IN-DEPTH REVIEWS

Recent Advances in Systemic Therapy for Malignant Melanoma

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ABSTRACT

Malignant melanoma is a major contributor of skin cancer related mortality with increasing incidence and resistance to therapy. Advanced melanoma has a poor prognosis with a median overall survival of 8-10 months and a 5-year survival rate of 10%. A better understanding of melanomagenesis and the tumor microenvironment has led to the development of several new therapies for metastatic melanoma. This review will provide a comprehensive overview of FDA approved novel systemic therapies for advanced melanoma as well as of other classes of molecules under clinical investigation. This paper will also identify the key signaling pathways these therapies target and briefly discuss the utility of biomarkers in guiding targeted therapy.

INTRODUCTION

Melanoma arises due to mutations in the pigment producing skin cells called melanocytes. It is the most aggressive and deadliest skin cancer but accounts for only 1% of all cutaneous cancers. Advanced melanoma has a poor prognosis with a median overall survival of 8-10 months and a 5-year survival rate of 10%.[1] Thus, early identification and treatment of melanoma is crucial in improving patient prognosis. In this article, we will discuss the various novel systemic treatments for advanced melanoma.

The skin's melanocytes rest in a microenvironment that is abundant in antigen-presenting dendritic cells, which helps facilitate activation of tumor specific cytotoxic T lymphocytes. These cytotoxic T lymphocytes (CTLs) are involved in immune checkpoints that optimize the strength and quality of the antitumor response. The immune response has inhibitory checkpoint molecules such as CTLA-4, PD-1 and LAG-3, and has stimulatory checkpoint molecules such as CD28, Ox-40, and 4-1BB. As tumor cells try to disrupt and evade the regulatory checkpoints, therapeutic blockade of these efforts restores robust effector T-cell response (helper T cells and CTLs). T cell activation or inhibition by the antigen presenting cells requires two signals. First, between the T cell receptor (TCR) and either the stimulatory checkpoint molecule CD28 or the inhibitory molecule CTLA-4.[2,3] Then this TCR complex interacts with the major histocompatibility complex (MHC) expressed on antigen presenting cells (APCs) to initiate the CTL response.

Immunotherapy helps CTLs perform two tasks in order to eradicate tumor cells: recognizing the presence of tumor cells and

killing them via tumor cell lysis. The Tumor Infiltrating Lymphocytes (TILs) capacity for proliferation, cytokine production, and tumor lysis is limited by the suppressive effects of the tumor microenvironment (TME).[4] TILs that are removed from the limiting TME and grown ex vivo redevelop their anti-tumor activity suggesting that the TME leads to T-cell exhaustion[5]. This notion drives the utility of adoptive T-cell therapy that aims to raise T-cells primed against melanoma ex vivo or in vitro and then injected back into patients to restore T-cell anti-tumor activity, such as tumor infiltration and lysis.

The US Food and Drug Administration has recently approved several novel immunotherapies, such as ipilimumab and nivolumab. These monoclonal antibodies restore robust infiltration of the TME by TILs, which leads to positive responses in some patients.[2] Immunotherapy is unique because it offers durable responses in patients, but the number of patients who respond are lower in comparison to other modalities of treatment.[6,7] These advances offer physicians several new options alongside traditional therapeutic approaches such as surgical resection, chemotherapy, radiotherapy, and photodynamic therapy.

Two types of barriers limit the efficacy of advanced melanoma therapy, and these include adverse events (AEs) and increased drug resistance. AE's most commonly lead to skin and gastrointestinal toxicity, due to the non-specific anti-tumor immune response.[8] As can be expected, AE's occur less frequently in monotherapy than in combination therapy, particularly when utilizing immunotherapy.

