

## Immunotherapy In Malignant Melanoma

Nepton Sheik Khoni<sup>1\*</sup>, Abdul Rahman El Kinge<sup>2</sup> and Gayathri Balasubramanian<sup>3</sup>

<sup>1</sup>Global Allied Pharmaceutical, Center for Excellence in Research & Development, USA

<sup>2</sup>Specialized Medical Center, Division of Hematology/Oncology, KSA

<sup>3</sup>Phamax Market Access Solutions, 5<sup>th</sup> Block, Koramangala, Bangalore, India

**\*Corresponding Author:** Nepton Sheik Khoni, Global Allied Pharmaceutical, Center for Excellence in Research & Development, 160 Vista Oak Dr. Longwood, FL 32779, USA.

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### Abstract

Malignant melanoma (MM) pertains to a state of malignancy that arises from the pigment producing cells called melanocytes. The incidence of melanoma is more than any other malignancy, but the mortality rate is comparatively low. It is estimated that Australia has the highest incidence of melanoma. MM is found more in white than black population, with men having slightly higher mortality rate than women. Other risk factors include environmental factors, socioeconomic conditions, skin type, genetic factors, age and skin pigmentation. However, intense sunlight exposure for a long duration prevails as a major risk factor. In recent years, advancing knowledge of the function of immune system has facilitated successful treatment of MM. Immunotherapy is one such promising development of late. Ipilimumab, nivolumab, trametinib, vemurafenib, dabrafenib, interferon-alfa-2b and aldesleukin are some of the FDA approved immunotherapies for the treatment of MM. Other potential treatment options under various phases of clinical trials for MM include immunotherapy using checkpoint inhibitors, Kinase Inhibitors, Cytokine Therapy, Monoclonal Antibodies, Adoptive T Cell Therapy, MDM2 Inhibitor, IDO inhibitor, Gene Therapy, mTOR Inhibitors, vaccines and other miscellaneous options such as usage of oncolytic virus. Better understanding of the tumor microenvironment has also led to trial of new modalities like combination therapies (chemotherapy with immunotherapy). Complete potential of such combination therapy will be unveiled over time and transform cancer care and improve patient outcomes.

**Keywords:** Malignant melanoma; Immunotherapy; Immuno-oncology; Ipilimumab; Nivolumab; Immunomodulators

### Abbreviations

ADCC: Antibody dependent cell mediated cytotoxicity; AdCD40L: A gene therapy vector of immunostimulatory origin; AKT: Protein kinase B; AMG 232: MDM2-p53 Inhibitor; APCs: Antigen presenting cells; ARAF: A Raf Proto Oncogene, Serine/Threonine Kinase; AUC: Area under the curve; B2M: Beta 2 Microglobulin; BCR-ABL: Breakpoint cluster region Abelson Tyrosine kinase inhibitor; BRAF: v-raf murine sarcoma viral oncogene homolog B1; CD27: Tumor necrosis factor receptor; CD28: Cluster of Differentiation 28; CDCL: Complement dependent cell lysis; CDK: Cyclin dependent kinase; CDKN2A and CDKN2B: Cyclin Dependent Kinase Inhibitor 2A and 2B; CDR: Complementarity determining regions; c-kit: 145 kD protein tyrosine kinase; Cmax: Maximum serum concentration of a drug; Cmin: Minimum blood plasma concentration of a drug; CML: Chronic myeloid leukemia; CNS: Central Nervous System; CSF1R: Colony stimulating factor 1 receptor; CTA: Cancer testis antigens; CTL: Cytotoxic T lymphocyte; CTLA4: Cytotoxic T lymphocyte associated protein 4; CTNNB1: Catenin Beta 1; DAF: Decay acceleration factor; DCs: Dendritic cells; ERK: Extracellular signal regulated kinase; FAK: Focal adhesion kinase; FAMMM: Familial Atypical Multiple Mole Melanoma Syndrome; FDA: Food and Drug Administration; FGFR3: Fibroblast growth factor receptor 3; FKBP-12: Immunophilin FK Binding Protein 12; FLT3: FMS like tyrosine kinase 3; G2/M: DNA damage checkpoint; GIST: Gastrointestinal stromal tumor; Gli1, Gli2, and Gli3: Glioma associated oncogene homologs; GliA: Glioma associated oncogene activated;

GliR: Glioma associated oncogene repressor; GPNMB: Glycoprotein NMB; HLA: Human leukocyte antigen; HRAS: Harvey Rat Sarcoma Viral Oncogene Homolog; hTERT: Human telomerase reverse transcriptase catalytic subunit; ICAM-1: Intracellular adhesion molecule 1; IDO: Indoleamine 2,3 dioxygenase; Ig: Immunoglobulin; IL-2: interleukin 2; ILD: Interstitial Lung Disease; INGN241: A non replicating adenoviral vector; IRM: Immune response modifier; KIT: Stem cell factor receptor; KRAS: Kirsten rat sarcoma; LAK: Lymphokine activated killer; LPV7: Long Peptide Vaccine 7; LVEF: Left Ventricular Ejection Fraction; M1: Macrophages; MAGE: Melanoma antigen; MAPK: Mitogen activated protein kinase; MDA7: Melanoma differentiation associated 7 gene; MDM2: Murine double minute 2; MDP: Melanocytic differentiation proteins; MEK/MAPK/ERK: Mitogen Activated Protein Kinase Kinase/Extracellular; MEN1: Multiple endocrine neoplasia type 1; MHC: Major Histocompatibility Complex; Mich1H6 and Mich2H6: Therapeutic genemodified human melanoma cell lines; MiTF: Microphthalmia associated transcription factor; MM: Malignant melanoma; MMAE: Mono methyl auristatin E; MoAb: Melanoma monoclonal antibodies; mTOR: Mammalian Target of Rapamycin; NK: Natural killer; NRAS, KRAS and HRAS: 3 Ras genes in humans; P13k/Akt pathway: Intracellular signaling pathway; p21/CIP1/WAF1: Cyclin dependent kinase inhibitor; PCTAIRE1: Cyclin dependent kinase family protein; PD-1: Programmed death 1 or programmed cell death 1; PDGF: Platelet derived growth factor; PDGF-R: Platelet derived growth factor receptor; Ph+: Philadelphia chromosome positive; PIP3: Phosphatidylinositol (3,4,5) trisphosphate; PTCH1: Protein patched homolog 1; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN: Phosphatase and tensin homolog; RAF/c: RAF proto oncogene serine/threonine protein kinase; Ras: Family of related proteins; Rb: Retinoblastoma; RTKs: Receptor tyrosine kinases; SCFR: Stem cell factor receptor; SGOT: Serum glutamic oxaloacetic transaminase; SHH: SONIC HEDGEHOG; SLPs: Synthetic long peptides; SMO: Smoothed receptor; TAA: Tumor Associated Antigen; TCR: T cell receptor; TFAP2A: Transcription Factor AP 2 Alpha; TGF: Transforming growth factor; TK: Tyrosine kinases; Tmax: The amount of time that a drug is present at the maximum concentration in serum; TP53: Tumor protein p53; TRP2: Tyrosinase related protein 2; UV: Ultraviolet; US: United States; vc: Valine citrulline; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; YMDGTMSQV: Tyrosinase peptide; 41BBL: Type 2 transmembrane glycoprotein belonging to the TNF superfamily

## Introduction/Epidemiology

Malignant melanoma (MM) pertains to a state of malignancy that arises from the pigment producing cells (melanocytes). About 90% of all the melanomas originate from cutaneous region. These are also located in the ears, gastrointestinal tract, eyes, oral and genital mucosa and leptomeninges [1,2]. The incidence of melanoma is more than any other malignancy, but the mortality rate is comparatively low [3]. In 2014, American cancer society estimated that 76,100 new cases (about 4.6 % of all newly diagnosed cancers) of MM were reported. Additionally, in the same year, 9710 cases of death (about 1.7% of all cancer deaths) were also reported in the United States due to MM. The five years survival rate of patients is 91.3% after the diagnosis of malignant melanoma (MM) and it is the fifth leading cancer [3]. Australia has the highest incidence rate compared to rest of the world (56 new cases per year per 100,000 for men and 43 for women) [4].

There is a predominance of male over female, with higher incidence rate observed in the age group of 55-64 years. Men have slightly higher mortality rates than women. The percentage of deaths from melanoma of the skin are highest in the age group range from 75-84 [3]. Female suffers with more melanocytic nevi on the limbs, while males have more melanocytic nevi on the trunk [4]. It rarely occurs before the puberty [5]. The new incidences and deaths from melanoma is higher in white people [3], but less common in non-whites. It is limited to non-pigmented body parts, such as the subungual regions, the palms of the hand, and the soles of the feet [5].

