BRAF Mutation in Cutaneous Melanoma

Subjects: Oncology

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In advanced melanoma, BRAF mutation testing is critical in predicting treatment response with targeted therapy (i.e., BRAF/MEK inhibitors). Certain features were identified in melanomas that harbor BRAF mutations (e.g., primary lesions located on the trunk, diagnosed in patients <50, visibly pigmented tumors and, at times, with ulceration or specific dermatoscopic features). For select advanced melanoma patients, delays in determining mutational status present a significant barrier to the prompt initiation of treatment. This can adversely impact patient outcomes, especially in the metastatic setting due to a rapidly progressive disease. Treatment in these cases needs to start promptly by a medical oncologist. Ordering BRAF testing by preceding members of the treating team will allow medical oncologists to initiate treatment at the first visit. According to poor survival outcomes, we propose that patients with thick tumors (>4.0 mm) or >2 mm tumors with ulceration (i.e., stage ≥IIB) should potentially be considered for systemic therapy, thus justifying reflex BRAF testing.

targeted therapy reflex testing BRAF inhibitor BRAF mutation MAPK pathway metastatic melanoma advanced melanoma stage II

1. Introduction

Melanoma incidence and mortality are continuously increasing in the United States, Canada, and other countries around the world [1][2][3][4]. Advances in our understanding of molecular pathways have led to improvements in the historically unfavorable prognosis of metastatic melanoma [5]. One of the most studied regulatory signaling pathways is the mitogen-activated protein (MAP) kinase pathway. In the early 2000s, it was discovered that many cases of metastatic melanoma exhibited inappropriate activation of this pathway through a mutated *BRAF* oncogene [6]. Since then, the development of targeted therapies to suppress this signaling have given *BRAF*-mutation status a critical role in the clinical decision making for the treatment of advanced melanoma.

Despite the importance of the MAP kinase pathway in the treatment of melanoma, there is no consensus at which time point *BRAF* mutation testing should take place during the workup of melanoma. For some patients, delays in determining mutational status present a significant barrier to the prompt initiation of treatment. This can adversely impact patient outcomes, especially in the metastatic setting, where patients may have rapidly progressive disease. Treatment in these cases needs to start promptly at the time of diagnosis.

2. *BRAF* Testing at the Time of Diagnosis

2.1. Overview of Diagnosis

The definitive diagnosis of melanoma requires histopathologic assessment of the tumor. Based on the eighth edition of the American Joint Committee on Cancer (AJCC) staging system, parameters of the primary tumor (T), lymph nodes and lymphatic drainage (N), and distant metastases (M) are used to determine the pathologic stage. Patients with primary tumors without spread are classified as stage I or II, depending on the tumor characteristics (tumor thickness and ulceration only). Tumors that have spread beyond the primary skin site as indicated by the presence of in-transit tumors, satellite tumors, or involvement of lymph nodes, but without distant metastases are classified as stage III. Patients with distant metastases are categorized as stage IV. Each stage carries a different risk of disease relapse and survival [7] (Table 1).

Table 1. Frequently reported features of melanoma found to be associated with *BRAF* mutation status.

Features/Outcomes	Primary Melanoma	Metastatic Melanoma
BRAF mutation prevalence	Primary melanoma: 33–47% [6]	Metastatic melanoma: 41–55% [6]
	Recurrent melanoma found to have higher frequency of *BRAF* mutation [8]	-
Patient Features		
Age of diagnosis	<50 [6][9]	Younger individuals [6]
UV exposure	High estimated lifetime exposure [10] and early-life exposure [10][11]	-
Total body nevus count	Patients with high number of nevi on back (>14) [10] more likely to harbor a *BRAF* mutation	-
Chronic sun-damaged skin	Fewer signs of chronic sun damage [9], such as lentigines [11] and solar elastosis [8][10]	Less chronic sun damage [6]
Melanoma Features		
Number of primary lesions	-	Occult or 1 lesion [6]
Location of primary melanoma	Truncal location [6][9][7]	Truncal location [6]
Melanoma subtype	Superficial spreading [9] or nodular [8]	-
Pigmentation	Presence of pigmentation on pathology and as detected by patient [11]	-
Breslow thickness (of primary)	*BRAF* mutation associated with increased tumor thickness compared to wildtype [12][13]	-

