immunotherapy may be improved in NRAS mutant melanoma.

Melanoma is the deadliest form of skin cancer and the incidence continues to rise in the United States and worldwide. Activating mutations in *RAS* oncogenes are found in roughly a third of all human cancers. Mutations in *NRAS* occur in approximately a fifth of cutaneous melanomas and are associated with aggressive clinical behavior. Cells harboring oncogenic *NRAS* mutations exhibit activation of multiple signaling cascades, including PI3K/Akt, MEK-ERK and RAL, which collectively stimulate cancer growth. While strategies to target N-Ras itself have proven ineffective, targeting one or more N-Ras effector pathways has shown promise in preclinical models. Despite promising preclinical data, current therapies for *NRAS* mutant melanoma remain limited. Immune checkpoint inhibitors and targeted therapies for *BRAF* mutant melanoma are transforming the treatment of metastatic melanoma, but the ideal treatment for *NRAS* mutant melanoma remains unknown. Improved understanding of *NRAS* mutant melanoma and relevant N-Ras effector signaling modules will be essential to develop new treatment strategies.

First draft submitted: 10 August 2015; Accepted for publication: 6 November 2015; Published online: 17 February 2016

Management of melanoma in 2016

Melanoma is a malignant transformation of melanocytes, pigment-producing neural-crest derived cells that exist in the epidermis and mucosal surfaces in various other anatomic sites [1]. The predominant risk factor for melanoma is ultraviolet light exposure, typically through excess sun exposure or use of tanning beds, and melanoma is more common in individuals with fair or light complexion, although other factors (e.g., family history, genetic predisposition, multiple nevi) also influence the

KEYWORDS

- BRAF • immunotherapy
- melanoma • MEK • NRAS
- PD-1

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risk of developing cutaneous melanoma. While early disease is cured with surgical resection in the majority of patients, advanced or metastatic disease is incurable and historically has been associated with an extremely poor prognosis with few treatment options. Melanoma remains the deadliest form of skin cancer, and the incidence is rising with 76,100 new cases and 9710 deaths are estimated for 2014 in the USA [1].

The last decade has seen a revolution in the treatment of metastatic melanoma, and since 2011, there have been six drugs approved by the FDA for melanoma [2]. These drugs comprise two classes of therapies:

- Drugs targeted at mediators of growth factors in the melanoma cell (vemurafenib, dabrafenib, trametinib); and
- Immunomodulatory monoclonal antibodies that neutralize immune checkpoints and thereby permit tumor control by the patient's own immune system (ipilimumab, pembrolizumab, and nivolumab) [2,3].

Approved targeted therapies for melanoma at present are available for melanomas harboring *BRAF* driver mutations, which are present in roughly half of melanomas. Over 90% of mutations in *BRAF* occur at Val600 in the catalytic domain resulting in 500-fold activation of BRAF activity, which in turn drives MEK-ERK signaling, and is capable of transforming immortalized melanocytes [4,5]. BRAF inhibitors and MEK inhibitors are active in *BRAF* mutant melanoma [6,7], but combination BRAF-MEK inhibition yields higher response rates and improved progression-free survival and overall survival [8–10]. Despite an impressive response rate for combination BRAF-MEK inhibition (70–80%) resistance develops in most patients within 1 year.

Immunotherapeutic drugs include ipilimumab, a monoclonal antibody targeting CTLA-4, and the PD-1 inhibitors pembrolizumab and nivolumab [3]. These agents function to neutralize inhibitory T-cell signaling. Ipilimumab was the first of the new generation of melanoma drugs, approved in 2011 based on an overall survival benefit compared with gp100 peptide vaccination [11]. Ipilimumab is associated with a response rate of 10–15% as a single-agent, though approximately 20% of patients treated are alive at 5 years [12]. PD-1 inhibitors (pembrolizumab and nivolumab) have higher response rates (30–40%) and a more favorable

toxicity profile compared with ipilimumab [13,14]. Recently, combination of CTLA-4 and PD-1 immune checkpoint inhibitors was shown to have an additive effect, improving response rates to up to 50–60% [13,15]. However, the toxicity was also markedly increased with combination therapy with >50% grade 3 or 4 adverse events, and it is not yet known if the combination of CTLA-4 and PD-1 improves overall survival compared with single agent therapy or sequential checkpoint blockade.

