Novel Therapies in Melanoma

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15.1 Introduction

The treatment of unresectable or metastatic melanoma was revolutionized by the advent of immunotherapy and targeted inhibitors of the Mitogen Activated Protein Kinase (MAPK) pathway. However, a high rate of resistance to the BRAF/MEK inhibitor combination has resulted in limited long-term benefit in the majority of patients receiving targeted therapy [1]. Additionally, while immunotherapy has unequivocally improved the outcome of advanced melanoma patients, overall survival at 5 years in patients treated with combined ipilimumab and nivolumab was 52%, leaving considerable room for improvement [1, 2]. Clinical trials are now focused on new therapeutics that may augment the effect of immune checkpoint and targeted inhibitors, with the ultimate goal of achieving more durable responses in a greater number of patients. This chapter aims to touch on some of the approaches that are currently being pursued to accomplish this goal.

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15.1.1 Updates on the Targeted Therapy Approach

15.1.2 Novel Combinations of Therapies

Early attempts to combine BRAF inhibitors with immunotherapy were hindered by dose-limiting toxicities and limited efficacy [3–5]. The combination of vemurafenib (BRAF inhibitor) and ipilimumab (CTLA-4 inhibitor) led to dose-limiting hepatotoxicity, necessitating trial discontinuation [3]. The inclusion of MEK inhibitors in the combination was better tolerated and appeared to potentiate anti-tumoral activity. Therefore, triple combination therapy with BRAF/MEK inhibitors and anti-PD-1 therapy has garnered considerable attention recently [4, 5]. A phase I study of 15 patients receiving dabrafenib (BRAF inhibitor), trametinib (MEK inhibitor), and pembrolizumab (PD-1 inhibitor) demonstrated an objective response rate of 73%, with 40% of patients having continued response after a median of 27 months [4]. However, 14/15 patients experienced toxicities necessitating dose modifications, and 10/15 patients experienced grade 3–4 adverse events (AEs), most notably transaminitis and pyrexia [4].

A phase II randomized trial comparing dabrafenib, trametinib, and pembrolizumab (triplet therapy) to placebo (doublet therapy: dabrafenib/

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trametinib) resulted in a numerically significant prolongation of progression-free survival (PFS; 16.0 vs. 10.3 months, $p = 0.043$) [5]. However, given the small sample size, the study did not reach its primary statistical end point. Interestingly, the overall response rate (ORR) was higher in the placebo vs. the experimental group (72% vs. 63%). Additionally, grade 3–5 AEs occurred in a greater proportion in the triplet group compared to doublet arm (70% vs. 45%, respectively), including one death in the triplet arm due to pneumonitis [5].

Following the results of a recent phase III randomized controlled trial (RCT), the triplet combination of vemurafenib, cobimetinib (MEK inhibitor) and atezolizumab (PD-L1 inhibitor) has been approved for unresectable, advanced melanoma that is BRAF-mutant. This is discussed in more detail in the "Systemic Therapy in Melanoma" chapter. Another similar phase III trial is currently underway evaluating dabrafenib plus trametinib with and without a novel PD-1 inhibitor (Spartalizumab, PDR001) for metastatic BRAF-mutant melanoma (NCT02967692) [6]. The early phase data from this combination demonstrated a CRR of 42% and an ORR of 75% [6]. Seventy-eight percent of patients experienced grade 3 or higher AEs, with 17% resulting in treatment discontinuation. Common AEs included pyrexia, chills, and fatigue [6].

The main criticism of the aforementioned triplet studies revolves around the choice of the control group. The trials do not address whether triplet therapy provides clinical benefit over immunotherapy alone (i.e., PD-1/CTLA-4 inhibitor combination or anti-PD-1 monotherapy). As addressed in the prior chapter, given its greater likelihood of durable effects, checkpoint inhibitor therapy is usually considered the preferred initial treatment choice in metastatic melanoma. This important question of whether combined BRAF/MEK and checkpoint inhibitor therapy is superior to checkpoint inhibition alone, unfortunately, remains unanswered. It is notable that triplet therapy has significant, high-grade toxicities, and more studies are warranted to determine whether the addition of BRAF/MEK inhibitors to immunotherapy yields further clinical benefit.