Systemic melanoma therapies are summarized in Tables 1 and 2 and are described in detail below:

Anti-Cytotoxic T Lymphocyte Associated Protein 4/ CTLA-4 Inhibitors

CTLA-4 is normally only active on the surface of specific regulatory T-cells, but in patients with melanoma, CTLA-4 is also activated on the surface of CTLs present in the TME. The CTLA-4 receptor signaling pathway plays a crucial role in inducing immunological tolerance of tumors by suppressing CTL activation.[9,10] Thus, CTLA-4 inhibits T-cell activation and evokes immune tolerance in the TME.

Ipilimumab is a monoclonal antibody that blocks CTLA-4, thereby enhancing the production of pro-inflammatory T-cell cytokines that promote clonal expansion and infiltration of tumors by CTLs. It was FDA-approved for treatment of metastatic melanoma in March, 2011. The approval was based on data from ten prospective and two retrospective studies that showed patients on ipilimumab had a median survival rate of 11.4 months and a 3-year survival rate of 22%, both significantly higher than the treatment in naïve or previously treated groups.[11] In another study on patients with resected stage III melanoma with high risk of recurrence, ipilimumab treated patients were recurrence free for a median of 26.1 months compared to the 17.1 months for the placebo group.[12] AEs observed in these trials include autoimmune alterations such as colitis, dermatitis, endocrinopathies, and neuritis. Current trials are investigating the efficacy of ipilimumab in conjunction with nivolumab, bevacizumab and others (NCT01673854, NCT02731729, NCT00790010).

PD-1 Inhibitors

Effector T cells express another immune checkpoint molecule called programmed cell death-1 (PD-1), which suppresses T-cell activation by binding to its ligands PD-L1 and PD-L2 present on APCs in the TME. Melanoma cells also express PD-1 when

induced by interferon-gamma, which is secreted by effector T cells. Once tumor cells express PD-1, it is taken up by APCs, which travel to and bind the PD-1 ligand on effector T cells. This coupling inactivates CTLs by changes to their metabolic signature and certain signaling cascades.[13,14] This serves as a potent mechanism for suppressing the immune response to tumor pathogenesis.

Effector T-cell response to tumor cells can be restored by monoclonal antibodies that target the binding of PD-1 to PD-L1. In 2014, Japan approved Nivolumab, a monoclonal antibody that targets PD-1,for the treatment of unresectable melanomas. In one study with multiple arms, 28% (26/94) of the patients with melanoma had a positive response, compared to 18% (14/76) for patients with non-small cell lung cancer, and 27% (9/33) for patients with renal cell carcinoma.[14] Interestingly, another study showed nivolumab to be more efficient than ipilimumab with a median progression-free survival (PFS) of 6.9 month versus 2.9 months for ipilimumab, and 11.5 months for combination with nivolumab and ipilimumab. This study suggested that immune checkpoint blockers have additive effects. However, it's important to note that 55.0% of patients in the nivolumab plus ipilimumab group experienced grade 3 or 4 treatment-related adverse events, higher than in the nivolumab (16.3%) or ipilimumab (27.3%) alone.[15] Thus, clinicians should pay close attention to the management of adverse events when using combination therapies. Other anti-PD-1 molecules being studied in trials include JS100, Pembrolizumab, Toripalimab, and Nivolumab in conjunction with other immunotherapy or chemotherapy (NCT03013101,NCT02608268, NCT02027961).

Additional Immune Checkpoints

Lymphocyte activation gene-3 (LAG-3) and T cell immunoglobulin and mucin domain-3 (TIM-3) are other inhibitory checkpoint molecules currently being studied as therapeutic targets. Both suppress co-stimulatory signals in effector T cells much like CTLA-4. Both are also expressed by T_{reg} cells and play vital roles in immunosuppression (Table2) (NCT03708328, NCT02817633).

Cancer Vaccines (Peptides and Dendritic Cell Therapy)

Cancer vaccines prime CTLs that recognize and attack antigenic tumor derived peptides presented on MHC molecules on the cancer cell surface. These vaccines can use peptides, proteins, mRNA, or DNA as the therapeutic target. Melanoma cells express gp100, which is a cell surface glycoprotein found only in melanocytes and cells in the retina. Epitopes in the cancer vaccine can amplify recognition of gp100 by CTLs and increase tumor infiltration. Preclinical data suggests limited efficacy of gp100 as monotherapy, but it may hold promise as an adjuvant therapy.[16,17] Combination therapies of gp100 with IL-2, MAGE-3, resiquimod and others are currently underway (NCT00470015,NCT01176461, NCT01176474, NCT02535078).