Despite these remarkable advances with the development of BRAF-MEK and immune checkpoint inhibitors, patients treated with targeted BRAF-MEK inhibitors eventually develop recurrent and refractory disease, and a limited number of patients respond to immunotherapy. Therefore, novel therapies and approaches to improve response rates and promote durable responses are needed. An attractive candidate for both the development of targeted therapies and the utilization and exploration of the impact of immune checkpoint inhibitors is *NRAS* mutant melanoma. While oncogenic Ras has historically been deemed 'undruggable', recent pre-clinical studies suggest that Ras may occupy a unique position in the oncogenic signaling network, making it an attractive candidate for both targeted agents and immune modulatory agents [16]. In this review, we will focus on the role of Ras-Raf signaling in melanoma, detail the unique clinical and pathologic features of *NRAS* mutant melanoma, and discuss clinical and preclinical approaches to treating *NRAS* mutant melanoma.

RAS & RAF signaling in melanoma

Three Ras proto-oncogenes have been identified, *KRAS*, *NRAS* and *HRAS* [17,18]. Oncogenic, activating mutations in *RAS* have been described in up to 30% of all human cancers [19–21]. Cellular homologues to these genes were first identified in the 1980s, initially with *NRAS* in neuroblastoma followed shortly thereafter by *KRAS* and *HRAS* [22,23]. These genes were later determined to encode small (low molecular weight) intracellular GTPases that mediate growth signals downstream of receptor tyrosine kinase activation and mediate diverse intracellular signal transduction cascades, including MAPK/ERK, PI3K/Akt, Ral-GDS and phospholipase-C-epsilon [17].

Ras proteins are active in the GTP-bound state, but are rendered inactive following GTP hydrolysis to GDP, a process promoted by RAS

GTPase-activating proteins (GAPs). Guanine nucleotide exchange factors (GEFs) promote formation of GTP from GDP. Ras signaling is subject to tight spatial and temporal control with multiple regulators of GTPase activity, distinct subcellular localizations for the different Ras proteins, distinct post-translational modifications, and multiple effector pathways have been described for the different Ras family members [18,24].

The majority of mutations in *RAS* are in codons 12, 13 and 61 which influence GTPase activity. In normal cells, Ras signaling requires upstream stimulation of a receptor-tyrosine kinase, but cancer cells harboring an oncogenic *RAS* mutation, the GTP-bound Ras protein is locked in the active position providing continuous activation of downstream signaling pathways. Mutations in codons 12 and 13 result in Ras proteins with impaired GAP-mediated hydrolysis, whereas mutations in codon 61 result in Ras proteins that cannot properly coordinate water molecules for the hydrolysis of GTP to GDP and are, therefore, locked in the GTP-bound state [25–27]. Codon 61 mutations have profound effect on intrinsic GTPase activity when Ras is bound to Raf [28].

Despite substantial homology, there appear to be non-overlapping roles for the different Ras isoforms. N-Ras and H-Ras are dispensable for cell survival, as double (and single) knockout *Hras* and *Nras* knockout mice are viable and have no reproductive defects, whereas *Kras*^{-/-} mice are embryonic lethal [29–32]. *Nras*-null mice appear to develop normally, but have impaired responses to viral pathogens owing to impaired T-cell activation [33]. Interestingly, NRAS overexpression has been shown to drive granulocytosis, T-cell expansion and early lethality [34].