## Etiology & Predisposing Factors

The following factors enhance the chance of developing malignant melanoma:

### Environmental factors

**1. Direct Sun exposure:** Intense sunlight is one of the main risk factors of melanoma. Direct sunlight exposure for a long duration throughout the year, makes a person more susceptible to melanoma. If anyone has suffered with sun burn in early age, he/she may be at the risk of melanoma [5].

**2.Artificial ultraviolet (UV) sources:** The high amounts of UV-A (Tanning beds) may be the risk factor for melanoma [5].

**3.Socioeconomic conditions:** From the various studies, the reports have suggested that people with high socioeconomic status are more prone to develop melanoma. An explanation for this finding may be that the people with high socioeconomic conditions usually travel more. However, more traveling in intense sunlight makes a person more susceptible to melanoma [5]. Another reason for enhanced risk of melanoma in people with high socioeconomic status is the reversal in the attitude towards the tanning of the skin and is a sign of leisure and good health among the people of high class [6].

**4.Skin type:** People with the presence of multiple moles are susceptible to melanoma. The most affected areas for men are trunk and for women are legs. Men with melanoma have more incidence rate and less survival rate than the women [3,4].

**5.Genetic factors:** Hereditary susceptibility is the major factor that affects the development of melanoma. A person, who has a first degree relative, suffering from melanoma has 50% more risk of developing melanoma than those who do not have. The condition where an individual belonging to the family of melanoma, develops atypical moles is called as Familial Atypical Multiple Mole Melanoma Syndrome (FAMMM). One third of the patients with melanoma are related to the previous genetic history [5]. People with FAMMM syndrome have higher risk of developing melanoma than those, who do not. BRAF gene has been identified as a major gene involved in causing melanomas and is found in approximately 50% of the cases. Other genes associated with the melanoma progression are p53, BRAF and most notably Cyclin Dependent Kinase Inhibitor 2A (CDKN2A) [7].

**6.Age:** Risk of melanoma increases with age in both men and women. High incidence of melanomas have been found at the average age of 60 years, and higher death rate is found at the average age of 80 years [4].

**7.Skin pigmentation:** The different skin type people, like Pale Caucasian skin (skin type 1 or 2), fair skin or a freckled complexion may be at higher risk of melanoma as compared to black people. Black people have 20 times less risk of getting malignant melanoma than white people and white men possess double the risk than women [3,4].

### Pathophysiology and molecular basis

There are various genes, which are involved in the development of malignant melanoma and there are two main classes of genes that have been detected in the molecular genetics of malignant melanoma, such as protooncogenes (CDK4, CTNNB1, MAPK) and tumor suppressor genes (B2M, CDC2L1, CDKN2A, CDKN2B, MEN1, RB1, PTEN, TFAP2A, TP53). There are several pathways, which are involved in the development of malignant melanoma and these are: RAS/RAF/MEK/ERK signaling, PI3K/PTEN/AKT signaling, HEDGEHOG (SHH)-GLI signaling, and the role of MiTF linked Erk1/2 kinase and p21CIP1/WAF1.

#### 1.RAS/RAF/MEK/ERK Signaling:

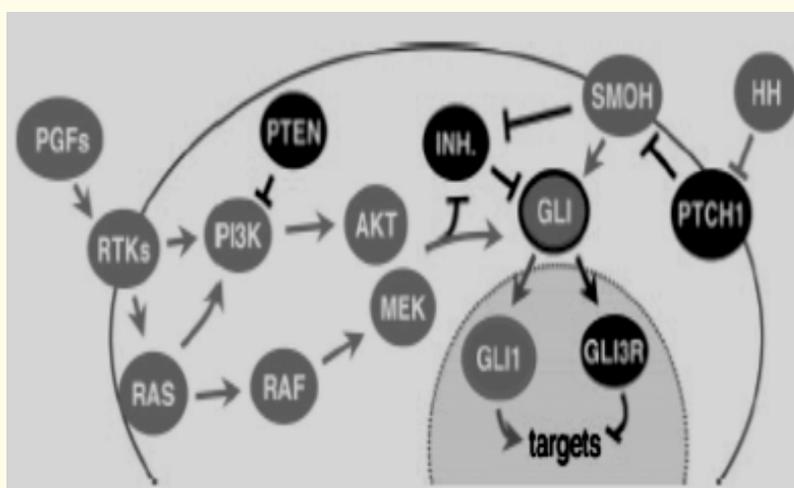
RAS is a small G-protein and has many subtypes in human, such as HRAS, KRAS, and NRAS. It is located in plasma membrane. It generally activates a downstream factor, RAF (ARAF, BRAF and CRAF in humans), followed by the sequential activation of MEK and ERK. Final transduced signal regulates the transcription in the nucleus. According to previous studies, growth factors exhibited over-activity of ERK in about 90% melanomas in humans. Mutation in *NRAS* and *BRAF* genes leads to their activation and results in the proliferation, survival, invasion, and angiogenesis of melanoma [8].

#### 2.PI3K/PTEN/AKT Signaling:

Phosphatase and tensin homolog (PTEN), phosphatidylinositol [3-5] trisphosphate (PIP3) and phosphorylated Protein kinase B (AKT) are involved in cell survival, proliferation, cancer development and anti-apoptotic signaling through mammalian Target of Rapamycin (mTOR) and NF- $\kappa$ B pathways in melanoma. RAS (which is involved in cancer development) is also involved in the binding and activating PI3K and increasing the activity of AKT. Moreover, Mouse double minute 2 homolog (MDM2) is used as a substrate for AKT. Various studies stated that MDM2 was expressed in 6% of dysplastic nevus, 27% of melanoma in situ, and 56% of invasive primary and metastatic melanomas. All these facts suggest the role of AKT/MDM2 pathway in melanoma progression [8].

### 3.RAS mediated HEDGEHOG (SHH) - GLI signaling

SONIC HEDGEHOG (SHH) - GLI signaling is found active in the matrix of human hair follicles. SHH-GLI signaling has an important role in the regulation of the proliferation and survival of human melanomas. This pathway is started by the Hh ligand, which binds to PTCH1 (present at the base of cilium on the cell membrane). This makes the Smoothed receptor (SMO) (present on endosomes) free, which further transmits signal. SMO, then migrates from the intracellular endosome to the cell membrane of the cilium. Then, the signal travels via various interacting proteins, and leads to the activation of the Gli family of zinc finger transcription factors (glioma-associated oncogene Gli1, Gli2, and Gli3). After this, the conversion of repressor (GliR) to activated GliA occurs. GliA moves into the nucleus and leads to transcription of the target genes [9]. Here, RAS signaling is connected to SHH-GLI signaling to suggest a new pathway in melanoma progression [10].



**Figure 1:** RAS mediated HEDGEHOG (SHH)-GLI signalling [8].

### 4.PCTAIRE 1

PCTAIRE1 is a cyclin-dependent kinase family protein. Its main role is in the spermatogenesis. PCTAIRE1 has an important role in the proliferation of some types of epithelial carcinoma cells, which is shown by a study, where PCTAIRE1 depleted cells showed apoptosis with mitotic arrest by dysregulation of centrosome. Furthermore, PCTAIRE1 suppress p27 by phosphorylation at Ser10. Knockdown of PCTAIRE1 leads to an increase in the expression of p27, which results in the suppression of tumor growth by apoptosis. It is suggested that PCTAIRE1 has an important role in tumor growth via p27 suppression. Moreover, Skp2 is also a suppressor of p27 and acts by increased proteosomal degradation. Studies suggested that PCTAIRE1 may be used as a target in the treatment of melanoma [11].

### 5.Role of adhesion molecule L1 (CD171) in melanoma progression:

The cell adhesion molecule L1 is a member of the immunoglobulin super family. It is mainly found in the primary melanomas and cutaneous metastases, which is associated with melanocytic nevus, and melanocytes. It is associated with metastatic spread. Recent studies suggest that the role of L1 is related with the activation of ERK in carcinomas and the upregulation of ERK-dependent motility and invasion-associated gene products, including avb3 integrin, small GTPases and proteases. The L1 has a key role in homophilic L1-L1 binding and heterophilic binding to integrins, including avb3 integrin. It functions not only as an adhesive molecule, but also as a signal transducing receptor. It suggests the role of L1 on the affected cell growth and migration by ligand binding and exhibit invasive tumor growth [12].

## 6.Role of MiTF links Erk1/2 kinase and p21<sup>CIP1/WAF1</sup> activation in melanoma:

The effect of UVC radiation is observed on normal human melanocyte and melanoma cells. UVC radiation is a DNA damaging agent and causes phosphorylation of MiTF at serine-73 through Erk1/2. This results in the degradation of proteasome mediated MiTF. The undegraded MiTF can activate p21<sup>WAF1/CIP1</sup> transcription and arrests the G1 cell cycle, temporarily. Cell lines showing that the high levels of MiTF expression have higher resistance to UVC-induced cell death than those with low-level MiTF [13].