15.1.3 NRAS-Targeted Therapies

Identification of driver mutations within overactive signaling pathways is crucial to the development of targeted therapies. The three RAS genes (KRAS, HRAS, and NRAS) are known to be involved in a wide array of malignancies, with NRAS mutations being the second most common mutation in melanoma after BRAF, occurring in approximately 20–30% of all melanomas [7]. While some have argued that the presence of NRAS mutations in T2b primary melanomas portends a more aggressive clinical course, the prognostic significance of an NRAS mutation in stage 4 disease is controversial [8–11]. Some authors believe that NRAS mutated stage 4 melanomas have a particularly high response rate to checkpoint inhibitor therapy [12]. While targeting the downstream effectors of RAS, BRAF, and MEK, dramatically improved melanoma outcomes, the development of NRAS-selective inhibitors has thus far been unsuccessful [7, 13].

Early attempts to target the RAS pathway by farnesyltransferase inhibitors (FTI) demonstrated no efficacy in clinical trials, despite promising preclinical studies [13]. Farnesyltransferase plays a critical role in the posttranslational modification of RAS, allowing its activation and membrane translocation. FTIs target this key regulatory step, inhibiting RAS' ability to mediate the stimulation of downstream effectors [13]. NRAS bypassed FTI inhibition effectively by utilizing substrates within a related group of enzymes. The lack of success of FTI inhibitors in clinical practice has unfortunately dissuaded the aggressive pursuit of other RAS-specific inhibitors for some time [1, 7, 13].

The difficulty of targeting RAS molecules has been attributed in part to the high affinity of GTP binding to RAS proteins, as well as the lack of deep hydrophobic pockets that allow tight binding of small molecules [7]. Nonetheless, recent progress has been made in targeting KRAS

G12C in patients with non-small cell lung cancer. Studies of the FDA-approved drug sotorasib have provided "proof of principle" that inhibition of RAS family members can in fact lead to important clinical responses (32% response rate) in tumors addicted to this oncogene [14]. The critical insight in this work was that the novel cysteine residue present in the G12C mutant KRAS molecule could serve as a reactive site for a drug that binds covalently at this site, thereby inhibiting KRAS by altering its conformation and ability to activate downstream effector molecules. Of some note, while certainly not the most common type of NRAS mutation, both G12C and G13C mutations have been reported in cutaneous melanomas, raising the possibility that this novel class of cysteine-targeted Ras oncogene-directed therapy may someday prove useful in a subset of patients with NRAS mutated melanomas as well [15].

As an alternate approach, a serine-threonine kinase (STK19) has been identified as a novel NRAS activator and a potential target in the treatment of NRAS-mutant melanoma [16]. In genetically engineered human melanocytes, STK19 was shown to activate NRAS via the MEK-ERK and PI3K-Akt pathways, contributing to its oncogenic potential [16]. Following STK19 inhibition in vitro and in vivo, NRAS-driven malignant transformation and melanoma growth were substantially inhibited [16]. The study offers preclinical proof of concept regarding the targeting of STK19 in melanomas with NRAS melanoma [16]. Validation of these results in clinical trials is the next step.

15.1.4 BET Inhibitors

In the first weeks of BRAF/MEK inhibitor therapy, the remaining tumor population undergoes an epigenetic-mediated change, resulting in increased expression of transcription factors and upregulation of various receptor tyrosine kinases (RTKs) critical to their survival [17, 18]. RTKs stimulate the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway, thereby bypassing BRAF/MEK inhibition [17]. Epigenetic regulators, such as the Bromodomain and Extraterminal Domain (BET) proteins BRD2, BRD3, and BRD4, were found to regulate cellular proliferation, and their inhibition reduced expression of RTKs, leading to decreased tumor cell survival [18]. In preclinical studies, NRAS-mutant melanomas, in particular, were found to be dependent on overexpression of BET proteins for survival, particularly BRD2/4 [18].

The addition of BET inhibitors (BETi) to BRAF/MEK inhibitors was also shown to offset treatment resistance and prolong survival in preclinical melanoma studies [18]. Unfortunately, early phase clinical studies have so far revealed the limited clinical benefit of BETi as monotherapy in patients with advanced solid tumors [19]. In one trial, no partial or complete responses were noted, and AEs were common (89% patients) [19]. Grade 3 or higher AEs were reported in 54% of patients, and 26% required dose discontinuation, most commonly due to thrombocytopenia, nausea, and fatigue [19]. The authors concluded that BETi is safe at doses up to 2 mg/kg but admitted that the clinical efficacy was limited with a narrow therapeutic window [19].