NRAS is the second most common mutation in metastatic melanoma (15–30% of human

melanomas) after *BRAF*, which is mutated in 40–50% of melanomas [4,21]. While mutations in *KRAS* are commonly found in lung adenocarcinoma, colon cancer and pancreatic cancer, mutations in *NRAS* occur in distinct tumor types including melanoma, acute myeloid leukemia and thyroid cancer [35] (Table 1). Oncogenic Ras stimulates downstream signaling through PI3K/Akt, MEK/ERK and Ral-GDS via RAF activation [36,37] (Figure 1). There are three Raf isoforms – A-Raf, B-Raf, and C-Raf – that are capable to activating MEK downstream of Ras activation, but have distinct cellular roles [38]. In normal melanocytes N-Ras signals through B-Raf dimers (rather than C-Raf/Raf-1), but Raf class-switching occurs in *NRAS* mutant melanoma whereby C-Raf signaling predominates as a consequence of ERK-mediated B-Raf inactivation [39]. While clinical data regarding the impact of BRAF inhibitors in *NRAS* melanoma are lacking, preclinical evidence indicates administration of BRAF inhibitors to *NRAS* mutant melanoma results in ERK activation and stimulation of tumor growth [40,41].

Clinical & pathologic features of NRAS mutant melanoma

Interest in targeting *NRAS* mutant melanoma has grown substantially in recent years [36,57–58]. *RAS* mutations have been detected in up to 1/3 of melanomas [21,42–43], and mutations in codons 12, 13 and 61 of *NRAS* occur in 15–20% of melanomas, with some series reporting a frequency up to 30% [44,45]. Interestingly, in melanoma, 80–90% of all *NRAS* mutations are in codon 61 (and more frequently involve Gln to Arg), whereas mutations in codons 12 and 13 predominate in colon, pancreatic, lung and ovarian cancer comprising roughly 90% of all *KRAS* mutations [19,48,59]. *NRAS* mutations have been identified in multiple subtypes of melanoma including acral lentiginous

Table 1. NRAS mutations in various malignancies.

Cancer type	Frequency (%)	Ref.
Melanoma	15–30	[42–45]
Acute myeloid leukemia	10	[46,47]
Colon cancer	1–2	[48]
Thyroid cancer	10	[49]
Acute lymphoblastic leukemia	10–15	[50–52]
Multiple myeloma	15–20	[53]
Myelodysplastic syndrome	5	[54]
Juvenile myelomonocytic leukemia/chronic myelomonocytic leukemia	20	[55,56]

Figure 1. N-Ras signaling and effector pathways. (A) In normal melanocytes, N-Ras is inactive until a stimulus (e.g., growth factor) binds its receptor tyrosine kinase, which promotes exchange of GDP to GTP by GEFs, thereby activating N-Ras. In normal melanocytes, activation of N-Ras signaling is mediated by the Raf-MEK-ERK pathway. (B) In *NRAS* mutant melanoma, the N-ras protein is unable to hydrolyze GTP to GDP, and remains in the GTP-bound, active state. Oncogenic N-Ras stimulates various Ras effector pathway, including Raf, PI3K/Akt, and RAL. Ras-Raf-MEK-ERK signaling drives cellular proliferation, as does the PI3K/Akt pathway. Downstream signaling can be disrupted via inhibition of MEK signaling, and in cooperation with MEK inhibition. Disruption of CDK4/6 signaling cooperates to disable tumor growth in *NRAS* mutant melanoma. There is some suggestion the response to immune checkpoint blockade is enhanced in patients with *NRAS* mutant melanoma, but the reports are limited and a proposed mechanism is lacking. Treatment of *NRAS* mutant melanoma (*) involves targeting MEK, PI3K/Akt, CDK4/6, or immune checkpoints (PD1, CTLA-4).

melanoma and sinonasal mucosal melanomas with a frequency of ~15% [60,61]. In an MD Anderson Cancer Center series, *NRAS* mutations were the most common genetic lesion in acral and mucosal melanomas, present in 20–25% of patient samples [44]. There are reports of regional differences in the incidence of *NRAS* and *BRAF* mutations, which may contribute to the variability in the frequency of *NRAS* mutations in various series [62,63].

Clinically, *NRAS* mutant melanoma is associated with thicker primary melanomas and a poorer prognosis compared with *BRAF* mutant melanoma, with 75% occurring in tumors >1 mm in thickness compared with 40% of *BRAF* mutant melanomas [64]. *NRAS* mutations are more commonly seen in melanomas arising on the extremities. They are also less likely to exhibit ulceration, but are associated with a higher mitotic rate compared with

BRAF mutant melanoma [64]. *NRAS* and *BRAF* mutant melanoma are both more likely to arise from skin not exposed to chronic sun damage, and both are associated with a higher rate of central nervous system metastases compared with patients with *NRAS* and *BRAF* wild-type melanoma [65]. *NRAS* mutation status has been reported as independent risk factor of shorter survival following a diagnosis of stage IV melanoma, but this has been challenged in other analyses [65,66].

