

## Cutaneous Malignant Melanoma and Targeted Therapy Based on the Biomarkers

### Abstract

Malignant melanoma is the most aggressive form of cutaneous malignancy. It accounts for more than 75% of cancer-related deaths among cutaneous malignancies. It accounts for <5% of cutaneous malignancy. Numerous biomarkers are used in malignant melanoma with varying clinical applications, including diagnostic purposes, prognosis, therapeutic purpose, and targeted therapy against melanoma. Systemic chemotherapy in malignant melanoma has little benefit compared to immunotherapy and targeted therapy. The observed overall survival with systemic chemotherapy is much less compared with targeted therapy in advanced or metastatic melanoma. Various targeted therapies are currently used in melanoma treatment including BRAF inhibitors such as vemurafenib and dabrafenib; MEK inhibitors such as trametinib; anti-CTLA-4 antibodies such as ipilimumab; and anti-programmed cell death 1 antibodies such as nivolumab, pembrolizumab, and pidilizumab. This study discusses the role of biomarkers and targeted therapies based on the biomarker.

**Keywords:** Anti-CTLA-4 antibodies, anti-programmed cell death 1 therapy, biomarkers, BRAF inhibitors, MEK inhibitors, melanoma, targeted therapy

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### Introduction

Malignant melanoma is the most aggressive form of cutaneous malignancy. It accounts for more than 75% of cancer-related deaths among cutaneous malignancies. It accounts for <5% of cutaneous malignancy. It mainly arises from melanocytes.<sup>[1]</sup> Sometimes, it is very difficult to differentiate between benign nevi from malignant melanoma. Numerous biomarkers are used in malignant melanoma with varying clinical applications, including diagnostic purposes, prognosis, therapeutic purpose, and targeted therapy against melanoma. Surgical excision is the primary treatment for cutaneous malignancy in early stage of melanoma, but in advanced stage, targeted therapy and immunotherapy play a significant role.

### Importance of Biomarkers in Melanoma

The incidence of melanoma is rising over the past few decades. It is one of the malignancies which showed increased incidence across the world. There are various causes for the increasing incidence

of melanomas such as prolonged exposure of ultraviolet (UV) rays, increased awareness of melanoma, and early detection of the malignancy.<sup>[2]</sup> Now, recent evidence showed that there is a significant increase in the incidence of melanomas all over the world. In the United States, the estimated death due to melanoma was 9710 in 2014.<sup>[3]</sup> Melanomas are most common in young adult age group of 25–29 years.<sup>[4]</sup> The 5-year survival in early stage (98%) of melanoma is much higher than advanced or metastatic disease (15%).

The fall of 5-year survival in advanced or metastatic stage is due to the late diagnosis or early spread of the tumor. Earlier diagnosis in melanoma has a significant survival advantage compared to late diagnosis.<sup>[5]</sup> In significant proportion of patients progress to advanced disease, even though they are treated early. The progression to advanced disease can be halted with targeted therapy against molecular biomarkers.

### Etiology of Melanoma

It can arise from a preexisting nevus or developed from melanocytes following UV radiation exposure or immunosuppression.<sup>[6]</sup> Malignant melanoma

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can be classified as cutaneous and noncutaneous malignant melanoma. The etiology of both cutaneous and noncutaneous malignant melanoma is different. Sunlight exposure with UV radiation is considered a cause for cutaneous malignant melanoma, but noncutaneous malignant melanomas such as anorectal melanoma and melanoma arising from choroid plexus cannot be explained with the above cause.

### Classification of Melanoma

Melanoma can be classified based on the following parameters:<sup>[7]</sup>

- a. Based on the type of melanoma
  1. Lentiginous malignant melanoma
  2. Superficial spreading melanoma
  3. Nodular melanoma.
  4. Acral lentiginous melanoma
- b. Based on organ involvement
  1. Cutaneous melanoma
  2. Mucous melanoma
  3. Ocular melanoma.

### Prognostic Factors in Melanoma

The following are considered important prognostic factors in melanoma, which includes depth of invasion, nodal metastasis, mitotic index, presence of ulceration, location of melanoma, type of melanoma and molecular markers of melanoma.<sup>[8]</sup>

Melanoma detected at a later stage or advanced stage has poor prognosis compared to early stage. In the above-mentioned prognostic factors, only few factors can be modified to improve better outcome to the patient. Molecular markers are one of the prognostic factors that can be intervened to improve prognosis. Molecular markers and their therapeutic target are one of the factors that can be modified for improved survival and outcome. Now, considerable research work is going on to identify molecular prognostic markers and targeted therapy against that prognostic factors.