One important caveat of the BETi clinical trials described above is that the BETi used thus far do not have significant isoform specificity, yet each of the BET isoforms has important and non-redundant functions in human physiology [20]. There is thus concern that BET inhibition may be prematurely dismissed as an attractive approach to the treatment of human malignancies such as melanoma due to off-target effects of relatively non-isoform specific agents. Once more selective BET inhibitors are developed, their activity in melanoma will need to be re-examined, and such studies are eagerly awaited.

15.1.5 CDK4/6 Inhibitors

The cyclin-dependent kinases, CDK4 and CDK6, regulate progression through the G1 phase of the cell cycle. When activated, CDK4/6 hyperphosphorylates retinoblastoma (Rb) protein, releasing it from the E2F transcription fac-

tor, thereby enabling cell cycle progression [21]. CDK4/6 activating mutations are frequently present in various malignancies, including melanoma, and inhibition of these mutant hyperactive kinases impedes the release of E2F, resulting in cell cycle arrest. CDK4/6 inhibitors such as ribociclib have emerged as an effective new class of anticancer drugs when combined with other agents that target the G0/G1 transition such as anti-estrogens in hormone receptor-positive breast cancer [21–24].

After preclinical studies demonstrated improved antitumoral activity, several early phase trials in melanoma have reported positive findings [23, 24]. A phase IB/II multicenter study recently evaluated the CDK4/6 inhibitor ribociclib in combination with the MEK inhibitor binimetinib for the treatment of 16 patients with NRAS-mutant melanoma [23]. Four patients (25%) developed a partial response, and seven patients (44%) had stable disease. Common grade 3–4 AEs included transaminitis (6–19%), nausea (19%), rash, and neutropenia [23]. A phase II trial is currently underway to further assess the antitumor activity of this combination [23].

Another phase Ib/II trial compared triple combination therapy with ribociclib, encorafenib (a BRAF inhibitor), and binimetinib to BRAF/MEK inhibition alone in patients with advanced BRAF-mutant melanoma [24]. The ORR was 52.4% (4 CR; 18 PR; 15 SD), and the median PFS was 9.0 months [24]. Ten patients (23.8%) discontinued treatment due to AEs, most commonly transaminitis in four patients. Other AEs included neutropenia and anemia [24]. The authors concluded that CDK4/6 inhibition is overall well-tolerated and may improve clinical response rates when used in combination with BRAF/MEK inhibitors [24].

15.1.6 ERK Inhibitors

Extracellular signal-regulated kinase (ERK) has been shown to play a pivotal role in acquired resistance to BRAF/MEK inhibitors [25]. The intracellular protein is the most distal kinase of the MAPK pathway, and its stimulation enables reactivation of the signaling pathway, resulting in continued gene expression and treatment evasion [25]. As a result, ERK inhibitors were developed as a potential strategy to overcome the high rates of resistance to targeted therapy, and they have demonstrated favorable results in early studies [25, 26].

Ulixertinib, a selective ERK1/2 inhibitor, was found to inhibit tumor growth in human xenograft models that were resistant to BRAF and MEK inhibitors [25]. The first-in-class phase I study evaluated ulixertinib in 135 patients with advanced solid tumors, including 53 with melanoma [26]. Out of 17 evaluable patients with NRAS-mutant melanoma, 3 (18%) achieved a PR, 6 had SD, and 8 had progressive disease (PD). In BRAF/MEK inhibitor-refractory BRAF-mutant melanoma, 3/19 patients (15%) had a PR [26]. Treatment discontinuation due to AEs were noted in 19% of patients. Acneiform eruptions, diarrhea, and fatigue were the most common AEs, and no patients experienced a grade 4 or 5 treatment-related AE [26]. The authors concluded that ulixertinib was safe and effective in the treatment of NRAS- and BRAF-mutant solid tumor malignancies. They recommended further evaluation of ERK inhibitors both as a single agent and in combination therapies [26].

