



## New Insights and Therapeutic Implications in Cutaneous Melanoma

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### Abstract

Melanoma is a highly aggressive tumour with poor prognosis in the metastatic stage that arises and evolves due to a myriad of genetic and epigenetic events. Among these, the interaction between epigenetic alterations (i.e., DNA methylation, histone modifications, mRNA silencing by miRNAs and nucleosome repositioning) has been recently identified as playing an important role in melanoma development and progression by affecting key cellular pathways such as cell cycle regulation, DNA repair, apoptosis, invasion and immune recognition. A number of new oncogene candidates such as MAPK1/2, ERBB4, GRIN2A, GRM3, RAC1, and PREX2 were identified. Their particular role in melanoma biology is currently under investigation. However, with the development of new immunotherapies (monoclonal antibodies specific for cytotoxic T lymphocyte-associated antigen 4 [anti-CTLA-4] and programmed death protein-1 [anti-PD1]) and small molecules interfering with intracellular pathways (BRAF inhibitors and mitogen-activated protein kinase kinase [MEK] inhibitors) the use of this approach is becoming a viable treatment strategy for locally advanced melanoma. In this review, the risk factors, pathogenesis, and innovations in treatment of melanoma, synthetic small molecule inhibitors, combined therapies and current progress in the development of phytochemical therapies and new targets will be mentioned.

### Keywords

Malignant melanoma, Immunomodulatory agents, BRAF inhibitor, MEK inhibitor, C-Kit inhibitor

### Introduction

Malignant melanoma (MM) is a mainly tumor of cutaneous origin which develops from melanocytes. Although it constitutes 5% of skin cancer, it is responsible for 75% of skin cancer-related deaths [1]. It also originates from oral, conjunctival or vaginal mucosa, uveal tract and leptomeninges [2]. Developmentally, melanocytic progenitor cells migrate from neural crest to the skin and malignant melanocytes forming melanoma also have migratory feature. High metastatic potential of melanoma could be explained by this feature [3]. Besides, it is known that large numbers of melanocytes are also arise from Schwann cell precursors of nerves innervating the skin [4]. Melanoma is diagnosed 10-15 years earlier with respect to more common cancers such as breast, lung and colon cancer. More than 35% of melanoma patients are below 45 years of age. In the United States, it is the most common type of cancer in young adults between the ages of 25-29 and the second most frequent type of cancer in

adolescents and young adults between the ages of 15-29 [5].

By the time, the increase in the incidence of MM has necessitated new treatment approaches. As factors in the pathogenesis were understood more clearly, targeted therapies have been raised and the studies have increased [3,5]. Although a new viewpoint does not mean an increase in survival, it may shed light on treatment decision and better treatment alternatives.

Traditional therapeutics and immunomodulatory agents have not shown much efficacy against metastatic melanoma. Agents that target the RAS/RAF/MEK/ERK (MAPK) signaling pathway - the BRAF inhibitors vemurafenib and dabrafenib, and the MEK1/2 inhibitor trametinib - have increased survival in patients with metastatic melanoma. Further, the combination of trametinib and dabrafenib has been shown to be superior to single agent therapy for the treatment of metastatic melanoma. However, resistance to these agents develops rapidly. Studies of additional agents and combinations targeting the MAPK, c-kit, PI3K/AKT/mTOR (PI3K), and other signaling pathways are currently underway. Furthermore, studies of phytochemicals have yielded promising results against proliferation, survival, invasion, and metastasis by targeting signaling pathways with established roles in melanomagenesis. The relatively low toxicities of phytochemicals make their adjuvant use an attractive treatment option [6].

The survival rates of melanoma, like any type of cancer, become worse with advancing stage. Spectrum theory is most consistent with the progression of melanoma from the primary site to the in-transit locations, regional or sentinel lymph nodes and beyond to the distant sites. Therefore, early diagnosis and surgical treatment before its spread is the most effective treatment. In addition to genomic profiling and sequencing will form the basis for molecular taxonomy for more accurate subgrouping of melanoma patients in the future. Sentinel lymph node biopsy has become a standard of care for staging primary melanoma without the need for a more morbid complete regional lymph node dissection. With recent developments in molecular biology and genomics, novel molecular targeted therapy is being developed through clinical trials [7].

