EDITORIAL

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Melanoma Management

Management of 'pan-negative' melanoma: current and emerging strategies

"…advancements in both immune-based therapies and molecularly targeted agents have greatly expanded the treatment options for patients with this disease."

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The incidence of melanoma in the USA has been increasing over the past decade, with an estimated 9480 deaths expected in 2013 [1]. Metastatic melanoma has historically carried an especially poor prognosis, with a median survival of 6–9 months. In recent years, however, advancements in both immune-based therapies and molecularly targeted agents have greatly expanded the treatment options for patients with this disease. The identification of molecularly defined cohorts in melanoma has facilitated many of these advances and enabled more effective treatment stratification. Oncogenic driver mutations in *BRAF* are present in 40–50% of advanced melanomas and confer sensitivity to selective BRAF inhibitors (vemurafenib and dabrafenib) and MEK inhibitors (trametinib). These molecularly targeted agents result in improved response rates and clinical outcomes compared with cytotoxic chemotherapy [2,3]. Although less common, *CKIT* mutations (largely identified in melanomas arising in the acral or mucosal surfaces) may predict benefit from imatinib [4]. Even melanomas with *NRAS* mutations (present in 15%), long considered to be an 'undruggable' target, may respond to recently developed MEK inhibitors [5]. Despite these advances, the remaining 30–40% of melanomas do not harbor identifiable driver mutations by conventional clinical assays and are considered to be 'pan-negative' [6]. The lack of clearly actionable mutations or available targeted therapies makes this a challenging cohort in the clinic.

Molecular testing

Defining a melanoma as 'pan-negative' is dependent upon the type of genetic profiling performed. Traditional *BRAF* cobas® testing (Roche, Basel, Switzerland), a frequently used clinical testing method, identifies only the most common $BRAF^{V600E}$ mutation [7]. Although highly sensitive and specific for this particular point mutation, this assay may not uncover alternative $BRAF^{V600}$ mutations that may confer sensitivity to the available BRAF or MEK inhibitors (i.e., V600K and V600R,

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among others). In addition, this testing method does not identify mutations in *CKIT* or *NRAS*, which may have therapeutic implications for approved or experimental agents. At our institution, we routinely use the SNaPshot platform as a high-throughput screen for 'hotspot' mutations in *BRAF*, *NRAS*, *KIT*, *GNAQ*, *GNA11* and *CTNNB1* [6]. If no mutations are identified with this approach, we consider obtaining targeted, next-generation sequencing (NGS) in order to identify additional potentially actionable genetic alterations. FoundationOne™ (Foundation Medicine, MA, USA) is a targeted NGS platform that sequences the entire coding region of 236 genes and 47 introns from 19 genes that are commonly rearranged in cancer. This assay detects point mutations, deletions, amplifications and gene fusions in numerous genes with potential relevance in melanoma [8]. This or other similar testing platforms may facilitate clinical trial enrollment by identifying actionable mutations and should be strongly considered for patients in need of additional therapy options.

Immune-based therapy

Immune-based therapies remain the only approved, effective treatment options for patients with 'pan-negative' melanoma. High-dose IL-2 is still considered to be a first-line option for patients with a good performance status and normal organ function. Despite its severe, acute toxicities, durable complete responses are observed in 6–7% of treated patients [9]. Ipilimumab is an immune checkpoint inhibitor that demonstrates improved overall survival compared with cytotoxic chemotherapy and may be used in the first-line setting and in pretreated patients, including those who cannot tolerate the side effects of IL-2. Although classically defined objective responses are uncommon, a pivotal Phase III trial demonstrated that overall survival at 3 years was twice that of an experimental peptide vaccine (22%) [10]. Newer immune checkpoint inhibitors targeting PD-1/PD-L1 appear to have more activity with less toxicity than the currently approved immune-based therapies and can be considered in a clinical trial. It is not clear whether genotype influences responses to immune-based therapies; there has been some suggestion that the 'pan-negative' group has lower response rates compared with other genetic cohorts [11,12]. This association has not been confirmed prospectively, and further studies are needed in order to identify biomarkers for predicting responses to the immune-based therapies.

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Cytotoxic chemotherapy

Chemotherapy is the other approved class of therapeutics for 'pan-negative' melanoma. Response rates for dacarbazine and temozolomide are in the range of 10% and no consistent improvement in overall survival has been demonstrated [13]. We therefore only use these agents in rare circumstances where no other approved or attractive experimental agents are available.