15.1.7 KIT Inhibitors (Imatinib, Sunitinib, Dasatinib, Nilotinib)

Mutations in KIT, a transmembrane receptor tyrosine kinase (RTK), have been detected in numerous melanoma subtypes, most notably acral, mucosal, and chronically sun-damaged skin melanoma [27]. Aberrations in KIT result in constitutive activation of several pathways, including MAPK, PI3K/AKT, and the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway, thereby promoting melanocytic oncogenesis [27]. Imatinib

has received the most attention in its class for the treatment of KIT-mutant melanoma, as discussed in the previous chapter "Systemic Therapies in Melanoma." However, various other KIT inhibitors are also being evaluated in this setting, with mixed results thus far [28–30].

Sunitinib was the first to be studied in ten evaluable patients with KIT aberrations [28]. One patient achieved a CR, three achieved PRs, and one SD [28]. The authors did note that KIT mutations were only present in 4/10 patients, whereas the rest had KIT amplification or overexpression. Among the four patients with KIT mutations, one achieved a CR and three achieved a PR, indicating that sunitinib may be efficacious in KIT-mutant melanomas [28]. The medication was not well-tolerated, however, as five patients required a dose reduction, and one patient required therapy discontinuation due to new-onset congestive heart failure [28].

Dasatinib has been investigated in advanced mucosal, acral, or vulvovaginal melanomas [29]. The medication demonstrated a low response rate (18%), with a median OS of 7.5 months, PFS of 2.1 months, and no CRs. Forty-four percent of patients experienced grade 3 AEs, including myocardial infarction in two patients and pleural effusions in four patients. Dasatinib was discontinued in 12% of patients due to AEs [29].

Lastly, nilotinib was investigated in a phase II study of 42 patients with KIT-mutated melanoma [30]. 400 mg twice daily was used, and the primary endpoint of ORR was 26.2% ($n = 11/42$; all 11 cases achieved PRs). Twenty patients had SD (47.6%) and ten patients had PD (23.8%). The median PFS was 4.2, and OSS was 18 months [30].

Overall, KIT inhibitors as a class achieved moderate clinical efficacy, and they should be considered for the treatment of KIT-mutant melanomas in the appropriate clinical setting. Clinicians and patients should be aware of the significant rate of high-grade AEs however, and close clinical monitoring of patients is recommended to identify and address any serious AEs that may arise while on treatment.

15.1.8 Angiogenesis Inhibitors

One promising strategy to enhance immune responses to cancers is to combine immune checkpoint inhibitors with inhibitors of angiogenesis [31]. Vascular endothelial growth factor (VEGF-A) has been shown to play a key role in promoting malignant cell growth and immunosuppression. Specifically, VEGF augments tumor angiogenesis and inhibits dendritic cell function and lymphocyte migration into the tumor microenvironment [32]. Levels of this growth factor have also been found to predict outcomes to ipilimumab therapy, with high VEGF levels correlating with less favorable outcomes [32]. VEGF inhibitors, such as bevacizumab, levatinib, and axitinib, were therefore developed to mitigate the tumor-promoting activities of VEGF [32–34].

A phase I study in metastatic melanoma patients combining bevacizumab and ipilimumab demonstrated an overall disease control rate (DCR) of 67.8% (8/46 PRs and 22/46 SDs) [32]. 13 patients experienced high-grade AEs, including giant cell arteritis, palpable purpura, and eosinophilic hepatitis. No treatment-related deaths were reported [32]. Tyrosine kinase inhibitors with various anti-angiogenic activities are also undergoing early phase studies for melanoma. Levatinib and axitinib both inhibit VEGF, in addition to various other receptors, such as KIT, platelet-derived growth factor (PDGF) and fibroblast growth factor receptor (FGFR1–4) [33, 34]. Levatinib demonstrated an overall DCR of 40.3%, with 5/29 patients experiencing PR [33]. The most common AEs were dose-limiting hypertension, fatigue, and proteinuria. Axitinib in combination with PD-1 inhibitor toripalimab achieved a 48.3% ORR with a median PFS of 7.5 months [34]. Grade 3 or greater AEs occurred in 39.4% of patients, including diarrhea, proteinuria, hand-foot syndrome, and fatigue [34].

