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Advanced Melanoma: Resistance Mechanisms to Current Therapies

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Summary

Despite the recent advances in melanoma treatment, resistance to currently available immune and targeted therapies remains the major barriers to long-term disease control in many patients. Most often, this is associated with intrinsic/innate resistance to immune checkpoint inhibitors and acquired resistance to BRAF and MEK kinase inhibitors. Mechanisms of resistance are diverse and will require innovative and perhaps personalized management strategies.

Keywords

immune checkpoint inhibitor; immunotherapy; resistance; BRAF inhibitor; MEK inhibitor; melanoma

Introduction

Advances over the last decade have brought about remarkable changes in the treatment of metastatic melanoma. The introduction of targeted therapies including BRAF and MEK inhibitors as well as immune checkpoint inhibitors (ICI) led to dramatically improved outcomes compared with chemotherapy, which had not been shown to improve survival in any large randomized trials. While impressive outcomes are seen in some patients, responses to these agents are heterogeneous. Initial robust responses to BRAF/MEK inhibitors are complicated by eventual disease progression in most treated patients. Immune checkpoint inhibitors may induce more durable long-term responses in some cases, yet a significant proportion of patients do not respond to these agents (innate resistance) or respond transiently followed by progression (acquired resistance). This review provides an overview

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of recent insights into innate and acquired resistance to both targeted therapies and checkpoint inhibition in metastatic melanoma. Further understanding of the mechanisms involved in resistance is warranted to inform optimal clinical decision making in individual patients as well as to drive therapeutic advances that continue to improve outcomes.

Discussion

Resistance to Checkpoint Inhibitors

Mutational and Neo-antigen Burden: Several studies have demonstrated a correlation between higher tumor mutational burden and response to checkpoint inhibition with either anti-PD-1/PD-L1 or anti-CTLA-4.[1–7] Malignant cells are often detected by immune surveillance through recognition of tumor-derived neoantigens that are foreign and thus immunogenic.[8] As these neoantigens are a result of somatic mutations, higher mutational load is thought to contribute to a wider and more robust spectrum of neoantigens that can be recognized by the immune system. Tumors deficient in mismatch repair, regardless of tissue of origin, are also particularly sensitive to immune checkpoint inhibition.[9–11] These tumors are characterized by a very high frequency of somatic alterations, with a mutational load 10 to 100-fold greater than those that are mismatch-proficient, which likely plays a role in response to ICI.[12]

Recognition of a specific neoantigen may be complicated by the spectrum of different mutations within clusters of malignant cells in each tumor. Clonal neoantigen burden has been associated with response to ICI while a high burden of sub-clonal alterations was seen in tumors that progressed on therapy.[13,14] Cytotoxic chemotherapy may potentiate production of sub-clonal neoantigens, possibly contributing to decreased efficacy of ICI in some patients despite an overall high mutational burden.[14] Neoantigens that arise earlier in tumorigenesis and are shared by a majority of cancer cells are likely to trigger a more robust immune response.

Most melanoma tumors are driven by UV-induced mutagenesis with a high frequency of somatic mutations. Subtypes of melanoma that are less likely to be UV-mediated, such as mucosal or acral melanoma, tend to have fewer point mutations but more frequent somatic structural or copy number aberrations.[15] Given the lower overall mutational burden and likely decreased neoantigen production, it is speculated that these subtypes may be less likely to respond to immune checkpoint inhibitors. Indeed, several studies report lower response rates in these non-cutaneous melanomas.[16–18] Conversely, desmoplastic melanomas, which have the highest mutational burdens of all melanomas, also have the highest response rates.[19] Miao et al stratified melanoma tumors based on mutational signature, which included UV-associated, those associated with exposure to alkylating agents and tumors not clearly associated with specific environmental exposures. When stratified based on dominant mutational signature, there was no significant difference in mutational burden between patients with progressive disease and those who responded to immunotherapy.[13] The authors thus postulated that mutational burden may actually serve as a marker of underlying pathobiology that promotes immunogenicity rather than a true mechanism of response to therapy in melanoma. In addition, most studies have suggested

threshold effects rather than a linear relationship between response and TMB within individual tumor types.