### Risk Factors of Malignant Melanoma

#### Ultraviolet radiation (UVR)

Intermittent excessive sunlight exposure and severe sunburns especially in childhood are major risk factors. Especially UVB radiation is more potent cause, its evidence is higher rates of

melanoma around the equator receives the most intense UVB. Artificial UV sources are also hazardous. The long-term cumulative UVR play a role in development of lentigo maligna melanoma. It has been showed that the regular use of sunscreens reduces the incidence of melanoma [2].

### Skin phototypes

People with skin phototype I or II, red or blond hair, blue or green eyes, or freckles are at increased risk [2].

### History of melanoma or multiple nevi

Individuals with personal or family history of melanoma, high number of nevi or dysplastic nevi, or large congenital melanocytic nevi, have higher risk [2].

### Genetics

Germline mutations in cyclin-dependent kinase inhibitor 2A (CDKN2A) located at chromosome 9p21 are responsible for about 40% of hereditary melanoma cases. These patients have also the risk of pancreatic cancer. CDKN2A encodes two proteins: First of them, P16INK4A is a cell-cycle regulator. P16INK4A binds and inhibits cyclin-dependent kinases, CDK4 and CDK6 thus causes G1 cell cycle arrest. If p16 loses its function or is inactivated by mutation, unrestrained CDK4 phosphorylates and inactivates retinoblastoma protein, and brings out transcription factor, E2-F, and thus causes the cells to enter the S-phase. In the absence of check-point regulation, increased cellular proliferation leads to melanoma formation. The second protein encoded by CDKN2A is P14ARF. This protein inhibits the cellular oncogen, HDM2 which accelerates the degradation of p53, a tumor-suppressor gene. The mutation of P14ARF causes melanomagenesis by dysfunction of p53. Thus germline mutations in CDKN2A lead to formation of tumor with both mechanisms. Somatic mutations in the BRAF gene are responsible for 66% of melanomas. For humans, BRAF is one of the three functional RAF proteins and one of the main components of mitogen-activated protein kinase (MAPK) pathway that is one of the key molecular pathways in melanoma formation. MAPK signaling pathway regulates cell development, proliferation and differentiation that occur in response to various growth factors, cytokines and hormones [8,9].

Growth factors that bind to tyrosine kinase associated receptors (c-KIT) localized to cell membrane activates this pathway. Activation of c-KIT leads to activation of RAS and then subsequent phosphorylation cascade activates RAF/MEK/ERK. Activated ERK translocates to the nucleus and activates transcription factors such as cyclin D1. These transcription factors control key cellular functions and could cause cancer if they are abnormally activated. Abnormal MAPK signaling leads to uncontrolled cell proliferation and melanoma formation. NRAS and BRAF mutations occur in the superficial spreading melanoma localized in intermittent sun damaged skin such as trunk. Therefore BRAF mutation is the most common mutation in melanoma. c-KIT mutations have been found in 40% of mucosal, 35% of acral, and 28% of lentigo maligna melanomas that arise in chronically sun damaged skin. GNAQ (guanine nucleotide-binding protein G) mutation which activates MAPK cascade is responsible for uveal melanomas, especially hereditary type (50%). One of the key pathways in the pathogenesis is PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B) pathway. Growth factors binding to tyrosine kinase receptors regulate mTOR signalling and block FOXO by the activation of PI3K that converts PIP2 into active PIP3 and its downstream effector AKT. mTOR signalling leads to increased cell proliferation and blockage of FOXO causes to decreased apoptosis. Thus tumorigenesis occurs. Loss of PTEN (phosphatase and tensin homologue) which is a tumor suppressor protein involved in the same pathway leads to the same result in the formation of PIP3. Loss of PTEN especially in the late stages of melanoma contributes to progression to the invasive melanoma. Other genes are following low penetrance susceptibility genes [7-10].

**MITF (Microphthalmia associated transcription factor) gene:** MITF protein is the main regulator of melanocyte differentiation. Amplification of MITF gene contributes to "lineage addiction", a novel mechanism to drive oncogenesis.

**MC1R (Melanocortin-1 receptor) gene:** MC1R polymorphisms cause to the synthesis of a high level of carcinogenic pheomelanin and lead to decreased eumelanin/pheomelanin ratio. These polymorphisms of MC1R are clinically associated with red hair colour, fair skin and 2 to 4-fold increased risk for development of melanoma and also contribute to the risk of non-melanoma skin cancer. MC1R variants have been associated with melanoma cells which contain BRAF-V600E.