NRAS mutations appear to be an early event in melanomagenesis. Mutational status of nevi-associated melanomas has demonstrated that the majority of samples share the same mutational profile, with 60–70% concordance between melanomas and associated nevi [67]. As a consequence, it has been postulated that mutations in *NRAS* (and *BRAF*) that are found in benign and malignant nevi result from UV damage. However, evaluation of specific base pair changes within the *NRAS* and *BRAF* genes do not demonstrate a mutational signature enriched for UV-induced cytosine to thymidine transitions [68]. Importantly, both *NRAS* mutant and NF1-inactivated melanomas demonstrate a high mutational burden with a strong UV signature [21]. *NRAS* and *BRAF* mutations are for the most part mutually exclusive, however coexisting mutations have been reported [69–71]. While *NRAS* mutations are rarely found in benign nevi, *BRAF* mutations are evident in the majority of benign nevi [72]. In contrast, mutations in *NRAS* have been identified in 80–90% of large and giant congenital melanocytic nevi (CMN) and 50–70% of small and medium congenital melanocytic nevi [73,74], while *BRAF* mutations are rare. Interestingly, development of neurocutaneous melanocytosis is seen in a minority of patients with CMN, but *NRAS* mutations were present in three-quarters of these patients [74].

NRAS mutations are diagnosed by sequencing of primary tumor samples, which is increasingly available as a part of many next-generation sequencing platforms [44]. Knowledge of a patient's *NRAS* mutational status does not currently impact management outside of the clinical trial setting, and therefore routine mutational profiling is rarely performed outside of academic centers. Most next-generation sequencing platforms or multiplexed PCR platforms evaluate for mutations in codons 12, 13 and 61. Immunohistochemical techniques for detection

of common *NRAS* mutations has recently been described, but are not widely available [75].

Aberrations in proteins that influence the activity/function of Ras, can also promote melanomagenesis. Neurofibromatosis type 1 results from mutations in neurofibromin (*NFI*) and is characterized by the development of tumors of neuroectodermal origin, particularly cutaneous neurofibromas. NF1 is a Ras-GTPase activating protein (Ras-GAP) that functions normally to restrict the activity of Ras proteins, and loss of NF1 signaling results in enhanced Ras signaling. NF1 is, therefore, considered a 'RAS-opathy' as the growth of these tumors results from aberrant Ras signaling [76]. Somatic deletions and mutations in *NFI* have been described in cutaneous melanoma and are associated with growth-dependent Ras-Raf-MEK signaling [77,78]. Recently, *NFI* mutations were found to be enriched in patients with desmoplastic melanoma [79]. Loss of NF1 has also been shown to mediate resistance to BRAF inhibitors [80].

In patients with *BRAF* mutant melanoma treated with vemurafenib or dabrafenib, mutations in *NRAS* and *KRAS* have been demonstrated in roughly 10–20% of resistant cancers [81,82]. In many cases this was due to acquisition of a codon 61 *NRAS* mutation that was not detected in the original tumor [83]. *RAS*, *NFI* and *BRAF* mutations are generally mutually exclusive and typically do not co-occur in human melanoma, but there is evidence to support the existence of *BRAF* mutant and *NRAS* mutant clones within the same tumor [21,69,71]. Acquired resistance to BRAF inhibitors occurs through several mechanisms, including *NRAS* mutation [83], upregulation of signaling through RTKs (including EGFR) [83], activation of COT (*MAP3K8*) [84], MEK mutations, loss of NF1 [80], amplification of *BRAF*, alternative *BRAF* splicing and stromal secretion of growth factors [85,86].