### Management of Melanoma

#### 1. Locoregional management

Locoregional management of melanoma depends on the depth of involvement and lymph node involvement. TNM staging, clinical staging and pathological staging highlighted from Tables 1-6.<sup>[9]</sup>

Depth of melanoma	management
1. Melanoma <i>in situ</i>	0.5 cm surrounding margin
2. Melanoma <2 mm thickness	1 cm clear margin
3. Melanoma >2 mm thickness	2 cm clear margin,

#### 2. Lymph node management

The management of lymph node in malignant melanoma depends on the status of lymph node. In clinically negative

**Table 1: TNM classification of malignant melanoma: Primary tumor (T)**

Classification	Tumor thickness (mm)	Ulceration/mitoses
TX	Primary tumor cannot be assessed (for example, curettaged or severely regressed melanoma)	
T0	No evidence of primary tumor	
Tis	Melanoma <i>in situ</i>	
T1	≤1.0	a: w/o ulceration and mitosis <1/mm <sup>2</sup> b: With ulceration or mitoses ≥1/mm <sup>2</sup>
T2	1.01-2.0	a: w/o ulceration b: with ulceration
T3	2.01-4.0	a: w/o ulceration b: with ulceration
T4	>4.0	a: w/o ulceration b: with ulceration

TNM: Tumor, node and metastasis

**Table 2: TNM classification of malignant melanoma: Regional lymph node (N)**

N classification	Number of nodal metastasis	Metastatic mass
NX	Patients in whom the regional nodes cannot be assessed (for example, previously removed for another reason)	
N0	No regional metastases detected	
N1	1 node	a: Micrometastasis b: Macrometastasis
N2	2-3 nodes	a: Micrometastasis b: Macrometastasis c: In transit met(s)/satellite(s) without metastatic nodes
N3	4 or more metastatic nodes, or matted nodes, or in transit met (s)/satellite (s) with metastatic node (s)	

**Table 3: TNM classification of malignant melanoma: Distance metastasis**

M stage	Metastasis
M0	No detectable evidence of distant metastases
M1a	Metastases to skin, subcutaneous, or distant lymph nodes
M1b	Metastases to lung
M1c	Metastases to all other visceral sites or distant metastases to any site combined with an elevated serum LDH

LDH: Lactate dehydrogenase

**Table 4: TNM classification of malignant melanoma: M stage based on serum lactate dehydrogenase levels**

M stage	Site	LDH level
M1a	Distant skin, subcutaneous, or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases distant metastasis	Normal Any Elevated

LDH: Lactate dehydrogenase

**Table 5: TNM classification of malignant melanoma: Clinical staging system**

Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage IIA	T2a	N0	M0
	T2b	N0	M0
Stage IIB	T3a	N0	M0
	T3b	N0	M0
Stage IIC	T4a	N0	M0
	T4b	N0	M0
Stage III	Any T	≥ N1	M0
Stage IV	Any T	Any N	M1

**Table 6: TNM classification of malignant melanoma: Pathological staging system**

0	Tis	N0	M0
IA	T1a	N0	M0
IB	T1b	N0	M0
	T2a	N0	M0
	T2b	N0	M0
IIB	T3a	N0	M0
	T3b	N0	M0
	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-4a	N1a	M0
	T1-4a	N2a	M0
IIIB	T1-4b	N1a	M0
	T1-4b	N2a	M0
	T1-4a	N1b	M0
	T1-4a	N2b	M0
	T1-4a	N2c	M0
	T1-4a	N2c	M0
IIIC	T1-4b	N1b	M0
	T1-4b	N2b	M0
	T1-4b	N2c	M0
IV	Any T	N3 Any N	M0 M1

lymph node should undergo sentinel lymph node biopsy and positive lymph node should undergo lymphadenectomy.<sup>[10]</sup>

### 3. Systemic therapy for metastatic melanoma

#### A. Cytotoxic chemotherapy

Dacarbazine, hydroxyurea, temozolomide, taxes, fotemustine, and platinum derivatives are the accepted

treatment options for advanced or metastatic melanomas by the Food and Drug Administration (FDA). The problems with the above-said agents are higher toxicity and it does not improve the overall survival or disease-free survival.<sup>[11]</sup>

### Melanoma Biomarkers and Its Use

Biomarkers are a group of proteins, enzymes, hormones, and growth factors used between tumor and host to identify diagnosis, treatment, and prognosis. Biomarkers helps in diagnosis, differentiation, prognosis and management of malignancy. Examples of biomarkers include receptors such as estrogens receptor, progesterone receptor, and androgen receptor; growth factors such as vascular endothelial growth factor (VEGF) and human epidermal growth factor; and enzymes such as lactate dehydrogenase (LDH).<sup>[12]</sup>

Apart from diagnosis, biomarkers also help in treatment purposes such as C-kit receptor antagonist imatinib, estrogen receptor (ER) antagonist tamoxifen, androgen receptor antagonist flutamide, and HER2/neu receptor antagonist trastuzumab. Routine use of biomarkers in melanoma is difficult, because it is a heterogeneous group of disorder with multiple defects or molecular mutation involved in various cellular processes such as cell signaling, chemotaxis, cell adhesion, cell migration, cell cycle regulation, cell differentiation, and cell apoptosis. Because of multiple gene mutation or defect, till now, a single biomarker is not identified as an ideal marker for diagnosis, differentiation, treatment, and prognosis. In today’s clinical practice, a combination of biomarkers are used in diagnosis, treatment, and prognosis.<sup>[13]</sup>

#### a. Diagnostic Biomarkers

##### Immunohistochemistry markers

Melanoma is one of the tumors which resembles histologically with other malignancies such as carcinoma, sarcoma, lymphoma, and neuroendocrine tumor. These tumors mimic melanoma histologically. These tumors must be differentiated from melanoma because of the different therapeutic approaches and prognosis.