Oncolytic Virus Therapy

In oncolytic virus therapy a nonpathogenic viral strain is directly injected into a metastatic melanoma nodule, which selectively targets cancer cells for viral replication and subsequent tumor lysis. The spilled cell content contains neo-antigens and cytokines such as interferon gamma that evoke intense proliferation and infiltration of other tumor cells by TCLs.[18,20]

Talimogene laherparepvec (T-VEC) is a genetically engineered oncolytic virus

derived from the herpes simplex family. It was approved by the FDA in October 2015 for the treatment of unresectable melanoma. Patients with metastatic melanoma were administered T-VEC in a phase II clinical trial, which displayed a 28% objective clinical response.[19] This drug demonstrates the potential to induce durable responses in some patients and current trials are evaluating its efficacy in conjunction with anti-CTLA-4 antibody and other immunotherapy (NCT02288897,NCT03259425, NCT03190824). In addition, some clinical trials are investigating similar monotherapies with coxsackievirus (NCT01227551), and in combination with pembrolizumab or ipilimumab (NCT02565992, NCT02307149).

Co-Stimulatory Agents

T-cell activation requires TCR signaling along with co-stimulatory signals such as glucocorticoid-induced TNRF-related gene (GITR). GITR is a stimulatory checkpoint molecule that prevents T-cell apoptosis thereby enhancing their activation and proliferation. In addition, when GITR is expressed by T_{reg} cells it weakens TME immunosuppression enabling T-cell infiltration and tumor lysis.[21] TRX518 is the monoclonal antibody for GITR that is currently being studied as monotherapy for metastatic melanoma (TRX518-001) or in combination with PD-1 inhibitors (NCT03277352).

Adoptive Cell Therapy

Adoptive cell therapy (ACT) increases the patient's innate antitumor immune response by utilizing either autologous or allogenic tumor-reactive T or NK cells. Although the exact mechanism of ACT's antitumor activity is not fully understood, it may include suppression of T_{regs} or changes in the TME cytokine profile that eliminates host immunosuppression.[22] T cells are primed against cancer cells by *in vitro* stimulation using neo-antigens that include cancer-antigenic peptides and tumor cells. These primed T cells are then injected into patients utilizing Rosenberg TIL transfer therapy, which involves lymphocyte removal by immunosuppressants and total body irradiation. For these patients, the response rate was above 70%.[23] Currently, several trials are investigating the efficacy of cloned TCRs primed specifically against melanocyte differentiation antigens (NCT02619058 & NCT02652455).

Inhibiting Immunosuppression within the Tumor Microenvironment

Metastatic melanoma patients are immunosuppressed by virtue of how cancer cells remodel their environment to allow for growth and proliferation.[24] Thus, the therapeutic value of cancer immunotherapy alone is limited, making it necessary to develop an approach for TME immunosuppression reversal. Below is a brief overview of systemic therapies for inhibiting immunosuppression in the TME, which include targeting the signaling pathways involving either BRAF, MEK, C-KIT, PI3K, or CCR4.

BRAF Inhibitors

BRAF mutations are present in up to 80% of melanoma patients and facilitate an immunosuppressive state within the tumor microenvironment by activating mitogen-activated protein kinase (MAPK) pathway. This pathway then stimulates malignant transformation and proliferation. The most common BRAF mutation occurs at amino acid 600, where valine is substituted by glutamic acid ($BRAF^{V600E}$ mutation). The MAPK signaling pathway leads to changes in the TME by disrupting dendritic cell production of anti-tumor cytokines IL-12 and tumor necrosis factor alpha (TNF-α), resulting in weakened stimulation of TILs.[45] Therefore, once the MAPK signaling cascade