Ras signaling is also central to one of the unique side effects of BRAF inhibitors. Treatment with vemurafenib or dabrafenib is associated with the development of secondary malignancies, most commonly keratoacanthomas and cutaneous squamous cell carcinomas due to paradoxical activation of MAPK signaling in keratinocytes. These occur in up to a quarter of patients usually within the first 3–6 months after initiating therapy, and are more common with increasing age [87]. *RAS* mutations have been identified in the majority of these squamous cell carcinomas, in particular mutations in *HRAS* [88,89]. The

incidence of these therapy-related squamous cell carcinomas is markedly reduced when BRAF inhibitors are given with MEK inhibitors.

Strategies for treating *NRAS* mutant melanoma

While *BRAF* mutant melanoma has been successfully targeted with BRAF inhibitors, and more recently with BRAF and MEK inhibitors in combination, no such strategy exists for *NRAS* mutant melanoma. Given the success of small molecules targeting BRAF and MEK in *BRAF* mutant melanoma, there is growing interest in developing small molecule inhibitors for the 50% of patients without *BRAF* mutations, especially those with *NRAS* mutations. Outside of a clinical trial, the treatment of *NRAS* mutant melanoma at this time remains no different than other *BRAF* wild-type melanomas [36,90]. Most current trial options are early phase trials, and there are no published large, randomized studies in *NRAS* mutant melanoma to date, although some Phase III trials are open and accruing. *NRAS* and *BRAF* mutant melanoma share a poor response systemic cytotoxic chemotherapy [66].

Despite decades of research since the initial discovery of oncogenic Ras, an effective therapy for *RAS* mutant cancer has remained elusive. The three primary approaches that have been evaluated for targeting Ras are: direct Ras inhibitors, Ras post-translational modification inhibitors, and Ras effector pathway inhibitors. A fourth potential option for treatment of *RAS* mutant cancers is immunotherapy, although it is not selective for Ras. Immune checkpoint inhibitors are now first-line for *BRAF* wild-type melanoma, and while predictors of response to immunotherapy remain poorly understood, there is some suggestion that *NRAS* mutant melanoma may have higher response rates to immunotherapeutic agents.

Targeting Ras itself, both using direct inhibitors and by disrupting key post-translational modifications have been largely unsuccessful. Efforts to develop direct enzyme inhibitors of Ras GTPases have been unsuccessful for two primary reasons [91]. First, given the low picomolar GTP concentration required for GTPase function relative to the high intracellular concentration of GTP, competitive small molecule inhibitors are not feasible. Second, targeting the molecular switch from GTP to GDP has proven challenging as GDP stabilizes the inactive molecule. In the 1990s, efforts to disrupt

RAS signaling by inhibiting enzymes responsible for key post-translational modifications to RAS were evaluated. RAS requires post-translational modification by farnesylation of critical cysteine residue in the C-terminus for proper membrane targeting [92]. Farnesyl transferase inhibitors were developed and showed promising activity in preclinical studies [93], but yielded disappointing results in early phase clinical studies [94]. There have been no further studies of inhibitors of Ras post-translational modifications.

Current approaches to targeting *RAS* mutant cancer, including *NRAS* mutant melanoma, now focus on targeting various downstream effector pathways, such as MEK-ERK, PI3K/Akt, RAL, and others (Figure 1). The only clinical trials to date that have evaluated any drugs prospectively in *NRAS* mutant melanoma have investigated the impact of MEK inhibitors, which have shown clinical activity in other *RAS* mutant cancers [95]. There have been a handful of early phase trials of various MEK inhibitors (reviewed in [57]), although none were specifically for *NRAS* mutant melanoma. Nevertheless, a phase II study of binimetinib (MEK162) demonstrated some clinical activity in patients with *NRAS* mutant melanoma. Although there were no complete responses, 6 of 30 (20%) patients had a partial response to MEK162 [96].

