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REVIEW

An Update on BRAF Inhibitors and Other New Molecular Targets for the Treatment of Malignant Melanoma of the Skin

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Abstract: Malignant melanoma of the skin originates from mutations in melanocytes and can be lethal if unrecognized or untreated in its earlier stages. Deaths from melanoma are increasing in the United States and around the world every year. The available treatments produce low rates of response with modest survival impact. Among potential molecular targets under investigation, which are mostly in the tyrosine kinase pathway, the BRAF (V-raf murine sarcoma viral oncogene homolog B1) gene is the best studied and most frequently reported mutation in melanoma. The molecular targets for melanoma treatment, promising drugs for future melanoma treatment as well as the new molecular entities that are approved are reviewed here. Approved by FDA in 2011, vemurafenib (Zelboraf) is the first personalized targeted therapy for treatment of metastatic melanoma that acts by selectively inhibiting $BRAF^{V600E}$. This has opened a new avenue for the discovery of targeted drug therapies for melanoma based on the principles of pharmacogenomics.

Keywords: Melanoma, BRAF inhibitor, molecular target, vemurafenib

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Introduction

Malignant melanoma, which originates from mutations in melanocytes, is a skin cancer that can be lethal, particularly if unrecognized or untreated in its earlier stages. Melanoma is characterized by stages 0 to IV based on the degree of cutaneous penetration, radial expansion, regional spread and invasion, and/or metastasis. While stage 0 melanoma or melanoma in situ is restricted to the superficial layers of the epidermis, ie, has not through the basement membrane, as the melanoma advances through subsequent stages, regional lymph nodes and tissues may be invaded. In stage IV melanoma, there is metastasis to distant organs and tissues, including the lung, liver, brain, and bone. The 2013 American Cancer Society Facts and Figures report estimates 76,690 new cases of melanoma of the skin and 9,480 deaths from melanoma in the United States. These estimates are significantly higher than the 2003 American Cancer Society Facts and Figures report of an estimated 54,200 new cases of melanoma and 7,600 deaths. In 2006, there were 60,000 new cases of cutaneous melanoma in the European Union and 13,000 deaths. The current incidence rates range from 15 to 60 per 100,000 people. Although the 2013 American Cancer Society Facts and Figures indicates that the 5-year relative survival rate for locally involved (early stage) melanoma is 98%, the 5-year survival rate falls to 15% for metastatic melanoma. More than 80% of deaths sby skin cancer are due to aggressive metastatic melanoma that is resistant to existing therapies.[1,2] Both molecular genetic factors (eg, 40% of melanoma-prone families have a mutation in the growth regulating CDKN2A gene) and environmental factors (eg, exposure to ultraviolet light and a history of severe sunburn, particularly early in life) are thought to be responsible for increasing risk for the development of melanoma.[3–5]

Molecular Targets for Melanoma

The current therapeutic options for metastatic malignant melanoma are limited. Until the recent approval of the targeted therapy vemurafenib,[6] a kinase inhibitor indicated for the treatment of patients with unresectable or metastatic $BRAF^{V600E}$-positive melanoma, available treatment options, including cytotoxic and immunologic therapies, sometimes produced low rates of response with modest survival impact. Traditional chemotherapeutic drugs, such as dacarbazine (Objective Response Rate (ORR) ~18%), temozolomide (ORR ~15%), paclitaxel (ORR ~13%), cisplatin (ORR ~23%), docetaxel (ORR ~11%), lomustine (ORR ~13%), or carboplatin (ORR ~16%) offer only limited, short lived benefit in a small fraction of patients (ORR overall less than 25%). Though the combination of drugs like dacarbazine with immune boosting drugs like ipilimumab have yielded higher 1 year survival rates of 47.3%, the three year survival rate remains poor (20.8%), reflecting the limited duration of response of such immuno-modulatory therapy. In addition, significant grade 3 to 4 toxicities are observed in a large fraction of patients (56.3%).[7]