BRAF plays an important role in this pathway, and the activation of BRAF or NRAS results in the activation of Mek1/2, followed by activation of Erk1/2, which leads to the phosphorylation of MiTF at serine-73. Erk1/2 activates its downstream kinase p90-RSK1, and then phosphorylation of MiTF at serine-409 occurs. Thus, phosphorylation of both the sites is triggered by c-Kit stimulation, which results in a signal event and further leading to pigment cell development [13].

### Immunotherapy

#### A.Checkpoint Inhibitors:

##### a.United States (US) Food and Drug Administration (FDA) approved checkpoint inhibitors:

**1.Ipilimumab:** [14] It is a recombinant human monoclonal antibody that binds to the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Ipilimumab is an IgG1 kappa immunoglobulin with an approximate molecular weight of 148 kDa. Ipilimumab is produced in mammalian (Chinese hamster ovary) cell culture. It blocks inhibitory marker CTLA-4 and activates the immune system along with induction of T cells to target the tumor.

**Indication and use:** It is indicated for the treatment of unresectable or metastatic melanoma.

**PD/PK:** The results of pharmacokinetic studies in patients with metastatic melanoma exhibited steady-state concentrations of ipilimumab and it was reached by the third dose; the mean C<sub>min</sub> at steady-state was 19.4 mcg/ml following repeated doses. Terminal half-life (t<sub>1/2</sub>) is found to be 15.4 days (34%) and CL is found to be 16.8 ml/h (38%).

**Contraindication:** None.

**Warnings:** Due to T-cell activation and proliferation, there can be severe and fatal immune-mediated adverse reactions. The most common severe immunemediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. Patients should be assessed for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy and should be evaluated by clinical chemistries, including liver function tests and thyroid function tests at the baseline and before each dose.

**Adverse Events:** The most common adverse events are fatigue, diarrhea, pruritus, rash, and colitis.

**2.Nivolumab [15]:** A fully human monoclonal antibody directed against the negative immunoregulatory human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1/PCD-1) with immunopotential activity. Nivolumab binds to and blocks the activation of PD-1, an Ig superfamily transmembrane protein, by its ligands PD-L1 and PD-L2, resulting in the activation of T-cells and cell-mediated immune responses against tumor cells or pathogens. Activated PD-1 negatively regulates T-cell activation and effector function through the suppression of P13k/Akt pathway activation.

**Indication and use:** It is indicated for the treatment of patients with unresectable or metastatic melanoma.

**PD/PK:** According to pharmacokinetic data of nivolumab, (CL) 9.5 ml/h (49.7%), (V<sub>ss</sub>) 8.0 L (30.4%), and (t<sub>1/2</sub>) 26.7 days (101%) are reported. Steady-state concentrations of nivolumab are reported to be reached within 12 weeks (at dose 3 mg/kg every 2 weeks).

**Contraindications:** None.

#### Warnings:

- Nivolumab should be discontinued in immunemediated pneumonitis, hepatitis and colitis.
- Nivolumab enhances the risk of renal dysfunction, so, it should be discontinued.
- Nivolumab can cause fetal harm. It should be avoided in pregnant women.

**Adverse Events:** The most common adverse events are rashes, fatigue, and diarrhea.

**3.Pembrolizumab: [16]** A humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1) with potential immunopotentiating activity. Upon administration, pembrolizumab binds to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells, and blocks the binding to and activation of PD-1 by its ligands, which results in the activation of T-cell-mediated immune responses against tumor cells. The ligands for PD-1 include PD-L1, which is expressed on antigen presenting cells (APCs) and overexpressed on certain cancer cells, and PD-L2, which is primarily expressed on APCs. Activated PD-1 negatively regulates T-cell activation through the suppression of the PI3K/Akt pathway.

**Indications and Uses:** Unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

**PD/PK:** Elimination  $t^{1/2}$  : 26 days. As the dose is increased in the dose range of 2 to 10mg/kg over 3 weeks, the Cmax, Cmin and AUC increases proportionally.

**Contraindications:** None.

**Warnings:**

- Immune-mediated adverse reactions: Based on the severity of the reaction, corticosteroids should be administered.
- 1.Immune-mediated pneumonitis:** In case of moderate pneumonitis, withhold the drug and in case of life-threatening or severe pneumonitis, it should be permanently discontinued.
  - 2.Immune-mediated colitis:** In case of moderate or severe colitis, withhold the drug and in case of life-threatening colitis, it should be permanently discontinued.
  - 3.Immune-mediated hepatitis:** The changes in the hepatic function must be monitored. The drug should be withheld or discontinued, based on the severity of liver enzyme elevations.
  - 4.Immune-mediated hypophysitis:** In case of hypophysitis, the drug should be withheld for moderate hypophysitis, withheld or discontinued for severe and discontinued in life-threatening cases.
  - 5.Immune-mediated nephritis:** The changes in the renal function must be monitored. The drug should be withheld for moderate nephritis and permanently discontinued in severe or life-threatening cases.
  - 6.Immune-mediated hyperthyroidism and hypothyroidism:** The changes in the thyroid function must be monitored. The drug should be withheld in case of severe hyperthyroidism and permanently discontinued in severe or life-threatening cases.
- Embryofetal Toxicity:** It may lead to fetal harm. The females of child-bearing potential must be advised about the potential risk to the fetus.

**Adverse Events:** The most common adverse reactions include nausea, constipation, cough, fatigue, rash, pruritis, reduced appetite, diarrhea and arthralgia.

**b.Non-FDA approved checkpoint inhibitors:** There are some checkpoint inhibitors that are not currently approved by FDA for malignant melanoma. However, the inhibitors that are under clinical trials are given in the table-1 below:

**1.Varlilumab:** A human agonistic monoclonal antibody (MoAb) specific for CD27, with potential immunostimulating and antineoplastic activity. Upon administration of CDX-1127, this MoAb binds to CD27 and may potentiate the immune response by increasing the cytotoxic T-lymphocyte (CTL) response against CD27-expressing tumor cells. This may lead to growth inhibition of CD27-expressing tumor cells. In addition, this agent may increase the proliferation and activation of antigen-specific T lymphocytes upon co-administration of Tumor Associated Antigen (TAA)-containing vaccines, such as dendritic cell vaccines. CD27, a co-stimulatory molecule and member of the tumor

necrosis factor family overexpressed in certain tumor cell types, is constitutively expressed on mature T-lymphocytes, memory B cells and natural killer cells and plays an important role in NK cell mediated cytolytic activity and T and B lymphocyte proliferation and activation.

**2.Tremelimumab:** A human IgG2 monoclonal antibody directed against the T-cell receptor protein CTLA4. Tremelimumab binds to CTLA4 and blocks the binding of the antigen-presenting cell ligands B7-1 and B7-2 to CTLA4, resulting in inhibition of B7-CTLA4-mediated down regulation of T-cell activation; subsequently, B7-1 or B7-2 may interact with another T-cell surface receptor protein, CD28, resulting in a B7-CD28-mediated T-cell activation unopposed by B7-CTLA4-mediated inhibition.

Checkpoint Inhibitors	Clinical trial identifier no.	PHASE	Study Design	Target
Varlilumab	NCT02335918	Phase 1/2	Safety/Efficacy Study; Open Label	CD-27 antigen
Tremelimumab	NCT02141542	Phase 1	Safety/Efficacy Study, Single Group Assignment Open Label	CTLA-4

**Table 1:** Non-FDA approved checkpoint inhibitors [17,18].

**Kinase Inhibitors:**

**a.FDA approved Kinase inhibitors:**

**1.Vemurafenib [19]:** An orally bioavailable, ATP-competitive, small-molecule inhibitor of BRAF(V600E) kinase with potential antineoplastic activity. Vemurafenib selectively binds to the ATP-binding site of BRAF(V600E) kinase and inhibits its activity, which may result in an inhibition of an over-activated MAPK signaling pathway downstream in BRAF(V600E) kinase-expressing tumor cells and a reduction in tumor cell proliferation. Approximately 90% of BRAF gene mutations involve a valine-to-glutamic acid mutation at residue 600 (V600E); the oncogene protein product, BRAF(V600E) kinase, exhibits a markedly elevated activity that over-activates the MAPK signaling pathway. The BRAF(V600E) gene mutation has been found to occur in approximately 60% of melanomas, and in about 8% of all solid tumors, including melanoma, colorectal, thyroid and other cancers. Vemurafenib tablets got the U. S. FDA approval in 2011 against unresectable or metastatic melanoma with the BRAF V600E mutation [12].

**Indications and uses:** It is a kinase inhibitor and it is indicated mainly for the treatment of unresectable or metastatic melanoma with BRAF V600E mutation. However, it is not indicated for the treatment of wild-type BRAF melanoma.

**PD/PK:**  $T_{max}$  : 3 hours,  $C_{max}$  :  $62 \pm 17 \mu\text{g/mL}$ , AUC:  $601 \pm 170 \mu\text{gh/ml}$ . The apparent volume of distribution is reported to be 106 L and apparent clearance is 31 L/day. The median elimination half-life is estimated to be 57 hours.