Tumors can demonstrate selective loss of neoantigens recognized by T-cells as a mechanism to avoid immune detection.[20,21] Malignant cells that express these neoantigens are selectively lost from the overall tumor population through the loss of mutant alleles or epigenetic silencing of implicated genes.[20] Neoantigens lost by tumor cells following ICI were noted to have higher affinity for MHC variants and result in stronger TCR responses in peripheral lymphocytes than those that were retained.[22] The loss of T-cell recognized neoantigens correlates with the development of neo-antigen specific reactivity amongst tumor infiltrating lymphocytes, suggesting that T-cells likely play a role in modulating immunoediting.[20]

PD-L1 Expression—PD-L1 expression in tumor cells or tumor-infiltrating immune cells has unsurprisingly been correlated with response to PD-1/PD-L1 blockade in melanoma.[23–26] PD-L1 negativity, however, is not a definitive marker of resistance and studies have consistently demonstrated durable responses in some patients with PD-L1 negative tumors.[24,25,27] PD-L1 status is used to identify patients appropriate for treatment with immunotherapy in some malignancies but is of uncertain utility in melanoma, which is also associated with relatively high rates of PD-L1 positivity.[28] PD-L1 expression is frequently heterogenous over time, within individual patients or even within the same tumor. Further, several different IHC stains and cut-off values were employed in early trials, further complicating assessment of PD-L1 status.[28] Expression of PD-1 and PD-L1 are also known to be dynamic and influenced by several complex and inter-related factors within the tumor microenvironment, including IFN or other cytokine signaling, genomic alterations or changes induced by radiation or targeted therapy.[12,29]

Specific Genomic Alterations—Tumors that are subject to immune surveillance may become enriched for a wide range of genomic alterations that aid in immune escape. Alterations in the gene encoding β−2-microglobulin (B2M), the invariant chain of MHC, have been shown to play a role in both innate and acquired resistance to checkpoint inhibition.[30–32] Decreased expression of MHC, whether brought about by genomic changes or other mechanisms, has been associated with lack of response to anti-PD1 (MHC-II) and anti-CTLA-4 (MHC-1).[33] Germline differences in HLA genotype may impact response to checkpoint inhibition, potentially due to a decreased spectrum of neoantigens presented by tumors with homozygous HLA alleles.[34]

Loss of function mutations in *JAK1/JAK2* have been associated with both innate and acquired resistance to PD-1/PD-L1 inhibitors in melanoma.[32,35] Tumors with *JAK1/JAK2* mutations demonstrate a lack of response to IFN-γ stimulation, including a lack of associated PD-L1 expression.[32,35] Several genomic defects in the IFN-γ pathway have also been identified amongst tumors that do not respond to CTLA-4 blockade.[36] Overall these mutations appear uncommon, and likely only explain a minor proportion of resistance.

Certain tumor signaling pathways have also been shown to suppress the recruitment and diversification of T cells within the tumor microenvironment.[37,38] Activation of the WNT/β-

catenin pathway in melanoma contributed to T cell exclusion in mouse models, evidenced by a complete lack of T-cell infiltrate in many tumors.[37] Single cell RNA sampling of melanoma tumor cells treated with ICI identified a specific transcription factor, TCF7, that served as a marker of response regardless of the extent of lymphocytic invasion. This transcription factor plays a role in the Wnt/β-catenin signaling pathway and is involved with cytotoxic T-cell auto-renewal, differentiation and persistence.[39] The loss of PTEN within tumor cells has been correlated with decreased T-cell infiltration, increased expression of immunosuppressive cytokines and overall inferior clinical response to ICI.[40,41]

