Targeted therapy in melanoma: the era of personalized medicine

Karen Naert
Ayman Al Habeeb
Craig Gedye
Danny Ghazarian

Abstract

Malignant melanoma is the most aggressive of all cutaneous tumours, with over 76, 000 new cases and 9700 deaths estimated for 2014 in the United States.[1] In Canada, both the incidence and mortality of melanoma are increasing, with a risk of developing melanoma being 1 in 59 for men and 1 in 73 for women.[2] The incidence of melanoma is higher in Australia, with a risk of 1 in 14 for males and 1 in 23 for females to age 85 reported for 2009.[3] Although early melanoma can be managed surgically, until recently there have been few advances in the treatment of advanced melanoma. However, with the introduction of molecular targeted therapies, the landscape of melanoma treatment has changed dramatically in the past five years, resulting in improved survival rates for patients with metastatic disease. In this review, we will discuss the molecular basis and implementation for some of these novel treatments with particular emphasis on BRAF and BRAF inhibitors.

Keywords Melanoma; targeted; therapy; molecular; BRAF; TILs

Introduction

The incidence and mortality of malignant melanoma are increasing. In the united states there are over 76,000 new cases and 9700 deaths estimated for 2014 (1). In Canada the risk of developing melanoma is 1 in 59 for men and 1 in 73 for women (2). Australia has one of the highest incidence rates in the word with a risk of 1 in 14 for men and 1 in 23 for women (3). As is the case for many malignancies, defined mutations in proto-oncogenes have been identified which act as drivers for melanoma development or progression. In melanoma, the mitogen-activated protein kinase (MAPK) pathway is the major site of mutations and source of potential therapeutic targets (Figure 1). Mutations in this pathway have been identified in the majority of melanomas, with mutations in *BRAF*, *NRAS* and *KIT* being the most common. These mutations result in MAPK pathway activation and increased proliferation and survival in melanoma[4] (see Figure 2).

BRAF is a serine–threonine kinase that has been found to be mutated in approximately 50% of melanomas,[5–7] and is also mutated in numerous other human tumours including papillary thyroid cancer and colorectal cancer. *NRAS* mutations have been found in up to 30% of cases.[6,7] *KIT* mutations are less common (<5%). Mutations in these three proto-oncogenes are typically mutually exclusive.[7]

Clinicopathologic correlations with mutation status

There is some correlation between the mutation profile and clinicopathologic features of melanoma (Table 1). Mutation frequency differs by anatomic site, with *KIT* mutations found more often in acral and mucosal locations[8] and *BRAF* mutations found in fewer than 20% of acral melanoma.[5] Nail apparatus melanomas share a mutational profile similar to acral sites.[9] *NRAS* mutations have also been found to occur in vulvar and vaginal melanomas.[10] *BRAF* and *NRAS* mutations are found more often on truncal locations[11,12] but can be found at a variety of anatomic sites. However, *BRAF* mutations are not typically found in mucosal, uveal, and leptomeningeal melanomas, and as mentioned are less common in acral sites.[5] Uveal melanomas, while not *BRAF* mutated, frequently share with blue nevi non-MAPK pathway mutations, particularly G-protein alpha-subunit (GNAQ) mutations.[13,14] Conjunctival melanomas resemble cutaneous melanoma more than uveal melanoma at the molecular genetic level, with *BRAF* and *NRAS* reported in 29% and 18% of cases, respectively, in one study.[15]

Related to site-specific variation in mutation frequencies is the finding of different mutational frequencies depending on geographic location, with populations wherein acral and mucosal melanoma are more common than cutaneous melanoma having lower frequencies of BRAF positive cases.[16]

Site-specific variation in mutation frequency may be attributable, at least in part, to degree and extent of sun-exposure. Both *BRAF* and *NRAS* mutations have been found to be associated with non-chronically sun-damaged skin,[6,12,17,18] with the majority of melanomas arising on chronically sun-damaged skin showing mutations in neither *BRAF* nor *NRAS*.[18] The association between younger age at diagnosis, non-chronically sun-damaged skin and *BRAF* mutation suggests that non-*BRAF* mutated tumours may require accumulation of additional UV damage over time.[17]

As mentioned above, *BRAF* mutated melanomas tend to be diagnosed at a younger age than those tumours wild type for *BRAF*.[11,17] *NRAS* mutated cases show a significantly higher age at diagnosis compared to *BRAF* mutated cases.[7]

Histology also varies by mutation status. *BRAF* mutations are found most often in cases superficial spreading melanoma[6,11] which show pagetoid scatter of melanocytes and nesting.[12] *BRAF* mutated tumours have also been shown to be more often associated with a pre-existing nevus and with increased tumour infiltrating lymphocytes.[7] *NRAS* mutated tumours tend to be thicker and more mitotically active than wild type tumours or *BRAF* mutated tumours[7,19] and show low pagetoid spread and better circumscription,[12] consistent with an association with nodular melanoma.[6] Spindle cell/desmoplastic melanomas show a lower frequency of *BRAF* mutation (30% in one study), with no

Karen Naert *MD FRCPC Department of Pathology, University Health Network, Toronto, Ontario, Canada. Conflicts of interest: none declared.*

Ayman Al Habeeb *MD FRCPC Department of Pathology, University Health Network, Toronto, Ontario, Canada. Conflicts of interest: none declared.*

Craig Gedye *BScHons MBChB FRACP PhD Staff Specialist Department of Medical Oncology Calvary Mater Newcastle Edith St Waratah NSW 2298.*

Danny Ghazarian *MD PhD FRCPC Department of Pathology, University Health Network, Toronto, Ontario, Canada. Conflicts of interest: none declared.*