Features/Outcomes	Primary Melanoma	Metastatic Melanoma
Ulceration (of primary)	*BRAF* mutation associated with the presence of ulceration [8][7][14][15]	No association [6]
Dermoscopy features	Irregular peripheral streaks [16], blue-white veil [17], and “peppering” [18]	-
Outcomes		
Stage at presentation	Presentation at a more advanced stage is associated with *BRAF* mutation [9][19]	
Response to chemotherapy	-	No association [6]
Response to BRAF/MEK inhibitor	-	Highly predictive of response to therapy [20]
Disease-free interval (primary diagnosis to first distant metastasis)	-	No association [6]
Outcome (survival)	No association [6]	Further investigation necessary

Importantly, variability exists in the published guidelines directing *BRAF* mutation testing. The NCCN guidelines recommend *BRAF* testing in patients for whom targeted therapy may be an option [21]. This includes patients with stage III melanoma at high risk for recurrence or patients presenting with loco-regional recurrence or stage IV disease. The NCCN panel does not recommend *BRAF* testing for resected pathologic stage I or II cutaneous melanoma unless the results may be used to direct participation in clinical trials. The European Society for Medical Oncology (ESMO) mandates mutation testing for all patients with advanced disease, which includes stages III or IV (resected or unresected) [22]. Contrary to the NCCN guidelines, ESMO recommends mutation testing for high-risk pathologic stage IIC melanoma patients.

As the landscape for treatment options expands, clear guidelines for biomarker testing ensure that high-risk patients receive the first-line treatment options for which they are eligible. As mentioned, there is currently a discrepancy between the published guidelines. In congruence with the ESMO guideline recommendation for testing, pathologic stage IIC should be recognized as high-risk melanoma, and these tumors should undergo mutation testing. This is supported by the evidence/clinical data reporting that stage IIC melanoma patients have paradoxically worse outcomes of overall survival (OS) and relapse-free survival (RFS), when compared to patients presenting at stage IIIA [23][24]. Specifically, 5-year survival rates for both stage IIB and stage IIC disease (87% and 82%, respectively) are lower than the 5-year survival rate of 93% for stage IIIA melanoma [7][23]. Although targeted or immunotherapies are not currently formally indicated in high-risk stage II patients, a number of ongoing clinical trials (e.g., MK-3475-716/KEYNOTE-716 and CheckMate76K trials) will aid to resolve the role of adjuvant therapy in pathologic stage IIB/C disease. Hence, patients with thick tumors (>4.0 mm) or >2 mm tumors with ulceration

should potentially be considered for systemic therapy, thus justifying reflex *BRAF* testing in this higher-risk patient population.

2.2 Methods of BRAF Mutation Testing

Many testing options are available to detect BRAF mutations, each with unique strengths and weaknesses to be taken into consideration. The current guidelines do not provide a detailed diagnostic testing algorithm. In clinical practice, some centers use immunohistochemistry (IHC) as a preliminary screening tool to initiate treatment. Confirmatory testing can then be performed using molecular techniques. Other centers prefer the use of real-time PCR (RT-PCR) or next-generation sequencing (NGS) approaches to detect mutations over IHC. Notably, in Canada, confirmatory/validation testing can be performed within a hospital testing center using a locally accepted technology. However, in the United States, only specific platforms certified by the Food and Drug Administration (FDA) can be used to confirm BRAF mutation status. In Europe, according to the ESMO guidelines, a validated test should be used only in an accredited (certified) institute that includes appropriate quality controls [25]. A summary of the diagnostic testing modalities is provided in Table 2.