is blocked in $BRAF^{V600E}$ tumor cells, the immunosuppressive state is then attenuated. Vemurafenib is an oral BRAF inhibitor approved by the FDA in 2011 that has shown promise by increasing infiltration of $CD8^+$ and $CD4^+$ T cells in melanoma tissues of patients with $BRAF^{V600E}$ mutations. Vemurafenib likely does this by stimulating expression of melanoma-specific antigens, which attracts effector T cells and NK cells, and reduces the production of immunosuppressive cytokines.[25] In comparison to chemotherapy, vemurafenib has shown 24% longer PFS and overall survival (OS) in clinical trials of $BRAF^{V600E}$ positive melanoma patients. Furthermore, in $BRAF^{V600E}$ positive patients who received vemurafenib, clinical studies showed 90% tumor regression.[26] Other BRAF inhibitors that are in clinical trials include dabrafenib and encorafenib as monotherapy (NCT01436656) and in combination with immunotherapy or chemotherapy(NCT02902042, NCT03235245, NCT02631447).

MEK Inhibitors

MEK is a downstream target of BRAF and has emerged as a target to overcome resistance to BRAF inhibitors. The FDA approved Trametinib, a selective MEK1/2 inhibitor, as monotherapy for patients with metastatic melanoma and BRAF mutations. The blocking of MEK 1/2 results in decreased tumor cell proliferation and reduced growth factor mediated cell signaling.[25,27] In comparison to chemotherapy, trametinib was seen to significantly improve clinical response rate, PFS, and OS.[28] In addition, a clinical trial studying trametinib and dabrafenib showed durable responses and the FDA approved this combination therapy in 2014 for patients with metastatic *BRAF* melanoma.[29] Current trials are evaluating its efficacy alongside cell cycle and immune checkpoint inhibitors (NCT02159066).

C-KIT Inhibitors

Patients with mucosal, acral lentiginous, or cutaneous melanoma arising in areas of sun damage may overexpress CKIT, a tyrosine kinase receptor. Mutations or gene amplifications in *CKIT* also result in the constitutive activation of both MAPK and PI3K/AKT pathways.[25,30,31] Imatinib is an oral CKIT inhibitor that has shown a 30% response rate and PFS of 3-4 months and is currently being studied in combination with chemotherapy and immunotherapy (NCT00667953 & NCT02812693).[32,33]

PI3K-AKT-mTOR Pathway Inhibitors

Activation of the mTOR pathway has been characterized in patients with *BRAF* mutations and is associated with poor prognosis. mTOR is activated by the PI3k/AKT pathway and thus plays a pivotal role in tumor development and progression.[33-35] Presently, clinical trials aim to curtail this pathway and suggest that the anti-tumor effects of mTOR may be enhanced when combined with the MAPK pathway inhibitors.[36] Thus, mTOR inhibitors such as everolimus or temsirolimus are being tested in combination with a BRAF inhibitor or chemotherapy (NCT01390818 & NCT01596140).

Anti-CCR4 Antibodies

CCR4 is a chemokine receptor expressed on T cells that helps regulate transport of leukocytes and is associated with a poor prognosis in T cell leukemia, renal cell carcinoma, and melanoma.[37] Currently, trials are evaluating the efficacy of the anti-CCR4 antibody for melanoma. It is suspected that since the receptor is expressed in T_{regs}, its blockade may restore the immune response by preventing cancer cells from binding T_{regs} and suppressing the effector T cell response.[38]

Chemotherapy

The selection of a chemotherapy regimen involves understandings the efficacy of dosing, timing, delivery, and the utility of drug combinations. Chemotherapy is a second line alternative to immunotherapy when treating patients with metastatic melanoma. The immunosuppressive effects of chemotherapies are varied and selective. Many anticancer drugs can weaken the immune system. For example, cyclophosphamide, gemcitabine, or docetaxel will reduce the number of T_{reg} cells; and gemcitabine, docetaxel, or doxorubicin will reduce the number of myeloid-derived suppressor cells (MDSCs).[39,40] Currently, dacarbazine is one of the most effective chemotherapy agents for melanoma patients. In a pooled analysis of numerous randomized controlled trials, dacarbazine monotherapy showed an objective response rate between 5.3% and 28%.[41] Although it showed poor efficacy alone, dacarbazine in combination with other modalities can cause durable responses in some patients.[42]