##### 1. Pmel/pm17/SILV/gp100

The gp100 is otherwise called as premelanosome protein (pmel)/pm17 or SILV. The gene involved in gp100 is PMEL. The major function of gp100 is formation of fibrillar matrix for polymerization of melanoma.<sup>[14]</sup> It is mainly detected using immunohistochemical marker HMB-45. The HMB-45 is 100 kD glycoprotein. The sensitivity of this biomarker varies from 72% to 100% and the specificity varies from 91% to 100% based on various studies. HMB-45 has decreased specificity for malignant melanoma in sentinel lymph node compared to Melan-A. HMB-45 has poor sensitivity for detecting desmoplastic melanoma.

##### 2. Melan-A

The Melan-A is otherwise called as an antigen recognized by T cells-1 or MART-1.<sup>[15]</sup> The gene involved in Melan-A is

MLANA. It is mainly located on the membrane protein located in melanosome, endoplasmic reticulum, and trans-Golgi network. It is mainly detected using immunohistochemical marker A103. The function of Melan-A in melanomas is expression, traffic, processing, and stability of pmel.<sup>[16]</sup> The sensitivity of this biomarker varies from 81% to 100% and the specificity varies from 81% to 98%. Melan-A expressed in cytoplasm of melanocytes, retinal pigment epithelium and melanoma. The advantage of this Melan-A is that it is one of the most sensitive markers used in the detection of melanoma in frozen section. The problem with Melan-A is that it is less sensitive for the detection of metastatic melanoma compared with primary melanoma and it shows lower sensitivity for desmoplastic melanoma. The use of this Melan-A is sometimes difficult because it is also positive in adrenal cortical cells, Leydig cell, granulosa cells, and theca ovary cells.

### 3. Tyrosinase

It is an enzyme that involves part of the biosynthesis of melanin expressed in melanocytes.<sup>[17]</sup> This enzyme is located in the melanosomes. The gene which involved in the synthesis of this biomarker is TYR. Tyrosinase is an important enzyme involved in the biosynthesis of melanin. It is mainly detected with the help of T311 in immunohistochemistry. Apart from immunohistochemistry, tyrosinase is also detected in the blood with the help of RT-PCR by detecting its mRNA levels. The problems with tyrosinase are it does not predict survival benefit and disease-free survival. The sensitivity and specificity of tyrosinase are 90%–100% and 97%–100%, respectively. It has a good sensitivity and specificity. The main advantage of tyrosinase is distinguishing melanoma from nonmelanocytic tumor. Like any other biomarkers in melanoma, it also has lower sensitivity in detecting desmoplastic melanoma.

### 4. Microphthalmia-associated transcription factor

Microphthalmia-associated transcription factor (MITF) is involved in melanocyte development and differentiation. It is essential for melanoblast differentiation from neural crest cells. The gene responsible for this factor is MITF gene.<sup>[18]</sup> Apart from melanocytes, development and differentiation also regulates the transcription of pmel, Melan-A, and tyrosinase. MITF stain is positive in histiocytes, lymphocytes, fibroblast, Schwann cells, and smooth muscle cells. It has a sensitivity of 100% and a specificity of 87%–100%. It is detected with immunohistochemical marker C5 and D5. MITF lacks sensitivity and specificity for desmoplastic or spindle cell melanoma.<sup>[19]</sup>

### 5. S100

S100 is an important protein involved in the development of malignant melanoma. The reason behind the name S100 is that it is 100% solubility in saturated ammonium sulfate. It consists of a family of more than 21 proteins.<sup>[20]</sup> S100 is also positive in glial cells, Schwann cells, melanocytes, Langerhans cells, and chondrocytes. The main function of

S100 is regulation of cell growth, cell cycle, cell motility, calcium homeostasis, transportation and differentiation, inflammatory response, and regulation of cytoskeletal components. It is mainly detected with the help of polyclonal antibody against S100. It has a sensitivity of 89%–100% and a specificity of 70%–79%. The main problem with S-100 is that it is less sensitive in frozen section. The advantage of S100 is that it has greater sensitivity compared to the above-mentioned biomarkers in desmoplastic melanoma.<sup>[21]</sup>

### 6. SM5-1

The gene responsible for SM5-1 is FN1. The main function of SM5-1 is that it contributes to cell adhesion and migration and metastasis.<sup>[22]</sup> Apart from melanoma, it is also positive in dendritic cells, plasma cells, and myofibroblasts. Even though it has lower sensitivity in metastatic melanoma, compared with other markers, it has higher sensitivity. It is detected with the help of SM5-1 IgG1 antibodies. It has a sensitivity of 95%–99% and a specificity of 100%.