Table 2. Summary of diagnostic testing modalities used to detect *BRAF*-mutated melanoma. IHC, immunohistochemistry; HRM, high-resolution melt; NGS, next-generation sequencing; RT-PCR, real-time polymerized chain reaction.

Features	IHC	RT-PCR		HRM	Sanger	Pyrosequencing	NGS
		Cobas®	THxID®				
Detection of mutations [26] [27]	VE1 antibody for V600E	V600E	V600E V600K	Indirectly detects mutations	Whole exon, detects rare mutations	Optimized for V600 mutations	Whole exon, detects rare mutations
Sensitivity	Up to 98.6% [28]	95% [29]	>96% (V600E) >92% (V600K) [26]	99% [27]	92.5% (for V600E) [29]	90 to 100% [27] [30]	99% [31]
Specificity	97.7% [28]	98% [26]	100% [26]	100% [27]	100% [27]	95 to 100% [27] [30]	100% [31]
Limit of detection (i.e., proportion of cells that are positive)	Few cells [32]	7% [27]	5% [26]	6.6% [26]	6.6% [33]	5.0% [34]	2% [27]
Turnaround	<1 day	1 day		1 day	Up to 3 days	2 days	Up to 5 days

Features	IHC	RT-PCR		HRM	Sanger	Pyrosequencing	NGS
		Cobas®	THxID®				
time [27]							
Cost [27]	Low	Medium		Low	Medium	High	Very high

The implementation of carefully developed disease-specific reflex testing criteria by a multidisciplinary team is important to avoid the futile use of valuable healthcare resources. For *BRAF* mutation in the context of melanoma, reflex testing criteria should include advanced disease characteristics, as these patients would benefit the most from rapid initiation of *BRAF/MEK* inhibitors. These features to a clinician/pathologist might include melanomas exhibiting clinical characteristics associated with *BRAF* mutation (Table 1), thick tumors of Breslow depth 2-4 or >4mm with or without ulceration (*i.e.,* stages IIB and IIC, respectively) and all patients with nodal involvement (i.e., stage III) or lymphatic progression (satellitosis or in transit metastasis). While systemic therapies are not approved for patients with pathologic stage II melanoma, considering the risk of disease progression in these individuals and decreased 5 and 10-year survival rates (82% and 75%, respectively for stage IIC and 87% and 82%, respectively for stage IIB disease), knowledge of the *BRAF* mutational status may prove useful for selection of future therapies. Furthermore, most often if stage IIB/C melanoma recurs, this usually occurs within 2 years of surgery. Advanced knowledge of the mutation status will help initiate treatment faster for newly metastatic or recurrent disease. While many tertiary care centers and specialized melanoma programs have or are actively implementing reflex *BRAF* mutation testing, it is paramount to promote this change across community hospitals as well, so that patients with high risk (stage ≥IIB) melanoma can consistently arrive to their first medical oncology appointment with this information at hand to make an informed treatment decision. This maybe critically important for those patients, who present to the multidisciplinary clinic with far more advanced melanomas than implied by the microstaging features of the primary tumor. For example, patients with large infiltrating tumors of dubious resectability or tumors that involve vital structures might benefit from neoadjuvant targeted therapy to facilitate their removal. Furthermore, as noted earlier, ongoing clinical trials (MK-3475-716/KEYNOTE-716 and CheckMate76K that enroll stage IIB and IIC patients) should answer the question whether these patients might benefit from adjuvant targeted therapy. The collective agreement on worrisome signs identifiable by dermatologists, surgeons, pathologists and oncologists will enable for cost-effective reflex *BRAF* testing and timely management for patients.

4. Conclusions

Exploitation of the MAP kinase signaling pathway has led to great improvements in the prognosis of metastatic melanoma. Mutational testing of high-risk melanoma gives patients the option of personalized treatment, which has been shown to provide a greater survival benefit than historical treatment modalities. Importantly, the implementation of standardized reflex testing criteria will allow for timely initiation of these treatment options. Further research identifying optimal use of therapies and new molecular targets will continue to improve the outlook for advanced melanoma.

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