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Melanoma: New Insights and New Therapies

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Abstract

Metastatic melanoma has historically been considered as one of the most therapeutically challenging malignancies. However, for the first time after decades of basic research and clinical investigation, new drugs have produced major clinical responses. The discovery of BRAF mutations in melanoma created the first opportunity to develop oncogene-directed therapy in this disease and led to the development of compounds that inhibit aberrant BRAF activity. A decade later, vemurafenib, an orally available and well-tolerated selective BRAF inhibitor, ushered in a new era of molecular treatments for advanced disease. Additional targets have been identified, and novel agents that impact on various signaling pathways or modulate the immune system hold the promise of a whole new therapeutic landscape for patients with metastatic melanoma. One of the major thrusts in melanoma therapy is now focused on understanding and targeting the network of signal transduction pathways and on attacking elements that underlie the tumor's propensity for growth and chemoresistance. In this article, we review the novel targeted anticancer approaches that are under consideration in melanoma treatment.

INTRODUCTION

Curative treatments for patients with metastatic melanoma remain elusive. The median survival time for melanoma patients with metastatic disease is 8–9 months, and the 3-year overall survival (OS) rate is less than 15% (Balch *et al.*, 2009). Until recently, clinical trials of chemotherapy, immunotherapy, and biochemotherapy have failed to significantly improve survival. Conventional chemotherapy with dacarbazine (DTIC) alone is associated with an objective response rate of, at most, 15%; moreover, nearly all of these responses are partial (Lui *et al.*, 2007). Immune-based therapies, such as IFN-α and IL-2, have yielded comparable response rates, but are associated with more intense toxicities and no clear impact on OS for the overall population of metastatic melanoma patients (Eggermont and Schadendorf, 2009). Therefore, there has been significant room for improvement with regard to both efficacy and toxicity of melanoma therapies.

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CONFLICT OF INTEREST

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Recent advances in molecular oncology have yielded new treatment strategies that target specific molecules and pathways expressed in the cancer cells. One of the first approaches of this therapeutic strategy was the development of Herceptin (trastuzumab), a mAb, for patients with *HER2*-overexpressing breast cancers (Baselga *et al.*, 1999). A second successful approach was the therapeutic use of a tyrosine kinase inhibitor, imatinib, in chronic myeloid leukemia, a disease that is characterized by a reciprocal translocation (Philadelphia chromosome; [t(9:22)(q34; q11)]), which constitutively activates the Abl tyrosine kinase (Mauro *et al.*, 2002). To date, several targeted therapies have been approved for the treatment of malignancies such as colorectal, breast, head and neck, non-small-cell lung, and renal cell cancer.

Melanoma is a heterogeneous disease, which suggests a richly complex etiology. Deep molecular analyses have revealed consistent genetic patterns among different melanoma subtypes. For instance, 50–60% of the more common forms of melanoma (i.e., superficial spreading) harbor *BRAF* mutations. In addition, *NRAS* mutations are observed in 15–30% of cutaneous melanomas and are mutually exclusive of *BRAF* mutations (Albino *et al.*, 1989; Tsao *et al.*, 2004). Loss of tumor suppressor genes (TSGs) have also been identified in melanoma, often accompanying mutated oncogenes within the same tumor. Experimental studies have shown that the cell cycle regulators, p16 and p14ARF (both derivative products of the *CDKN2A* locus), are frequently inactivated in melanomas arising on chronically exposed skin (Sharpless and Chin, 2003). Finally, *KIT* alterations (mutations and/or amplifications) are found more frequently in melanomas from acral, mucosal, and chronic sun-damaged sites (Curtin *et al.*, 2006), whereas uveal melanomas uniquely harbor activating mutations in the α-subunit of a G protein of the Gq family, *GNAQ* and *GNA11* (Van Raamsdonk *et al.*, 2009, 2010). The clinical challenge today is whether effective therapies can specifically target the aberrant functionalities associated with these somatic mutations (Figure 1).

TARGETING SIGNALING MOLECULES IN MELANOMA

c-Kit

c-Kit is the receptor tyrosine kinase for stem cell factor. Activation of c-KIT by ligand binding results in the stimulation of the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)-AKT1, and JAK-STAT signaling pathways, thereby producing proliferative and survival effects. c-KIT is ubiquitously expressed in mature melanocytes, but tends to be reduced or lost in invasive or metastatic melanoma (Natali *et al.*, 1992). In unselected melanomas, the proportion of tumors retaining c-KIT overexpression is less than 3% (Curtin *et al.*, 2006). Recent studies reported *KIT* mutations in 21% of mucosal, 11% of acral, and 17% of chronic sun-damaged melanomas; if *KIT* amplifications are included, the rates of *KIT* aberrations are 39% for mucosal, 36% for acral, and 28% for chronic sun-damaged melanomas (Curtin *et al.*, 2006). The mutations are frequently located in the juxtamembrane (exons 9, 11, and 13) domain rather than in the catalytic domain.