**XP (xeroderma pigmentosum) genes:** Genetic defects in seven XP repair genes lead to increased mutagenesis and early carcinogenesis. XP is an autosomal recessive genodermatosis. XP patients are at 600 to 1000-fold increased risk of skin cancer, including melanoma.

**BRCA2 (breast cancer susceptibility gene):** BRCA2 mutation carriers have 2.8 times greater risk for melanoma. However, the mechanism of genetic connection is not clear.

### Melanoma Treatment

According to staging system developed by American Joint Committee on Cancer (AJCC), patients with MM are evaluated in three categories. These are localized disease (stage I-II), regional disease (stage III) and metastatic disease (stage IV). Wide surgical excision is the primary treatment for localized disease. The recommended surgical margins for melanomas measuring 1.00 mm or less, 1.01 to 2 mm and more than 2 mm in thickness are 1 cm, 1 to 2 cm and 2 cm, respectively. Wide surgical excision of primary tumor and complete lymph node dissection should be performed to patients with regional disease. After surgical treatment, adjuvant treatment options such as IFN- $\alpha$ , high dose ipilimumab, biochemotherapy and radiotherapy could be preferred to minimize risk of recurrence. Treatment options for stage III patients with in transit metastasis include local therapy such as complete surgical excision to clear margins, intralesional Talimogene laherparepvec (T-VEC), BCG, IFN or IL-2 injection, local ablation therapy, topical imiquimod for superficial dermal lesions and radiotherapy, regional therapy (i.e. isolated limb perfusion with melphalan) or systemic therapy. If feasible, surgical resection is recommended for patients with limited metastatic disease. The options for patients with disseminated distant metastatic disease are systemic therapy with anti-PD1 antibodies, BRAF inhibitors, MEK inhibitors, anti-CTLA4 antibodies, Kit inhibitors, cytotoxic agents, high dose IL-2 or biochemotherapeutic agents, intralesional T-VEC injection, radiotherapy, participation in a clinical trial or supportive care [11]. The systemic treatment options are discussed in more detail below.

### Systemic treatment options

These treatment options include clinical trial or systemic therapy with high-dose interleukin-2 (IL-2), ipilimumab or vemurafenib. Other regimens are temozolomide, dacarbazine, imatinib (for c-Kit-mutant melanoma), paclitaxel (paclitaxel monotherapy or combined with carboplatin), dacarbazine/temozolamide-based combination chemotherapy or biochemotherapy (with cisplatin, vinblastine, dacarbazine, IL-2 and IFN- $\alpha$ ). In the last 30 years, none of several alternative systemic treatment options could exceed the success of classic dacarbazine/temozolamide regimen or IL-2 treatment.

First of these systemic treatment agents, ipilimumab which is an immunotherapeutic agent was approved by FDA in March 2011. Then BRAF inhibitor, vemurafenib received FDA approval in August 2011. Thereafter two agents which are dabrafenib, a BRAF inhibitor and trametinib, a MEK inhibitor were approved by FDA on May 29, 2013. These agents could prolong the survival and could be summarized as gene targeting drugs and immunotherapeutics [12].

### Chemotherapy/Biochemotherapy regimens

Although temozolomide, dacarbazine, paclitaxel (paclitaxel monotherapy or combined with carboplatin), dacarbazine/

temozolamide-based combination chemotherapy or biochemotherapy (with IL-2 and IFN- $\alpha$  or cisplatin and vinblastine alone) are still among the options, response rates of chemotherapeutics do not exceed 20%. According to the meta-analyses, biochemotherapeutics improve the response rates but do not increase the overall survival. Choosing the first-line systemic therapy agents is based on many factors such as course of the disease, presence of BRAF mutations and melanoma-related symptoms. Asymptomatic patients with low tumor burden who have time to generate anti-tumor immune responses are good candidates for immunotherapeutics, especially ipilimumab. By contrast, vemurafenib should be considered for symptomatic, BRAF-mutated patients with high tumor burden patients progressed despite immunotherapy. The best alternative for eligible patients is to participate in clinical trials. Due to low efficiency, the above-mentioned chemotherapy and biochemotherapy regimens can be selected when they are mandatory [12,13].

