New Therapies in Melanoma: Current Trends, Evolving Paradigms, and Future Perspectives

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PRACTICE **POINTS**

- Immune checkpoint inhibition has resulted in a paradigm shift for the treatment of metastatic melanoma.
- Alternative therapies with novel targets such as lymphocyte-activated gene 3 aim to overcome resistance to the usual immune targets such as PD-1/PD-L1 and CTLA-4.
- Newer promising tools such as nanotechnology are being added to the growing armamentarium of melanoma treatment strategies.

Melanoma is an aggressive skin cancer with increasing incidence and mortality worldwide. For many years the therapeutic strategies were limited to surgery, radiotherapy, and chemotherapy. Recent advances in immunology and cancer biology have led to the discovery and development of novel therapeutics, such as immune checkpoint inhibitors (ICIs) and targeted therapies, which have revolutionized the clinical care of patients with metastatic melanoma. Despite recent successes with ICIs, many melanoma patients do not experience long-term benefits from ICI therapies, highlighting the need for alternative treatments with novel targets such as lymphocyte-activated gene 3 (LAG-3). In this review, we explore new therapeutic agents and novel combinations that are being tested in early-phase clinical trials. We discuss newer promising tools such as nanotechnology to develop nanosystems that act as drug carriers and/or light absorbents to potentially improve therapy outcomes. Finally, we also highlight challenges such as management after resistance and intervention with novel immunotherapies and the lack of predictive biomarkers to stratify patients to targeted treatments after primary treatment failure.

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Cutaneous malignant melanoma represents an aggressive form of skin cancer, with 132,000 new cases of melanoma and 50,000 melanoma-related deaths diagnosed worldwide each year.[1] In recent decades, major progress has been made in the treatment of melanoma, especially metastatic and advanced-stage disease. Approval of new treatments, such as immunotherapy with anti–PD-1 (pembrolizumab and nivolumab) and anti–CTLA-4 (ipilimumab) antibodies, has revolutionized therapeutic strategies (Figure 1). Molecularly, melanoma has the highest mutational burden among solid tumors. Approximately 40% of melanomas harbor the BRAF V600 mutation, leading to constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway.[2] The other described genomic subtypes are mutated RAS (accounting for approximately 28% of cases), mutated NF1 (approximately 14% of cases), and triple wild type, though these other subtypes have not been as successfully targeted with therapy to date.[3] Dual inhibition of this pathway using combination therapy with BRAF and MEK inhibitors confers high response