Recent evidence has suggested that the MAPK pathway may play a role in immune escape through upregulation of VEGF and other immunosuppressive cytokines in addition to other unknown mechanisms.[42,43] BRAF inhibitors have been shown to increase intra-tumoral T-cell infiltration and result in a more favorable TME with fewer immunosuppressive cytokines, fewer myeloid-derived suppressor stem cells and decreased PD-L1 expression, while other studies have suggested a low $CD8^+$ T cell infiltrate at the time of progression on BRAF inhibitors.[44–48] Acquired resistance to BRAF inhibition has been associated with upregulation of PD-L1 on tumor cells and treatment with MAPK inhibitors can induce transcriptional signatures similar to the IPRES signature associated with ICI resistance.[47,49] It remains unclear if and how changes induced by BRAF inhibition impact subsequent response to ICI in melanoma yet molecular evidence is suggestive of overlapping resistance mechanisms to both classes of therapy.[50,51] Retrospective analysis found higher response rates to first-line immunotherapy amongst *NRAS*-mutant melanoma compared with *BRAF* or *BRAF/NRAS* wild-type melanoma although these data have not been validated extensively.[52] The impact of MAPK pathway alterations in the development of ICI resistance is yet to be defined.

Tumor Microenvironment—Malignant cells employ a variety of mechanisms to evade immune destruction, many of which involve alterations in the surrounding tumor microenvironment that create an immunosuppressive barrier. The presence of tumor infiltrating lymphocytes within the TME is associated with improved outcomes across wide spectrum of malignancies.[53] Higher numbers of intra-tumoral CD8+ T cells, both in the core and at the invasive margin, are predictive of response to anti-PD-1 therapy in melanoma, particularly if they express PD-1.[27,54] Functional analysis of TILs that expressed high amounts of both PD-1 and CTLA-4 revealed a partially exhausted CD8+ T-cell phenotype that could be restored to a fully activated state upon PD-1/PD-L1 inhibition.[27]

Persistent antigen exposure and activation is complicated by eventual T-cell exhaustion both in the setting of ICI therapy and natural immune surveillance. The interaction between PD-1 and PD-L1 contributes significantly to the loss of effector T-cell function exhibited by exhausted CD8+ T-cells.[55] Blockade of PD-L1/PD-1 can re-invigorate exhausted cytotoxic T-cells; an abundance of partially exhausted CD8+ T-cells within the TME has been shown to correlate with response to ICI.[27]

These re-invigorated T-cells, however, will revert back to an exhausted state in the face of continued and persistent antigen exposure and do not exhibit a memory T-cell phenotype upon antigen clearance.[56] Recent studies have uncovered a hardwired epigenetic profile

unique to exhausted T-cells that may limit prolonged response to checkpoint inhibition and contribute to acquired resistance.[57,58] Epigenetic therapies have been proposed as a potential mechanism to overcome T-cell exhaustion and are being evaluated as an adjunct to ICI in refractory patients in ongoing trials.

The PD-1/PD-L1 interaction drives adaptive resistance through several additional mechanisms, many of which are the result of negative feedback interactions within the TME that upregulate PD-L1 expression.[12] PD-L1 plays a role in induction and maintenance of regulatory T-cells and immunosuppressive myeloid cells within the TME, which are of uncertain significance in predicting response to ICI.[59,60] CTLA-4 inhibitors have been shown to result in upregulation of CD4(+)Foxp3(−) T cells within the tumor microenvironment, which express PD-1 but lack cytotoxic function and express Treg-associated markers.[61] Treatment with both PD-1/PD-L1 and CTLA-4 antibodies notably mitigates this effect in many cases and persistence of these cells after anti-PD-1 correlates with poor prognosis.[62]

A group of 26 transcriptomic signatures, collectively referred to as the innate anti-PD-1 resistance (IPRES) signature, were found to be co-enriched in pre-treatment ICI-resistant melanomas.[63] This signature donates upregulation of genes involved with mesenchymal transition, angiogenesis and wound healing. Epithelial to mesenchymal transition has been associated with an increased presence of various immune checkpoint molecules, regulatory T cells and immunosuppressive cytokines.[64–66] The epithelial to mesenchymal transition signature is notably associated with TNFα, which promotes phenotypic plasticity and upregulation of an innate resistance signature through translational reprogramming.[67]

Both type I and II interferon signaling play an important role in T-cell activation required for initial response to checkpoint inhibition yet prolonged IFN signaling has also been associated with immune supression.[68] Interferon signaling accordingly plays a complicated role in modulating response to checkpoint inhibition. Increased expression of IFN-γ and IFN-related genes has been associated with response to anti-PD-1 in melanoma and defects in IFN signaling pathways have been associated with acquired resistance.[32,69] Gene expression studies have also identified an interferon-γ related GEP that was necessary, but not always sufficient, for clinical benefit across tumor types exposed to ICI.[70]