Emerging targets

Despite the lack of *BRAF*^{V600} or *NRAS* mutations in 'pan-negative' melanomas, the MAPK pathway is dysregulated in the majority of these melanomas through alterations in *NF1*, *KRAS*, *CRAF*, *MAP2K1*, atypical *BRAF* mutations and others [14]. Targeting this pathway, therefore, will likely remain a critical component of targeted therapeutic strategies for this cohort. A Phase I study of trametinib, a MEK inhibitor, provides clinical support for this idea: 20 'pan-negative' patients were treated and four had objective responses (20%) [15]. Mutations in *BRAF* at loci other than V600 appear to constitute a subgroup that may particularly benefit from MEK inhibitor monotherapy based on preclinical studies [16]. These activating mutations occur in exons 11 and 15 (particularly codons 469, 597 and 601) and comprise approximately 10% of the 'pan-negative' cohort. In case reports and in early trials, several patients with atypical *BRAF* mutations had prolonged clinical responses to trametinib and TAK-733 (an experimental MEK inhibitor) [15,16]. Although selective BRAF inhibitors do not appear to be active in preclinical data, a single case of *BRAF*^{L597}-mutant melanoma that responded to vemurafenib has also been reported [17]. *BRAF* gene fusions are another recently identified alteration that probably includes up to 5% of 'pan-negative' melanomas, although the frequency has not been well defined. *BRAF* fusions induce the robust activation of MAPK signaling and are highly sensitive to MEK inhibition in laboratory studies [18]. We are planning a clinical trial of trametinib in advanced melanoma with atypical *BRAF* mutations and gene fusions.

Although a subset of patients may respond to single-agent MEK inhibition, combination strategies are probably needed for the majority of patients with 'pan-negative' melanoma. The

PI3K–Akt pathway is also frequently activated in melanoma, most commonly in the loss of *PTEN* (20–40% of tumors); point mutations in *PIK3CA*, *AKT3* and other regulatory components also occur in small percentages of patients [14]. Furthermore, mutations in upstream signaling mediators, such as receptor tyrosine kinases, *NF1*, *GNAQ* and *GNA11*, are predicted to activate the PI3K–Akt pathway. Combining inhibitors of this pathway with MEK inhibitors is an attractive potential strategy in 'pan-negative' tumors. Currently, this approach is being evaluated in several clinical trials.

Alterations in cell cycle regulation, particularly in *CDKN2A*, *CDK4* and *CCND1*, have also been uncovered in a large majority of melanomas [14]. Loss of *CDKN2A* (which encodes p16INK4A) or activating mutations in *CDK4* or *CCND1* induce aberrant cell cycle progression by inhibiting Rb1 function, a critical cell cycle checkpoint. Recently, preclinical data in *NRAS*-mutated melanomas showed enhanced sensitivity with combined inhibition of MEK and CDK4/6 [19]. A trial of MEK162 and LEE011 (experimental MEK and CDK4/6 inhibitors) is now being conducted and is showing great promise in *NRAS*-mutant melanoma. Among the first 21 patients treated, seven experienced objective partial responses (33%), with nearly all patients experiencing some degree of tumor regression [20]. Trials in the 'pan-negative' population are being planned with these agents, as well as with trametinib and palbociclib (CDK4/6 inhibitor). Given the frequency of MAPK and cell cycle changes, this combination in 'pan-negative' melanoma is a promising approach.

Other targets have various degrees of clinical and preclinical support. Inhibitors of angiogenesis may have some activity alone or in combination with other agents. Although bevacizumab did not improve survival in combination with carboplatin and paclitaxel compared with chemotherapy alone, axitinib has demonstrated an overall response rate of almost 20% (genotyping for responding patients was not available) [21]. Evaluation of VEGF inhibitors in combination with immune-based therapies is ongoing. In addition, inhibitors of ERK, the final step in classical MAPK signaling, are being developed. Although these are still in early stages of development, they are predicted to have activity in the majority of melanomas (including 'pan-negative' melanomas). Other possible targets with preclinical support include ERBB4, MERTK and mediators of apoptosis.

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Conclusion

'Pan-negative' melanoma comprises approximately 35% of this malignancy and is a genetically diverse cohort. Immune-based therapies, including IL-2, ipilimumab and anti-PD-1/PD-L1, are effective treatment options for these patients. Extended genetic profiling with NGS platforms may identify targets that facilitate clinical trial enrollment and should be considered in patients without other available treatment options. MEK inhibition is a promising strategy in some patients with 'pan-negative' melanoma. Further studies are needed in order to clarify the role of MEK inhibitors as monotherapies and in combination with other cell signaling/cell cycle inhibitors.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J. Clin.* 63, 11–30 (2013).

2 Chapman PB, Hauschild A, Robert C *et al.* Improved survival with vemurafenib in melanoma with *BRAF* V600E mutation. *N. Engl. J. Med.* 364(26), 2507–2516 (2011).