### Palliative radiation therapy

Radiation therapy can alleviate the symptoms of metastatic disease. It provides symptomatic relief of 40% in the central nervous system metastases and 70-80% in others. 46 Specific indications are pain control, stabilization of bone metastases, local control of spinal cord compression, brain metastasis (whole brain radiotherapy or stereotactic radiosurgery) and skin involvement [2].

### Adjuvant therapy

The adjuvant therapy is given for reducing relapse risk due to occult disease after surgical treatment. Nowadays, the only FDA approved therapy for patients whose estimated risk of recurrence exceeds 30%; i.e. patients with stage IIB (Breslow of 2-4 mm ulcerated (T3b) or > 4 mm nonulcerated (T4), without lymph node metastasis) disease and stage III disease with lymph node metastasis or in-transit disease is adjuvant high-dose INF- $\alpha$ -2b for one year. The positive impacts of adjuvant IFN therapy on relapse-free survival has been shown by numerous publications and meta-analysis. Although survival advantage of high-dose IFN has been shown in two different studies, this benefit decreases after 10 years. Conventional high-dose IFN therapy is applied for a total of 1 year, 20 MU/m<sup>2</sup> intravenously 5 days a week for 4 weeks followed by 10 MU/m<sup>2</sup> subcutaneously 3 days a week for 48 weeks [14].

**Pegylated-interferon alpha (Peg-IFN- $\alpha$ ):** Peg-IFN- $\alpha$  was obtained by attachment of polyethylene glycol (PEG) polymer chains to IFN. PEGylation protects IFN from proteolytic degradation and extends the half-life of IFN. Peg-IFN- $\alpha$  is an alternative to high-dose IFN therapy for stage III melanoma patients with positive sentinel node or clinically positive nodes following complete resection. However it is not an option for stage III patients with in-transit metastases. Peg-IFN- $\alpha$  has a positive effect on relapse-free survival and has been approved by FDA. It is applied subcutaneously one day a week for 3 to 5 years [14].

**Adjuvant radiotherapy:** Adjuvant radiotherapy could be used to control nodal recurrences in patients with cervical lymph node involvement (2 or more nodes and/or 2 cm or more in diameter), macroscopic extra-capsular lymph node extension, a great number of lymph nodes involvement (4 or more) or large lymph nodes (3 cm or more in diameter). Hypofractionated radiotherapy seems to be as effective as the conventional schedule. If melanoma recurs, adjuvant radiotherapy is indicated again after resection [13].

Finally, the goal of treatment in stage III patients with satellite or in-transit metastases is to maintain local control. For skin metastases, surgery may be considered. Complete excision with negative surgical margin of one or a few in-transit metastases is an ideal approach. However, it could not be applied in numerous or extensive lesions. If in-transit metastases, especially dermal lesions, is not eligible for surgical excision, other alternatives are intralesional BCG or IFN- $\alpha$  injections or topical imiquimod [13]. When multiple lesions are localized to the limb and surgery is inadequate, application of isolated limb perfusion with melphalan has been shown to be successful. It has

**Table 1:** Gene targeting drugs.

BRAF inhibitors	Vemurafenib, Dabrafenib, Encorafenib
MEK inhibitors	Trametinib, Selumetinib, Cobimetinib
Kit inhibitors	Imatinib, Sunitinib, Nilotinib, Dasatinib, Masitinib
PI3K Inhibitors	LY294002, Wortmannin
Mammalian target of rapamycin (m-TOR) inhibitors	Rapamycin

**PI3K:** *phosphatidylinositol 3-kinase*

been reported that it has high response rates and provides long-term survival [15].

### Treatment of metastatic melanoma

Treatment of metastatic melanoma is based on limited or disseminated disease [11]. 1. For limited metastatic disease, resection may provide significant palliation and may even prolong survival. Surgery has been the main treatment for localized melanoma. Surgical excision of subcutaneous or distant lymph node metastases provides local control and reduce morbidity [2,3]. However, according to some authors, the administration of systemic therapy prior to surgery is a more appropriate approach for solitary visceral metastasis. As an alternative, limited metastases could also be treated with systemic therapy with the standard treatment regimen or the treatments within the scope of clinical trials. If complete resection could not be carried out, limited metastasis should be treated as disseminated disease [13]. Treatment of disseminated disease is supportive therapy.