Before the identification of *KIT* mutations in melanoma, two Phase II studies of imatinib, a tyrosine kinase inhibitor that targets BCR-ABL, c-Kit, and platelet derived growth factor receptor (PDGFR)-α and -β, failed to suggest any clinical benefit (Ugurel *et al.*, 2005; Wyman *et al.*, 2006). In retrospect, only a few patients enrolled into these trials would have been expected to harbor KIT mutations based on chance alone. Soon after the identification of KIT mutations in melanoma, two case reports (Hodi *et al.*, 2008; Lutzky *et al.*, 2008) quickly established the potential promise of KIT-targeted therapy in these patients, and two Phase II studies evaluating imatinib in the context of *KIT*-mutated metastatic melanoma have further explored this possibility (Carvajal *et al.*, 2011; Guo *et al.*, 2011). In the Carvajal

trial, the authors showed a 16% overall durable response rate (median OS of 46.3 weeks), with the better response rates occurring in cases with mutations affecting recurrent hotspots (c-KIT^{K642E} or c-KIT^{L576P}) or with a mutant-to-wild type allelic ratio of more than 1—significance measure of potential KIT dependence. In the Guo trial, 43 patients were treated with 400mg per day imatinib and experienced a median progression-free survival (PFS) of 3.5 months with a 6-month PFS rate of 36.6%. Eighteen patients (41.9%) demonstrated shrinkage of tumor mass, and the 1-year OS rate was 51.0%. These studies confirm the potential clinical utility of c-KIT suppression, although the full effects require Phase III trials. Other c-KIT inhibitors (Table 1) are currently under study. A significant response to another receptor tyrosine kinase inhibitor, dasatinib, has also been reported in two metastatic melanoma patients with the c-KIT^{L576P} mutation (Woodman *et al.*, 2009). Nilotinib, a second-generation tyrosine kinase inhibitor of c-KIT, PDGFR, and BCR-ABL, is currently being tested in patients with *KIT*-altered melanomas who are resistant or intolerant in other tyrosine kinase inhibitors. A randomized Phase III trial is comparing the efficacy of nilotinib vs. dacarbazine in the treatment of metastatic and/or inoperable melanoma harboring a *KIT* mutation (NCT01028222). Although limited in numbers thus far, these early clinical findings confirm that *KIT* inhibition in the proper genetic context can be a potentially valuable therapeutic alternative. There is some evidence that some c-KIT mutations are more amenable to targeting with the available drugs than others.

RAS/RAF/MAPK/ERK PATHWAY

RAS—The RAS signaling network has gained much attention in melanoma. This signaling cascade promotes proliferation, survival, and invasion through two distinct pathways, the MAPK pathway and the PI3K pathway (Hocker *et al.*, 2008). Activation of MAPK signaling by oncogenic mutations has been found in up to 90% of melanoma cases. Therefore, therapies specifically aimed at the MAPK pathway components are likely essential treatment strategy aiming to antagonize pathogenic signal transduction pathways in melanoma (Figure 1).

The first component found to be activated in this pathway was *NRAS* (Padua *et al.*, 1984, 1985). *NRAS* is mutated in 15–20% of all melanomas, with the most common change occurring at Glutamine 61 (Brose *et al.*, 2002). Substitutions at this codon impair GTP hydrolysis, and thus the NRAS protein is constitutively active (Dahl and Guldberg, 2007). Although RAS is considered an ideal therapeutic target for melanoma and many other cancers, specific anti-RAS therapies have been elusive.

Farnesyltransferase inhibitors, such as tipifarnib and lonafarnib, block RAS activation by inhibiting posttranslational farnesylation of the protein, thereby preventing translocation of RAS to the plasma membrane. This transition to the membrane is required for RAF dimerization and further downstream signaling (Purcell and Donehower, 2002). A single-agent, single-arm Phase II trial of tipifarnib for patients with metastatic disease, including those with melanoma, showed a lack of response among the first 14 patients; this led to early closure of the trial (Gajewski *et al.*, 2006). Nevertheless, there is some evidence that RAS antagonism might enhance the effectiveness of other chemotherapeutic agents and may thus be used as part of a combination regimen. *In vitro* studies using human and mouse melanoma cell lines showed that the combination of cisplatin and lonafarnib (SCH66336) markedly enhanced the level of cisplatin-induced apoptosis, an effect that was associated with an enhanced G2/M cell cycle arrest (Smalley and Eisen, 2003; Morgillo and Lee, 2006).

More recently, Niessner *et al.* (2011) demonstrated that the combination of lonafarnib and sorafenib (a nonselective kinase inhibitor) synergistically inhibited melanoma cell growth,

significantly enhanced sorafenib-induced apoptosis, and completely suppressed invasive tumor growth in mono-layer and organotypic cultures, respectively. Lonafarnib did not affect MAPK and AKT but did affect mammalian target of rapamycin (mTOR) signaling (Niessner *et al.*, 2011). These findings suggest that lonafarnib may have stronger inhibitory effects on mTOR signaling and may sensitize melanoma cells to sorafenib-induced apoptosis. Barring the availability of selective RAS inhibitors, this evidence suggests that partial modulation of RAS activation with farnesyltransferase inhibitors may contribute efficacy in combination treatment regimens.

RAF—The most common oncogene to be mutated in melanoma is BRAF. Approximately 60% of all melanomas harbor activating mutations in BRAF, making this gene a prime therapeutic target (Davies *et al.*, 2002). So far, over 50 distinct mutations in *BRAF* gene have been identified (Garnett and Marais, 2004). The most prevalent change is the c.T1799A transversion, which results in a p.V600E substitution (i.e., $BRAF^{V600E}$; Garnett and Marais, 2004). This gain-of-function BRAF mutation accounts for more than 90% of the BRAF alterations described in melanoma, with alternative point mutations at the same position (p.V600D, p.V600K, p.V600R) contributing another 5–6% of the total (Davies *et al.*, 2002). The p.V600E change occurs in the CR3 domain of BRAF and leads to constitutive activation of the down-stream protein kinases (i.e., MEK and ERK) and heightened proliferation of melanoma cells.