**Contraindication:** None

**Warnings:**

- Vemurafenib should be discontinued in patients, who suffer from hypersensitivity reactions, including generalized rash, hypotension, and erythema.
- When patients experience dermatological reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, then it should be permanently discontinued.
- Patients experiencing hepatotoxicity and photosensitivity during the treatment with vemurafenib should discontinue its use.
- Vemurafenib can cause fetal harm. It should be avoided in pregnant women.

**Adverse Events:** The most common adverse events are arthralgia, rash, alopecia, fatigue, photosensitivity reaction, nausea, pruritus, and skin papilloma.

**2.Dabrafenib [20]:** An orally bioavailable inhibitor of B-raf (BRAF) protein with potential anti-neoplastic activity. Dabrafenib selectively binds to and inhibits the activity of B-raf, which may inhibit the proliferation of tumor cells which contain a mutated BRAF gene.

**Indications and Uses:** Unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test. It is used in combination with trametinib for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations as detected by an FDA-approved test. The use in combination is based on the demonstration of durable response rate.

**PD/PK:** Mean absolute bioavailability is 95%.  $T_{max}$  is 2 hours after oral administration. Terminal half-life of dabrafenib is 8 hours.

**Contraindications:** None.

**Warnings:**

- Cutaneous as well as non-cutaneous, new primary malignancies may occur, if dabrafenib is administered as a single agent or in combination with trametinib.
- Increased proliferation of cells can occur with the use of BRAF inhibitors.
- Major hemorrhagic events might occur with its use, either as single therapy or in combination with trametinib. Monitor for bleeding signs and symptoms.
- In patients receiving dabrafenib and trametinib, deep vein thrombosis and pulmonary embolism may occur.
- Monitor Left Ventricular Ejection Fraction (LVEF) before treatment with dabrafenib and trametinib in combination, after one month and then after every 2 to 3 months.
- Ophthalmologic evaluation must be done for any visual disturbances.
- Dabrafenib and trametinib, in combination, might lead to the increased incidence and severity of pyrexia.
- Skin toxicities and secondary infections might occur. If Grade 2, Grade 3 or Grade 4 rashes are present that do not improve within 3 weeks, even after interruption of dabrafenib, discontinue its use.
- In patients with already existing diabetes or hyperglycemia, monitor the serum glucose level.
- Deficiency of glucose-6 phosphate dehydrogenase. Monitor for hemolytic anemia.
- Might cause harm to the fetus. Hormonal contraception or other methods of contraception should be used by the women of child-bearing potential.

**Adverse Events:** As a single agent, the most common adverse events include: hyperkeratosis, papilloma, pyrexia, headache, palma-plantar erythrodysesthesia syndrome, arthralgia and alopecia. In combination with trametinib, the adverse events includes: nausea, vomiting, fatigue, chills, rashes, diarrhea, abdominal pain, arthralgia, cough, headache, constipation, peripheral edema, night sweats, myalgia and reduced appetite.

**3.Trametinib [21]:** An orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in the inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers.

**Indications and Use:** It is a kinase inhibitor indicated as a single agent and in combination with dabrafenib for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations as detected by an FDA-approved test. The use in combination is based on the demonstration of durable response rate. Improvement in disease-related symptoms or overall survival has not been demonstrated for trametinib in combination with dabrafenib.

Trametinib as a single agent is not indicated for treatment of patients who have received prior BRAF-inhibitor therapy.

**PD/PK:**  $T_{max}$  is reported to be 1.5 hours after dose administration. The bioavailability of a 2mg tablet is found to be 72%. Elimination half-life is reported to be 3.9 to 4.8 days, estimated according to the population PK model.

**Contraindications:** None.

**Warnings:**

- New cutaneous and non-cutaneous, primary malignancies may occur, when trametinib is used in combination with dabrafenib. Prior to initiation of therapy, patients must be monitored for new malignancies, while on therapy and after discontinuation of the combination treatment.
- Major hemorrhagic events might occur with its use in combination with trametinib. Monitor for bleeding signs and symptoms.
- In patients receiving trametinib along with dabrafenib, deep vein thrombosis and pulmonary embolism may occur.
- Monitor LVEF before treatment, after one month and then after every 2 to 3 months.
- **Ocular Toxicity:** In case of visual disturbances, perform ophthalmologic evaluation. It should be permanently discontinued, in case of Retinal Vein Occlusion (RVO).
- **Interstitial Lung Disease (ILD):** Trametinib must be withheld for new or progressive pulmonary symptoms. It should be permanently discontinued for ILD, associated with treatment-related ILD or pneumonitis.
- Trematinib, in combination with dabrafenib, can lead to serious febrile reactions.
- **Serious Skin Toxicity:** Skin toxicities and secondary infections might occur and should be monitored. If Grade 2, Grade 3 or Grade 4 rashes are present that do not improve within 3 weeks, even after interruption of trametinib, discontinue its use.
- **Hyperglycemia:** In patients with pre-existing diabetes or hyperglycemia, monitor the serum glucose levels.
- **Embryofetal toxicity:** Might cause harm to the fetus. Advise females of childbearing potential about the potential risk to the fetus.

**Adverse Events:** As a single agent, the most common adverse events associated with trametinib include rash, diarrhea and lymphedema. In combination with dabrafenib, the adverse effects include: nausea, vomiting, fatigue, chills, rash, diarrhea, abdominal pain, arthralgia, cough, headache, constipation, peripheral edema, night sweats, myalgia and reduced appetite.

**4. Cobimetinib:** [22] It is a reversible, selective, allosteric, oral inhibitor that blocks the mitogen-activated protein kinase (MAPK) pathway by targeting the mitogen-activated extracellular signal-regulated kinase (MEK) 1 and MEK 2 which results in inhibition of phosphorylation of the extracellular signal-regulated kinase (ERK) 1 and ERK 2. It blocks the cell proliferation induced by the MAPK pathway through inhibition of the MEK1/2 signalling node.

**Indications and uses:** It is indicated for use in combination with vemurafenib for the treatment of adult patients with unresectable or metastatic melanoma with a BRAF V600 mutation.

**PD/PK:** Following oral dosing of 60 mg in cancer patients, cobimetinib showed a moderate rate of absorption with a median  $T_{max}$  of 2.4 hours. The mean steady-state  $C_{max}$  and AUC<sub>0-24</sub> were 273 ng/mL and 4340 ng.h/mL respectively. The mean accumulation ratio at steady state was approximately 2.4-fold.

**Contraindication:** Hypersensitivity to the drug

**Warnings:**

- The should be discontinued in patients, who suffer from hypersensitivity reactions, including generalized rash, hypotension, and erythema.
- Serous retinopathy has been observed in patients treated. The majority of events were reported as chorioretinopathy or retinal detachment.
- Patients experiencing hepatotoxicity and photosensitivity during the treatment and should discontinue its use.
- Decrease in LVEF from baseline has been reported in patients receiving the drug.

**Adverse Events:** The most common adverse reactions observed with a higher frequency in the Cobimetinib plus vemurafenib use were diarrhoea, rash, nausea, pyrexia, photosensitivity reaction, increased alanine aminotransferase, increased aspartate aminotransferase, increased blood creatine phosphokinase, and vomiting.

**b.Non-FDA approved Kinase Inhibitors:** The kinase inhibitors that are under clinical trials have been given in Table-2 below:

**1.Masitinib:** A multi-targeted protein tyrosine kinase inhibitor with potential antineoplastic activity. Masitinib selectively binds to and inhibits both the wild-type and mutated forms of the stem cell factor receptor (c-Kit; SCFR); platelet-derived growth factor receptor (PDGFR); fibroblast growth factor receptor 3 (FGFR3); and to a lesser extent, focal adhesion kinase (FAK). As a consequence, tumor cell proliferation may be inhibited in cancer cell types that overexpress these receptor tyrosine kinases (RTKs).

**2.Binimetinib (MEK162):** An orally available inhibitor of mitogen-activated protein kinase kinase 1 and 2 (MEK1/2) with potential antineoplastic activity. Binimetinib, non-competitive with ATP, binds to and inhibits the activity of MEK1/2. Inhibition of MEK1/2 prevents the activation of MEK1/2-dependent effector proteins and transcription factors, which may result in the inhibition of growth factor-mediated cell signaling. This may eventually lead to an inhibition of tumor cell proliferation and an inhibition in production of various inflammatory cytokines, including interleukin-1, -6 and tumor necrosis factor. MEK1/2 are dual-specificity threonine/tyrosine kinases that play key roles in the activation of the RAS/RAF/MEK/ERK pathway and are often upregulated in a variety of tumor cell types.