Long-term responses were achieved in approximately 5% of patients using the immunotherapy agent interleukin 2, however the low response rates and toxicities excluded this agent in the majority of patients with melanoma. Vemurafenib and ipilimumab have been approved by US Food and Drug Administration for metastatic malignant melanoma. These agents have significantly improved the survival rates of melanoma patients. Many new agents are in development. However, the majority of long term survivors of metastatic melanoma who respond to targeted therapies develop resistance and disease progression. Combinations of targeted therapies are needed to prevent resistance as well as to improve survival rates. Currently, ipilimumab is the most promising drug. Although it has effective response rates, its use is limited by severe reactions [16].

### Gene targeting drugs

Gene targeting drugs are summarized (Table 1).

**BRAF-Inhibitors:** Shortly after the compelling discovery of BRAF mutations in melanoma, BRAF mutations were also found to be common in melanocytic nevi, including both acquired and congenital nevi, as well as melanoma [17,18]. However, it is not sufficient alone for development of melanoma. In addition, the absence of germline B-RAF mutations in patients with sporadic and familial melanoma suggests that B-RAF mutation does not play an important role in susceptibility to development of melanoma. This finding implicates mutation of the BRAF gene and activation of the MAPK pathway as crucial steps in the development of not only melanoma but also benign melanocytic neoplasia. Notably, melanocytic nevi are normally indolent for decades despite the presence of oncogenic BRAF mutations. Therefore, it is conceivable that growth arrest of melanocytic nevi results from oncogene induced senescence acting as an effective cellular brake against BRAFV600E mediated oncogenic signalling. This hypothesis is supported by a recent investigation showing that sustained BRAFV600E expression in human melanocytes induces cell cycle arrest, which is accompanied by the induction of both p16INK4a and senescence-associated acidic beta-galactosidase activity, a commonly used senescence marker. The expression of p16INK4a and senescence-associated acidic beta-galactosidase has also been demonstrated in in vivo samples of melanocytic nevi.

These genetic differences may result in varying responses to therapeutic agents. As more molecular-targeted therapies are being developed, identification of molecular signatures and classification of melanoma subtypes based on their genetic profiles will be crucial in determining the therapeutic regimen [18].

**Vemurafenib:** Vemurafenib is a selective inhibitor of V600E mutated BRAF. It was approved in August 2011. The recommended dose is 960 mg, orally twice daily. It is indicated for patients with positive BRAF V600E mutation test (Cobas 4800 or THxID BRAF test). The average survival time in patients with metastatic melanoma is 6 to 10 months and could be lengthened to 18 months by vemurafenib. Clinical responses to vemurafenib are quick (occurs in days to weeks) but short-lived (limited to 5-6 months). Dose modifications may be necessary due to side effects such as squamous cell carcinoma (20-30%), arthralgia (20%), severe photosensitivity (12%), keratoacanthoma (10%), verruca vulgaris, acneiform eruption, follicular hyperplasia, palmar/plantar hyperkeratosis, panniculitis, alopecia. Recently, it has been reported that it causes radiosensitivity. The formation of secondary skin tumors could be explained by activation of MAPK signaling pathway which is paradoxically activated by the inhibition of BRAF and induces RAS mutations and the increase of ERK signaling. Therefore RAS mutation testing is necessary to determine and follow carefully the patients who are susceptible to squamous cell carcinoma [19]. The activity of vemurafenib has been proven and the usage of this drug in stage III (without distant metastases) has also been planned. NCRN442 BRIM 8 is a phase III, randomized, double-blind, placebo-controlled study of vemurafenib adjuvant therapy in patients with surgically resected, cutaneous BRAF-mutant melanoma at high risk for recurrence and the study is open [13,19-22].