A better understanding of the underlying molecular changes in spontaneous malignant melanoma has led to the development of targeted small molecules. In particular, the mitogen activated protein kinase (MAPK) pathway has been described to be aberrant and of oncogenetic relevance in melanoma. However, a sole aberration in this pathway, which is also aberrant in many benign nevi, has not been described be to be sufficient for malignant transformation. Along with c-Kit mutations, BRAF (V-raf murine sarcoma viral oncogene homolog B1) gene is the best studied and is reported to be the most frequently mutated of all spontaneous metastatic melanomas (about 50%–70%). In these patients, responses in about half of all patients can be observed.

Other potential targets that are being studied include components of the Raf/Ras/MAPK pathway, phosphoinositide 3-kinase (PI3K)/AKT pathway, tyrosine kinases, angiogenesis, poly (ADP ribose) polymerases, surviving and heat shock protein 90, metastatic pathways, Rho GTPase signaling, integrin activity, and actomyosin contractility.[3,8] In some investigations, treatments based on two or more targets have been considered. For example, NVP-BEZ235, a potent and stable dual PI3K/mTOR inhibitor, has entered Phase I/II clinical trials in patients with advanced solid tumors and shows potential in the treatment of metastatic melanoma.[9] An excellent review on emerging molecular targets in melanoma invasion and metastasis has been published recently.[8]

Melanoma and BRAF

Several members of RAS and RAF family in the ERK/MAPK pathway, including BRAF, NRAS, HRAS

and KRAS, undergo mutations and associated with melanoma. BRAF, a serine/threonine kinase of the RAF family in this pathway, regulates cell growth, survival and differentiation and is activated by membrane-bound receptors including tyrosine kinases and G-protein-coupled receptors. Mutations in BRAF lead to hyper-activation of ERK causing enhanced melanoma proliferation and accounts for 50%–70% of melanomas.[10] The PI3K is another pathway signaled by oncogenic RAS thereby affecting cell proliferation, survival, migration, and invasion. The BRAF and NRAS mutations are mutually exclusive.[1,11]

The most frequent BRAF mutation is a glutamic acid for valine substitution at position 600 ($BRAF^{V600E}$) and accounts for >90% of BRAF-positive mutant melanomas.[10,12] Other minor, but important mutations that can activate MEK and ERK signaling include G465A, K600E, and A727V and are thus also important molecular targets in melanoma invasion.[12–14] Mechanistically, $BRAF^{V600E}$ plays a dual role in melanoma initiation and metastatic progression is the most appealing target for the development of drugs to treat primary and metastatic melanoma. Several potent BRAF inhibitors have been developed as potential treatments for melanoma. However, no selective inhibitors of NRAS have been developed. Tibifarnib (R115777), a RAS farnesyltransferase inhibitor, entered clinical trials but, perhaps due to lack of specificity, did not yield promising results. However, since RAS is a key element in MEK/ERK and PI3K signaling, combination therapies involving the inhibitor of these pathways are considered promising therapeutic options.[15,16] A selected number of molecularly targeted agents in the treatment of malignant melanoma are summarized in Table 1.

Selective BRAF Inhibitors

The structures for selective BRAF inhibitors are shown in Figure 1. Vemurafenib (Zelboraf), a 7-azaindole derivative, is a highly selective oral, small molecule, inhibitor of $BRAF^{V600E}$ kinase activity and has been recently approved by the US Food and Drug Administration (FDA) for the treatment of $BRAF^{V600E}$ mutation-positive melanoma. It is co-developed by Roche and Plexxikon, a member of the Daiichi Sankyo Group, and is co-promoted in the US by Genentech and Daiichi Sankyo. Compared to dacarbazine chemotherapy, vemurafenib reduced the risk of death by 56% in patients with previously untreated $BRAF^{V600E}$ mutation-positive, unresectable (inoperable) or metastatic melanoma in the clinical trial. Vemurafenib also reduced disease progression or death by 74% compared to those who received chemotherapy.[17]