Persistent IFN signaling induces epigenomic and genomic alterations within tumors that dampen immune response, including expression of PD-L1 as well as upregulation of multiple additional T-cell inhibitory receptors.[71] IFN-γ may also play a role in expediting cytotoxic T-cell dependent immunoediting.[72] As noted above, response to anti-PD-1 has been associated with a T-cell rich inflammatory TME, characterized by an abundance of TILs and PD-L1 expression. A responsive TME is also likely one that is “adaptive immune resistant” and further categorization of the cellular and molecular changes associated with adaptive immunity may help inform treatment decisions or uncover new therapeutic targets.[70]

Proteomic profiling found immunotherapy-responsive tumors to be enriched for proteins involved with oxidative phosphorylation and lipid metabolism compared with non-

responsive samples. These samples were also enriched for antigen presentation and IFN signaling. Functional studies demonstrated that increased lipid metabolism led to upregulation of antigen expression by melanoma cells, increasing immunogenicity and potential for ICI response.[73]

The Host Microbiome—Increasing evidence suggests that the intestinal microbiome plays an important role in a wide variety of inflammatory or immune-mediated conditions. Increased microbial biodiversity within the host microbiome has been associated with response to PD-1 inhibition in melanoma.[74] Specific bacterial species have also been noted to be prevalent amongst responders while others are associated with a lack of response.[74–76] Xenografted germ-free mice transplanted with stool samples from responding patients demonstrated improved outcomes with anti-PD-1 compared with those transplanted from non-responders.[76] Concurrent and prior antibiotic therapy has also been correlated with decreased response to ICI.[77,78] High dietary fiber intake has been shown to contribute to an immunostimulatory landscape within the microbiome and patients with melanoma who self-reported a high fiber diet were significantly more likely to respond to anti-PD-1 agents.[79,80]

Overcoming ICI Resistance—Several strategies have been proposed to augment cytotoxic T-cell priming and tumor infiltration integral to ICI response, which were in part mentioned above. The full spectrum of current combination therapy approaches are beyond the scope of this review. Radiation therapy broadens the spectrum of T-cell-receptors amongst tumor-infiltrating lymphocytes, which may help to overcome innate or acquired mechanisms of resistance involving T-cell exclusion.[81] Melanoma vaccines may also augment the effect of checkpoint inhibition, particularly when directed towards particular neoantigens associated with a robust response to ICI.[7,82] The combination of checkpoint inhibitors and cancer vaccines, including individualized vaccines directed at neoantigens shown to demonstrate immunogenicity within a specific tumor, are currently under investigation in early phase trials.[82]

Certain oncolytic viruses capitalize on defective interferon signaling to enter and replicate within cells and may therefore be used to target IFN- γ deficient clones and overcome associated resistance.[83] Agonists of the stimulator for interferon genes (STING) receptor may increase sensitivity to checkpoint inhibition by increasing PD-L1 expression through upregulation of the JAK/STAT pathway.[84,85] A resistance program associated with T cell exclusion and immune evasion identified by single cell RNA sequencing was also found to be repressed by CDK4/6 inhibition when given in combination with ICI in mouse models.[86] Other approaches, including co-stimulation of additional immune checkpoints, toll-like receptor agonists, and tumor infiltrating lymphocytes may also have promise. Antibodies targeting the T-cell inhibitory receptor lymphocyte activation gene-3 (LAG-3) showed promise when used with nivolumab in melanoma refractory to anti-PD-1/PD-L1 and are being evaluated with anti-CTLA-4 in this setting as well as with anti-PD-1/PD-L1 in ICI-naïve patients (NCT03978611, NCT03743766).[87] Engagement of toll-like receptors within the TME promotes innate immune activation and associated pro-inflammatory cytokine production that can increase intra-tumoral T-cell infiltration and potentiate response to ICI.[88] Early phase trials have shown encouraging results in anti-PD-1/PDL-1 refractory patients

treated with an intra-tumoral TLR9 agonist and ipilimumab with an overall response rate of 47% (7 of 15 patients).[89] Adoptive cell therapy with autologous tumor-infiltrating lymphocytes has been attempted and refined in melanoma for several decades with more recent trials limited to those who have progressed on standard therapies. TIL therapy optimized with pre-infusion lymphodepletion and a post-transfer IL-2 regimen resulted in durable response in some refractory patients with an ORR of 36.4% and a median duration of response that was not reached after 17 months.[90]