Sorafenib is a small-molecule, nonselective RAF inhibitor that has been shown to abrogate MAPK signaling biochemically and to harbor antimelanoma effects *in vitro* (Karasarides *et al.*, 2004). Besides RAF, sorafenib also inhibits receptor tyrosine kinases, including the vascular endothelial growth factor (VEGF), c-KIT, and PDGF receptors, and the tyrosine kinase FLT3. Early clinical trials have failed to show any activity of sorafenib as monotherapy in patients with metastatic melanoma (Eisen *et al.*, 2006). The combination of sorafenib and DTIC or temozolamide was tested in randomized trials but failed to prove any clinical benefit for metastatic melanoma patients (Hauschild *et al.*, 2009).

Currently, other more selective BRAF inhibitors (SBIs) have been developed and are currently being evaluated in clinical trials. The first SBI to be developed in the clinical setting is vemurafenib (PLX4032). Vemurafenib is an orally available, potent inhibitor of BRAF with an approximately 30-fold selectivity for the p.V600E mutated form compared with wild-type BRAF. In the Phase I trial, there was an 80% response rate to vemurafenib among 32 genotype-selected metastatic melanoma patients treated at the maximum tolerated dose of 960 mg twice daily. Overall, 26 patients showed an objective response including two complete responses (Flaherty *et al.*, 2010). The estimated median PFS among all patients was greater than 8 months. The impact of vemurafenib on OS has been recently evaluated in a Phase III trial comparing vemurafenib with dacarbazine in 675 patients with previously untreated, metastatic melanoma harboring the $BRAF^{V600E}$ mutation. At 6 months, OS was 84% in the vemurafenib group and 64% in the dacarbazine group. In the interim analysis for OS and final analysis for PFS, vemurafenib was associated with a relative reduction of 63% in the risk of death ($P<0.001$) and of 74% in the risk of either death or disease progression ($P<0.001$), as compared with dacarbazine (Chapman *et al.*, 2011).

A rather novel side effect noted with vemurafenib was the development of keratoacanthomas (KA) and invasive squamous cell carcinoma (SCC), which may be due to compensatory signaling through RAS/CRAF (Heidorn *et al.*, 2010). Although these tumors can be easily recognized and treated, the surveillance strategy could be more complex in the adjuvant setting if duration of treatment becomes more of an issue.

There are several additional BRAF inhibitors in clinical development. GSK2118436 is an SBI with a >100-fold selectivity for cell lines that harbor $BRAF^{V600E}$ mutation. Early results of a Phase I clinical trial have been recently reported (Kefford *et al.*, 2010). The response rate was comparable to vemurafenib even before the maximum tolerated dose was defined. Notably, 8 of 10 patients with asymptomatic brain metastases exhibited a partial response to GSK2118426. A Phase II study has been designed to assess the efficacy of GSK2118436 administered to patients with $BRAF^{V600E/V600K}$ mutation-positive metastatic melanoma to the brain (NCT01266967).

Despite the vanguard studies that therapeutically validated BRAF inhibition, there were also several challenges—complete responses were rare, occasional patients were refractory to treatment, and most cases ultimately relapsed through secondary resistance. An elucidation of the mechanisms underlying resistance to vemurafenib has emerged as a major research objective. Unlike imatinib in KIT-mutated gastrointestinal stromal tumor, in which secondary mutations in the target account for acquired resistance, no gatekeeper BRAF mutations have been identified in melanoma patients with acquired resistance to vemurafenib (Nazarian *et al.*, 2010). However, there are early studies that show compensatory activation of NRAS or upregulation of PDGFR-β (Nazarian *et al.*, 2010), induction of insulin-like growth factor (Villanueva *et al.*, 2010), and activation of MEK1 (Wagle *et al.*, 2011). In a genome-wide screen, overexpression of CRAF and COT1 also appears to render cells resistant to BRAF inhibitors (Johannessen *et al.*, 2010).

MEK—MEK kinases lie immediately downstream of BRAF and have been considered another important target, particularly in the setting of activating BRAF mutations. Several MEK inhibitors have been tested in clinical trials in patients with metastatic melanoma. AZD6244 is a selective, non-ATP competitive inhibitor of MEK1 and MEK2 that has been subjected to Phase I/II trials (Adjei *et al.*, 2008; Haura *et al.*, 2010). In the Phase II trial of AZD6244 for patients with $BRAF^{V600E}$-mutated melanoma, 12% of the patients experienced significant, but incomplete, regression. This relatively modest activity was reproduced in a larger randomized Phase II study comparing AZD6244 with temozolamide; in this trial, the AZD6244 arm did not show any significant benefit in terms of response rates or impact on PFS (Dummer *et al.*, 2008), although five of six responding patients had $BRAF^{V600E}$-mutated tumors.

On a molecular level, it has been shown that MEK inhibitors achieve much of their apoptotic effect through suppression of the anti-apoptotic Bcl-2 member, Mcl-1, and that melanoma lines that are resistant to MEK inhibitors do not experience Mcl-1 suppression in response to MEK inhibitors (Wang *et al.*, 2007). Thus, as is the case with other genes in the MAPK pathway, a better understanding of the cross-talk that occurs with the Bcl-2 apoptotic network will likely be crucial in the development of rational treatment regimens involving MEK inhibitors.

The fact that restoration of MEK signaling is sufficient to confer resistance to BRAF inhibitors raises the intriguing question as to whether MEK inhibitors can be used to overcome resistance to SBIs. Studies are under way to clinically test this approach, including the combination of a MEK inhibitor (GSK1120212) and a BRAF inhibitor (GSK2118436) in a Phase II study involving patients with $BRAF^{V600E}$ tumors (NCT01072175); early evidence suggests that this combination may yield fewer SCCs/KAs and skin eruptions compared with each agent alone (Infante *et al.*, 2011). There is also another trial testing the co-inhibition of both MAPK and PI3K/AKT pathways by MEK inhibitor AZD6244 and AKT inhibitor MK2206 in patients with $BRAF^{V600E}$ melanomas who previously failed an SBI (NCT01021748).