### 7. CSPG4/high molecular-weight melanoma-associated antigen

CSPG4 is otherwise called as a high molecular-weight melanoma-associated antigen (MAA). It is found in melanocytes, endothelial cells, and pericytes.<sup>[23]</sup> It is produced from gene CSPG4. The main function of CSPG4 is that it promotes cell adhesion, motility and growth, and metastasis. CSPG4 has lower sensitivity for acral lentiginous melanoma. The CSPG4 has higher sensitivity (>90%) for metastatic lesions, better than Melan-A, S-100, and HMB-45. CSPG4 also has higher sensitivity for both primary and metastatic desmoplastic melanomas compared to HMB-45 and Melan-A. It is detected with the help of mouse monoclonal antibodies 763.74, VF1-TP41.2, and VT 80.12.

### 8. P16

It helps distinguishing Spitz nevi from melanoma.

### 9. Other markers – MUM-1, Mel-5, melanocortin-1, and PNL2

Melan-A, HMB-45, and tyrosinase all show diminishing sensitivity with advanced stage of disease. None of these biomarkers are able to distinguish malignant from nonmalignant melanocytic lesions. Lewis *et al.* showed Melan-A, CD63 and Budding uninhibited by benzimidazoles1 homolog (BUBI) helps in distinguish between melanoma and non melanocytic lesion.

## b. Prognostic Markers

### 1. Mitotic rate

Mitotic rate is one of the important prognostic markers useful in predicting the outcome of malignant melanomas. Tumors with a higher mitotic index have worst prognosis compared with tumors with a low mitotic index.<sup>[24]</sup> Mitotic



index otherwise predicts tumor turnover. Mitotic index is second most important marker for predicting survival in malignant melanoma.

## 2. Ki-67

Ki-67 is third most important marker for predicting survival in malignant melanoma. It is detected with the help of antibody Ki-67 (MIB-1 clone). It is produced from the gene MKI-67. The main function of Ki-67 in melanoma is nuclear antigen expression during proliferation. Patients who express higher Ki-67 showed worst prognosis compared with patients who express lower Ki-67. Ki-67 is a nuclear antigen and marker of proliferation, expressed during active phases of the cell cycle. Ki-67 correlate with prognosis of melanoma irrespective of thickness of melanoma.<sup>[25]</sup>

## 3. MCAM

MCAM is also known as MUC18 and CD146 molecule. It weighs approximately 113 kDa. MCAM is a cell adhesion molecule responsible for tumor cell migration and metastasis. It is produced from the gene MCAM.<sup>[26]</sup> It is detected with the help of anti-MCAM antibody. Tumor showed higher expression, leading to poor prognosis in melanoma. It is expressed in endothelial and smooth muscle cells. MCAM is used as an independent predictor for prognosis in melanoma.

## 4. Metallothionein I and II

Metallothioneins belong to the family of heavy metal-binding low molecular-weight proteins. Both metallothionein I and II are responsible for homeostasis of heavy metal ions and protection against oxidative stress. Patients with higher expression showed poor prognosis compared with patients showing lower level.<sup>[27]</sup> It is produced from the gene on chromosome 16q13. It is detected with the help of monoclonal antibodies against Metallothionein I and II. Overexpression of metallothioneins is associated with hematogenous metastasis.

## 5. CD10

CD10 is zinc-dependent endopeptidases. CD10 helps in degradation of enkephalin which is known to suppress melanoma tumor growth. CD10-positive tumors are associated with a shorter 5-year survival.

## c. Serological Biomarkers

- A. Current investigation phase
  1. MAA
  2. Melanin-related metabolites
  3. Adhesion molecules
  4. Angiogenesis factors
  5. Cytokines.
- B. Markers on already use.

### 1. Lactate dehydrogenase

LDH is one of the strongest prognostic indicators for predicting tumor burden. LDH is mostly related to death

of cell or high tumor turnover. Cytoplasmic enzyme helps in conversion of pyruvate to lactate. The cancer cells replicate via anaerobic or glycolytic mechanism.<sup>[28]</sup> LDH elevation is due to upregulation of LDH by tumor cells and tumor cell necrosis, causing spillover of the enzymes in the bloodstream. Elevated LDH is associated with poor prognosis. The pitfall of LDH is not specific for malignancy, but also elevated in hemolysis, infection, infarction, and inflammation.<sup>[29]</sup> LDH is less sensitive in early stages. The advantage of LDH is that it is indicative of tumor progression or metastatic relapse.

### 2. S100

In S100 family proteins, S100B is particularly found in patients with melanoma. Elevated levels of S100B are associated with poor prognosis and decreased disease-free survival and decreased overall survival. The disadvantage of S100 is false positive seen in the brain, liver, and renal injury as well as infectious disease. It is used as both immunohistochemical and serological biomarkers. An elevated level of S100B is associated with advanced disease, metastasis, and relapse and decreased overall survival. S100B is not superior to LDH in monitoring prognosis and survival in Stage III and Stage IV disease. Another problem with S100B is that it is also elevated in other conditions such as ischemic stroke, cerebrovascular accident, and following bypass surgery.