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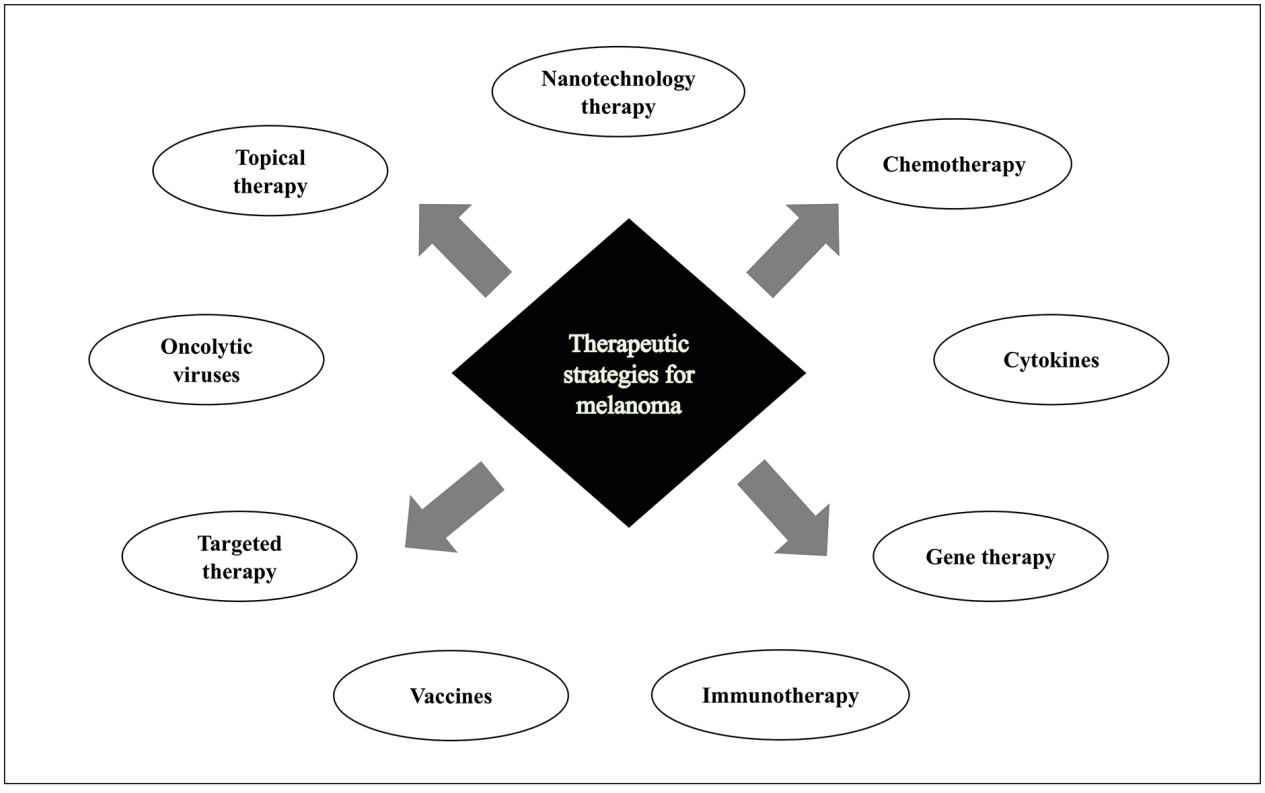

FIGURE 1. Schematic representation of various therapeutic strategies for the treatment of melanoma.

rates and survival benefit, though efficacy in metastatic patients often is limited by development of resistance. The US Food and Drug Administration (FDA) has approved 3 combinations of targeted therapy in unresectable tumors: dabrafenib and trametinib, vemurafenib and cobimetinib, and encorafenib and binimetinib. The oncolytic herpesvirus talimogene laherparepvec also has received FDA approval for local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with recurrent melanoma after initial surgery.[2]

In this review, we explore new therapeutic agents and novel combinations that are being tested in early-phase clinical trials (Table). We discuss newer promising tools such as nanotechnology to develop nanosystems that act as drug carriers and/or light absorbents to potentially improve therapy outcomes. Finally, we highlight challenges such as management after resistance and intervention with novel immunotherapies and the lack of predictive biomarkers to stratify patients to targeted treatments after primary treatment failure.

Targeted Therapies

Vemurafenib was approved by the FDA in 2011 and was the first BRAF-targeted therapy approved for the treatment of melanoma based on a 48% response rate and a 63% reduction in the risk for death vs dacarbazine chemotherapy.[4] Despite a rapid and clinically significant initial response, progression-free survival (PFS) was only 5.3 months, which is indicative of the rapid development of resistance with monotherapy through MAPK reactivation. As a result, combined BRAF and MEK inhibition was introduced and is now the standard of care for targeted therapy in melanoma. Treatment with dabrafenib and trametinib, vemurafenib and cobimetinib, or encorafenib and binimetinib is associated with prolonged PFS and overall survival (OS) compared to BRAF inhibitor monotherapy, with response rates exceeding 60% and a complete response rate of 10% to 18%.[5] Recently, combining atezolizumab with vemurafenib and cobimetinib was shown to improve PFS compared to combined targeted therapy.[6] Targeted therapy usually is given as first-line treatment to symptomatic patients with a high tumor burden because the response may be more rapid than the response to immunotherapy. Ultimately, most patients with advanced BRAF-mutated melanoma receive both targeted therapy and immunotherapy.