The PI3K pathway

PI3K is a downstream effector of RAS and the lead-off enzyme for another arm of the RAS pathway. PI3K phosphorylates a second messenger, phospatidylinositol-4,5-biphosphate, thereby generating phospatidylinositol-3,4,5-triphosphate, which in turn leads to activation of the pathway's major downstream effector, AKT. Activated AKT has several different enzymatic substrates, including Hdm2, NF-κB, mTOR, and p27—all of which promote cell growth and survival. This pathway is negatively regulated by the PTEN protein. At the molecular level, PTEN downregulates PI3K signaling by dephosphorylating phospatidylinositol-3,4,5-triphosphate, thereby inducing cell cycle arrest and apoptosis (Damen *et al.*, 1996).

Although alterations in the PI3K pathway have been reported in up to 60% of cutaneous melanomas (Zhou *et al.*, 2000), attempts to therapeutically extinguish either PI3K or AKT have not been forthcoming, given the lack of robust clinically relevant inhibitors against these targets. Thus, investigators have focused on downstream targets such mTOR (Kumar *et al.*, 2001). Recently, a series of rapamycin analogs have been synthesized and evaluated for use in melanoma, such as temsirolimus (CCI-779) and everolimus (RAD001). A Phase II trial of temsirolimus was terminated after only one objective response among 33 melanoma patients was observed. In addition, no objective responses were recorded in a Phase II trial of everolimus in patients with metastatic melanoma, although 7 of 20 patients enrolled in the study were progression-free at 16 weeks (Rao *et al.*, 2006).

Tsao *et al.* (2004) found genetic evidence for cooperativity between BRAF mutagenesis and PTEN inactivation, indicating a need to simultaneously activate MAPK and PI3K pathways, respectively; this interaction has been substantiated in an animal model of melanoma (Dankort *et al.*, 2009). It has also been shown that the combination of sorafenib or MEK inhibitors (U0126 or PD98059) and rapamycin potentiated growth inhibition in melanoma cell lines. Moreover, sorafenib in combination with rapamycin completely suppressed invasive melanoma growth in organotypic cultures. These effects were associated with complete downregulation of the anti-apoptotic proteins Bcl-2 and Mcl-1. A Phase I/II study is currently underway testing temsirolimus in combination with sorafenib in stage III/IV melanoma (NCT00349206).

Werzowa *et al.* (2011) has also studied the effect of targeting the PI3K/mTORC1/mTORC2 pathway by PI-103 (an inhibitor of PI3K class IA and mTORC1/mTORC2) and rapamycin. In cultured melanoma cells and in a human melanoma xenograft model, PI-103 induced apoptosis and cell cycle arrest, and suppressed the viability of melanoma cells *in vitro. In vivo*, the combination of PI-103 and rapamycin significantly reduced the tumor growth compared with both agents independently. These data support dual targeting of the PI3K/mTORC1/mTORC2 pathway to maximize suppression. Newer inhibitors that inhibit both PI3Kand mTOR (XL765) have also proved to be well tolerated in Phase I studies (Papadopoulos *et al.*, 2008). It remains to be determined whether targeting PI3K, AKT, or mTOR will result in a single-agent activity in any subset of melanoma, or whether efficacy can only be observed when targeting this pathway in conjunction with others, particularly the MAP kinase pathway.

Restoring tumor suppression function

Epigenetic events in cancer development have attracted much attention. This refers to any changes in gene expression without alteration of the DNA sequence. Epigenetic silencing has been shown to functionally inactivate several TSGs including *PTEN*, *CDKN2A*, and *APAF-1*. For example, whereas mutations and deletions of *PTEN* have been observed in up to 60% of melanoma cell lines, only about 10% of uncultured samples contain genetic

alterations. These observations have led to speculations that *PTEN* inactivation may predominantly occur through epigenetic programs. Two particular mechanisms of gene regulation that have undergone therapeutic manipulation include DNA methylation and histone modification. DNA methylation is mediated by DNA methyltransferases (DNMTs), which are responsible for the formation of a covalent attachment of a methyl group to cytosine residues at CpG dinucleotides. Aberrant hypermethylation of TSGs likely contributes to tumor promotion (Herman and Baylin, 2003). As the promoter must be re-methylated during each cycle of DNA replication (Herman and Baylin, 2003), DNMT inhibitors can be used to nonselectively reactivate TSGs. One such DNMT inhibitor, 5-aza-2′-deoxycytidine (decitabine), is currently approved for patients with myelodysplastic syndrome. DNMT inhibitors have also shown some promise in melanoma. Decitabine has been safely administered with high-dose IL-2 and appears to enhance the activity of IL-2 with reported objective responses in 31% of melanoma patients (Gollob *et al.*, 2006).

The primary enzyme responsible for histone modification is histone deactylase (HDAC). HDAC inhibitors are also currently being studied as a possible treatment against melanoma. In the M14 human melanoma cell line, valproate, an HDAC inhibitor, has been shown to induce p16INK4a and a dose-dependent G_0/G_1 phase arrest, apoptosis, and sensitization to cisplatin and etoposide (Valentini *et al.*, 2007). Melanoma patients are eligible for an ongoing trial with the HDAC inhibitor, vorinostat (NCT006670820).