**Dabrafenib:** Dabrafenib is a selective BRAF kinase inhibitor that has an activity similar to vemurafenib in patients with V600E BRAF mutation. In the phase I and II trials, it has been shown that the median progression-free survival was 5.5 months and the response rate was 78%. These results have also been observed in patients with brain metastases [23]. Because of the potential neurotoxic effects, dabrafenib was developed not to cross the blood-brain barrier. However this drug could reach the tumor in the brain due to disruption of the normal blood-brain barrier in the presence of melanoma macrometastases. With dabrafenib, Falchook et al could extend the average survival time from 5 months to 12 months in patients with brain metastases. The recommended dose is 150 mg, orally twice daily and its activity starts within 2 weeks. However, phase I trial of dabrafenib and MEK inhibitor trametinib combination therapy has been started due to the development of resistance to dabrafenib in 5.5 months. Safety and efficacy are comparable with vemurafenib; there is no photosensitivity but severe fever is more common. 55 Cutaneous side effects are seen in approximately 90% of patients. The most common finding was reported as keratotic hyperproliferative lesions (verruiform keratoses (49%), squamous cell carcinoma (20%) and acute plantar hyperkeratosis (22%)). Besides Grover's disease (27%), seborrheic keratosis, epidermal cysts, acneiform eruption, hair loss and changes in hair structure are also seen [23]. FDA approval was taken on May 09, 2013.

**Encorafenib:** Encorafenib (LGX818) is a new and potent BRAF inhibitor that has selective anti-proliferative and apoptotic activity in patients with V600E BRAF mutation. It has a similar side effect profile to vemurafenib and dabrafenib. The COLUMBUS trial which is an open label, multi-center, phase III study comparing the efficacy and safety of encorafenib plus binimetinib to vemurafenib and encorafenib monotherapy in patients with locally advanced unresectable or metastatic melanoma with BRAF V600 mutation is still ongoing [24].

**MEK-Inhibitors (Trametinib, Selumetinib and Cobimetinib):** MEK, a member of the MAPK signaling pathway is usually active in melanoma. MEK inhibitors inhibits cell proliferation and induces apoptosis. In phase I trial of trametinib, the median progression-free survival was 5.7 months and MEK has been reported as a valid therapeutic target. 56 In subsequent phase II trial, it has been reported

that well tolerated dose of trametinib was 2 mg, orally once daily but minimal activity was observed in the group treated with previously BRAF inhibitors and BRAF inhibitor resistance mechanisms confer resistance to MEK-inhibitor monotherapy. Progression free survival time up to 4 months was observed in patients treated with only chemotherapy and/or immunotherapy [25]. However the usage of trametinib with BRAF inhibitors has come up, with the aim of providing efficiency and preventing secondary skin tumors. In an open-label study involving 247 patients with metastatic melanoma, dabrafenib (150 mg) and trametinib (1 or 2 mg) have been administered as monotherapy or in combination. Progression-free survival time has been found as 9.4 months in the combination group and 5.8 months in the monotherapy group. Partial or complete response rates have been 76% in the combination group and 54% in the monotherapy group. It has been concluded that combined therapy with dabrafenib and trametinib at full monotherapy doses was safe, increased the rate of pyrexia, reduced the rate of proliferative skin lesions and prolonged progression-free survival [26]. Trametinib has received FDA approval on May 29, 2013 as a monotherapy agent.

**Selumetinib (AZD6244),** another MEK inhibitor is a novel agent for metastatic uveal melanoma. In phase II trial included 98 patients with uveal melanoma, selumetinib and temozolomide have been randomized. In temozolamide group, tumor regression was 11% and progression-free survival was 7 weeks whereas in selumetinib group, tumor regression was 50% and progression-free survival was 16 weeks. At the 2013 ASCO (American Society of Clinical Oncology) Annual Meeting, it has been reported that selumetinib is the first systemic therapy, which can be really effective, in uveal melanoma. Finally, cobimetinib (GDC-0973) has strongly inhibited MEK in mutant tumor models and is currently under investigation [27].

**KIT-Inhibitors (Imatinib, Sunitinib, Nilotinib, Dasatinib, Masitinib):** In melanoma, it appears that c-kit is highly expressed in the in situ and in the junctional component of invasive lesions, but expression is lost once the melanoma becomes invasive and metastatic [28-30]. Laboratory studies suggest that this is because the loss of c-kit expression allows the melanoma cells to escape from kit ligand (stem cell factor) - induced apoptosis [31].