Resistance to BRAF/MEK Inhibitors

Mutations in *BRAF* occur in almost half of all melanomas and thus represent a major therapeutic target. A majority of *BRAF*-mutant melanomas harbor a substitution at codon 600 of the *BRAF* gene, which results in constitutive kinase activation and downstream activation of the MAPK pathway. The MAPK pathway plays a role in a wide spectrum of intracellular processes, including differentiation, stress response, and cell survival, which it also regulates via physiologic negative feedback mechanisms. Constitutive activation of the MAPK pathway results in unregulated cell growth and proliferation that drives tumorigenesis.

The development of small molecule inhibitors specific to the *BRAF*-mutant kinase (BRAFi) represented a major breakthrough in the treatment of melanoma. These agents were associated with significantly improved overall response rates, PFS and OS when compared with chemotherapy in patients with *BRAF* V600 mutant melanoma.[91,92] Primary resistance to BRAF inhibition is relatively rare, with impressive initial response in many patients, yet these regimens are frequently complicated by acquired resistance. Unlike many other malignancies treated with other targeted kinase inhibitors, which often develop resistance through “gate-keeper” mutations that impede interaction between the inhibitor and the mutated kinase, *BRAF*-mutated melanomas employ several complex alternative mechanisms to upregulate the downstream MAPK pathway and bring about resistance.

Evidence that re-activation of the MAPK pathway was involved with acquired resistance to BRAFi led to the introduction of combined therapy with both BRAF and MEK inhibition (MEKi). MEK is directly down-stream from BRAF in the MAPK cascade and MEKi was independently associated with superior outcomes compared with chemotherapy in patients with *BRAF* V600 mutant melanoma.[93] Targeting multiple sites along the MAPK pathway with the addition of MEKi was proposed in an attempt to mitigate eventual resistance as well as to promote a more robust response to therapy. Multiple trials demonstrated significant improvement in response rate, PFS and OS with both BRAF and MEK inhibitors compared with BRAFi monotherapy and combination therapy became standard of care in *BRAF* V600 mutant melanoma.[94–96]

Despite significant improvements with combination therapy, BRAF/MEK inhibition is still frequently complicated by eventual resistance, most often via upregulation of MAPK signaling. Although many studies have focused on categorizing BRAF inhibitor resistance in melanoma, resistance to the BRAF/MEKi combination is due to similar mechanisms.[97–99] Eventual resistance to BRAF/MEKi should be expected in most patients, yet recently published follow-up data from the COMBI-v and COMBI-d trials suggests the potential for

long-term benefit in a minority of patients treated with BRAF + MEK inhibitiors.[100] Patients who demonstrated a complete response to therapy (109 of 563, 19%) had a 5-year overall survival of 71% compared with 34% in the overall cohort. Several baseline factors were also found to be associated with prolonged progression-free survival including older age, female sex, normal lactate dehydrogenase level, and less than three organ sites with distant metastasis. Patients who demonstrated a complete response shared similar baseline factors. PFS at 5 years was observed in approximately 15% of the population.[100] Longer-term follow-up of patients with sustained response to BRAF/MEKi as well as further molecular and clinical characterization of this sub-group is warranted.

The categorization of BRAF/MEKi resistance is complicated by a diverse array of mechanisms with significant heterogeneity between patients and within individual tumors. While a single predominant resistance mechanism may be identified in one resistant tumor sample, additional biopsies from the same patient often demonstrate distinct or unknown drivers of resistance.[99] Melanoma clones emerging after BRAFi therapy demonstrate branched evolution and some tumors can proliferate in the setting of BRAFi in the absence of any clear genomic driver.[101] Intra-tumor heterogeneity can be explained in part by a suspected multi-step pattern of resistance acquisition. This starts with adaptive transcriptional reprogramming that allows for cell survival in the presence of BRAFi via phenotypic plasticity as well as increased signaling in alternative RTK pathways that frequently converge with the MAPK pathway. This adaptive transcriptional state allows tumor cells to survive long enough to acquire “fixed” mediators of resistance, which are often genomically mediated.