Given the limited activity of single agent MEK inhibition in other cancers as well as *BRAF* mutant melanoma, newer trials are evaluating the efficacy of a MEK inhibitor in combination with other agents. Preclinical work has identified CDK4 as a resistance mechanism to single-agent MEK inhibition, and demonstrated that combined treatment was more effective than single agent MEK in xenograft models of *NRAS* mutant melanoma [97]. The combination of MEK and CDK4/6 inhibition moved into phase I studies in the preliminary data were presented at ASCO 2014. In this study, patients with metastatic *NRAS* mutant melanoma were treated with combination binimetinib (MEK162) and LEE011 (a CDK4/6 inhibitor) in which 7 of 22 patients had a clinical response and 11 patients had stable disease [98]. A separate, phase I/II, dose-escalation study evaluating a different MEK + CDK4/6 combination (trametinib with palbociclib) is currently enrolling patients (NCT02065063). Combination of MEK inhibition with PI3K/Akt/mTOR inhibition is another strategy under investigation based on compelling preclinical data, and further supported by the high levels of Akt3 overexpression in *RAS* mutant

melanoma in the TCGA analysis [21,99,100]. There are now several trials underway evaluating various drug combinations for patients with *NRAS* mutant melanoma (Table 2), and many more pre-clinical studies evaluating novel drugs and drug combinations (Table 3).

Immunotherapy in *NRAS* mutant melanoma

In *BRAF* wild-type melanoma, immunotherapy with either CTLA-4 inhibition or PD-1 inhibition is now first-line therapy, with recent reports suggesting PD-1 immune checkpoint inhibitors have higher response rates with a lower incidence of toxicity. Moreover, combination immune checkpoint blockade with ipilimumab and nivolumab demonstrated higher response rates than either drug alone, albeit with a much higher rate of grade 3 or 4 toxicities. Immunotherapy offers the possibility of prolonged and durable responses in the metastatic setting, but only in a small subset of patients. Efforts are underway to increase the number of patients who respond to immune checkpoint inhibitors. Presently, there are no biomarkers established for routine clinical use, patient characteristics, or pathologic features that are strongly predictive of response to immunotherapies, but this remains an area of active investigation.

Increased expression of PD-L1 (the ligand expressed on some tumor cells that interacts with PD-1) is associated with increased response rates to PD-1 inhibition in many cancers. In melanoma, and some other cancers, higher expression of PD-L1 is associated with higher response rates [127,128]. However, PD-L1 positivity (or negativity) has not been predictive of response to PD-1 inhibitors equally across all studies and cancer types, prompting many to seek out other determinants and biomarkers to predict response to these agents.

BRAF status does not significantly influence the response to immune checkpoint inhibition, as response rates and progression-free survival data are comparable in patients with *BRAF* wild-type and *BRAF* mutant melanoma [13]. Differential expression of PD-L1 by specific driver mutation has been evaluated in cell culture, and failed to demonstrate a clear difference in PD-L1 expression based on *BRAF* and *NRAS* status in melanoma although PD-L1 expression appears to be variably affected by *BRAF* and *MEK* inhibitors [129].

Two, small retrospective studies suggest that responsiveness to immunotherapy may be influenced by *NRAS* mutational status. A retrospective analysis of patients that received high-dose IL-2 demonstrated a response rate of 47% in

Table 2. Clinical trials for *NRAS* mutant melanoma.

Clinical trial number	Phase	Population	Status	Drug(s)/notes
NCT01781572	Ib/II	Locally advanced or metastatic <i>NRAS</i> mutant melanoma	Currently recruiting	Binimetinib + ribociclib (LEE011) [101]
NCT02065063	I/II	Multiple cancers	Currently recruiting	Trametinib + palbociclib [102]
NCT01363232	Ib/II	Multiple cancers	Ongoing, no longer recruiting	PI3K inhibitor BKM120 + the MEK1/2 inhibitor MEK162 [103]
NCT01337765	Ib	Multiple cancers	CLOSED	PI3K/mTOR inhibitor BEZ235 in combination with the MEK1/2 inhibitor MEK162 [104]
NCT01449058	Ib	Multiple cancers	Currently recruiting	BYL719 plus MEK162 [105]
NCT01941927	II	<i>BRAF</i> wild-type mutation melanoma	Ongoing, no longer recruiting	Trametinib (2 mg) in combination with uprosertib GSK2141795 (25 mg) oral daily [106]
NCT01693068	II	<i>NRAS</i> mutant locally advanced or metastasis malignant cutaneous melanoma	Ongoing, no longer recruiting	Pimasertib (MEK 1/2 inhibitor) vs dacarbazine [107]
NCT01763164	III	Metastatic or unresectable cutaneous melanoma (<i>NRAS</i> Q61 mutant only)	Currently recruiting	NEMO: Phase III trial of binimetinib (MEK162) vs dacarbazine [108]
NCT01352273	Ib	Adult patients with advanced solid tumors harboring <i>RAS</i> or <i>BRAF</i> V600E mutations	Completed	MEK162 and RAF265 in adult patients with advanced solid tumors harboring <i>RAS</i> or <i>BRAF</i> V600E mutations [109]