Unlike the more genetically precise targeted treatments, both DNMT and HDAC inhibitors restore gene expression, including TSGs, but in a nonspecific manner. Thus, cells with evidence of deleterious injury at TSG loci would probably not benefit from these agents. Moreover, the effects of non-selective re-induction of genes may yield unpredictable phenotypes.

Targeting apoptosis

Therapeutic agents that target the apoptotic pathways have also been widely analyzed. It has been shown that the overexpression of a number of anti-apoptotic proteins, such as Bcl-s, Bcl-xL, and Mcl-1, may lead to resistance to chemotherapy. Oblimersen is an 18-base antisense agent that targets Bcl-2. An international randomized controlled trial of 771 melanoma patients comparing DTIC and oblimersen with DTIC alone resulted in a higher and durable objective response rate, an increased median PFS, but no significant difference in OS (Bedikian *et al.*, 2006). It was never adequately established that this agent modulated Bcl-2 sufficiently to render cells more susceptible to cytotoxicity (Jansen *et al.*, 2000).

Another therapeutic target is Bcl-xL, a molecule that is considered to serve many of the same functions as Bcl-2. Tumor cells are able to switch expression from Bcl-2 to Bcl-xL and, in many cases, Bcl-2 and Bcl-xL are expressed in a reciprocal manner (Han *et al.*, 1996; Arriola *et al.*, 1999). Encouraging early human studies have simultaneously targeted Bcl-xL and other anti-apoptotic Bcl-2 family members using small-molecule inhibitors such as obatoclax (Nguyen *et al.*, 2007; Wolter *et al.*, 2007). Mcl-1 is a structurally distinct member of the anti-apoptotic Bcl-2 family, is strongly expressed at all stages of disease (Tang *et al.*, 1998; Leiter *et al.*, 2000), and is highly selective for BAK inhibition (Zhai *et al.*, 2008). Nguyen *et al.* (2007) found that obatoclax disrupted the interaction between MCL-1 and BAK in intact mitochondrial outer membrane and in intact cells, and overcame MCL-1-mediated resistance to both Bcl-2 inhibitor ABT-737 and the proteasome inhibitor bortezomib. Thallinger *et al.* (2003) showed that the combination of DTIC plus antisense oligonucleotide against Mcl-1-sensitized melanomas to DTIC in a SCID mouse model. Recent data have also shown that MEK inhibitors achieve much of their apoptotic effect through Mcl-1 suppression (Wang *et al.*, 2007). Taken together, these data suggest that dual MEK/Mcl-1 inhibition could be an effective means of improving clinical response.

As p53 is preserved but functionally inactivated by p14ARF loss in melanoma, restoration of p53 function represents another attractive means of throwing the switch from cytostasis to cytotoxicity. Ji *et al.* (2011) demonstrated that Hdm2 antagonism using nutlin-3 strongly induced p53 protein and activity levels in melanoma cells, reduced viability *in vitro*, and enhanced apoptosis in cell lines treated with a MEK inhibitor.

Targeting angiogenesis

Angiogenesis is an essential process in the development of most human tumors, including melanomas (Hanahan and Weinberg, 2011). Melanoma cells elaborate a wide variety of angiogenic factors *in vitro*, including VEGF, bFGF, IL-8, and PDGF, and the importance of these mediators in promoting melanoma angiogenesis and metastasis has been confirmed in tumor xenotransplant models (Rofstad and Halsor, 2000). Serum levels of VEGF in melanoma patients increase with clinical stage, and high serum levels of VEGF represent an adverse prognostic feature (Ugurel *et al.*, 2001). On the basis of these findings, several inhibitors of angiogenesis have been tested in melanoma patients and some have demonstrated activity against melanoma, including sunitinib (Chan *et al.*, 2008), vatalanib (Cook *et al.*, 2010), axitinib (Fruehauf *et al.*, 2008), and aflibercept (Tarhini *et al.*, 2009). Bevacizumab is a humanized IgG antibody that binds to the most common VEGF isoform, VEGF-A. Small studies of bevacizumab have documented modest responses in conjunction with other agents (Gonzalez-Cao *et al.*, 2008; Perez *et al.*, 2009; Vihinen *et al.*, 2010). One possible explanation is that VEGF-A/VEGFR-2 blockade leads to transient vessel remodeling and normalization of the tumor vasculature. This results in vessel stabilization and reduced vascular permeability, which facilitates access of co-administered chemotherapeutic drugs. Moreover, it has been shown that exposure of melanoma cells to chemotherapy induces VEGF overproduction, which, in turn, may allow melanoma cells to evade cell death and acquire resistance. Most recently, a multicenter Phase II trial of temozolomide and bevacizumab for stage IV melanoma patients showed promising results with an OS of 9.3 months and PFS of 4.2 months. Interestingly, response rates were higher in patients with $BRAF^{V600E}$ wild-type patients compared with those with mutated tumors (Dummer *et al.*, 2010; von Moos *et al.*, 2011). Other trials that have evaluated angiogenesis inhibitors in combination with chemotherapy have reported mixed results. In a randomized Phase II trial, patients with metastatic melanoma received first-line treatment with the combination of paclitaxel and carboplatin, with or without bevacizumab. Despite some encouraging early results, this trial ultimately failed to demonstrate a significant PFS and OS advantage (O'Day *et al.*, 2009). However, a similar Phase III trial adding sorafenib instead of bevacizumab to the combination of paclitaxel and carboplatin as a second-line treatment in patients with unresectable melanoma did not show any improvement in PFS or OS in the sorafenib group (Hauschild *et al.*, 2009).