Chemotherapies induce immunogenic cell death (ICD), which is different from necrosis or apoptosis in that ICD produces a more robust antitumor response via increased uptake of cancer cell antigens by dendritic cells (DCs) and increased activation of APC's and T cells. ICD also stimulates expression of calreticulin on the cell surface of tumor cells, which promotes phagocytosis by DCs. Cell death also causes adenosine triphosphate (ATP) to be released in the extracellular space as a signal to attract immune cells to the tumor. ATP also primes the inflammasome by binding to a purine receptor and inducing IL-1B production. This promotes T cell activation and proliferation. DCs are induced in a similar fashion with the release of high-mobility group box 1 protein (HMGB1) from tumor cells which bind to Toll-like receptor 4 ligands on DCs. In addition to these extracellular chemokines, mitochondrial heat shock proteins such HSP70 and HSP90 are leaked, increasing antigen uptake by DC's through complex formation with tumor derived antigens.[43]

IDO Inhibitors

Indoleamine 2,3 dioxigenase 1 (IDO1) is an enzyme that converts tryptophan into kynurenine. IDO1 is expressed by many tumor cells in addition to DCs and macrophages and tends to indicate a poor prognosis. In the TME, tumor cells expressing IDO1 stimulate Cytotoxic T Lymphocytes (CTLs) conversion of tryptophan into kynurenine. This depletion of tryptophan in CTLs weakens their anti-tumor activity and results in an immunosuppressed environment, thereby presenting IDO inhibition as a potential target for immunosuppression reversal.[44] IDO inhibitors are currently being studied in combination with checkpoint inhibitors to test their efficacy against metastatic melanoma (NCT02073123).

Anti-CSF1R Antibodies

Colony-stimulating factor 1 (CSF-1) receptor is a cell surface receptor, and both CSF-1 and IL-34 help tumor-associated macrophages (TAM) with survival. TAM's play a pivotal role in maintaining the tumor immunosuppressive environment; thus, tumor growth can be limited by inhibiting TAM's (NCT03101254).

Anti-VEGF Antibodies

Vascular endothelial growth factor (VEGF) is a signal protein that stimulates the formation of blood vessels, which helps to mediate tumor growth and immune suppression. VEGF inhibitors aim to directly suppress the metastasis and growth of tumor cells.[46,47] Bevacizumab is an anti-VEGF monoclonal antibody that challenges tumor growth. A multicenter phase II trial showed

bevacizumab and temozolomide had an objective response rate of 16%, a PFS of 4.2 months, and an OS of 9.6 months.[48] In another phase II clinical trial, bevacizumab in combination with IFN α-2b showed a PFS rate of 4.8 months and an OS rate of 17 months. Although these studies failed to validate its use as monotherapy in metastatic melanoma, current trials are ongoing and studying bevacizumab use in combination with immunotherapy and chemotherapy (NCT00026221,NCT01048554, NCT03175432,NCT02681549, NCT03167177).

Predictive Biomarkers for Immune Checkpoint Blockade

The identification of biomarkers can help guide patient therapy towards specific treatments that show the greatest promise in producing a durable response. The BRAF V600E mutation, for example, is a predictive marker for response to BRAF inhibitors. However, many of these patients develop resistance over a variable period of time and thus require additional treatment options.[49,50] Treatment resistance can be divided into genomic, epigenetic, and immunologic factors. Patients with melanoma who possess markers like *NRAS/KRAS* mutations, *BRAF* mutations, low levels of PD-1L in the TME, or harmful epigenetic changes tend to become resistant to both chemotherapy and targeted therapy.[51] Thus, certain biomarkers not only inform selection of therapy but also the likelihood of developing resistance.