Increasing evidence suggests melanoma cells exposed to BRAF inhibition may capitalize on an innate stress reaction that promotes transition to a “slow-growth” phenotypic state associated with oncogene-induced senescence and de-differentiation as well as changes in chromatin remodeling and histone deacetylase activity.[102–104] Transcriptomic analysis of BRAF/MEKi resistant tumors demonstrated recurrent involvement of specific genes and pathways, which frequently demonstrated differential methylation of tumor cell-intrinsic CpG sites.[105]

The early adaptive “persister” state adopted by some melanoma cells in response to BRAF inhibition has also been associated with phenotypic transition to a more de-differentiated mesenchymal state.[106,107] Overexpression of the transcription factor c-JUN has been associated with a mesenchymal gene signature and an EMT-like phenotypic transition signature in melanoma cells.[108,109] Inhibition of c-JUN, either via direct silencing or upstream inhibition of c-JUN amino-terminal kinase (JNK), has been shown to increase overall cell death and decrease the population of “persister” cells when used in combination with BRAF inhibitors.[106,108,109] Microphthalmia associated transcription factor (MITF), a transcription factor that controls multiple genes integral to melanocyte function, has been repeatedly implicated in BRAF/MEK inhibitor resistance. While some studies have implicated MITF up-regulation as a mechanism of BRAF resistance, a majority have demonstrated the emergence of “MITF-low” populations early in the course of acquired resistance.[110–113]

In addition to re-activation of MAPK signaling, increased signaling in the PI3 kinase/AKT pathway has also been implicated in de novo and acquired resistance to BRAF/MEK inhibition in almost 20% of melanoma patients.[114] MAPK pathway inhibition has been shown to induce upregulation of AKT signaling in resistant cells and high levels of AKT activity have been correlated with a lack of response to MEK inhibition in patients with *BRAF*-mutant melanoma.[114–116] Despite encouraging pre-clinical data, inhibitors of PI3K or downstream PI3K pathway effector molecules have largely failed to offer additional clinical benefit when used in combination with BRAF/MEK inhibitors in early stage trials.[117]

Increased signaling in the MAPK and PI3K pathways in resistant cells is modulated by multiple additional RTK pathways that are frequently up-regulated in the setting of BRAF/MEKi resistance.[118–123] Evidence suggests that treatment with BRAFi more likely results in the coordinated upregulation of multiple RTKs in individual tumor cells rather than selective upregulation of specific receptors that may be amenable to therapeutic intervention.[117] Additional RTK pathways shown to be upregulated in resistant cells include epidermal growth factor receptor (EGFR), ERBB3, hepatocyte growth factor receptor (c-MET), platelet derived growth factor (PDGFR)-a, and the insulin like growth factor (IGF)-1 receptor.[118–123] Some studies have demonstrated an inverse correlation between MITF expression in resistant samples and upregulation of multiple RTKs, including the RTK AXL, which is over-expressed in many advanced malignancies and often associated with acquired resistance to chemotherapy.[110–112,124]

Melanoma cells capable of adapting to MAPK inhibition will eventually acquire permanent genomic alterations that confer resistance to therapy. Genomic profiling studies have demonstrated a wide spectrum of genetic drivers associated with MAPKi resistance with significant intra-patient and intra-tumoral heterogeneity. As expected, a majority of identified mutations are associated with increased signaling within the MAPK pathway, which is restored in an estimated 80% of patients resistant to dabrafenib or vemurafenib.[125] These include activating mutations in NRAS and/or MEK1/2 as well as *BRAF* V600 amplifications and *BRAF* splice site variants.[99,118,126] Non-MAPK pathway alterations most frequently involve increased signaling through the PI3K pathway.[99,101,127] Copy number variations in CDKN2A and CCND1 and inactivation of Rb have also been implicated in decreased response to BRAF/MEK inhibition although it is unclear whether this is due to more aggressive disease overall or a specific mechanistic link to resistance.[128,129]