Axitinib is an oral inhibitor of VEGFR-1,-2, and -3, c-KIT, PDGFR-α, and PDGFR-β. In a Phase II study of 32 patients with stage IV melanoma, treatment with axitinib resulted in an overall response rate of 16%, a median PFS of 2.3 months, and a median OS of 13 months (Fruehauf *et al.*, 2008). Dovitinib, an inhibitor of FGFR, VEGFR, PDGFR, and other tyrosine kinases, has demonstrated clinical activity and acceptable toxicity in a Phase I study in 19 patients with advanced melanoma (Kim *et al.*, 2008). Vatalanib (PTK787/zk222584), an inhibitor of VEGFR-1,-2, and -3, has shown efficacy in stabilizing metastatic melanoma in a Phase II study (Corrie *et al.*, 2008; Cook *et al.*, 2010).

Targeting the immune system

Melanoma is one of the most immunogenic tumors, as supported by the observed spontaneous regression of the primary tumor, the prognostic significance of tumor infiltration by lymphocytes, and the detection of tumor antigen–specific antibodies in the

peripheral blood of melanoma patients (Lee *et al.*, 1999). Immunological approaches that have shown some activity in patients with advanced melanoma include the use of high-dose IL-2 and IFN-α, autologous and allogeneic cellular vaccines, or cytokines. Furthermore, multiple novel immunomodulatory agents with activity against melanoma are in development. However, only recently was a clear survival benefit achieved by two different immune-directed approaches in metastatic melanoma (Hodi *et al.*, 2010). The first approach includes ipilimumab, a fully human mAb against cytotoxic T-lymphocyte antigen 4 (CTLA-4). CTLA-4 is a co-inhibitory molecule that functions to regulate T-cell activation. In resting T cells, CTLA-4 is expressed intracellularly; however, upon T-cell activation, the protein is transported to the immune synapse where effector T cell and the antigen-presenting cell make physical contact. Monoclonal antibodies that bind to CTLA-4 can block the interaction between B7 and CTLA-4 and can enhance immune responses, including antitumor immunity. A Phase III randomized trial of ipilimumab with or without a gp100 peptide vaccine vs. gp100 peptide vaccine alone in previously treated stage IV melanoma patients showed an OS advantage in the ipilimumab groups (hazard ratio for death in the comparison with gp100 alone, 0.66; P=0.003; Hodi *et al.*, 2010). The impact of ipilimumab therapy on OS was further supported in a recent Phase III study of ipilimumab with dacarbazine vs. dacarbazine alone in 502 previously untreated stage IV melanoma patients. The trial showed a significant OS benefit in the group receiving ipilimumab plus dacarbazine than in the group receiving dacarbazine plus placebo (11.2 vs. 9.1 months), with higher survival rates in the ipilimumab–dacarbazine group at 1 year (47.3 vs. 36.3%), 2 years (28.5 vs. 17.9%), and 3 years (20.8 vs. 12.2%; Robert *et al.*, 2011). After positive results in advanced disease, the adjuvant role of ipilimumab has been examined in two studies: EORTC18071, where ipilumimab is compared with placebo, and ECOG E1609, where it is compared with high-dose IFN-α. Finally, a trial of ipilimumab and vemurafenib (NCT01400451) will be open in the near future for patients with $BRAF^{V600}$ mutations.

Programmed death-1 is an inhibitory receptor expressed on activated T cells that also suppresses antitumor immunity. Anti-programmed death-1 blockage is thus related to, but distinct from, ipilimumab. In a Phase I trial, 39 patients with advanced metastatic melanoma, colorectal cancer, prostate cancer, non-small-cell lung cancer, or renal cell carcinoma received a single intravenous infusion of anti-programmed death-1 (0.3, 1, 3, or 10mg kg^{-1}), followed by a 15-patient expansion cohort at 10mg kg^{-1}. One durable complete response and two partial responses (melanoma, RCC) were reported with anti-programmed death-1, although there was one serious adverse event (inflammatory colitis) in a patient with melanoma (Brahmer *et al.*, 2010).

The second immune-targeted approach includes the combination of high-dose IL-2 and the gp100:209–217 (210 M) peptide vaccine vs. IL-2 alone. A Phase III trial including 185 patients with advanced melanoma showed that the vaccine/IL-2 group had a higher response rate (16 vs. 6%, P=0.03), and a 9% complete response rate in the vaccine/IL-2 group vs. 1% in the IL-2 alone group. Median PFS (2.2 vs. 1.6 months; P=0.008) and median OS (17.8 vs. 11.1 months; P=0.06) were also improved (Schwartzentruber *et al.*, 2011).

These recent trials with immune-based therapies have added tremendous balance to the pipeline of molecular treatments that have emerged. One of the major advantages of immunologically directed therapies is the application of these treatments to patients who are ineligible for anti-BRAF regimens. However, some immune-based approaches do require specific host profiles, such as HLA haplotypes.

CONCLUSION

Melanoma remains the deadliest form of skin cancer and, until recently, there have been only few therapeutic options for patients with metastatic disease. At present, genotyping metastatic tissue is of paramount importance as BRAF/c-KIT status dictates the eligibility for treatment with vemurafenib and imatinib, respectively. Although targeted therapies have produced major clinical responses, their impact on OS and cures is still under investigation. It is now clear that melanoma is not a singular, homogeneous disease with a common set of genetic alterations. Hence, the selection of treatment will likely be dictated by distinct molecular signatures. Future efforts will need to focus on targeting multiple coexistent aberrations in different pathways, and addressing the mechanisms that underlie the tumor's propensity for growth and chemoresistance. The greatest challenge lies in the elucidation of mechanisms by which resistance develops. This in turn will lead to a rational basis for combination therapy or second-generation agents aimed at circumventing resistance.