Mutations of KIT (encoding proto-oncogene receptor tyrosine kinase) activate intracellular MAPK and phosphatidylinositol 3-kinase (PI3K) pathways (Figure 2).[7] KIT mutations are found in mucosal and acral melanomas as well as chronically sun-damaged skin, with frequencies of 39%, 36%, and 28%, respectively. Imatinib was associated with a 53% response rate and PFS of 3.9 months among patients with KIT-mutated melanoma but failed to cause regression in melanomas with KIT amplification.[8]

Overview of Various Therapeutic Strategies for Melanoma With Corresponding Mechanisms of Action and Clinical Indications

Treatment modality	Mechanism of action	Drug	Clinical indications
Chemotherapy	Cytotoxic via alkylation	Dacarbazine, temozolomide	Advanced metastatic melanoma
Targeted therapy	Blocks tumor proliferation and growth by inhibiting the RAF/MEK/ERK pathway on tumor cells and reduces tumor angiogenesis by inhibiting VEGFR and PDGFR signaling	Sorafenib	Unresectable melanoma with BRAF V600E mutation
	Interrupts the BRAF/MEK step on the BRAF/MEK/ERK pathway	Vemurafenib	Unresectable melanoma with BRAF V600E mutation
	Competitive and selective BRAF inhibitor by binding to its ATP pocket	Dabrafenib	Unresectable melanoma with BRAF V600E mutation
	Reversible, highly selective, allosteric inhibitor of MEK1 and MEK2; by binding to unphosphorylated MEK1 and MEK2 with high affinity, trametinib blocks the catalytic activity of MEKs	Trametinib	Unresectable melanoma with BRAF V600E mutation
Immunotherapy			
CTLA-4 inhibitor	Dynamic competition for the B7 ligand, which subsequently interferes with the CD28/B7 costimulatory pathway	Ipilimumab	Unresectable advanced metastatic melanoma
PD-1 inhibitor	Blockade of the PD-1/PD-L1–signaling axis restores the function of exhausted T cells to mediate antitumor immunity	Nivolumab	Advanced metastatic melanoma, including ipilimumab refractory melanoma
		Pembrolizumab	Unresectable stage III and stage IV melanoma
Topical therapy	Acts on innate and adaptive immune responses both directly and indirectly; direct action is by binding to TLR7 and TLR8 of macrophages, monocytes, and dendritic cells and by induction of apoptosis; indirect action occurs by imiquimod inducing the release of immunomodulatory cytokines	Imiquimod	Melanoma in situ
Cytokines	Antiangiogenic, potent immunoregulatory, differentiation-inducing, antiproliferative, and proapoptotic effects	IFN-α, PEGIFNα2b, IL-2	Patients with stage III melanoma who are cancer free but are at a high risk of recurrence; patients with stage IIB or IIC melanoma who have primary lesions thicker than 4 mm

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TABLE. (continued)

Treatment modality	Mechanism of action	Drug	Clinical indications
Oncolytic viruses	Enhances antigen loading of MHC class I molecules and expresses GM-CSF to increase tumor-antigen presentation by dendritic cells	Talimogene laherparepvec	Unresectable advanced melanoma (stage IIIB, IIIC, or IV)
Vaccines	Activates immune system by binding fibronectin on keratinocytes within the epidermis and inducing the release of proinflammatory cytokines, such as IL-1α and TNF-α	BCG vaccine	Inoperable stage III in-transit metastatic melanoma

Abbreviations: ATP, adenosine triphosphate; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGFR, platelet-derived growth factor receptor; PEGIFNα2b, pegylated interferon α2b; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; TVEC, talimogene laherparepvec; VEGFR, vascular endothelial growth factor.