PLX4720 is another 7-azaindole derivative that is highly selective $BRAF^{V600E}$ kinase inhibitor in both biochemical and cellular assays. Thus it potently inhibits ERK phosphorylation in $BRAF^{V600E}$-bearing tumor cell lines but not in cells lacking this oncogenic $BRAF^{V600E}$. When dosed orally, PLX4720 caused significant tumor growth delays, including tumor regressions, without evidence of toxicity in $BRAF^{V600E}$-dependent tumor xenograft models.[18]

GDC-0879 is an indane derivative and is highly selective, potent, orally bioavailable $BRAF^{V600E}$ kinase inhibitor. It is also very effective in reducing cellular viability of $BRAF^{V600E}$-mutant inoperable or metastatic melanoma cells thus improving the survival of the mouse with $BRAF^{V600E}$ tumors.[19] AZ628 is a potent tyrosine protein inhibitor for wild-type CRAF and $BRAF^{V600E}$ and also suppresses activity of VEGFR2, DDR2, Lyn, Flt1, and FMS. By causing cell cycle arrest, it triggers apoptosis in melanoma cell lines harboring $BRAF^{V600E}$ and due to inhibition of VEGFR2 it is anti-angiogenic.[20] SB590885 is a selective inhibitor of BRAF, with significant activity only against CRAF and has minimal off-target activity.[21]

Other Kinase Inhibitors

The structures of several potent kinase inhibitors are shown in Figure 2. The biaryl urea derivative sorafenib (Nexavar) is a multi-kinase inhibitor that targets both wild type BRAF ($BRAF^{WT}$) and mutated $BRAF^{V600E}$, CRAF, VEGF receptors 1, 2 and 3, PDGF receptor, Flt-3, p38, c-Kit, and FGFR-1 and thus inhibits tumor growth, angiogenesis and metastatic spread. Its monotherapy is inefficient in melanoma possibly due to survival escape routes provided by other pathways such as tumor-necrosis factor-IX when BRAF is inhibited.[22] Regorafenib (BAY 73-4506) is also a multi-kinase inhibitor targeting c-KIT, VEGFR2, and BRAF and is orally bioavailable. The inhibition of tumor cell signaling kinases (RET, KIT, PDGFR, and RAF) may result in the inhibition of tumor angiogenesis and tumor cell proliferation and shows potent activity in a wide variety of preclinical xenograft models.[23] RAF265 is an oral, highly selective RAF and VEGFR kinase

Table 1. A summary of emerging molecularly-targeted agents for melanoma.

Drug/compound	Molecular/cellular target(s)	Clinical status
Vemurafenib	$BRAF^{V600E}$	Approved for treatment of $BRAF^{V600E}$ mutation-positive melanoma.
PLX4720	$BRAF^{V600E}$	In preclinical studies, induces cell cycle arrest and apoptosis in $BRAF^{V600E}$ mutation-positive melanoma.
GDC-0879	$BRAF^{V600E}$	In preclinical studies, alters survival of cell line- and patient-derived $BRAF^{V600E}$ tumor cells.
AZ628	$BRAF^{V600E}$, $CRAF^{WT}$	In preclinical studies, cytotoxic against $BRAF^{V600E}$ mutation-positive melanoma.
SB590885	BRAF, CRAF	In preclinical studies, decreases growth of BRAF mutation-positive cell lines.
Sorafenib	$BRAF^{WT\ and\ V600E}$, CRAF, VEGFR1, 2 and 3, PDGFR, Flt3, p38, c-Kit, FGFR1	Shown to be ineffective as monotherapy in Phase I, II, and III trials.
Regorafenib	c-Kit, VEGFR2, BRAF	Responses reported in Phase I colorectal cancer trial.
RAF265	RAF, VEGFR	Inhibits the growth of metastatic melanoma related to $BRAF^{WT}$, $c\text{-}KIT^{L576P}$, $NRAS^{Q61R}$, and $BRAF^{V600E/K}$ mutations (Phase I/II).
Sunitinib	Multi-kinase inhibition	In Phase II trial, inconclusive responses reported in melanoma patients with Kit mutations.
NVP-BHG712	EphB4, VEGFR2, CRAF, c-src and c-Abl kinases	Preclinical studies show inhibition of VEGF-dependent angiogenesis.
ZM336372	CRAF BRAF, SAPK2/p38, PKA, PKC, p42 MAPK, CDK1, SAPK1/JNK	Preclinical status.
Perifosin	Akt	Not promising in a single dose Phase II trial.
Dabrafenib and trametinib	BRAF, MEK	Responses reported for combination therapy in a Phase II trial.
Bevacizumab and everolimus	Angiogenesis, mTOR	Well tolerated in the treatment of metastatic melanoma patients in a Phase II trial.
Sorafenib with temsirolimus or tipifarnib	Multiple pathway targeting	Not promising in a Phase II trial.
1,3-bis(3,5-dichlorophenyl) urea (COH-SR4)	Inhibit proliferation and activates apoptosis through multiple pathway targeting	Promising in preclinical study.

