Commentary

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Melanoma Management

Combating NRAS mutant melanoma: from bench to bedside

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"Oncogenic NRAS plays a critical role in melanoma initiation and maintenance; however, to date there are no effective ways to directly block the activity of mutant NRAS."

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NRAS is the second most common oncogenic driver in melanoma, mutated predominantly at codon 61 in almost 30% of all melanomas [1]. Tumors bearing *NRAS* mutations are highly aggressive and are associated with shorter patient survival [2]. Despite the prevalence of *NRAS* mutations and the severity of the resulting disease, treatment for NRAS mutant melanoma has lagged far behind BRAF-mutant tumors. Here, we summarize the status of the most promising strategies, highlighting the successes and the gaps that remain to be filled.

Under normal physiological conditions, NRAS acts as a molecular switch, cycling between an inactive GDP-bound state and an active GTP-bound state. The hydrolysis of GTP is stimulated by GTPase activating proteins (RasGAPs) and suppressed by guanine-nucleotide exchange factors (RasGEFs) [3]. In contrast, the mutant form of NRAS is refractory to GAPs, leading to constitutive NRAS activation and persistent intracellular signaling that triggers uncontrolled cell proliferation and tumor cell survival.

Oncogenic NRAS plays a critical role in melanoma initiation and maintenance; however, to date there are no effective ways to directly block the activity of mutant NRAS. Developing small molecules that bind directly to NRAS has been extremely challenging, due to the lack of hydrophobic pockets deep enough to properly fit a small molecule, the high affinity of NRAS for GTP, and the high intracellular concentration of GTP, among other factors [3]. While direct targeting of mutant NRAS has been elusive, novel preclinical strategies targeting KRAS including RAS mimetics that prevent RAS/effector interaction, and monobodies specific for the dimerization interface of KRAS are showing promise [4,5]. Should these strategies be proven effective, they may pave the way for comparable approaches against NRAS.

Indirect approaches to combat NRAS tumors include blocking the horizontal and vertical signaling networks hyperactivated in NRAS-mutant melanoma, mainly RAF/MEK/ERK and PI3K/AKT/mTOR [6]. Since, NRAS mutant melanoma is refractory to BRAF inhibitors, as they induce RAF dimerization, enzyme transactivation, and paradoxical MAPK activation [6], pan-RAF and RAF dimer inhibitors such LY3009120, have been tested and shown antitumor activity in KRAS-, NRAS- and BRAF-mutant tumors (NCT02014116) [7]. Currently, a combination of the pan-Raf inhibitor LHX254 and the anti-PD-1 antibody PDR001 is being evaluated in clinical trials (NCT02607813). In the last two decades, many MEK inhibitors (MEKi) have been developed and tested. To date, the most effective MEKi for melanoma are trametinib and binimetinib, with confirmed overall response rates of 29 and 15% (NCT01763164) [8,9]. However, MEKi have not shown significant efficacy in NRAS mutant melanoma patients as single agents [8]; hence, MEKi-based combinations are now being explored. The most promising has been the combination of the MEKi binimetinib and the CDK4 inhibitor ribociclib, with 33% of patients achieving partial response and 52% of patients with stable disease (NCT01781572) [10]. Cotargeting parallel pathways by combining MEK and PI3K/mTOR1/2 inhibitors has synergistic activity in preclinical models, but limited success in clinical trials, and is often associated with high toxicity (NCT01390818) [6]. For example, combinations of the MEKi trametinib and the PI3K/mTORi omipalisib (GSK2126458) showed moderate activity in NRAS mutant melanoma cells, but failed to demonstrate efficacy in a trial for patients with advanced solid tumors, including

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melanoma (NCT01248858). MEKi have also been combined with inhibitors of receptor tyrosine kinases, which are upstream of NRAS and often activated following inhibition of NRAS effectors. A Phase I clinical trial using the METi tivantinib and the multikinase inhibitor sorafenib led to an overall response rate of 20% in NRAS mutant melanoma patients and median progression-free survival of 5.4 months with no unforeseen toxicities [11]. Notably, 28% of the treated patients had high expression of MET with 4/4 patients with high MET levels achieving disease control, but response rates were not significantly different between MET-high and MET-low melanoma patients. Future studies with larger patient cohorts could assess whether the expression of MET might predict the likelihood of response to this combination. Another approach has involved targeting ERK, a downstream effector of MAPK; for example, the ERK inhibitor MK-8353 (SCH772984) has shown efficacy in NRAS-mutant melanoma cells [12]. Other ERK inhibitors undergoing clinical evaluation include LTT462 (NCT02711345), and GDC-0994, which is being tested in combination with cobimetinib in a Phase I trial (NCT02457793).