**3.PLX3397:** A capsule formulation containing a small-molecule receptor tyrosine kinase (RTK) inhibitor of KIT, CSF1R and FLT3 with potential antineoplastic activity. Multitargeted tyrosine kinase inhibitor, PLX3397, binds to and inhibits phosphorylation of the stem cell factor receptor (KIT), colony-stimulating factor-1 receptor (CSF1R) and FMS-like tyrosine kinase 3 (FLT3), which may result in the inhibition of tumor cell proliferation and down-modulation of macrophages, osteoclasts and mast cells, involved in the osteolytic metastatic disease. FLT3, CSF1R and FLT3 are overexpressed or mutated in many cancer cell types and play major roles in tumor cell proliferation and metastasis.

**4.Pazopanib:** A small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits vascular endothelial growth factor receptors (VEGFR)-1, -2 and -3, c-kit and platelet derived growth factor receptor (PDGF-R), which may result in the inhibition of angiogenesis in tumors, in which these receptors are upregulated.

**5.Nilotinib:** An orally bioavailable aminopyrimidine-derivative Bcr-Abl tyrosine kinase inhibitor with antineoplastic activity. Designed to overcome imatinib resistance, nilotinib binds to and stabilizes the inactive conformation of the kinase domain of the Abl protein of the Bcr-Abl fusion protein, resulting in the inhibition of the Bcr-Abl-mediated proliferation of Philadelphia chromosome-positive (Ph+)

chronic myeloid leukemia (CML) cells. This agent also inhibits the receptor tyrosine kinases platelet-derived growth factor receptor (PDGF-R) and c-kit, a receptor tyrosine kinase mutated and constitutively activated in most gastrointestinal stromal tumors (GISTs). With a binding mode that is energetically more favorable than that of imatinib, nilotinib has been shown to have an approximately 20-fold increased potency in kinase and proliferation assays compared to imatinib.

**6.Axitinib:** An orally bioavailable tyrosine kinase inhibitor. Axitinib inhibits the proangiogenic cytokines vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor (PDGF), thereby exerting an anti-angiogenic effect.

**7.Imatinib:** It binds to an intracellular pocket located within tyrosine kinases (TK), thereby inhibiting ATP binding and preventing phosphorylation and the subsequent activation of growth receptors and their downstream signal transduction pathways. This agent inhibits TK encoded by the bcr-abl oncogene as well as receptor TKs, encoded by the c-kit and platelet-derived growth factor receptor (PDGFR) oncogenes. Inhibition of the bcr-abl TK results in decreased proliferation and enhanced apoptosis in malignant cells of Philadelphia-positive (Ph+) hematological malignancies such as CML and ALL; effects on c-kit TK activity inhibit mast-cell and cellular proliferation in those diseases, overexpressing c-kit, such as mastocytosis and gastrointestinal stromal tumor (GIST).

**8.Alisertib:** A second-generation, orally bioavailable, highly selective small molecule inhibitor of the serine/threonine protein kinase Aurora A kinase with potential antineoplastic activity. Alisertib binds to and inhibits Aurora A kinase, which may result in disruption of the assembly of the mitotic spindle apparatus, disruption of chromosome segregation, and inhibition of cell proliferation. Aurora A kinase localizes to the spindle poles and to spindle microtubules during mitosis, and is thought to regulate spindle assembly. Aberrant expression of Aurora kinases occurs in a wide variety of cancers, including colon and breast cancers.

**9.Buparlisib:** An orally bioavailable specific oral inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases with potential antineoplastic activity. Buparlisib specifically inhibits class I PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway in an ATP-competitive manner, thereby inhibiting the production of the secondary messenger phosphatidylinositol-3,4,5-trisphosphate and activation of the PI3K signaling pathway. This may result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a variety of antineoplastic agents.

**10.LGX818:** An orally available Raf kinase inhibitor with potential antineoplastic activity. LGX818 specifically inhibits Raf kinase, a serine/threonine enzyme in the RAF/mitogen-activated protein kinase kinase (MEK)/ extracellular signal-related kinase (ERK) signaling pathway. By inhibiting the activation of the RAF/MEK/ERK signaling pathway, the administration of LGX818 may result in a decrease in proliferation of tumor cells. The Raf mutation BRAF V600E is frequently upregulated in a variety of human tumors and results in the constitutive activation of the RAF/MEK/ERK signaling pathway that regulates cellular proliferation and survival.

**11.LEE011:** An orally available cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. CDK4/6 inhibitor LEE011 specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

**12.Palbociclib:**An orally available cyclin-dependent kinase (CDK) inhibitor with potential antineoplastic activity. Palbociclib selectively inhibits cyclin-dependent kinase 4 (CDK4) and 6 (CDK6), thereby inhibiting retinoblastoma (Rb) protein phosphorylation early in the G1 phase leading to cell cycle arrest. This suppresses DNA replication and decreases tumor cell proliferation. CDK4 and 6 are serine/threonine kinases that are upregulated in many tumor cell types and play a key role in the regulation of cell cycle progression.

Kinase Inhibitors	Clinical trial identifier no.	PHASE	Study Design	Target
Masitinib	NCT01280565	Phase-3	Safety/ Efficacy Study, Open Label	c-kit, SCFR, PDGFR, FGFR3
Binimetinib	NCT01763164	Phase-3	Safety/Efficacy Study, Open Label	MEK1/2
PLX3397	NCT02071940	Phase 2	Efficacy Study, Open Label	KIT, CSF1R and FLT3
Pazopanib	NCT01107665	Phase 2	Safety/ Efficacy Study, Open Label	VEGFR-1,2,3
Nilotinib	NCT01168050	Phase-2	Efficacy Study, Open Label	Bcr-Abl
Axitinib	NCT01321437	Phase 2	Safety/ Efficacy Study, Open Label	VEGF
Imatinib	NCT01782508	Phase 2	Efficacy Study, Open Label	c-kit and PDGFR
Alisertib	NCT01316692	Phase 2	Efficacy Study. Open Label	Aurora A kinase
Buparlisib	NCT01512251	Phase-1/2	Safety/Study, Open Label	Class 1 PIK3
LGX-818	NCT01543698	Phase – 1/2	Safety/Efficacy Study, Open Label	Raf Kinase
LEE011	NCT01781572	Phase 1/2	Safety/Efficacy Study, Open Label	CDK4/6
Palbociclib	NCT02202200	Phase 1/2	Safety/Efficacy Study, Open Label	CDK4/6

**Table 2:** Non-FDA approved kinase inhibitors [23,34].

### C.Cytokine Therapy:

#### a.FDA approved Cytokine Therapy:

**1.Interferon alfa-2b [35]:** Alfa interferons bind to specific cell-surface receptors, resulting in the transcription and translation of genes, whose protein products mediate antiviral, antiproliferative, anticancer, and immune-modulating effects.

**Indications and Use:** It is indicated as an adjuvant to surgical treatment in patients 18 years of age or older with malignant melanoma, who are free of disease, but at high risk for systemic recurrence, within 56 days of surgery.

**PD/PK:** The maximum serum concentrations were found to be 18 to 116 IU/ml after a single dose of 5 million IU/m<sup>2</sup> given subcutaneously, intramuscularly and 30-minute intravenous infusion. The elimination half-life is reported to be 2 to 3 hours after intramuscular and subcutaneous injection.

#### Contraindications: It is contraindicated in patients with:

- Hypersensitivity to interferon alfa or any of its constituents.
- Autoimmune hepatitis.
- Decompensated liver disease.
- In combination with ribavarin, it is contraindicated in:
- Patients with hypersensitivity to ribavarin or any of its components.
- Pregnant women.
- Men, who have pregnant female partners.
- Patients with hemoglobinopathies, such as sickle cell anemia and thalassemia major.
- Patients, whose creatinine clearance lies below 50ml/min.