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Abbreviations

DNMT	DNA methyltransferase
MAPK	mitogen-activated protein kinase
mTOR	mammalian target of rapamycin
OS	overall survival
PFS	progression-free survival
PI3K	phosphatidylinositol 3-kinase
TSG	tumor suppressor gene

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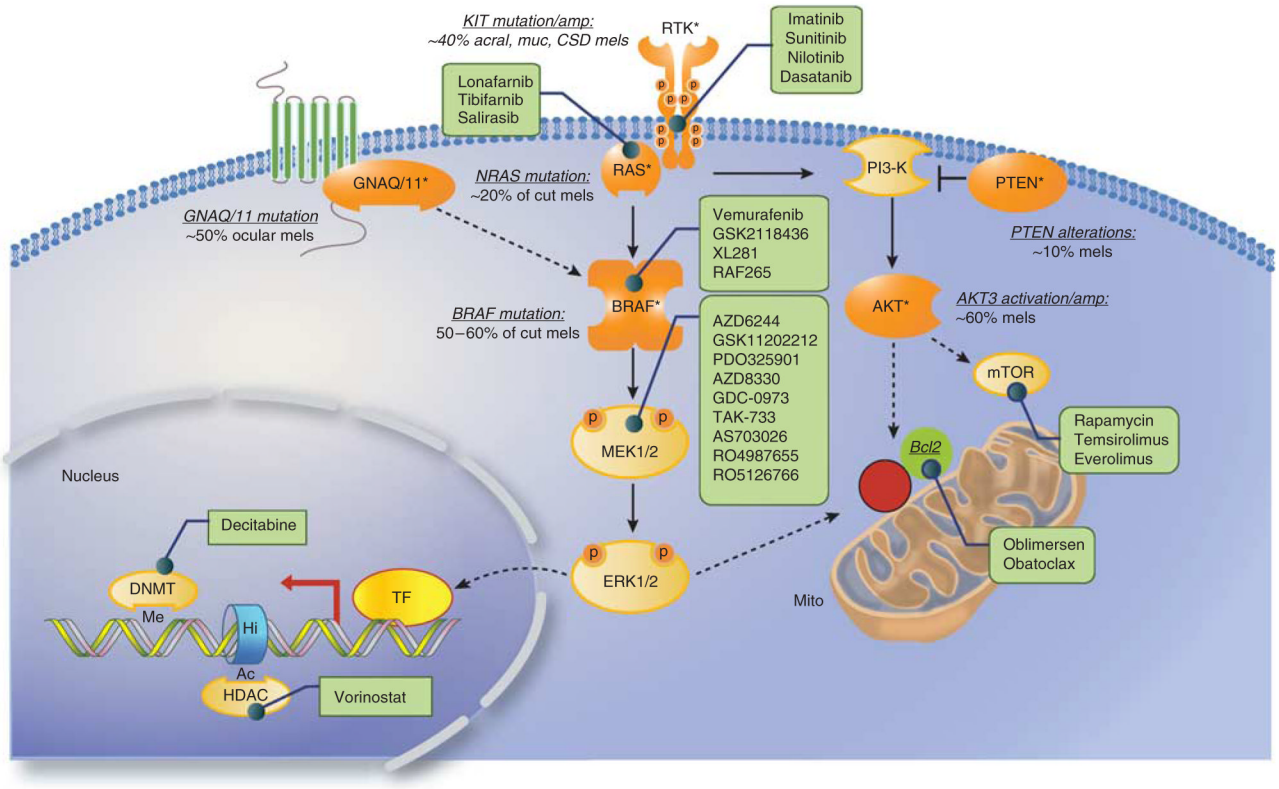

Figure 1. Key pathways and therapeutic targets in melanoma

Activation of the receptor tyrosine kinase (RTK)-NRAS-BRAF-MEK-ERK signaling stream is central in a large proportion of melanomas (mels), with BRAF and NRAS being the most commonly activated oncogenes. Upstream of RAS, KIT is amplified or activated in a substantial fraction of melanomas from acral, mucosal (muc), and chronic sun-damaged (CSD) sites. Stimulation of the phosphatidylinositol 3-kinase (PI3K) pathway also occurs in melanomas either through loss of PTEN or activation of AKT3. In addition, GNAQ and GNA11, which encode G-α proteins, are preferentially mutated in ocular melanomas. Downstream effectors of the activated signaling network lead to increased transcription of survival genes by transcription factors and heightened prosurvival signals in the mitochondria (Mito) via regulation of apoptotic proteins (red, proapoptotic; green, prosurvival). In the nucleus, epigenetic silencing of tumor suppressor genes occurs through DNA methylation and/or histone acetylation, which are mediated by DNA methyltransferase (DNMT) and histone deactylase (HDAC), respectively. Targeted agents listed in the light purple boxes inhibit the central pathogenetic pathways at specific points of action and potentially have a therapeutic impact on melanoma. Ac, acetylation (of Histone, Hi); cut, cutaneous; Me, methylation (of DNA); TF, transcription factor.