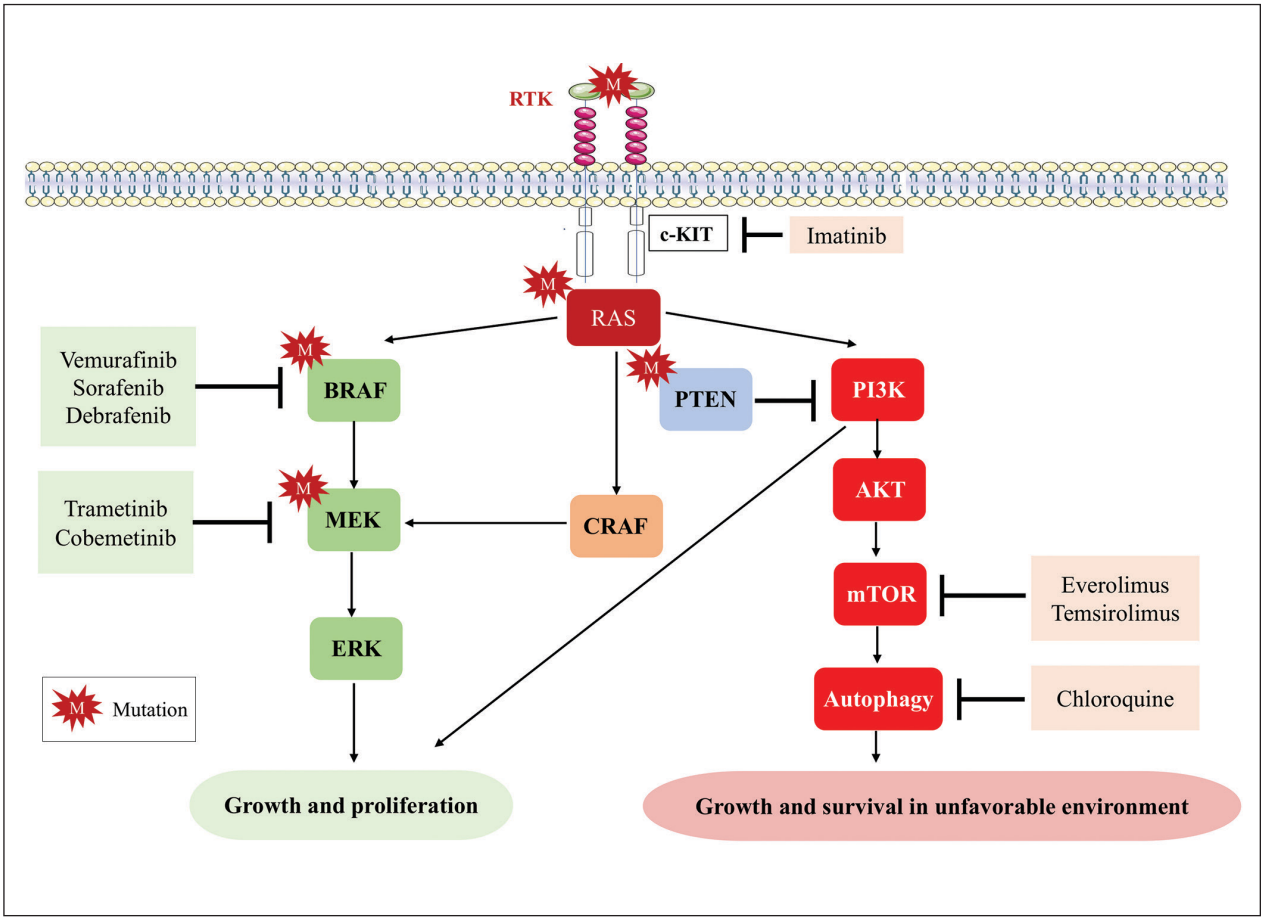

FIGURE 2. Binding of ligands to receptors with tyrosine kinase activity (eg, c-KIT) promotes the activation of downstream signaling pathways, including RAS, CRAF, MEK, ERK (extracellular signal-regulated kinase), PI3K (phosphoinositide 3-kinase), and AKT. Inhibition by imatinib or by different BRAF and MEK inhibitors represents clinically relevant strategies. mTOR indicates mammalian target of rapamycin; PTEN, phosphotase and tensin homolog deleted on chromosome 10; RTK, receptor tyrosine kinase.