**Warnings:**

- **General:** Fever and flu-like symptoms might develop, hence, it should be given cautiously in patients with pulmonary disease or diabetes mellitus, who are especially prone to ketoacidosis. Care must be taken in patients with thrombophlebitis, pulmonary embolism or severe myelosuppression.
- **Cardiovascular disease:** Hypotension, arrhythmia, tachycardia of 150 beats per minute or greater, myocardial infarction and cardiomyopathy have been observed. Close monitoring must be done in patients with a history of myocardial infarction or previous or present arrhythmic disorders.
- **Cerebrovascular Disorders:** Ischemic and hemorrhagic cerebrovascular events have been observed.
- **Neuropsychiatric Disorders:** Depression and suicidal behavior, which includes suicidal ideation, suicidal attempts, completed suicides, homicidal ideation and aggressive behavior has been reported. Hence, it should be used with caution in patients with psychiatric disorders. Treatment should be discontinued in patients developing suicidal tendencies while on-treatment. Narcotics, hypnotics or sedatives can be given concurrently with caution and patients must be monitored, until the adverse effects have resolved.
- **Bone Marrow Toxicity:** Bone marrow suppression, cytopenias including aplastic anemia may occur. Complete blood counts must be checked prior to the therapy and monitored on regular basis. It should be discontinued in patients, who have neutrophil count less than  $0.5 \times 10^9/L$  or platelet count less than  $25 \times 10^9/L$ .
- **Ophthalmologic Disorders:** Decreased or loss of vision, retinal artery or vein thrombosis, retinopathy including macular edema, retinal hemorrhages; optic neuritis, serious retinal detachment and papilledema may be induced or aggravated. All patients must be monitored regularly and baseline eye examination must be conducted. It should be discontinued in patients, who develop new or a worse form of ophthalmologic disorders.
- **Endocrine Disorders:** Hypothyroidism or hyperthyroidism might develop. Patients with already existing thyroid disorders and whose thyroid function cannot be maintained within limits should not be treated with interferon. Serum TSH levels should be monitored prior to therapy.
- **Gastrointestinal disorders:** Hepatotoxicity, including fatality has been reported. Close monitoring and discontinuation of therapy must be done, if appropriate.
- **Pulmonary Disorders:** Pulmonary infiltrates, dyspnea, broncholitis obliterans, interstitial pneumonia, sarcoidosis and pulmonary hypertension might occur. Chest X-ray should be done for the patients, who develop fever, cough or dyspnea. In case of pulmonary impairment, close monitoring should be done or treatment should be discontinued.
- **Autoimmune Disorders:** Thrombocytopenia, rheumatoid arthritis, vasculitis, Raynaud's phenomenon, rhabdomyolysis and lupus erythematosus have been observed.

**Adverse Events:** The most common adverse events include: fatigue, depression, diarrhea, nausea/vomiting, alopecia, depression, altered taste sensation, dizziness/vertigo, chills, headache and anemia. Life-threatening adverse reactions include: neutropenia, fever, chills, headache, myalgia, increased SGOT, Grade 4 fatigue and depression and lethal hepatotoxicity.

**2.Aldesleukin [36]:** A recombinant analog of the endogenous cytokine interleukin-2 (IL-2) with immunoregulatory and antineoplastic activities. Aldesleukin binds to and activates the IL-2 receptor, followed by heterodimerization of the cytoplasmic domains of the IL-2R beta and gamma(c) chains; activation of the tyrosine kinase Jak3; and phosphorylation of tyrosine residues, located on the IL-2R beta

chain, resulting in an activated receptor complex. Various cytoplasmic signaling molecules are recruited to the activated receptor complex and become substrates for regulatory enzymes that are associated with the receptor complex. This agent enhances lymphocyte mitogenesis; stimulates long-term growth of human IL-2 dependent cell lines; enhances lymphocyte cytotoxicity; induces lymphokine-activated killer (LAK) cell and natural killer (NK) cell activities; and induces expression of interferon-gamma. Aldesleukin may induce T cell-mediated tumor regression in some tumor types.

**Indications and Use:** It is indicated for the treatment of adults with metastatic melanoma.

**PD/PK:** Upon administration, approximately 30% of administered dose is detected in the plasma. The distribution and elimination half-life is reported to be 13 and 85 minutes, respectively.

**Contraindications:** It is contraindicated in patients with altered or impaired pulmonary function tests, thallium stress test and those with organ allografts. Patients with following drug related toxicities are contraindicated to undergo retreatment with aldesleukin:

- Persistent ventricular tachycardia ( $\geq 5$  beats).
- Cardiac arrhythmias, which are uncontrolled or non-responsive to the management.
- Cardiac tamponade.
- Intubation for  $>72$  hours.
- Renal failure that requires dialysis  $>72$  hours.
- Coma or toxic psychosis lasting  $>48$  hours.
- Bowel ischemia or perforation.
- GI bleeding requiring surgery.

**Warnings:**

- Aldesleukin therapy should be given only to those patients, who have normal cardiac and pulmonary functions, evaluated through thallium stress testing and formal pulmonary function testing.
- It should be administered under the surveillance of a qualified physician, who has a great experience in the field of anticancer agents. It can lead to hypotension, reduced organ perfusion, cardiac arrhythmia, myocardial infarction, angina, respiratory insufficiency, renal insufficiency, GI bleeding or infarction, edema and change in mental status.
- **Capillary leak Syndrome (CLS):** It is characterized by the vascular tone loss and plasma protein and fluid extravasation.
- Impaired neutrophil function, with an increased risk of disseminated function, along with sepsis and bacterial endocarditis may be observed.
- Aldesleukin should be withheld, if a patient develops moderate to severe lethargy or somnolence. If the administration is continued, it may result in coma.
- Aldesleukin may exacerbate the pre-existing autoimmune and inflammatory disease. Exacerbation of arthritis, scleroderma, Crohn's disease, oculo bulbar myasthenia gravis, diabetes mellitus, cholecystitis, cerebral vasculitis, Stevens-Johnson syndrome, IgA glomerulonephritis and bullous pemphigoid have been reported.
- Neurologic symptoms, such as mental status, difficulty in speech, hallucinations, coma, cortical blindness, agitation, limb or gait ataxia and obtundation may occur. Prior to therapy, evaluation and treatment of CNS metastasis should be done.

**Adverse Events:** Various adverse events associated with aldesleukin include: chills, fever, malaise, asthenia, infection, abdominal pain, enlarged abdomen, hypotension, tachycardia, vasodilation, supraventricular tachycardia, cardiovascular disorder, arrhythmia, diarrhea, nausea, vomiting, stomatitis, anorexia, nausea, vomiting, leucopenia, thrombocytopenia, anemia, bilirubinemia, increase in creatinine levels, peripheral edema, increased SGOT, weight gain, edema, acidosis, hypomagnesemia, hypocalcemia, alkaline phosphatase increase, confusion, somnolence, anxiety, dizziness, dyspnea, lung and respiratory disorder, increased cough, rhinitis, rash, pruritis, exfoliative dermatitis and oliguria.

**b.Non-FDA approved Cytokine Therapy:** The cytokine-based agents that are under clinical trials have been given in Table-3 below:

**1.Imiquimod:** A synthetic agent with immune response modifying activity. As an immune response modifier (IRM), imiquimod stimulates cytokine production, especially interferon production, and exhibits antitumor activity, particularly against cutaneous cancers. Imiquimod’s proapoptotic activity appears to be related to Bcl-2 overexpression in susceptible tumor cells.

Cytokines	Clinical trial identifier no.	PHASE	Study Design	Target
Imiquimod	NCT01720407	Phase-3	Efficacy Study, Open Label, Treatment	Interferon

*Table 3: Non-FDA approved cytokine therapy [37].*

**D.Monoclonal Antibodies:**

**a.Non-FDA approved monoclonal antibodies:** The non-FDA approved monoclonal antibodies that are under clinical trials have been given in Table-4 below:

**1.Glembatumumab vedotin:** An antibody-drug conjugate, consisting of the fully human monoclonal antibody CR011 directed against glycoprotein NMB (GPNMB) and conjugated via a cathepsin B-sensitive valine-citrulline (vc) linkage to the cytotoxic agent mono-methyl auristatin E (MMAE), with potential antineoplastic activity. Upon administration, the monoclonal antibody CR011 moiety binds to glycoprotein nmb (GPNMB), expressed on the surfaces of a variety of cancer cell types; upon endocytosis, the synthetic dolastin analogue MMAE is released via enzymatic cleavage into the tumor cell cytosol, where it binds to tubulin and inhibits tubulin polymerization, which may result in G2/M phase arrest and apoptosis. The vc linkage system is highly stable in serum, rendering the cytotoxicity of glembatumumab vedotin specific for GPNMB-positive cells. GPNMB is a transmembrane protein overexpressed on the surfaces of various cancer cell types, including melanoma, breast, and prostate cancer cells.

**2.Ofatumumab:** A fully human, high-affinity IgG1 monoclonal antibody, directed against the B-cell CD20 cell surface antigen with potential antineoplastic activity. Ofatumumab binds specifically to CD20 on the surfaces of B cells, triggering complement-dependent cell lysis (CDCL) and antibody-dependent cell-mediated cytotoxicity (ADCC) of B cells overexpressing CD20. The CD20 antigen, found on over 90% of B cells, B cell lymphomas, and other B cells of lymphoid tumors of B cell origin, is a non-glycosylated cell surface phosphoprotein that acts as a calcium ion channel; it is exclusively expressed on B cells during most stages of B cell development.

**3.Bevacizumab:** A recombinant humanized monoclonal antibody directed against the VEGF, a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGFR binding, thereby preventing the growth and maintenance of tumor blood vessels.

**4.Fresolimumab:** A panspecific, recombinant, fully human monoclonal antibody directed against human transforming growth factor (TGF) -beta 1, 2 and 3 with potential anti-neoplastic activity. Fresolimumab binds to and inhibits the activity of all isoforms of TGF-beta, which may result in the inhibition of tumor cell growth, angiogenesis, and migration. TGF-beta, a cytokine often over-expressed in various malignancies, may play an important role in promoting the growth, progression, and migration of tumor cells.