### 3. C-reactive protein

C-reactive protein (CRP) belongs to the member of pentraxin protein family. It activates the complement pathway. It is an acute-phase reactant associated with inflammation infection and tissue injury. Elevated CRP has been associated with poor prognosis in melanoma.<sup>[30]</sup>

### 4. Melanoma-inhibiting activity

Melanoma-inhibiting activity (MIA) is secreted by melanoma cells. MIA belongs to 11 kDa soluble protein. Actually, the name is a misnomer; MIA increased invasiveness and metastasis of melanoma cells. Serum level of MIA not only correlates with disease stage but also determines the response to therapy.<sup>[31]</sup> MIA is not only elevated in melanoma, it also elevated in squamous cell carcinoma, pregnancy and in children. It is involved in cell-to-cell contact by interacting with the extracellular matrix.

### 5. Vascular endothelial growth factor

VEGF is known to induce tumor-associated angiogenesis. Only a few studies showed that VEGF was an independent prognostic marker, but most of the studies fail to confirm the above findings.<sup>[32]</sup> Angiogenic cytokine regulates endothelial proliferation, differentiation, and survival. VEGF is not only secreted by melanoma cells but also secreted by lymphocytes and platelets. Its role as a biomarker in melanoma is questionable.

## 6. BRAF V600E mutation

Serum BRAF V600E DNA levels are not correlating with disease progression.

## 7. Serum miRNA

Circulating miRNA shows diagnostic and prognostic utility in different types of cancer, but yet to be investigated in melanoma.<sup>[33]</sup>

## 8. Osteopontin

Osteopontin is a phosphoprotein involved in suppression of apoptosis, tumor growth and recruitment of tumor promoting cells from bone marrow. The disadvantage of osteopontin is that it is also elevated in autoimmune diseases. Osteopontin in combination with S100 can help in differentiating patients who are likely to develop relapse and recurrence.<sup>[34]</sup>

## 9. YKL-40

YKL-40 is a glycoprotein secreted by cancer cells, macrophages, and neutrophils.

## 10. Interleukin-8

Interleukin-8 (IL-8) is also known as CXCL8. It is a chemokine produced by malignant cells. It can induce neutrophil chemotaxis and promote tumor angiogenesis. IL-8 correlates with tumor burden and stage of disease, overall survival, and response to therapy with BRAF inhibitor.

## Targeted Therapy Based on Biomarkers

Systemic chemotherapy in malignant melanoma has little benefit compared to immunotherapy and targeted therapy. The observed overall survival in systemic chemotherapy is much less compared with targeted therapy in advanced or metastatic melanoma.<sup>[35]</sup>

BRAF is a serine/threonine protein kinase that activates the mitogen-activated protein kinase (MAPK) pathway. BRAF is one of the common mutations detected in melanoma, but only 40%–50% of melanoma expresses BRAF mutation. In BRAF mutation, more than 90% of mutation occurs at codon 600 with replacement of glutamic acid for valine (BRAF V600E).

### a. BRAF inhibitors

BRAF is an oncoprotein involved in evasion of senescence, evasion of apoptosis, uncontrolled proliferation, tissue invasion, evasion of immune system, angiogenesis and metastasis. The above-said drugs are oncogenic BRAF V600 protein kinase inhibitors [Table 7]. BRAF inhibitors mainly used in BRAF positive unresectable or metastatic tumors. BRAF is located on the long arm of chromosome 7. The pathway for melanoma cell survival and proliferation is RAS-RAF-MEK-ERK [Figure 1].<sup>[36]</sup> BRAF inhibitor

Vemurafenib showed improved survival in BRAF positive mutation.

### i. Vemurafenib

Vemurafenib is approved for BRAF V600E mutation-positive metastatic melanoma.<sup>[37]</sup> Vemurafenib is maximum beneficial in patient with stage M1c melanoma with increased lactate dehydrogenase concentration. After administration of vemurafenib, most of the tumors showed rapid response and tumor burden decreased in due course. It blocks both forms of BRAF mutation such as v600E (glutamine for valine) and V600k (lysine for valine).

Even though vemurafenib showed rapid response on tumor burden, the effect never lasted longer due to the development of resistance. The development of resistance in vemurafenib is due to either MAPK pathway dependent or MAPK pathway independent.<sup>[38]</sup> BRAF mutation is more common in patients with cutaneous melanoma compared with other types of melanoma such as ocular and mucous melanoma. Tumor with less number of BRAF mutation rarely responds to BRAF inhibitors.