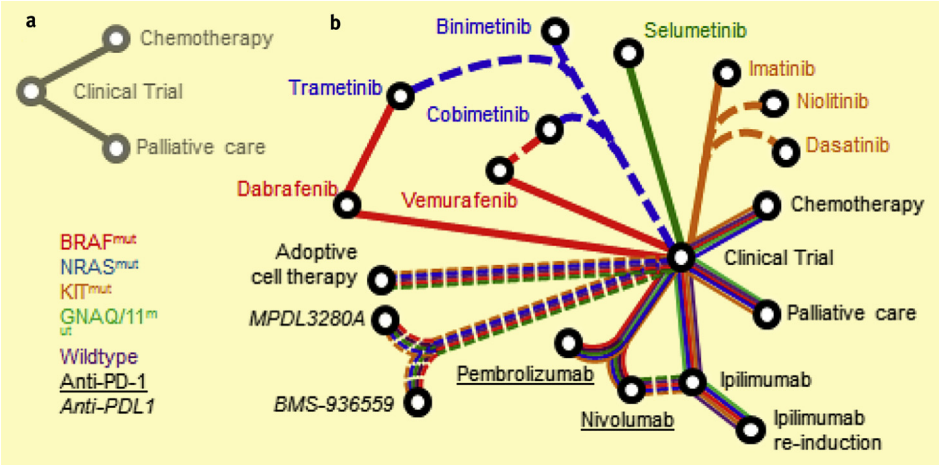

Figure 1 (**a**). In the absence of a clinical trial, chemotherapy (often dacarbazine) was the standard of care for metastatic melanoma. (**b**). Since the approval of ipilimumab and vemurafenib in 2011 there has been a flurry of activity with the development of small molecule inhibitors targeting oncogenic mutations (coloured) and monoclonal antibodies that activate the immune system by inhibiting checkpoint molecules (anti-CTLA4, ipilimumab; anti-PD1, underlined; anti-PDL1, *italics*). Combinations of targeted therapies, and combinations of immunotherapies, are already generating further survival improvements in clinical practice (solid) or promise improved outcomes in ongoing clinical trials (dashed lines).

KIT or *NRAS* mutations found.[20] Lentigo maligna melanomas may have *BRAF* mutations, but at a lower frequency (approximately 20%).[21] Lentigo maligna appears to have a higher frequency of a variant *BRAF* mutation compared to other cutaneous melanomas (discussed below).

Prognostic significance of mutation status

The data are not uniform regarding prognostic significance of mutation status. The presence of an *NRAS* mutation has been associated with shorter survival compared to wild type tumours.[19,22] *NRAS* mutation has been shown to be an adverse prognostic factor in one study that showed no prognostic significance for *BRAF* mutation,[19] whereas another study showed that *BRAF* mutated tumours not treated with a BRAF inhibitor had shorter median survival compared to wild type tumours.[11] Another study showed no association between either *BRAF* or *NRAS* mutation on overall survival.[7] In a multivariate analysis, BRAF/NRAS wild type melanomas appeared to have poorer disease free intervals from primary to metastatic disease, but survival from diagnosis of metastases, and responses to chemotherapy were not associated with mutation status.[23]

BRAF mutation testing as a diagnostic tool?

It is important to remember that the presence of a *BRAF* mutation in a given melanocytic lesion does not imply behaviour. Benign nevi adjacent to melanomas, as well as individual benign nevi have also been found to harbour *BRAF* mutations. Likewise, nodal nevi have been found to have BRAF mutations in >40% of cases.[24] Therefore, molecular techniques designed to identify the presence of a *BRAF* mutation should not be relied upon for diagnostic purposes in a given lesion. Detection of $BRAF^{V600}$ mutations in circulating serum DNA may assist in rapidly personalizing treatment though this requires further validation.[25]

BRAF inhibitors and melanoma

The most common *BRAF* mutation causes a substitution of valine with glutamic acid at the 600th position (V600E), which is found in greater than 70% of BRAF mutated melanomas.[5,11,26] A valine to lysine substitution (V600K) constitutes the second most common mutation, seen in approximately 20% of mutated cases.[5,11,26] Other mutations are seen rarely.

As alluded to above, V600K-mutated melanomas tend to have special clinicopathologic features including older age, male gender, head and neck primary, and worse prognosis including shorter time to metastasis and shorter overall survival compared to V600E mutated cases, as well as wild type and *NRAS* mutated cases.[21,26,27]

The identification of *BRAF* mutations in melanoma has facilitated the introduction of therapy targeted at mutant BRAF, with two specific inhibitors currently approved by the FDA: vemurafenib and dabrafenib. These agents have shown significantly increased response rates to treatment compared to older non-specific agents such as dacarbazine, as well as increases in progression free survival and overall survival.[28–31] Adverse effects are seen with BRAF inhibitors and include rash, photosensitivity, pyrexia, arthralgia, fatigue, alopecia and panniculitis,[32,33] as well as neoplasms, particularly squamoproliferative lesions such as well-differentiated squamous cell carcinoma and keratoacanthoma.[32] Interestingly, the majority of these lesions have been negative for HPV in one study.[34] BRAF inhibitor therapy also induces changes in pre-existing benign melanocytic lesions and second primary melanomas have been reported in patients on BRAF inhibitors.[32] (see Table 2).

Mutant *BRAF* in a given melanoma can be identified by numerous molecular techniques, described in detail elsewhere.[35] Of particular importance to practicing surgical pathologists is the availability of immunohistochemical antibodies to detect mutant BRAF protein, of which several are currently available.[36] The antibodies VE1 and Anti-B-Raf have been shown in various studies to have high sensitivity and specificity for $BRAF^{V600E}$ mutations, but have lower predictive