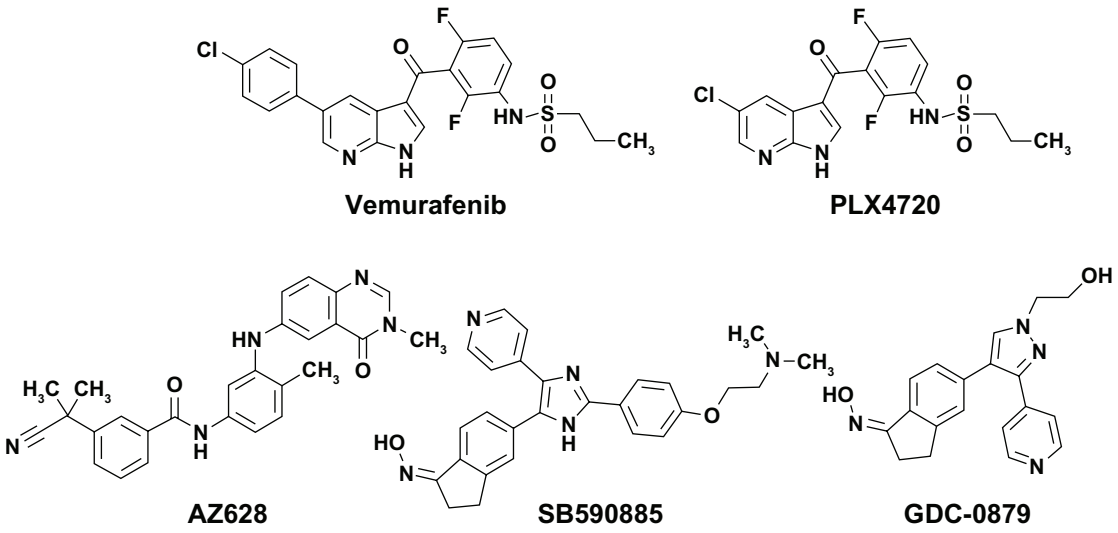

Figure 1. Structures of selective BRAF inhibitors.