MABs	Clinical trial identifier no.	Phase	Study Design	Target
Glambatimumab vedotin	NCT02302339	Phase 2	Efficacy Study, Open Label	Glycoprotein NMB (GPNMB)
Ofatumumab	NCT01376713	Phase 2	Efficacy Study; Open Label	CD20 antigen
Bevacizumab	NCT02065466	Phase ½	Open Label; Treatment	VEGF
Fresolimumab	NCT00356460	Phase 1	Non-Randomized, Safety Study, Open Label	TGF-β

**Table 4:** Non-FDA approved MABs [38-41].

**E.Vaccines:**

**a.Non-FDA approved vaccines:** There are some vaccines that are not currently approved by FDA for malignant melanoma. However, many vaccines are under clinical trials in phase I, II, and III as in the table-5 below:

**1.MELITAC 12.1 Peptide Vaccine:** A peptide cancer vaccine consisting of an emulsion of a mixture of 12 class I Major Histocompatibility Complex (MHC)-restricted melanoma peptides and a class II MHC-restricted tetanus toxoid helper peptide, with potential immunostimulating and anti-neoplastic activities. Upon administration, the MELITAC 12.1 peptide vaccine may stimulate the host immune system to mount a cytotoxic T-cell response against tumor cells expressing the melanoma peptide antigens, resulting in tumor cell lysis. The melanoma peptides contained in the vaccine are upregulated in melanoma cancer cells.

**2.CDX-1401 and Poly-ICLC Vaccine:** The cancer vaccine CDX-1401 attaches to a protein that is made in tumor cells. The vaccine helps the body to recognize the tumor to fight the cancer. The CDX-301 vaccine may help the body make more of the tumor fighting cells, known as dendritic cells. The poly-ICLC vaccine stimulates the immune system and may help these dendritic cells to mature so that they can recognize the tumor.

**3.Dorgenmeltucel-L:** The alpha (1,3)-gal stimulates an immune response against melanoma-specific antigens in the tumor cell lines. The patient’s immune system, then targets their own melanoma cells and destroys them.

**4.A2/4-1BB ligand-expressing melanoma vaccine:** An allogeneic melanoma cell vaccine derived from a cell line with high expression of melanoma associated antigens and genetically modified to express both HLA-A2 and 4-1BB ligand, with potential immunostimulating and anti-neoplastic activities. Upon administration, the 4-1BB ligand of the allogeneic HLA-A2/4-1BB ligand-expressing melanoma vaccine binds to 4-1BB on activated T-lymphocytes, which induces a strong immune response against HLA-A2 positive melanoma cells.

**5.IL15-DC Vaccine:** Interleukin-15 is a new generation of the dendritic cell vaccines. This vaccine has improved potency to work against the cancerous cells. IL15-DC is a most promising vaccine for the treatment of malignant melanoma.

**6.HLA-A1-binding Melanoma antigens 1 and 3 (MAGE-1/MAGE-3) multi-peptide-pulsed autologous dendritic cell vaccine:** A cell-based cancer vaccine composed of autologous dendritic cells (DCs) pulsed with human leukocyte antigen (HLA)A1-binding melanoma-associated antigen peptides MAGE-1 and MAGE-3 with potential immunomodulating and anti-neoplastic activity. Upon vaccination, HLA-A1-binding MAGE-1/MAGE-3 multi-peptide-pulsed autologous dendritic cell vaccine may stimulate the host immune system to mount an anti-tumoral cytotoxic T lymphocyte (CTL) and antibody responses against MAGE1- and MAGE-3-expressing cancer cells, resulting in tumor cell lysis. HLA-A1 is an MHC class I molecule that presents antigenic peptides to CD8+ T cells; epitope design restricted to epitopes that bind most efficiently to HLA-A1 may improve antigenic peptide immunogenicity.

**7.POL-103A:** It is a newly developed investigational vaccine composed of antigens associated with multiple melanoma. These are derived from 3 human melanoma cell lines, which are admixed with alum that acts as an adjuvant. Due to the presence of various antigens, the induction of immune response against the tumor is maximized and hence, prevents the ability of tumor cell to escape the immune response.

**8.Survivin Peptide Vaccine:** A peptide vaccine comprised of synthetic HLA-A1, -A2 and -B35 restricted survivin epitopes combined with the adjuvant Montanide ISA-51 with potential antineoplastic activity. Upon administration, HLA-A1, A2, B35-restricted survivin peptides/Montanide ISA-51 vaccine may stimulate a cytotoxic T cell response against tumor cells that overexpress survivin, resulting in tumor cell lysis. Montanide ISA-51, also known as incomplete Freund's adjuvant or IFA, is a stabilized water-in-oil emulsion adjuvant containing mineral oil with mannide oleate added as a surfactant that non-specifically stimulates cell-mediated immune responses to antigens.

**9.AGI-101H:** A cancer vaccine derived from two genetically modified human melanoma cell lines with potential antineoplastic activity. Allogeneic melanoma vaccine AGI-101H consists of a 1:1 mixture of cells from two genetically modified human melanoma cell lines, designated as Mich1H6 and Mich2H6, that have been gamma-irradiated to render the cells non-proliferative. Upon administration, this vaccine may stimulate a cytotoxic immune response against melanoma tumor cells.

**10.SCI-B1:** A proprietary DNA-based cancer vaccine that encodes a melanoma antigen tyrosinase-related protein 2 (TRP2) cytotoxic T-lymphocyte (CTL) epitope and a modified monoclonal antibody, a chimera of human IgG1/murine IgG2a with T cell mimotopes expressed within the complementarity-determining regions (CDR) of the antibodies, with potential immunostimulating and antineoplastic activities. Upon intramuscular injection and electroporation, melanoma TRP2 CTL epitope vaccine SCIB1 expresses the modified antibody. Subsequently, the Fc component of the engineered antibody targets and binds to the CD64 receptor on the dendritic cells (DCs); upon processing by DCs, the cellular immune system may be activated to induce helper T-cell and CTL immune responses against tumor cells expressing the TRP2 antigen.

**11.MAGE-3.1A vaccine:** A synthetic peptide cancer vaccine consisting of human leukocyte antigen HLA-A1-restricted peptide derived from MAGE-3 with potential immunostimulating and antineoplastic activities. Upon administration, MAGE-3.1 A peptide vaccine may stimulate the immune system to mount a cytotoxic T-cell (CTL) response against tumor cells expressing MAGE-3, resulting in tumor cell lysis. MAGE-3, a TAA, is overexpressed by a variety of cancer cell types.

**12.Theravac:** It is a recombinant adenylate cyclase toxin, which is obtained from Bordetella pertussis that has undergone the process of detoxification by mutation of its catalytic domain and is further coupled to the Tyrosinase.A2 epitope, YMDGTMSQV.

**13.NY-ESO1 vaccine:** A cancer vaccine consisting of an immunogenic peptide derived from the cancer-testis antigen (NY-ESO-1), an antigen found in normal testis and various tumors. Vaccination with NY-ESO1 vaccine may stimulate the host immune system to mount a humoral and cytotoxic T lymphocyte (CTL) response to cells expressing NY-ESO1 antigen, resulting in tumor cell lysis.

**14.Long Peptide Vaccine 7 (LPV7):** A peptide vaccine consisting of a combination of seven synthetic long peptides (SLPs), which are each about 30 amino acids in size, and derived from cancer-testis antigens (CTA) and melanocytic differentiation proteins (MDP), with potential immunostimulating and antitumor activities. Upon administration, long peptide vaccine 7 may stimulate the host immune system to mount a cytotoxic T-cell lymphocyte (CTL) response against tumor cells expressing these peptides. CTA and MDP are overexpressed in a variety of cancer cell types.

**15.UV1 vaccine:** A synthetic, peptide cancer vaccine directed against the human telomerase reverse transcriptase catalytic subunit (hTERT) with potential immunomodulating activity. Vaccination with the UV1 telomerase peptide may stimulate cytotoxic T-cells to recognize and kill telomerase-expressing cells. Telomerase, a reverse transcriptase normally repressed in healthy cells, is overexpressed in most cancer cells and plays a key role in cellular proliferation.