Phase II study results are variable and the patients with KIT mutations, especially in exon 11 and 13 are more likely to respond to KIT inhibitors. Trials of sunitinib, sorafenib, nilotinib, dasitinib and masitinib as monotherapy agent have been initiated. The only phase III trial in KIT mutant melanoma is the trial of nilotinib versus dacarbazine (DTIC). Then the trial was modified to a single-arm phase II trial of nilotinib alone. Another strategy for patients who developed resistance is combining KIT inhibitors with chemotherapy, immunotherapy, and other targeted therapies. Immunotherapeutics are anti-CTLA-4 (ipilimumab), anti-PD-1 (programmed cell death protein 1) antibody (nivolumab and lambrolizumab), anti-PD-L1 (programmed cell death ligand 1) antibody (MPDL3280A), TVEC and the combination of anti-CTLA-4 (ipilimumab) with GM-CSF and anti-PD-1 antibody [32].

**PI3K (phosphatidylinositol 3-kinase) Inhibitors:** The PI3K (phosphatidylinositol 3-kinase)-AKT pathway is one of the most important signaling networks in cancer. There is growing evidence that activation of this pathway plays a significant role in melanoma, frequently in the setting of concurrent activation of RAS-RAF-MEK-ERK signaling. This evidence includes the identification of genetic and epigenetic events that activate this pathway in melanoma cell lines and clinical specimens. In addition, functional experiments have demonstrated important roles for the PI3K-AKT pathway in both melanoma initiation and therapeutic resistance. The availability of many inhibitors against the PI3K-AKT pathway is rapidly leading to the development of trials that will ultimately determine its clinical significance in this disease. The rational development of such therapies will be facilitated by strategies that utilize the growing understanding of the complexity of the regulation and roles of this pathway [33].

In a cell culture study performed by Sinnberg *et al.*, it was

**Table 2:** Immunotherapeutic drugs.

Anti-CTLA-4	Ipilimumab
Anti-PD-1 Antibody	Nivolumab, Pembrolizumab, MPDL3280A
TVEC	

**CTLA-4:** cytotoxic T-lymphocyte antigen 4; **PD-1:** Programmed Cell Death Protein 1

determined that rapamycin, wortmannin or LY294002 combined with cisplatin or temozolomide increased the apoptosis of melanoma cells compared to treatment with rapamycin, wortmannin, LY294002 or chemotherapeutics alone [34]. Similarly, temozolomide or cisplatin combined with LY294002 or rapamycin in another study. It was shown that these combinations sensitize melanoma cells to apoptosis induced by chemotherapeutics and the most potent combination is temozolomide and rapamycin [35].

**m-TOR inhibitors:** Rapamycin (sirolimus) is a natural fungicide isolated from *Streptomyces hygroscopicus*. Previously it has been used to prevent rejection in organ transplantation. Afterwards, it was shown that rapamycin has antitumor activities. m-TOR is an atypical serine/threonine kinase and plays a key role in proliferation, migration and differentiation of cells. Rapamycin inhibits the activity of m-TOR and thus it causes cell cycle arrest, apoptosis, growth inhibition, inhibition of cell migration and invasion, reduce in protein synthesis and changes in the cytoskeleton [36]. Two cases with metastatic melanoma who showed significant remission after combination of carboplatin and paclitaxel with rapamycin have been reported [37]. In a recent cell culture study, it was shown that rapamycin in combination with multikinase inhibitor sorafenib inhibits growth and survival of metastatic melanoma cells [38].

## Immunotherapeutic drugs

Immunotherapeutic drugs are summarized (Table 2).

**Anti-CTLA-4 (Ipilimumab) :** CTLA-4 (cytotoxic T-lymphocyte antigen 4) is an immune checkpoint receptor which is expressed on T cells and suppresses immune system. T-cell activation requires both the stimulation of the T-cell receptors and co-stimulatory signals that are caused by binding B7, expressed on antigen-presenting cells to CD28 on T-cells. CTLA-4 is a homologue of CD28 and increases after T-cell activation. Its function is to connect to B7 with racing CD28, thus it prevents the costimulatory signals and suppresses T-cell activation. Ipilimumab is a monoclonal antibody that binds to CTLA-4. It leads T-cell activation due to the inhibition of natural braking mechanisms which suppresses the immune system. After demonstrating the prolongation of overall survival in 676 patients with metastatic melanoma in randomized phase III trial, ipilimumab has received FDA approval in March 2011 [11,13].