The tumor microenvironment is another important indicator of drug potential and efficacy. The TME presents opportunities for therapeutic targets to induce infiltration by CTLs and suppress cancer growth. Interestingly, patients who respond the best to immunotherapy display robust tumor infiltration at presentation. In patients undergoing PD-1 therapy, those with $CD8^+$ infiltration of the TME at presentation had the greatest PFS and OS.[53] Another observation in these patients is that high numbers of somatic mutations prime tumor cells to express more neo-antigens, creating a host environment that may benefit greatly from targeted therapy.[54-56] Thus, to inform the selection of ideal therapy, clinicians should consider testing patients for important biomarkers and align this data with the patients genetic and immune status.

CONCLUSION

Elucidation of essential mechanisms in melanomagenesis has led to the development of novel targets in treatment. The introduction of immunotherapies targeting CTLA-4 and PD-1 checkpoint inhibitors has ushered in a new era of melanoma treatment. Utilizing melanoma patients' genetic status and stratifying their anti-tumor immune responses may help guide first-line targeted therapy. Despite these advances, malignant melanoma remains a lethal disease, especially when diagnosed at a late stage. Further investigation of melanomagenesis and mechanisms of resistance represent a central goal for cancer research and may help more patients obtain durable responses in the future.

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Table 1. Classification of systemic melanoma treatments

Function	Target	Agents	Development
Checkpoint Blockers	CTLA-4	Ipilimumab Tremelimumab	FDA approved Phase III
	PD-1	Nivolumab Pembrolizumab PDR001	FDA approved FDA approved Phase III
	PD-L1	Atezolizumab Durvalumab Avelumab	Phase II Phase II Phase Ib
	LAG-3	Imp321 LAG525	Phase I Phase II
Co-Stimulatory agents	GITR	TRX518	Phase I
Immunomodulators	VEGF	Bevacizumab	Phase II
	BRAF	Vemurafenib	FDA Approved
	MEK	Trametinib	FDA Approved
	mTOR	Everolimus	Phase II
	IDO	Indoximod Epacadostat	Phase I-II
Chemotherapy	Methylating guanine at the O-6 and N-7 positions	Dacarbazine	FDA Approved

Abbreviations: CTLA-4, cytotoxic T lymphocyte-associated antigen 4; GITR, glucocorticoid-induced TNFR-related gene; IDO, indoleamine 2,3-dioxigenase; LAG-3, lymphocyte activation gene-3; PD-1, programmed cell death-1; PD-L1, programmed cell death-1 ligand-1; TIM-3, T-cell immunoglobulin and mucin domain-3; VEGF, vascular endothelial growth factor; BRAF, proto-oncogene *BRAF*; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of Rapamycin.

Table 2. Outcomes from key clinical trials for systemic treatments for melanoma

Target	Treatment	**ORR** (%)	**PFS** (months)	**OS** (months)
CTLA-4[58]	Ipilimumab + DTIC or dacarbazine	15.2 10.3	3 (all)	11.2 9.1
PD-1[59]	Nivolumab or Dacarbazine	40 13.9	5.1 2.2	Not Reached
CTLA-4 + PD1[60]	Ipilimumab or Nivolumab or I+N	19 43.7 57.6	2.9 6.9 11.5	Not Reached
BRAF[61]	Vemurafenib or DTIC	48 5	5.3 1.6	13.6 9.7
MEK[62]	Trametinib or DTIC	22 8	4.8 1.5	81 67
MEK + BRAF[63]	Trametinib or Dabrafenib + Trametinib	51 64	7.3 11.4	18 25.6

Abbreviations: CTLA-4, cytotoxic T lymphocyte-associated antigen 4; PD-1, programmed cell death-1; BRAF, proto-oncogene *BRAF*; MEK, mitogen-activated protein kinase kinase; VEGF, vascular endothelial growth factor; DTIC, dacarbazine

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