Melanoma driver mutations and immune therapy

Douglas B. Johnson1,1,*, Christine M. Lovly1,1, Ryan J. Sullivan2, Richard D. Carvajal3, and Jeffrey A. Sosman1

1Vanderbilt University Medical Center and Vanderbilt-Ingram Cancer Center, Nashville, TN; 2Massachusetts General Hospital, Boston, MA; 3Columbia University Medical Center, New York, NY

These authors contributed equally.

Introduction

Melanoma treatment approaches have been revolutionized by the development of effective genetically-targeted therapies and novel immune therapies. BRAF and/or MEK inhibitors induce tumor regression and improve survival in the 40–50% of patients that harbor recurrent BRAF6600 mutations. Novel MEK inhibitors as single agents and in combination may be effective for those with NRAS-mutated melanoma (15–20%), a cohort with inferior survival rates in historical series.1,2 Although these mutations clearly impact clinical outcomes for patients treated with genetically-targeted therapies, their interaction with immune therapy is far less well characterized.

Immune checkpoint inhibitors, including agents targeting CTLA4 (cytotoxic T lymphocyte antigen-4, ipilimumab) and PD-1/PD-L1 (programmed cell death-1/ligand; nivolumab, pembrolizumab, MPDL3280A), produce durable responses in an increasing proportion of patients. Furthermore, the toxicities of these agents are largely tolerable, making them attractive treatment options. Identifying predictive biomarkers has been a challenge and remains a critical priority to optimize treatment selection, minimize costs, and inform clinical trial design. Several candidate biomarkers have been identified, including tumor or stromal expression of PD-L1, profiles and diversity of infiltrating T cells, and detection of tumor neoantigens induced by somatic mutations.3–6 Despite these promising approaches, the link between the immune response and well-characterized oncogenes (such as BRAF and NRAS) has remained poorly understood. Studies in this arena have largely focused on the impact of BRAF/MEK targeted therapies on the immune microenvironment.7 One retrospective study, however, has suggested that NRAS mutations may correlate with response to interleukin-2 (IL-2).8

NRAS Mutations and Response to Immune Therapy

In our study, we assessed whether common oncogenic driver mutations (in BRAF and NRAS) would correlate with response to immune therapy in patients with advanced melanoma.9 We conducted retrospective clinical data review to assess the NRAS and BRAF mutation status of 229 patients from 3 cancer centers who received immune therapy (the vast majority with immune checkpoint inhibitors). We observed that patients with NRAS-mutated melanoma had superior response rates to first-line immune therapy (28% vs. 16%, p = 0.04) and superior clinical benefit (objective responses plus stable disease lasting >6 months; 45% vs. 26%, p < 0.01). The NRAS cohort also trended toward improved progression-free survival compared to those with other genotypes (BRAF-mutated or NRAS/BRAF wild type) although overall survival was similar in these groups. Given the poor historical outcomes for NRAS-mutant melanoma, we speculated that the lack of an overall survival benefit for this group may have been driven by rapid progression and lack of additional effective therapies for patients that failed to respond (such as BRAF-targeted therapy in BRAF-mutant patients). Indeed, the non-responders with NRAS mutations did have inferior survival and poor outcomes compared to other genetic subtypes.

We assessed whether improved response rates in the NRAS group occurred across immune therapies or whether there were therapy specific differences. Patients with NRAS mutations had higher rates of clinical benefit to ipilimumab (42% vs. 19%), but no difference in objective response rates (19% vs. 11%) and no difference in outcomes to high-dose interleukin-2. Strikingly, we noted a 64% response rate for patients with NRAS-mutated melanoma treated with anti-PD-1. In view of these unexpectedly high rates of objective response, we hypothesized that PD-L1 expression might be higher in melanomas with NRAS mutations, either through direct promotion of PD-L1 by mutated NRAS or through co-occurrence of these factors on a more immunogenic subset of melanomas. To investigate, we performed PD-L1 staining on a cohort of 32 melanoma samples. The NRAS group had seemingly more frequent expression of PD-L1

*Correspondence to: Douglas Johnson; E-mail: douglas.b.johnson@vanderbilt.edu
Received: 05/08/2015; Accepted: 05/08/2015
http://dx.doi.org/10.1080/2162402X.2015.1051299
compared to BRAF-mutated and NRAS/BRAF wild type samples (50% vs. 30%; 6 of 12 samples vs. 6 of 20 samples with ≥5% expression), but this was not statistically significant.

**Future Directions and Outstanding Questions**

Although our study strongly suggests a link between NRAS mutated melanoma and improved response to immune therapy, we were not able to conclusively identify a mechanism for this observation. Mutated NRAS may directly mediate immunogenicity through dysregulation of intracellular pathways that induce upregulation of PD-L1, melanoma-specific antigens, or cause cytokine/chemokine production. Conversely, NRAS mutations may simply occur more frequently on melanomas that harbor high numbers of somatic mutations and resultant peptide neoantigens. Two intriguing recent studies have demonstrated a strong correlation between mutation burden and response in melanoma (with ipilimumab) and in nonsmall cell lung cancer (with pembrolizumab).6,10 We are currently assessing whether total numbers and types of mutations (other than NRAS) influence response to anti-PD-1 through targeted next generation sequencing approaches.

This study may have applications for various immune and targeted therapy combinations. With the advent of effective MEK inhibitor-based regimens, combining these agents and other experimental therapeutics with anti-PD-1 appears feasible. The NRAS subset may be particularly likely to benefit, although toxicities and lymphocyte function will require close monitoring.

In conclusion, developing clinically accessible biomarkers for immune therapies remains a major need. As anti-PD-1 therapies are used across an increasingly wide range of cancer types, this need will grow in importance. In melanoma, NRAS mutations could potentially serve as a marker of response in combination with other predictive molecular or clinical features.

**Funding**

This work was supported by K12 CA0906525 (DBJ and CML).

**References**

1. Ascierto PA, Schadendorf D, Berking C, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. The lancet oncology 2013;14:249–56.
2. Jakob JA, Bassett RL, Jr., Ng CS, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. Cancer 2012;118:4014–23.
3. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443–54.
4. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563–7.
5. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568–71.
6. Snyder A, Makarov V, Mergnhoub T, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. The New England journal of medicine 2014.
7. Hu-Lieskovan S, Robert L, Homer Moreno B, Ribas A. Combining Targeted Therapy With Immunotherapy in BRAF-Mutant Melanoma: Promise and Challenges. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014.
8. Joseph RW, Sullivan RJ, Harrell R, et al. Correlation of NRAS mutations with clinical response to high-dose IL-2 in patients with advanced melanoma. J Immunother 2012;35:66–72.
9. Johnson DB, Lovly CM, Flavin M, et al. Impact of NRAS Mutations for Patients with Advanced Melanoma Treated with Immune Therapies. Cancer immunology research 2015;3:288–95.
10. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in nonsmall cell lung cancer. Science 2015.