Anti–CTLA-4 Immune Checkpoint Inhibition

CTLA-4 is a protein found on T cells that binds with another protein, B7, preventing T cells from killing cancer cells. Hence, blockade of CTLA-4 antibody avoids the immunosuppressive state of lymphocytes, strengthening their antitumor action.[9] Ipilimumab, an anti–CTLA-4 antibody, demonstrated improvement in median OS for management of unresectable or metastatic stage IV melanoma, resulting in its FDA approval.[8] A combination of ipilimumab with dacarbazine in stage IV melanoma showed notable improvement of OS.[10] Similarly, tremelimumab showed evidence of tumor regression in a phase 1 trial but with more severe immune-related side effects compared with ipilimumab.[11] A second study on patients with stage IV melanoma treated with tremelimumab as first-line therapy in comparison with dacarbazine demonstrated differences in OS that were not statistically significant, though there was a longer duration of an objective response in patients treated with tremelimumab (35.8 months) compared with patients responding to dacarbazine (13.7 months).[12]

Anti–PD-1 Immune Checkpoint Inhibition

PD-1 is a transmembrane protein with immunoreceptor tyrosine-based inhibitory signaling, identified as an apoptosis-associated molecule.[13] Upon activation, it is expressed on the cell surface of CD4, CD8, B lymphocytes, natural killer cells, monocytes, and dendritic cells.[14] PD-L1, the ligand of PD-1, is constitutively expressed on different hematopoietic cells, as well as on fibroblasts, endothelial cells, mesenchymal cells, neurons, and keratinocytes.[15,16] Reactivation of effector T lymphocytes by PD-1:PD-L1 pathway inhibition has shown clinically significant therapeutic relevance.[17] The PD-1:PD-L1 interaction is active only in the presence of T- or B-cell antigen receptor cross-link. This interaction prevents PI3K/AKT signaling and MAPK/extracellular signal-regulated kinase pathway activation with the net result of lymphocytic functional exhaustion.[18,19] PD-L1 blockade is shown to have better clinical benefit and minor toxicity compared to anti–CTLA-4 therapy. Treatment with anti-PD1 nivolumab in a phase 1b clinical trial (N=107) demonstrated highly specific action, durable tumor remission, and long-term safety in 32% of patients with advanced melanoma.[20] These promising results led to the FDA approval of nivolumab for the treatment of patients with advanced and unresponsive melanoma. A recent clinical trial combining ipilimumab and nivolumab resulted in an impressive increase of PFS compared with ipilimumab monotherapy (11.5 months vs 2.9 months).[21] Similarly, treatment with pembrolizumab in advanced melanoma demonstrated improvement in PFS and OS compared with anti–CTLA-4 therapy,[22,23] which resulted in FDA approval of pembrolizumab for the treatment of advanced melanoma in patients previously treated with ipilimumab or BRAF inhibitors in BRAF V600 mutation–positive patients.[24]

Lymphocyte-Activated Gene 3–Targeted Therapies

Lymphocyte-activated gene 3 (LAG-3)(also known as CD223 or FDC protein) is a type of immune checkpoint receptor transmembrane protein that is located on chromosome 12.[25] It is present on the surface of effector T cells and regulatory T cells that regulate the adaptive immune response.[26] Lymphocyte-activated gene 3 is reported to be highly expressed on the surface of tumor-infiltrating lymphocytes, thus the level of LAG-3 expression was found to corelate with the prognosis of tumors. In some tumors involving the kidneys, lungs, and bladder, a high level of LAG-3 was associated with a worse prognosis; in gastric carcinoma and melanoma, a high level of LAG-3 indicates better prognosis.[27] Similar to PD-1, LAG-3 also is found to be an inhibitory checkpoint that contributes to decreased T cells. Therefore, antibodies targeting LAG-3 have been gaining interest as modalities in cancer immunotherapy. The initial clinical trials employing only LAG-3 antibody on solid tumors found an objective response rate and disease control rate of 6% and 17%, respectively.[25,26,28] Given the unsatisfactory results, the idea that combination therapy with an anti–PD-L1 drug and LAG-3 antibody started gaining attention. A randomized, double-blind clinical trial, RELATIVITY-047, studying the effects of a combination of relatlimab (a first-in-class LAG-3 antibody) and nivolumab (an anti–PD-L1 antibody) on melanoma found longer PFS (10.1 months vs 4.6 months) and a 25% lower risk for disease progression or death with the combination of relatlimab and nivolumab vs nivolumab alone.[28] The FDA approved the combination of relatlimab and nivolumab for individuals aged 12 years or older with previously untreated melanoma that is surgically unresectable or has metastasized.[29] Zhao et al[30] demonstrated that LAG-3/PD-1 and CTLA-4/PD-1 inhibition showed similar PFS, and LAG-3/PD-1 inhibition showed earlier survival benefit and fewer treatment-related adverse effects, with grade 3 or 4 treatment-related adverse effects occurring in 18.9% of patients on anti–LAG-3 and anti–PD-1 combination (relatlimab plus nivolumab) compared with 55.0% in patients treated with anti–CTL-4 and anti–PD-1 combination (ipilimumab plus nivolumab)(N=1344). Further studies are warranted to understand the exact mechanism of LAG-3 signaling pathways, effects of its inhibition and efficacy, and adverse events associated with its combined use with anti–PD-1 drugs.