#### How to overcome the resistance?

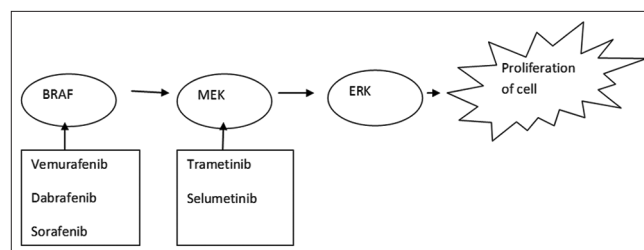
To overcome the resistance to vemurafenib, we can use two methods. First, we can use a combination of BRAF inhibitors along with MEK inhibitors.<sup>[39]</sup> Another method is intermittent use of vemurafenib.

#### Advantage of vemurafenib

Both vemurafenib and dabrafenib showed a significant impact on the treatment of metastatic melanoma to the brain. Both agents showed a significant improvement in BRAF-mutant melanoma with brain metastases than whole-brain irradiation for melanoma.

**Table 7: BRAF inhibitors**

Agent	Dose	Adverse effects
Vemurafenib	960 mg twice daily	Photosensitivity, squamous cell carcinoma
Dabrafenib	150 mg twice daily	Pyrexia
Sorafenib	400 mg twice	High blood pressure, hair loss
Trametinib	2 mg only daily	Cutaneous rash, Gastrointestinal Side effects
Selumetinib	75 mg twice daily	Rash, fever



**Figure 1: Mechanism of action of BRAF and MEK inhibitors**

## ii. Dabrafenib

Dabrafenib is another BRAF inhibitor. The response rate to dabrafenib depends on the type of BRAF mutation. The response rate to BRAF V600E is approximately around 60% whereas BRAF V600K showed a response rate of around 13%. Hence, it is mandatory to check the type of BRAF mutation before giving dabrafenib as a targeted therapy. Like vemurafenib, dabrafenib also showed a significant improvement in response rate and progression-free survival in BRAF-mutant metastatic melanoma compared to conventional chemotherapy.<sup>[40]</sup>

## iii. Sorafenib

Sorafenib is also a kinase inhibitor that acts on inhibiting kinase at multiple targets. It acts by inhibiting VEGF receptors VEGFR1, VEGFR2, and VEGFR3 and the platelet-derived growth factor receptor PDGFR. It also acts by inhibiting FLT3, C-Kit, and BRAF. There are many studies conducted to detect the use of sorafenib in melanoma. The results of most of the studies showed sorafenib as a monotherapy or combined chemotherapy of limited use. According to the study, only few subpopulations showed benefit in response to sorafenib.

### Adverse effects of BRAF inhibitors

The safety profile and side effect profile of both vemurafenib and dabrafenib are same. However, the incidence of photosensitivity is much higher in vemurafenib and pyrexia is more in dabrafenib. There are increased incidence of squamous cell carcinoma following administration of vemurafenib, particularly keratoacanthoma. The reason for the development of squamous cell carcinoma is due to paradoxical activation of the MAPK pathway in nonmutant BRAF (wild-type BRAF).<sup>[41]</sup> Otherwise, both the drugs are safe for the administration of advanced or metastatic BRAF-mutant melanoma.

## b. MEK inhibitors

### iv. Trametinib

Trametinib is also a targeted therapy similar to vemurafenib and dabrafenib. It acts by inhibiting MEK which is the only known substrate of BRAF, which, in turn, leads to decreased cell signal and growth of malignant cells [Figure 1]. It is useful in both BRAF V600E/K-mutant and unresectable metastatic melanoma.<sup>[42]</sup> The net effect of trametinib persists in patients who are already given chemotherapy or immunotherapy, but this effect is lost in patients who have already undergone BRAF inhibitor therapy. Hence, the selection of patients as well as screening of patients with mutation is very important before initiating trametinib therapy.<sup>[43]</sup>

### v. Selumetinib

Selumetinib is an MEK inhibitor which is also involved in MAPK pathway. A Phase III trial using selumetinib as a monotherapy or combined with chemotherapy fails

to improve disease-free survival or progression-free survival in patients with melanoma. As per the current recommendations, selumetinib is not recommended for advanced or metastatic melanoma.

### Adverse effect of MEK inhibitors

MEK inhibitors are well tolerated compared with BRAF inhibitors. Most of the side effects of MEK inhibitors belong to cutaneous side effects such as rash and gastrointestinal side effects such as diarrhea and gastrointestinal upset.

### Major limitations of BRAF inhibitors

Kinase inhibitors targeting RAS/RAF/MEK pathway are useful in only 40%–50% of patients with malignant melanoma, because BRAF mutation is detected only in 40%–50% of patients with melanoma.<sup>[44]</sup>

## Mechanism of Combination Targeted Therapy

### Up regulation of melanoma antigen

After administration of BRAF inhibitors, most of the melanoma cells express melanoma antigens such as MART-1, gp100, c-kit, and class I MHC antigen. MEK inhibitors also increase melanoma antigen expression in both BRAF-mutant and BRAF nonmutant melanoma.