Patients treated with ipilimumab should be monitored carefully for potentially lethal immune-related adverse events, seen in 60% of patients. The most common of these are rash and diarrhea. Colitis is generally mild or moderate, but sometimes, can be very serious. Hypophysitis, hepatitis, pancreatitis, iridocyclitis, lymphadenopathy, neuropathy and nephritis have also been reported. After completion of the 12 months induction therapy, if progression occurs in patients with partial or complete response or stable disease for three months, ipilimumab at 3 mg/kg can be administered every 3 weeks for up to 4 treatments [13]. Preclinical studies have shown that targeted therapies work optimally with an active immune system. Therefore, it has been aimed to increase the success of treatment using consecutively ipilimumab and vemurafenib. For this aim, ipilimumab treatment followed by vemurafenib and vemurafenib treatment followed by ipilimumab have been administered to different groups in the E1612 study conducted by ECOG (Eastern Cooperative Oncology Group) [19,39].

Combination of Ipilimumab with GM-CSF: GM-CSF (Sargramostim) is a growth factor used to increase leukocytes after chemotherapy or stem cell transplantation. When combined with ipilimumab, increase of 13% in overall survival and decrease in

the incidence of side effects, especially related to pulmonary and gastrointestinal toxicity have been reported [40].

**Anti-PD-1 (Programmed Cell Death Protein 1) Antibody:** PD-1 (programmed cell death protein 1) is another immune checkpoint receptor that is expressed on activated T cells, B cells and macrophages and further suppresses the immune system compared with CTLA-4. Tumor cells use PD-1 to protect themselves against the immune system attacks. Anti-PD1 antibodies block the biochemical shield formed by tumor cells.

**Nivolumab:** Nivolumab is a human IgG4 monoclonal antibody that targets PD-1. Anti-tumor activity and safety of these agent have been shown. At the ASCO annual meeting, median survival time of metastatic melanoma patients treated with nivolumab has been reported as 20 months. Side effect profile of nivolumab has been found to be more favorable compared to ipilimumab. Advanced phase III clinical studies of 3 mg/kg dose are planned. In a new phase I trial, nivolumab and ipilimumab has been used as concurrent or sequenced. In the concurrent-regimen group, response rates were higher and tumor reduction was 80% or more in 53% of patients. Side effects were not frequent or severe when compared with nivolumab or ipilimumab alone. It has been reported that the combination of these antibodies targeting two different immune checkpoint promises exciting therapeutic results [41].

**Lambrolizumab:** Lambrolizumab is another human IgG monoclonal antibody that targets PD-1. At the ASCO annual meeting, it has been reported that lambrolizumab exterminates the tumor in 85 of 294 patients (35%). Besides its anti-tumor activity, it is well-tolerated and has manageable side effects [42].

**MPDL3280A:** MPDL3280A is a new monoclonal antibody that targets PD-L1. Anti-PDL1 antibodies are thought to be more selective than the anti-PD1 antibodies. MPDL3280A has been reported to has good response rates in 31% of patients with metastatic melanoma [43].

**TVEC:** TVEC is an oncolytic immunotherapeutic, genetically modified version of herpes simplex type-1. It is injected directly into tumors every 2 week, selectively replicates in tumors and stimulates anti-tumor immune response by producing GM-CSF. According to the first Phase III trial, TVEC destroys the tumor for at least 6 months and provides an increase of overall survival [44]. On 2015, October 27<sup>th</sup>, TVEC approved for the treatment of melanoma lesions in the skin and lymph nodes by FDA [45].

## Follow up for Melanoma

The principles of treatment can be summarized as only dermatological examination for stage 0, physical examination and lymph node ultrasound in addition to dermatological examination for stage IA-IIA, chest x-ray, computed tomography (CT) and/or positron emission tomography (PET) and magnetic resonance imaging (MRI) scans of the brain in addition to former recommendations for stage IIB-IV.

## Conclusion

Fully understanding of the disease is necessary to provide more accurate, personalized preventive and therapeutic approaches. Thus cancer research is focusing on understanding the roles of signal transduction pathways and developing new targeted therapies. When developing new therapies, to generate personalized treatment alternatives, to reveal drug activities by melanoma cell culture studies and to perform controlled comparative studies with larger groups of patients should be aimed. This review pays attention on etiological factors and molecular pathways of melanoma and novel targeted therapies for melanoma. These targeted therapy agents that have higher response rates compared to the standard chemotherapy can provide promising therapeutic approaches. However, additional studies are needed to define the ideal therapy for melanoma.

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