Table 3. Select preclinical studies and early phase clinical trials for NRAS mutant melanoma.

Target	Agent(s)	Details	Ref.
MEK + PI3K/mTOR	Multiple	MEK and PI3K/mTOR1,2 inhibition is synergistic in xenograft model of <i>NRAS</i> melanoma	[110]
PI3K/mTOR + BRAF + MEK	Omipalisib (GSK2126458)	PI3K/mTOR (with dabrafenib + trametinib)	[111]
PKC delta	B106	Novel PKC delta inhibitors demonstrated activity in <i>NRAS</i> melanoma cell lines and triggers apoptosis (B106, most selective and potent)	[112]
c-MET	PHA665752	c-Met phosphorylation elevated in <i>NRAS</i> melanoma primary tumors	[113]
RAF	PRi	Enhanced efficacy of combination pan-RAF inhibitor (Amgen) with MEK inhibitor (trametinib) in <i>NRAS</i> melanoma cell lines	[114]
RAF	PLX7904	Novel Raf inhibitors – PLX7904 showed activity in <i>BRAF</i> mutant melanoma with a secondary <i>NRAS</i> Q61 resistance mutation	[115]
ERK	SCH772984	ERK 1/2 inhibitor, SCH772984, has single agent activity in both <i>BRAF</i> and <i>NRAS</i> mutant melanoma	[116]
Multitarget	Amuvatinib (MP-470)	Multitarget kinase inhibitor (activity against c-Kit, Axl, PDGFRA, Rad61) inhibited growth of <i>NRAS</i> (but not <i>BRAF</i>) mutant melanoma cell lines	[117]
HSP90	XL888	HSP90 inhibitor XL888 downregulated Wee1, AKT, and CDK4 in <i>NRAS</i> mutant melanoma <i>in vitro</i> and <i>in vivo</i>	[118]
GRM1 + mTOR	Riluzole	(GRM1 – metabotropic glutamate receptor 1 inhibitor) with mTOR inhibitor slowed melanoma growth regardless of <i>BRAF</i> status	[119]
MEK + TBK1		Overexpression of TBK1 (an effector of the RAS-RAL pathway) promoted invasive behavior and inhibition/depletion of TBK1 decreased invasive features, which was enhanced with a MEK inhibitor	[120]
mTOR + Wee1	MK-1775 + Torin 1	Wee1 inhibition potentiated inhibition of mTOR in <i>NRAS</i> mutant leukemia and melanoma	[121]
MEK/ERK + ROCK	GSK269962A + trametinib	MEK inhibitor (or ERK inhibitor) + ROCK inhibitor resulted in decreased proliferation and cell death <i>in vitro</i>	[122]
MEK + Plk1	JTP-74057 + BI 6727	Plk1 overexpressed in <i>NRAS</i> melanoma. MEK and Plk1 inhibition demonstrated activity <i>in vitro</i> and in xenograft model of <i>NRAS</i> melanoma (MEK inhibitor – JTP-74057 and Plk1 inhibitor – BI 6727)	[123]
MEK	PD325901	GAB2 induces angiogenesis in <i>NRAS</i> mutant melanoma (and is sensitive to MEK inhibition)	[124]
Akt/NF-kB	BI-69A11	BI-69A11 (inhibitor of Akt/sphingosine kinase-NF-kB) Inhibited tumor growth in a murine model of <i>NRAS</i> melanoma	[125]
MEK	Trametinib + metformin	Combination metformin and trametinib decreased <i>NRAS</i> melanoma growth <i>in vitro</i> and in xenograft model	[126]

patients carrying *NRAS* mutations compared with 23% for those with *BRAF* mutations, and 12% that were *NRAS-BRAF* wild-type [130]. While the finding was statistically significant and compelling, it was a retrospective evaluation of a small patient population. Out of the 208 patients identified that were identified in the initial review, only 108 had available tissue for testing, there were only 7 out of 15 patients with *NRAS* mutations. Furthermore, there was no improvement in progression-free survival or overall survival in patients harboring *NRAS* mutations.