Nanotechnology in Melanoma Therapy

The use of nanotechnology represents one of the newer alternative therapies employed for treatment of melanoma and is especially gaining interest due to reduced adverse effects in comparison with other conventional treatments for melanoma. Nanotechnology-based drug delivery systems precisely target tumor cells and improve the effect of both the conventional and innovative antineoplastic treatment.[27,31] Tumor vasculature differs from

normal tissues by being discontinuous and having interspersed small gaps/holes that allow nanoparticles to exit the circulation and enter and accumulate in the tumor tissue, leading to enhanced and targeted release of the antineoplastic drug to tumor cells.[32] This mechanism is called the enhanced permeability and retention effect.[33]

Another mechanism by which nanoparticles work is ligand-based targeting in which ligands such as monoclonal antibodies, peptides, and nucleic acids located on the surface of nanoparticles can bind to receptors on the plasma membrane of tumor cells and lead to targeted delivery of the drug.[34] Nanomaterials used for melanoma treatment include vesicular systems such as liposomes and niosomes, polymeric nanoparticles, noble metal-based nanoparticles, carbon nanotubes, dendrimers, solid lipid nanoparticles and nanostructures, lipid carriers, and microneedles. In melanoma, nanoparticles can be used to enhance targeted delivery of drugs, including immune checkpoint inhibitors (ICIs). Cai et al[35] described usage of scaffolds in delivery systems. Tumor-associated antigens, adjuvant drugs, and chemical agents that influence the tumor microenvironment can be loaded onto these scaffolding agents. In a study by Zhu et al,[36] photosensitizer chlorin e6 and immunoadjuvant aluminum hydroxide were used as a novel nanosystem that effectively destroyed tumor cells and induced a strong systemic antitumor response. IL-2 is a cytokine produced by B or T lymphocytes. Its use in melanoma has been limited by a severe adverse effect profile and lack of complete response in most patients. Cytokine-containing nanogels have been found to selectively release IL-2 in response to activation of T-cell receptors, and a mouse model in melanoma showed better response compared to free IL-1 and no adverse systemic effects.[37]

Nanovaccines represent another interesting novel immunotherapy modality. A study by Conniot et al[38] showed that nanoparticles can be used in the treatment of melanoma. Nanoparticles made of biodegradable polymer were loaded with Melan-A/MART-1 (26–35 A27L) MHC class I-restricted peptide (MHC class I antigen), and the limited peptide MHC class II Melan-A/MART-1 51–73 (MHC class II antigen) and grafted with mannose that was then combined with an anti–PD-L1 antibody and injected into mouse models. This combination resulted in T-cell infiltration at early stages and increased infiltration of myeloid-derived suppressor cells. Ibrutinib, a myeloid-derived suppressor cell inhibitor, was added and demonstrated marked tumor remission and prolonged survival.[38]

Overexpression of certain microRNAs (miRNAs), especially miR-204-5p and miR-199b-5p, has been shown to inhibit growth of melanoma cells in vitro, both alone and in combination with MAPK inhibitors, but these miRNAs are easily degradable in body fluids. Lipid nanoparticles can bind these miRNAs and have been shown to inhibit tumor cell proliferation and improve efficacy of BRAF and MEK inhibitors.[39]