3 Flaherty KT, Infante JR, Daud A *et al.* Combined BRAF and MEK inhibition in melanoma with *BRAF* V600 mutations. *N. Engl. J. Med.* 367(18), 1694–1703 (2012).

4 Carvajal RD, Antonescu CR, Wolchok JD *et al. KIT* as a therapeutic target in metastatic melanoma. *JAMA* 305(22), 2327–2334 (2011).

5 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. *Lancet Oncol.* 14(3), 249–256 (2013).

6 Lovly CM, Dahlman KB, Fohn LE *et al.* Routine multiplex mutational profiling of melanomas enables enrollment in genotype-driven therapeutic trials. *PLoS ONE* 7(4), e35309 (2012).

• **Discusses the frequency of driver mutations in melanoma as identified on a melanoma SNaPshot panel.**

7 Halait H, Demartin K, Shah S *et al.* Analytical performance of a real-time PCR-based assay for V600 mutations in the *BRAF* gene, used as the companion diagnostic test for the novel BRAF inhibitor vemurafenib in metastatic melanoma. *Diagn. Mol. Pathol.* 21(1), 1–8 (2012).

8 Frampton GM, Fichtenholtz A, Otto GA *et al.* Development and validation of a clinical

cancer genomic profiling test based on massively parallel DNA sequencing. *Nat. Biotechnol.* 31(11), 1023–1031 (2013).

• **Describes the validation and results of FoundationOne™ (Foundation Medicine, MA, USA), a targeted, next-generation sequencing platform that may potentially identify actionable alterations in 'pan-negative' melanoma.**

9 Atkins MB, Lotze MT, Dutcher JP *et al.* High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J. Clin. Oncol.* 17(7), 2105–2116 (1999).

10 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).

11 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.* 35(1), 66–72 (2012).

12 Johnson DB, Lovly CM, Flavin M *et al.* *NRAS* mutation: a potential biomarker of clinical response to immune-based therapies in metastatic melanoma (MM). *J. Clin. Oncol.* 31, 9019 (2013).

13 Middleton MR, Grob JJ, Aaronson N *et al.* Randomized Phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. *J. Clin. Oncol.* 18(1), 158–166 (2000).

14 Hodis E, Watson IR, Kryukov GV *et al.* A landscape of driver mutations in melanoma. *Cell* 150(2), 251–263 (2012).

•• **Demonstrates the frequency of genetic alterations in melanoma, highlighting the frequency of alterations which promote MAPK signaling and cell cycle dysregulation.**

15 Falchook GS, Lewis KD, Infante JR *et al.* Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a Phase 1 dose-escalation trial. *Lancet Oncol.* 13(8), 782–789 (2012).

•• **Demonstrates that the approved MEK inhibitor trametinib has activity in a subset of 'pan-negative' melanomas. Highlighted are the responses in patients with alternative *BRAF* mutations.**

16 Dahlman KB, Xia J, Hutchinson K *et al.* *BRAF* L597 mutations in melanoma are associated with sensitivity to MEK inhibitors. *Cancer Discov.* 2(9), 791–797 (2012).

17 Bahadoran P, Allegra M, Le Duff F *et al.* Major clinical response to a BRAF inhibitor in a patient with a *BRAF* L597R-mutated melanoma. *J. Clin. Oncol.* 31(19), e324–e326 (2013).

18 Hutchinson KE, Lipson D, Stephens PJ *et al.* *BRAF* fusions define a distinct molecular subset of melanomas with potential sensitivity to MEK inhibition. *Clin. Cancer Res.* 19(24), 6696–6702 (2013).

• ***BRAF* fusions may define a subset of 'pan-negative' melanomas that may respond to MEK inhibitors.**

19 Kwong LN, Costello JC, Liu H *et al.* Oncogenic *NRAS* signaling differentially regulates survival and proliferation in melanoma. *Nat. Med.* 18(10), 1503–1510 (2012).

• **Highlights the preclinical promise of MEK/CDK4 inhibition in *BRAF* wild-type melanoma. It remains to be defined whether this combination will have efficacy in the 'pan-negative' population.**

20 Sosman JA, Kittaneh M, Lolkema MPJ *et al.* A Phase 1b/2 study of LEE011 in combination with binimetinib (MEK162) in patients with *NRAS*-mutant melanoma: early encouraging clinical activity. *J. Clin. Oncol.* 32, 9009 (2014).

21 Fruehauf J, Lutzky J, McDermott D *et al.* Multicenter, Phase II study of axitinib, a selective second-generation inhibitor of vascular endothelial growth factor receptors 1, 2, and 3, in patients with metastatic melanoma. *Clin. Cancer Res.* 17(23), 7462–7469 (2011).