### Increased tumor-infiltrating lymphocyte function

After administration of BRAF inhibitors, there is an increased expression of tumor-infiltrating lymphocyte in tumor specimen. Patients who develop resistance or disease progression following BRAF inhibitors showed decreased TILs, which was restored following combination therapy with BRAF and MEK inhibitors.<sup>[45]</sup>

### Block immune suppression

Evasion of immune system occurs following secretion of oncogenic protein by BRAF-mutant cells. BRAF inhibitors decrease these immune suppression agents, thereby increasing T cell infiltration.

### Combined targeted therapy

The combination of BRAF and MEK inhibitors showed a significant improvement in progression-free survival and overall survival.<sup>[45]</sup> Another advantage of combined therapy is delay in the onset of BRAF resistance and decreased toxicity including lower incidence of squamous cell carcinoma due to blocking of paradoxical MAPK activation.<sup>[46]</sup>

### CRLA-4 inhibitors

Ipilimumab is an antibody against cytotoxic T-lymphocyte antigen-4 (CTLA-4) approved for unresectable metastatic melanoma [Table 8]. The main function of CTLA-4 is that it downregulates T cell against immune response against self-antigen and prevents autoimmunity. Ipilimumab helps in melanoma by blocking CTLA-4, thereby activating T cell against tumor antigen cell [Figure 2].<sup>[47]</sup>

**Advantage of ipilimumab**

The action of ipilimumab depends on the CTLA-4 expression, rather than genetic mutation or expression of cells. Hence, it is effective in all tumors in a patient. The overall survival of this drug does not depend on age or sex of the patient, serum LDH level, stage of the disease, and previous chemotherapy.

The effect of ipilimumab persists even after cessation of treatment. Retreatment after disease progression may reactivate the immune system, which was already primed by ipilimumab. Ipilimumab is administered at the rate of 3 mg/kg irrespective of the type of melanoma. Like BRAF inhibitors, ipilimumab also penetrates the blood-brain barrier and mounts an immune response against metastases.

**Adverse effects of ipilimumab**

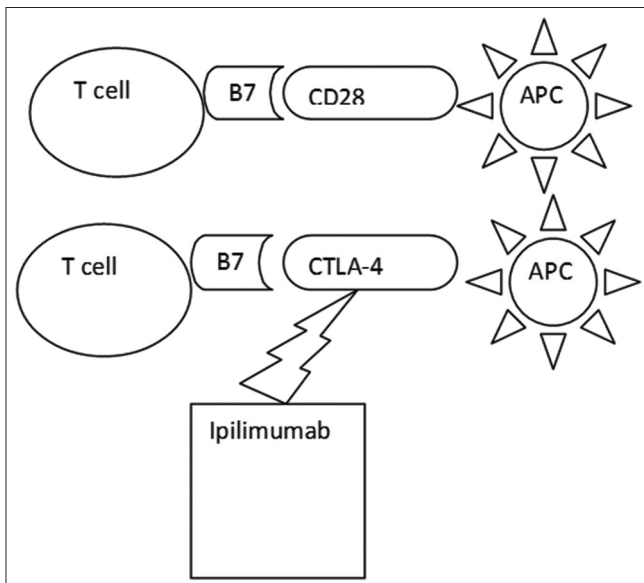
Ipilimumab is the drug that mainly acts on the tumor based on the immune expression, so it is obvious that after administration of ipilimumab, immune reactions are expected. Most of the immune reactions affect the gastrointestinal tract, central nervous system, cutaneous lesions, and endocrine systems. Most of the adverse events following ipilimumab can be cured with oral or parenteral steroids for a period of 6–8 weeks.

**Combination therapy**

The combination therapy with ipilimumab and vemurafenib has showed a significant increase in hepatic injury and hepatotoxicity. This hepatotoxicity is

**Table 8: CTLA-4 inhibitors**

Agent	Dose	Side effects
Ipilimumab	3-10 mg/kg	Autoimmune resection



**Figure 2: Mechanism of action of CTLA-4 inhibitors**

due to increased paradoxical activity of MAPK pathway in liver cells with wild-type BRAF following BRAF inhibitors.

**d. Anti-PD-1 antibodies and Anti-PD-L1 antibodies**

Immune system has constant surveillance on foreign bodies as well as tumor cells. However, the tumor cells have their own pathway or mechanism for evading immune system. Immune regulatory mechanism is one of the targeted therapies for melanoma. The programmed death 1 is an inhibitory T cell receptor, which has an effect on tumor cell through its ligand called programmed death ligand 1. Programmed cell death 1 (PD-1) is 288 amino acid-containing receptor [Figure 3].<sup>[48]</sup>