Triple-Combination Therapy

Immune checkpoint inhibitors such as anti–PD-1 or anti–CTLA-4 drugs have become the standard of care in treatment of advanced melanoma. Approximately 40% to 50% of cases of melanoma harbor BRAF mutations, and patients with these mutations could benefit from BRAF and MEK inhibitors. Data from clinical trials on BRAF and MEK inhibitors even showed initial high objective response rates, but the response was short-lived, and there was frequent acquired resistance.[40] With ICIs, the major limitation was primary resistance, with only 50% of patients initially responding.[41] Studies on murine models demonstrated that BRAF-mutated tumors had decreased expression of IFN-γ, tumor necrosis factor α, and CD40 ligand on $CD4^+$ tumor-infiltrating lymphocytes and increased accumulation of regulatory T cells and myeloid-derived suppressor cells, leading to a protumor microenvironment. BRAF and MEK pathway inhibition were found to improve intratumoral $CD4^+$ T-cell activity, leading to improved antitumor T-cell responses.[42] Because of this enhanced immune response by BRAF and MEK inhibitors, it was hypothesized and later supported by clinical research that a combination of these targeted treatments and ICIs can have a synergistic effect, leading to increased antitumor activity.[43] A randomized phase 2 clinical trial (KEYNOTE-022) in which the treatment group was given pembrolizumab, dabrafenib, and trametinib and the control group was treated with dabrafenib and trametinib showed increased medial OS in the treatment group vs the control group (46.3 months vs 26.3 months) and more frequent complete response in the treatment group vs the control group (20% vs 15%).[44] In the IMspire150 phase 3 clinical trial, patients with advanced stage IIIC to IV BRAF-mutant melanoma were treated with either a triple combination of the PDL-1 inhibitor atezolizumab, vemurafenib, and cobimetinib or vemurafenib and cobimetinib. Although the objective response rate was similar in both groups, the median duration of response was longer in the triplet group compared with the doublet group (21 months vs 12.6 months). Given these results, the FDA approved the triple-combination therapy with atezolizumab, vemurafenib, and cobimetinib. Although triple-combination therapy has shown promising results, it is expected that there will be an increase in the frequency of treatment-related adverse effects. In the phase 3 COMBi-I study, patients with advanced stage IIIC to IV BRAF V600E mutant cutaneous melanoma were treated with either a combination of spartalizumab, dabrafenib, and trametinib or just dabrafenib and trametinib. Although the objective response rates were not significantly different (69% vs 64%), there was increased frequency of treatment-related adverse effects in patients receiving triple-combination therapy.[43] As more follow-up data come out of these ongoing clinical trials, benefits of triple-combination therapy and its adverse effect profile will be more definitely established.

Challenges and Future Perspectives

One of the major roadblocks in the treatment of melanoma is the failure of response to ICI with CTLA-4 and PD-1/PD-L1 blockade in a large patient population, which has resulted in the need for new biomarkers that can act as potential therapeutic targets. Further, the main underlying factor for both adjuvant and neoadjuvant approaches remains the selection of patients, optimizing therapeutic outcomes while minimizing the number of patients exposed to potentially toxic treatments without gaining clinical benefit. Clinical and pathological factors (eg, Breslow thickness, ulceration, the number of positive lymph nodes) play a role in stratifying patients as per risk of recurrence.[45] Similarly, peripheral blood biomarkers have been proposed as prognostic tools for high-risk stage II and III melanoma, including markers of systemic inflammation previously explored in the metastatic setting.[46] However, the use of these parameters has not been validated for clinical practice. Currently, despite promising results of BRAF and MEK inhibitors and therapeutic ICIs, as well as IL-2 or interferon alfa, treatment options in metastatic melanoma are limited because of its high heterogeneity, problematic patient stratification, and high genetic mutational rate. Recently, the role of epigenetic modifications and miRNAs in melanoma progression and metastatic spread has been described. Silencing of *CDKN2A* locus and encoding for $p16^{INK4A}$ and $p14^{ARF}$ by DNA methylation are noted in 27% and 57% of metastatic melanomas, respectively, which enables melanoma cells to escape from growth arrest and apoptosis generated by Rb protein and p53 pathways.[47] Demethylation of these and other tumor suppressor genes with proapoptotic function (eg, *RASSF1A* and tumor necrosis factor–related apoptosis-inducing ligand) can restore cell death pathways, though future clinical studies in melanoma are warranted.[48]

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