More recently a retrospective analysis of patients treated with immunotherapy (including high dose-IL2, ipilimumab, and PD-1/PD-L1 antibodies) was performed to evaluate response rate by mutational status [131]. Sixty of 229

patients had *NRAS* mutations (most in codon 61), and this cohort had a statistically significant higher response rate (28 vs 16%) and clinical benefit rate (50 vs 31%), with a trend towards significance in progression-free survival, overall survival and response to any-line of immunotherapy. Subgroup analysis revealed that the *NRAS* mutant cohort had a particularly impressive response to immune checkpoint inhibitors, most notably with respect to the clinical benefit rate and response rate to PD1/PD-1L therapy. Clinical benefit was seen in 8 out of 11 patients (73%) with *NRAS* mutant melanoma treated with PD-1/PD-1L therapy compared with 13 out of 37 (35%) in the non-*NRAS* mutant group (WT and *BRAF* mutant). The proportion of PD-L1-positive cells was also higher in *NRAS* mutant melanoma samples (from

an independent cohort of archived samples), although this result was not statistically significant. It is conceivable that enhanced responses to immune checkpoint inhibition in this patient population is due to increased neoantigen formation in the background of a higher mutational burden of associated with *NRAS* mutant melanoma [21]. However, this is purely speculation and future studies in larger populations will be required to substantiate these findings, and to determine the mechanism whereby *NRAS* mutant melanoma might predict improved response rate to immunotherapies.

Conclusion & future perspective

The incidence of melanoma in the USA and worldwide has risen over the last two decades. Aberrant signaling through the Ras-Raf pathway is present in up to three-quarters of all melanomas, and anywhere from 15–30% of melanomas harbor mutations in *NRAS*. While the treatment of melanoma has been revolutionized over the last decade, there are no specific targeted agents for patients with *NRAS* mutant melanoma. Efforts to target *NRAS* mutant melanoma have failed to yield substantial clinical benefit to date, but there is a growing body of preclinical evidence identifying new potential drug targets. Moreover, several additional drug combinations are currently under investigation for *NRAS* driven melanoma. As the molecular understanding of *NRAS* mutant melanoma continues to evolve, new therapies are likely to emerge. *NRAS* mutant melanoma may have higher response rates to immunotherapies, and in particular PD-1/PD-L1 inhibition, but this will require validation in larger, prospective studies.

Improved understanding of the molecular basis of melanomagenesis, metastasis, clonal evolution, as well as identification of factors that predict both response and resistance will

inform the evaluation of strategies to target *NRAS* mutant melanoma. Paired analysis of primary tumors and sites of metastases to better understand mutational stability and acquisition of new mutations will likely provide additional insight into mechanisms of resistance and response [132], as will identification of molecular determinants of resistance [82]. Once a successful drug or drug combination is identified, characterizing exceptional responders may identify additional factors that can guide selection of therapies [85]. In addition to profiling tumors for acquired mutations that influence response to therapies, identification of germline mutations or variants in genes that influence inflammatory response may also inform our understanding of the basis of response to immune-targeted agents, as was recently demonstrated with a patient with lung adenocarcinoma that had an exceptional response to anti-PD-L1 therapy and was found to have a germline *JAK3* mutation [133]. With better understanding of both *NRAS* melanoma and determinants of immunotherapy response, combinations of targeted agents with immune checkpoint inhibitors may be viable option to improve both response rates and durability of responses, as has been suggested for *BRAF* mutant melanoma [134,135], although rational selection of agents and close monitoring with be essential to minimize both toxicity [136] and loss of efficacy.

Financial & competing interests disclosure

RW Jenkins has served as a consultant for Novartis and Astex Pharmaceuticals. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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