Overcoming BRAF/MEK Inhibitor Resistance—Given the role of persistent MAPK signaling in resistance to BRAF/MEKi, inhibition farther downstream in the pathway was proposed as a potential mechanism to mitigate MAPK re-activation. Mitogen-activated extracellular-signal regulated kinase (ERK) is the final effector in the MAPK cascade and acts within the nucleus to promote proliferation, growth and survival. Pre-clinical studies have suggested that ERK inhibitors can independently induce regression in *BRAF*-mutant melanoma and may also reverse resistance to BRAF/MEKi.[130] ERK inhibitors have produced responses in BRAF/MEKi resistant patients as well as treatment naïve *BRAF* and *NRAS* mutant melanomas in small early clinical trials.[131,132]

Re-introduction of BRAF/MEKi in patients who previously progressed on these agents has notably led to significant responses in some cases.[133] Intermittent dosing of BRAFi has also induced more durable responses in mouse models compared with continuous dosing with regression of BRAF-amplified resistant tumors following BRAFi discontinuation.[114,134] Evidence suggests that some BRAF/MEKi resistant melanomas may become “inhibitor addicted” and regress with short-term drug withdrawal.[135] Intermittent dosing strategies may improve outcomes in these patients and are being evaluated in early phase trials (NCT02196181). However, one study recently presented negative results with a regimen of 5 weeks on and 3 weeks off dabrafenib and trametinib compared with continuous dosing.[136]

The AXL receptor is a target of interest in many malignancies, particularly in the setting of refractory or advanced disease.[137] Several therapeutic agents are under investigation in early trials, including established multi-targeted kinase inhibitors shown to inhibit AXL and novel more specific small molecule inhibitors.[138] AXL-directed antibody-drug-conjugates (ADC) have also been developed in an attempt to more specifically target AXL-expressing cell populations. When used in combination with BRAF/MEKi in patient-derived xenografts of melanoma with heterogeneous cell populations, an ADC containing the antimitotic agent monomethyl auristatin E (AXL-107-MMAE) eliminated tumor cells in the AXL-high population while BRAF/MEKi were effective in AXL-low cell lines. AXL-107-MMAE was also shown to potentiate the effect of BRAF/MEKi in AXL-low populations by exploiting BRAF/MEKi-induced transcriptional upregulation of AXL, suggesting a potential for benefit in BRAF/MEKi naïve patients. A phase I trial evaluating an AXL-specific ADC in advanced or relapsed/refractory solid tumors is ongoing (NCT02988817).

Inhibitors of heat shock protein-90 (HSP90), a chaperone that supports many RTKs and intracellular proteins involved in tumor growth and progression, have also been suggested as an adjunct therapy that may mitigate BRAF/MEKi resistance in melanoma. A phase trial I evaluating the HSP90 inhibitor XL888 and vemurafenib in BRAFi naïve patients with *BRAF*-mutant melanoma demonstrated a notable 75% response rate (15 of 20 evaluable patients) in addition to a tolerable toxicity profile.[140] Multiple phase I trials are evaluating the use of these agents with dual BRAF/MEKi (NCT02721459, NCT02097225).

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Key Points

1. Novel targeted therapies and immune checkpoint inhibitors have transformed the management of advanced melanoma, although resistance limits this benefit.
2. Immune checkpoint inhibitors are often associated with intrinsic resistance and primary progressive disease, which may be due to lack of immune recognition, T cell exclusion, or alternative causes of T cell exhaustion.
3. Targeted therapies are more often associated with acquired resistance, which may in part be due to reactivation of MAP kinase signaling and transcriptomic reprogramming.

Synopsis

Novel therapeutic agents introduced over the last decade, including immune checkpoint inhibitors and targeted therapies, have revolutionized the management of metastatic melanoma and significantly improved patient outcomes. While robust and durable responses have been noted in some cases, treatment is often limited by innate or acquired resistance to these agents. This review provides an overview of known and suspected mechanisms involved with acquired resistance to BRAF/MEK inhibitors as well as developing insights into innate and acquired resistance to checkpoint inhibitors in patients with melanoma.