Since, therapies targeting solely the RAF/MEK/ERK (i.e., MAPK) pathway have not produced meaningful effects in NRAS-mutant melanoma, there is an urgent need to identify novel actionable targets and to develop more effective therapeutic strategies. One potential approach is to identify targets that when blocked are synthetic lethal with oncogenic NRAS, using chemical or genetic (shRNA/siRNA or CRISPR) screens and/or computational methods. An *in vivo* shRNA screen of patient-derived tumors identified *KMT2D* as a critical driver of cell migration and *in vivo* growth of NRAS mutant melanoma [13]. CRISPR screens hold great promise for discovering novel therapeutic targets; for example, CRISPR screens led to the identification of the chromatin-binding proteins TADA2B and TADA1 (members of the STAGA transcription coactivator histone acetyltransferase complex) as two novel genes whose loss confers resistance to BRAF inhibitors [14] in BRAF mutant melanoma cells. These studies underscore the relevance of epigenetic alterations in malignant melanoma as disease drivers and novel druggable targets. Additionally, using computational approaches, Segura *et al.* demonstrated high mRNA levels of the epigenetic reader BRD4, a member of the BET family of proteins, in melanoma [15]. Subsequently, BETi/BRDi have been tested in melanoma, revealing great potential for clinical application [16]. To achieve optimal efficacy and translational potential, combinations of inhibitors of epigenetic drivers, like BETi, with small molecules targeting critical melanoma pathways will likely be needed. It would be worth pursuing this avenue in NRAS mutant melanoma.

Due to the success of immunotherapy and the lack of selective therapies for NRAS mutant melanoma, most patients with NRAS mutant tumors receive anti-CTLA-4, anti-PD-1 or anti-PD-L1 as first-line therapy. A retrospective study found that 50% of patients with NRAS-mutant melanoma achieved clinical benefit (complete response, partial response or stable disease) and had better outcomes when receiving any type of immunotherapy compared with BRAF-mutant or triple-WT patients [17]. High PD-L1 expression in NRAS-mutant melanoma compared with other genotypes suggests a potential link with superior treatment response in this cohort [17]. Nonetheless, not all patients benefit from immunotherapies, many experience severe adverse events, and tumors often recur. Factors underlying variability of response and immune-related adverse events need further investigation. In addition to PDL1 levels, low mutational load, and JAK signaling, poor response to PD-1 blockade has been recently shown to be associated with activation of the PI3K pathway via loss of PTEN [18]. Hence, combining PI3Ki with anti-PD-1 may improve anti-PD-1 efficacy; however, isoform selective inhibitors may be needed to achieve optimal antitumor activity with an acceptable therapeutic index (NCT02646748). Additionally, inhibitors of BET family epigenetic readers modulate PD-L1 expression through inactivation of NF-B in melanoma cells, hence offering an alternative strategy to target PD-L1.

Since immunotherapies have dramatically improved melanoma management, combination strategies are currently being tested in patients, including combinations with targeted therapies, which can modulate immune responses, with anti-PD-1 or anti-PD-L1. A Phase II trial is evaluating the efficacy of anti-PD-1 plus trametinib in NRAS mutant melanoma (NCT02910700). Similarly, anti-PD-1 plus the DNA methyltransferase inhibitor azacitidine is in Phase II trials (NCT02816021). The rationale for this combination is that alterations in the epigenome can modulate multiple components of the immune system, thereby enhancing immune responses.

To test whether host–antitumor immune responses could be further enhanced, a series of clinical trials are evaluating strategies modulating the tumor microenvironment; for example, and on-going trial is testing the TLR9 agonist IMO-2125 in combination with anti-CTLA-4 or anti-PD-1 (NCT02644967). So far, IMO-ipilimumab has led to clinical responses in anti-PD-1 refractory patients [19]. Adoptive T-cell therapies, aimed at breaking tumor-related immune tolerance, are also being tested. One intervention consists of lympho-depleting chemotherapy, infusion of T cells, and high or low doses of IL-2; participants who achieve stable or partially responding disease then

receive pembrolizumab (NCT02500576). In another, an infusion of CD8+ T cells targeting mutant $KRAS^{G12D}$ produced antitumor efficacy and 9-month partial response in a colorectal cancer patient; a similar strategy could be used to target NRAS mutant melanoma [20]. The recent approval of CAR T-cell therapies for acute lymphoblastic leukemia will likely spur the development of novel approaches using T cells to target NRAS mutant tumors.

The prognosis for melanoma patients is radically changing due to novel treatments, particularly combination therapies. A better understanding of the molecular intricacies of oncogenic NRAS is critical to develop rational combination therapies targeting NRAS-mutant melanoma. A limitation to test novel strategies is the scarcity of suitable preclinical models to identify synergistic combinations and dissect their complex mechanism of action. Testing the effect of treatment sequence on tumor growth, survival time and toxicities would be necessary. Successful strategies likely will need to target not only the tumor cells but also the microenvironment, harnessing its interactions with the immune system. Additionally, resistance to treatment, both intrinsic and acquired, remains a major challenge. While there is still a long road ahead, we are a step closer to novel and more efficacious strategies that can be translated into the clinic to improve the lives of people with NRAS mutant melanoma.

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