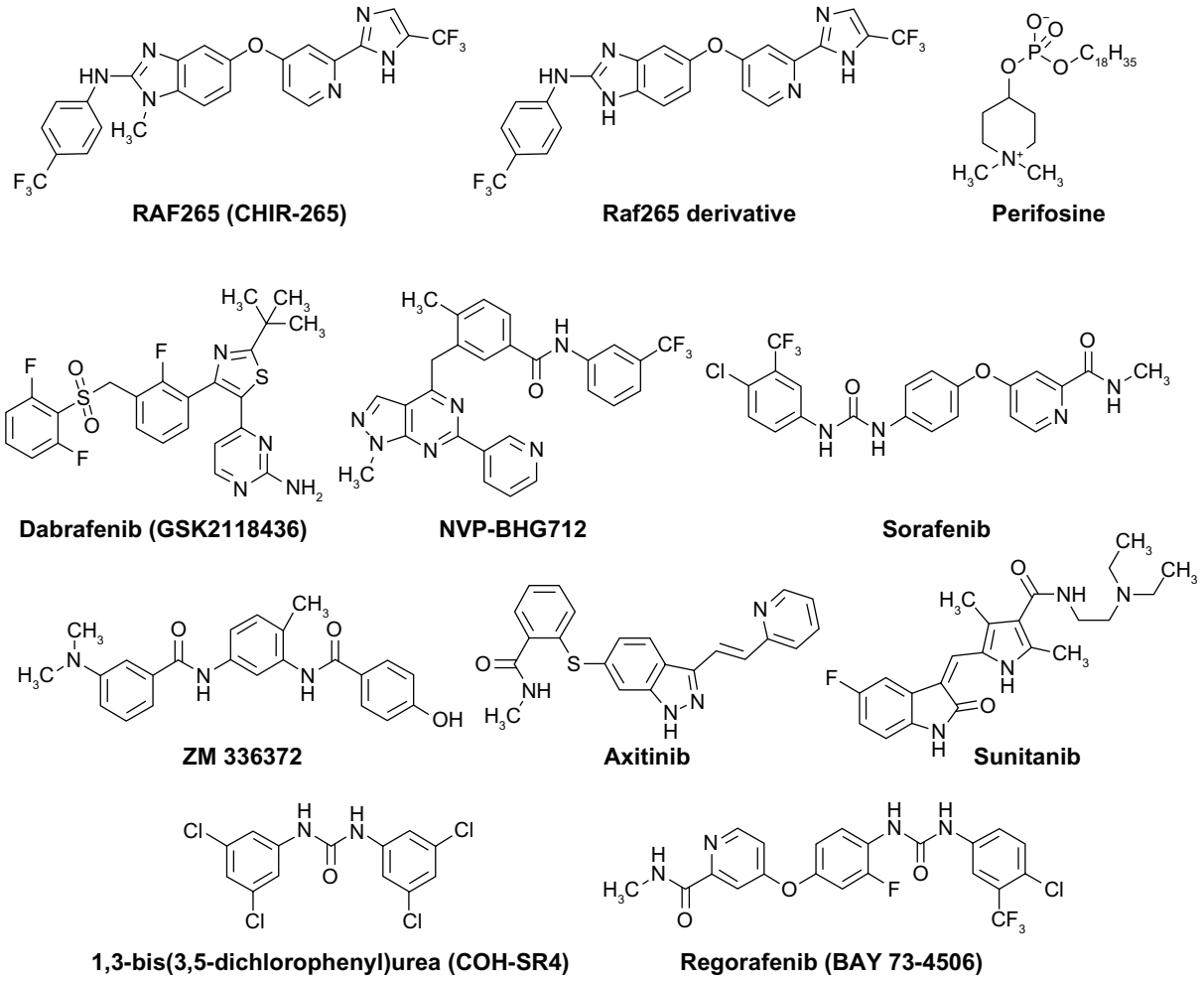

Figure 2. Structures of kinase inhibitors identified as potential treatments for melanoma.

inhibitor and thus exhibits anti-angiogenic activity.[24] In a preclinical study it was found to inhibit the growth of advanced human melanoma tumors related to $BRAF^{WT}$, $c\text{-}KIT^{L576P}$, $NRAS^{Q61R}$, or $BRAF^{V600E/K}$ mutations.[25] The indolone derivative sunitinib, a kinase inhibitor, was found to respond well, but inconclusively, in melanoma patients with KIT mutations in a clinical study.[26]