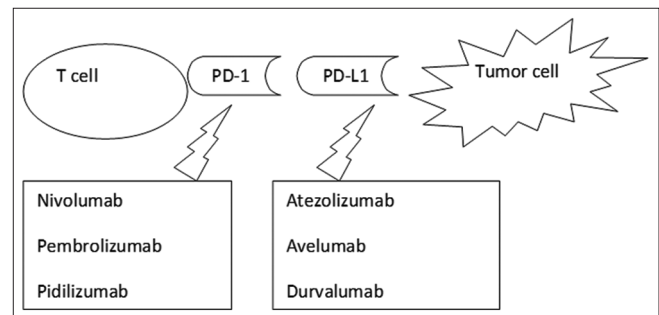
Blockage of PD-1 or programmed death-ligand 1 (PD-L1) with antibodies has a significant effect on tumor control because it specifically acts on tumor-specific antigen. Because of its specific effect on T cell, PD-1 and PD-L1 antibodies have a significantly higher effect on tumor cell with lesser side effects.<sup>[49]</sup>

PD-1 downregulates T cells by sending inhibitory signals to its ligand PD-L1. Programmed death receptor 1 is expressed in variety of tissues including normal cells and numerous cancerous cells. The mechanism of PD-1 action depends on the inhibitory action on the T cells, thereby preventing T cell action on the tumor cell. PD-1 plays an important role in immunity against chronic infection and tumor antigen.

**Mechanism of action of anti-programmed cell death 1 and anti-programmed death-ligand 1**

The tumor cells located in our body have to develop some mechanism to evade from our immune system. One of the most important evading systems is called adaptive immune resistance where tumor tries to express PD-L1 to protect tumor cells against cytotoxic T cell destruction.

Anti-PD-1 antibodies have higher tumor activity and lesser side effect compared with CTLA-4 inhibitors [Tables 9 and 10]. The antitumor response by anti-PD-1 depends on multiple factors, but most of the studies showed long-lasting antitumor effects. All anti-PD-1 antibodies developed from IgG4 humanized antibody.



**Figure 3: Mechanism of action of PD-1 and PD-L1 inhibitors**



**Table 9: Anti-PD-1 antibodies**

Agent	Dose	Side effects
Nivolumab	3 nmol/l	Pneumonitis, diarrhea
Pembrolizumab	<100 pmol/l	Pneumonitis, diarrhea
Pidilizumab	20 nmol/l	Pneumonitis, diarrhea

**Table 10: Anti-PD-L1 antibodies**

Agent	Dose	Side effects
Atezolizumab	0.3-10 mg/kg	Diarrhea, arthralgia, rash, nausea, pruritus, and headache
Avelumab	0.3-10 mg/kg	Diarrhea, arthralgia, rash, nausea, pruritus, and headache
Durvalumab	0.3-10 mg/kg	Diarrhea, arthralgia, rash, nausea, pruritus, and headache

### i. Nivolumab

Nivolumab is fully humanized monoclonal antibody against PD-1 approved by the FDA in metastatic or advanced melanoma. Another significant advantage of nivolumab is that it is effective even in tumor not responding to other drugs such as ipilimumab or BRAF inhibitors. Nivolumab showed highest tumor response in the dose of 3 mg/kg body weight. It showed a significant overall survival of 63% at 1 year and 48% at 2<sup>nd</sup> year.

### ii. Pembrolizumab

Pembrolizumab is another monoclonal antibody used against PD-1. It also got approval in patients with advanced or metastatic melanoma and those patients who are not responding or pretreated with ipilimumab or BRAF inhibitors. The overall survival of patients with melanoma following pembrolizumab is slightly higher than nivolumab (69% vs. 63%).

### iii. Pidilizumab

Pidilizumab is another anti-PD-1 antibody. It is being evaluated for metastatic melanoma. It is compared with other anti-PD-1 antibodies. The overall response rate following administration is lower than other drugs.

### Adverse effects

As we already discussed, most of the anti-PD-1 antibodies are well tolerated because of being specific and more precise action. However, still there are reports of immune-related adverse events such as pneumonitis, diarrhea, and liver injury.

### Biomarkers for anti-programmed cell death 1 therapy

The tumor response for the administration of anti-PD-1 is assessed using immunohistochemical analysis. Expression of PD-1 varies from tumor to tumor due to tumor microenvironment and immunogenicity. PD-1 cannot be used as prognostic marker, because melanoma with negative PD-1 also respond anti PD-1 therapy. Hence, based on the above findings, it is not mandatory to express

PD-1 for giving anti-PD-1 therapy. Hence, PD-1 is not a marker for better or poor prognosis. The cutoff value of PD-1 expression varies from studies to studies. Most of the studies showed tumor expression of 5% or more than 5% of PD-1 expression for nivolumab or 1% for pembrolizumab treatment.

### Combination of anti-programmed cell death 1 therapy with other immunotherapy therapy

The combination of anti-PD-1 with anti-CTLA-4 showed improvements and long-lasting response.<sup>[50]</sup> This is because the combination of these two drugs acts on different mechanisms, so those tumors unlikely develop resistance. Another advantage of the combination of the above two drugs is that it maintains tumor response even after discontinuation of both drugs. Tumor with positive PD-1 express excellent response compared with tumor with negative PD-1 receptors.<sup>[51]</sup>

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### Conflicts of interest

There are no conflicts of interest.

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