Vaccines	Clinical trial identifier no.	Phase	Study Design	Target
MELITAC 12.1 Peptide Vaccine	NCT01961115	Phase 2	Efficacy Study Open Label	IDO1 inhibitor
CDX-1401 and Poly-ICLC Vaccine	NCT02129075	Phase 2	Randomized Efficacy Study Open Label	Melanoma cells
Dorgenmeltucel-L	NCT02054520	Phase 2	Randomized Safety/ Efficacy Study, Open Label	Melanoma cells
A2/4-1BB ligand-expressing melanoma vaccine	NCT01898039	Phase 1, 2	Safety/ Efficacy Study, Open Label	HLA-A2
IL15-DC Vaccine	NCT01189383	Phase 1, 2	Safety/Efficacy Study, Open Label	Melanoma cells
HLA-A1-binding MAGE-1/ MAGE-3 multi peptide-pulsed autologous dendritic cell vaccine	NCT01082198	Phase 1, 2	Open Label	MAGE1/3
POL-103A	NCT01546571	Phase-3	Safety/Efficacy Double blind	Tumor cells
Survivin peptide vaccine	NCT00108875	Phase – 1/2	Safety/ Efficacy Study, Open Label	T-cells
AGI-101 H	NCT00716495	Phase-1/2	Safety Study, Open Label	Tumor cells
SCIB1	NCT01138410	Phase-1/2	Safety/ Efficacy Study; Open Label	CTL epitope
MAGE-3.1A vaccine	NCT01191034	Phase-1/2	Safety/ Efficacy Study, Open Label	CTL cells
Theravac	NCT01331915	Phase 1/2	Safety Study, Open Label	Tumor cells
NY-ESO1	NCT01584115	Phase-1/2	Safety/ Efficacy Study, Open Label	CTL
LPV7	NCT02126579	Phase-1/2	Safety/ Efficacy Study, Open Label	CTL
UV1 vaccine	NCT02275416	Phase 1/2	Safety Study, Open Label	hTERT

**Table 5:** Non-FDA approved vaccines [42-56].

**F.Adoptive T Cell Therapy:**

**a.Non-FDA approved Adoptive T-cell Therapy:** In adoptive T-cell therapy, T-cells are removed from a patient, genetically modified or treated with chemicals to enhance their activity, and then reintroduced into the patient with the goal of improving the immune system’s anti-cancer response. The following immunotherapeutics are in phase I, II, and III of clinical trials as in the table-6.

**1.CD62L+ Derived T Lymphocytes:** Human autologous CD62L-positive T-lymphocytes transduced with a retroviral vector encoding a T cell receptor (TCR) specific for the cancer-testis antigen NY-ESO-1, with potential anti-neoplastic activity. Following leukapheresis, isolation of lymphocytes, expansion ex vivo, transduction, and re-sintroduction into the patient, the anti-NY-ESO1 TCR-transduced autologous CD62L+-derived T lymphocytes bind to NY-ESO-1-overexpressing tumor cells. This may result in cytotoxic T-lymphocyte (CTL)-mediated elimination of NY-ESO-1-positive cancer

2.cells. NY-ESO-1, a TAA, is found in normal testis and on the surface of various tumor cell types. CD62L, also called L-selectin, is a lymphoid homing receptor and differentiation marker and is expressed on a subset of CD8-positive T-lymphocytes; it is involved in the migration of T-lymphocytes to lymph nodes and may improve the efficacy for ex vivo-expanded T-cells following adoptive cell therapy.

**3.4-1BB tumor infiltrating cells:** 4-1BB (CD137) binds to the 4-1BB ligand (4-1BBL) that is expressed on APC such as macrophages, dendritic cells (DC) and activated B cells, and in response to binding to 4-1BBL or to legating antibody, it delivers a signal for T-cell activation, survival and growth.

Adoptive Therapy	Clinical trial identifier no.	Phase	Study Design	Target
CD62L+-Derived T Lymphocytes	NCT02062359	Phase 2	Non-Randomized Safety/ Efficacy Study Single Group Assignment Masking: Open Label	NY-ESO-1 molecule
4-1BB tumor infiltrating cells	NCT02111863	Phase 2	Non-Randomized Efficacy Study Single Group Assignment Open Label	Melanoma cells

**Table 6:** Non-FDA approved adoptive T-cell therapy [57,58].

**G.MDM2 Inhibitor:**

**a.Non-FDA approved MDM2 Inhibitor:** The MDM2 inhibitors that are under clinical trials have been given in Table-7 below:

**1.AMG232:** An orally available, piperidinone inhibitor of MDM2, with potential antineoplastic activity. Upon oral administration, MDM2 inhibitor AMG-232 binds to MDM2 protein and prevents its binding to the transcriptional activation domain of the tumor suppressor protein p53. By preventing this MDM2-p53 interaction, the transcriptional activity of p53 is restored. This leads to p53-mediated induction of tumor cell apoptosis. MDM2, a zinc finger protein and a negative regulator of the p53 pathway, is overexpressed in cancer cells; it plays a key role in cancer cell proliferation and survival.

MDM2 inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
AMG232	NCT02110355	Phase 1/2	Safety/Efficacy Study, Open Label	MDM2

**Table 7:** Non-FDA approved MDM2 inhibitors [59].

**H.Indoleamine 2,3-dioxygenase (IDO) inhibitor:**

**a.Non-FDA approved IDO inhibitor:** The IDO inhibitors that are under clinical trials have been given in Table-8 below:

**1.Indoximod:** A methylated tryptophan with anti-immunosuppressive activity. Indoximod inhibits the enzyme IDO, which degrades the essential amino acid tryptophan, and may increase or maintain tryptophan levels important to T cell function. Tryptophan depletion is associated with immunosuppression involving T-cell arrest and anergy.

IDO inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
Indoximod	NCT02073123	Phase I/II	Safety Study, Open Label	IDO

**Table 8:** Non-FDA approved IDO inhibitors [60].

**I.Gene Therapy:**

**a.Non-FDA approved Gene Therapy:** The adenovectors that are under clinical trials have been given in Table-9 below:

**1.INGN241:** A non-replicating adenoviral vector (adenovector) encoding the melanoma differentiation-associated 7 gene (MDA7) with potential antineoplastic activity. After intratumoral injection and adenovector-mediated gene transfer of MDA7 into tumor cells, the expressed MDA7 transgene may inhibit tumor cell proliferation and induce tumor cell apoptosis.

**2.AdCD40L:** It is a gene therapy vector of immunostimulatory origin. It is based on an adenovirus serotype 5, which is replication deficient and can transfer human CD40 ligand gene into the tumor cell. It actively stimulates the dendritic cells, which further activates T-cells, NK cells and M1 macrophages that are directed against the tumor.

Adenovectors	Clinical trial identifier no.	Phase	Study Design	Target
INGN241	NCT00116363	Phase II	Safety/ Efficacy Study, Open Label	Tumor cells
AdCD40L	NCT01455259	Phase I/II	Safety/Efficacy Study, Open Label	CD40L

**Table 9:** Non-FDA approved Adenovector Gene Therapy [61,62].

**J.mTOR Inhibitors:**

The mTOR inhibitors that are under clinical trials have been given in Table-10 below:

**1.Everolimus:** A derivative of the natural macrocyclic lactone sirolimus with immunosuppressant and anti-angiogenic properties. In cells, everolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of the mTOR, a key regulatory kinase. Inhibition of mTOR activation results in the inhibition of T lymphocyte activation and proliferation associated with antigen and cytokine (IL-2, IL-4, and IL-15) stimulation and the inhibition of antibody production.

mTOR inhibitors	Clinical trial identifier no.	Phase	Study Design	Target
Everolimus	NCT01252251	Phase II	Safety/ Efficacy Study, Open Label	FKBP-12

**Table 10:** Non-FDA approved mTOR Inhibitors [63].

**K. Other miscellaneous therapies:**

**Coxsackievirus A21 [64]:** A naturally occurring enterovirus with potential antitumor activity. Upon intratumoral administration, coxsackievirus A21 targets and binds to intracellular adhesion molecule 1 (ICAM-1) and decay acceleration factor (DAF), which are the cell surface molecules and are overexpressed on certain malignant cells. After entering the cells, coxsackievirus A21 replicates in these cancer cells, thereby causing cancer cell lysis. This results in a reduction of tumor cell growth.

**Oncolytic virus therapy [65]:** The US FDA approved Imlygic (talimogene laherparepvec), the first FDA-approved oncolytic virus therapy, for the treatment of melanoma lesions in the skin and lymph nodes. Imlygic, a genetically modified live oncolytic herpes virus therapy, is used to treat melanoma lesions that cannot be removed completely by surgery. Imlygic is injected directly into the melanoma lesions, where it replicates inside cancer cells and causes the cells to rupture and die.

**Conclusion**

Malignant melanoma is a type of skin cancer. It is found more in white than black population. Australia accounts for the highest incidence of melanoma. Its main risk factor is intense sunlight exposure for a long duration. Men have slightly higher rate of mortality than women. Immunotherapy has proven to be effective in the treatment of malignant melanoma. Ipilimumab, nivolumab, trametinib, vemurafenib, dabrafenib, interferon-alfa-2b and aldesleukin are some FDA approved immunotherapeutics, in the treatment of malignant melanoma. Our success in treatment of malignant melanoma is increasing and advancing with the knowledge of the function of the immune system. Immunotherapy has been a promising development in the past few years. The recent activities have increased our understanding of the tumor microenvironment, various immunotherapeutic modalities or combination therapy (like chemotherapy with immunotherapy). Additionally, the effects of such modalities in combination with immunotherapy are still exploratory phase. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

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