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Table 1

List of currently investigated targeted therapies in advanced melanoma (information obtained from www.clinicaltrials.gov)

Target	Molecular eligibility	Drug	Phase	NCT ID	Conditions
RTK	ERBB4 mutation positive	Lapatinib	Phase II	NCT01264081	Unresectable metastatic melanoma
RTK	KIT alteration present	Nilotinib	Phase II	NCT01099514	Unresectable metastatic melanoma with KIT aberration
RTK	KIT alteration present	Sunitinib	Phase II	NCT00631618	Unresectable metastatic melanoma
RTK	KIT alteration present	Nilotinib	Phase II	NCT00788775	Unresectable metastatic melanoma, which failed other TKIs
RTK	KIT alteration present	Nilotinib	Phase II	NCT01168050	Unresectable metastatic melanoma
RTK	KIT alteration present	Sunitinib	Phase II	NCT00577382	Unresectable metastatic mucosal, acral/ lentiginous melanoma
RTK	KIT mutation of exon 9, 11, 13, or exon 17 (Y822D and mutations D820Y, Y823D)	Nilotinib vs. dacarbazine	Phase III	NCT01028222	Unresectable metastatic melanoma
RTK (c-Kit selective)	KIT juxtamembrane mutation	Masitinib vs. dacarbazine	Phase III	NCT01280565	Unresectable metastatic melanoma
TK	KIT alteration present	Imatinib mesylate	Phase II	NCT00470470	Unresectable metastatic melanoma
TK	BRAF mutation positive	Lenvatinib (E7080)	Phase II	NCT01136967	Unresectable metastatic melanoma
TK	KIT mutations of exon 11 or 13	Dasatinib	Phase II	NCT01092728	Unresectable metastatic melanoma
BRAF	BRAF V600E- or V600K-mutation positive	GSK2118436	Phase II	NCT01266967	Unresectable metastatic melanoma to the brain
BRAF+MEK	BRAF mutation positive	GSK2118436+GSK1120212	Phase I	NCT01072175	Unresectable metastatic melanoma
BRAF	BRAF V600E mutation positive	GSK2118436 vs. dacarbazine	Phase III	NCT01227889	BRAF mutant unresectable metastatic melanoma
BRAF	BRAF V600 mutation positive	RO5212054 (PLX3603)	Phase I	NCT01143753	Colorectal cancer, malignant melanoma
BRAF	BRAF V600 mutation positive	Vemurafenib	Phase II	NCT01378975	Unresectable metastatic melanoma (brain metastases)
BRAF	BRAF V600E mutation positive	Vemurafenib	Phase III	NCT01307397	Unresectable metastatic melanoma
BRAF+MEK	BRAF V600 mutation positive	Vemurafenib+GDC-0973	Phase I	NCT01271803	Unresectable metastatic melanoma
MEK	BRAF WT	Docetaxel+selumetinib (AZD6244); docetaxel+placebo	Phase II	NCT01256359	Unresectable metastatic melanoma
MEK	BRAF V600E- or V600K-mutation positive	GSK1120212 vs. dacarbazine or paclitaxel	Phase III	NCT01245062	BRAF mutant unresectable metastatic melanoma
MEK	BRAF V600E or NRAS mutation positive	MEK162	Phase II	NCT01320085	BRAF or NRAS mutant unresectable metastatic melanoma

Target	Molecular eligibility	Drug	Phase	NCT ID	Conditions
MEK	BRAF V600E- or V600K-mutation or a NRAS mutation (codons 12, 13, or 61) positive	Selumetinib (AZD6244)	Phase II	NCT00866177	Unresectable metastatic melanoma
MEK	No GNAQ/GNA11 restrictions	Selumetinib (AZD6244) vs. temozolomide	Phase II	NCT01143402	Unresectable metastatic uveal melanoma
MEK	BRAF V600E mutation positive	TAK-733	Phase I	NCT00948467	Unresectable metastatic melanoma
CDK	CDK4 mutation or amplification	PD 0332991	Phase II	NCT01037790	Advanced cancers
mTOR	BRAF V600E mutation positive	Temsirolimus+selumetinib (AZD6244)	Phase II	NCT01166126	Unresectable metastatic melanoma
RTK (c-MET)	No restrictions	ARQ197+sorafenib	Phase I	NCT00827177	Advanced cancers
RTK (VEGFR and c-MET)	No restrictions	Cabozantinib	Phase II	NCT00940225	Advanced cancers
RTK	No restrictions	Pazopanib+paclitaxel	Phase II	NCT01107665	Unresectable metastatic melanoma
RTK	No restrictions	Sunitinib	Phase II	NCT01216657	Chemorefractory melanoma
RTK	No restrictions	Sunitinib+cisplatin+tamoxifen	Phase II	NCT00489944	High-risk ocular melanoma
RTK	No restrictions	Sunitinib+dacarbazine	Phase I/II	NCT00859326	Unresectable metastatic melanoma
RTK	No restrictions	Sunitinib+hydroxychloroquine	Phase I	NCT00813423	Chemorefractory melanoma
TK	No restrictions	Lenvatinib (E7080)+dacarbazine	Phase I/II	NCT01133977	Unresectable metastatic melanoma
RAF	No restrictions	RAF265	Phase I	NCT00304525	Unresectable metastatic melanoma
RAF	No restrictions	XL281±famotidine	Phase I	NCT00451880	Advanced cancers
RAF (and other kinases)	No restrictions	Sorafenib+cisplatin+tamoxifen	Phase II	NCT00492505	High-risk stage III melanoma
RAF (and other kinases)	No restrictions	Sorafenib vs. placebo	Phase II	NCT01377025	Unresectable metastatic uveal melanoma
MEK	No restrictions	RO4987655	Phase I	NCT00817518	Advanced cancers
CDK	No restrictions	Dinaciclib	Phase I/II	NCT01026324	Unresectable metastatic melanoma
PI3K+MEK	No restrictions	BKM120+MEK162	Phase I/II	NCT01363232	Unresectable metastatic melanoma
PI3K/mTOR+MEK	No restrictions	BEZ235+MEK162	Phase I/II	NCT01337765	Unresectable metastatic melanoma

Abbreviations: CDK, cyclin-dependent kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor; WT, wild type.