NVP-BHG712 is an isopurine derivative and an orally effective inhibitor of multiple Eph receptor kinases. It is highly selective for EphB4, relatively less selective for VEGFR2, CRAF, c-src and c-Abl kinases, and dose dependently inhibits VEGF stimulated tissue formation and vascularization.[27] ZM336372 is a potent, selective CRAF inhibitor and also inhibits BRAF, SAPK2/p38 over 17 other protein kinases including PKA, PKC, p42 MAPK, CDK,1 and SAPK1/JNK.[28] It markedly suppresses cellular proliferation and induces cell cycle inhibitors p21 and p18 in carcinoid tumor cells.[29] In a phase II clinical trial perifosin, an alkylphosphocholine analogue that targets Akt, did not show any promise for further development as a single dose therapy for metastatic melanoma, although no major toxicity was observed at the dose levels tested.[30]

Therapy Targeting Multiple Pathways

The BRAF targeted drugs increasingly develop resistance due to reactivation of the MAPK pathway. Moreover, secondary resistance develops almost inevitably, with reactivation of other components of the MAPK pathway or alternative pathway, including but not restricted to the PTEN/Akt pathway. This reflects that multiple pathways seem to be simultaneously of relevance in malignant melanoma and that simultaneous inhibition with active, low toxicity agents holds great promise in the primary treatment of malignant melanoma and in cases with secondary resistance to novel targeted agents. In this context, new candidate drugs capable of targeting multiple critical nodes of

melanoma signaling assume significance. Recent report from Beckman Research Institute, City of Hope National Medical Center: COH-SR4 (Fig. 2), a dichlorophenyl urea compound developed using an SAR strategy has been shown to inhibit proliferation and activates apoptosis in melanoma and has demonstrated significant activity using in vitro cultured cells (NCI-60 panel), as well as both syngeneic and nude mouse models of melanoma.[31]

Combination therapy involving BRAF and MEK inhibitors may be more effective in the treatment of metastatic melanoma. The combination of dabrafenib and trametinib, targeting BRAF and MEK respectively, in patients with metastatic melanoma and $BRAF^{V600E}$ mutations has been shown to improve progression-free survival and provide a favorable safety margin in an open-label study.[32] The combination of bevacizumab, an inhibitor of angiogenesis, and everolimus, an inhibitor of mTOR, was found to have moderate activity and was well tolerated in the treatment of patients with metastatic melanoma in a phase II trial of the Sarah Cannon Oncology Research Consortium.[31] However, in another phase II trial, the combination of sorafenib with temsirolimus or tipifarnib did not show sufficient activity to justify further use. It was suggested based on these results that newer agents by characterization of the molecular targets in individual tumors would show greater promise.[33]

Conclusion

Approved by FDA in 2011, vemurafenib (Zelboraf) is the first personalized targeted therapy for treatment of metastatic melanoma that acts by selectively inhibiting $BRAF^{V600E}$. It causes programmed cell death specifically in $BRAF^{V600E}$ mutation-positive melanoma cell lines by interrupting the BRAF/MEK/ERK pathway. About 60% of melanomas have this mutation and the FDA requires a positive test for the $BRAF^{V600E}$ mutation to be eligible for treatment with this medication. This finding has opened a new avenue for the discovery of targeted drug therapies for melanoma based on the principles of pharmacogenomics.

Overall, safer and effective treatment options for melanoma are scarce. However, there is a rapid pace of discovery and development in the field of molecular targeted drug therapies. It is a challenging task for biomedical scientists and clinicians to track and comprehend the volumes of information regarding the nature of molecular targets and the potential impact on therapeutic outcomes. As a result, it is important for interested groups to regularly communicate in the literature with reviews and editorials in order to provide concise summaries of the molecular and functional nature of targets and whether there are significant clinical trial findings which hold promise for positive patient outcomes.

Author Contributions

Wrote the first draft of the manuscript: MOFK. Contributed to the writing of the manuscript: MOFK, CLR. Jointly developed the structure and arguments for the paper: MOFK, CLR. Made critical revisions and approved final version: MOFK, CLR. All authors reviewed and approved of the final manuscript.

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Disclosures and Ethics

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