The authors believe the aforementioned studies provide enough evidence to support further investigation of VEGF inhibitors in the treatment of melanoma, especially in combination with

immune checkpoint blockade [32–34]. RCTs are currently underway to confirm the clinical efficacy noted in early phase studies.

15.2 Novel Immune Therapy

15.2.1 Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitors

The impressive efficacy of anti-PD-1 and anti-CTLA-4 therapies has led to efforts to discover other immune-based therapies. Indoleamine 2,3 dioxygenase (IDO) is an intracellular enzyme that converts tryptophan to its metabolites, depleting local stores of tryptophan [35]. Decreased tryptophan induces T-cell apoptosis and cell cycle arrest, leading to local immunosuppression in the tumor microenvironment [35]. As a result, IDO inhibitors, such as epacadostat, were developed to potentially circumvent this method of immunosuppression [36, 37].

After promising preclinical studies, clinical trials have yielded mixed results for IDO1 plus PD-1 inhibitor combination therapy [36, 37]. A phase III RCT comparing epacadostat plus pembrolizumab to monotherapy with pembrolizumab demonstrated no significant difference in PFS or OS [36]. The trial was therefore terminated after a median follow-up 12.4 months [36]. The rate of AEs and treatment modification was also similar between both groups, except for hepatitis, which was more frequent in the combination group [36].

Epacadostat was also investigated in combination with nivolumab in 50 patients with advanced melanoma [37]. The ORR was 62% (9 CR, 22 PR) and DCR of 80% (32/40). The rate of grade 3 or more AEs was 48% with 300 mg BID of epacadostat and 13% with 100 mg BID. Pneumonitis was the only grade 3 or higher AE, and no treatment-related deaths were reported [37]. Phase III studies are underway.

A phase I study of epacadostat + ipilimumab demonstrated poor tolerability overall, with five treatment-related deaths, 28% high-grade AE, and a 40% treatment discontinuation rate [38]. 10/39 immunotherapy-naive patients had an objective response rate (3 CR, 7 PR, 15 SD), whereas none of the 11 patients previously treated with immunotherapy experienced an objective response (4 SD, 5 PD, and 2 missing) [38]. Phase II studies were suspended due to the emerging success of PD-1 inhibitors [38].

15.2.2 Histone Deacetylase Inhibitors

A delicate balance of acetylation and/or phosphorylation of histones and other proteins within normal cells is integral to the process of regulating gene transcription. Tumors can exploit this equilibrium resulting in imbalanced gene transcription that favors repression of tumor suppressor genes. Histone deacetylase (HDAC) is known to play a critical role in this process and shows increased expression in many cancers [39]. In accordance with this, inhibitors of HDAC are being developed as potential anticancer therapies [39–42].

Panabinostat is a pan-deacetylase inhibitor that demonstrated promising results in preclinical studies in melanoma patients. Based on this data, the drug was utilized in a phase I trial in unresectable stage III and stage IV melanoma [40]. However, monotherapy with panobinostat did not corroborate the early results and was unable to demonstrate any clinical activity as a single agent in the treatment of metastatic melanoma [40].

A subset of patients in the trial showed increased MHCI staining and CD8+ T-cell tumor infiltration, suggesting a role for the combination of panabinostat with immune checkpoint inhibitors [40]. Subsequently, 17 patients with advanced melanoma were treated with panobinostat and ipilimumab combination, yielding a 12% ORR and 35% SD rate [41]. PFS and OS were 2.2 months and 21.0 months, respectively. 1/6 patients on the 5 mg and 3/9 patients on the 10 mg dose developed a dose-limiting toxicity (hydronephrosis, rash, diarrhea, and thrombocytopenia) [41].

Entinostat is a class I selective HDAC inhibitor. In an early phase trial, 53 patients with advanced melanoma resistant to PD-1 inhibitors

were treated with entinostat in combination with pembrolizumab [42]. The confirmed ORR and DCR were 19% and 32%, respectively, with 1 CR, 9 PRs, and 7 SDs. PFS was 4.2 months, and the median duration of response was 12.5 months. Five patients (9%) experienced grade 3/4 iRAE, including rash, colitis, pneumonitis, and hepatitis [42]. The study showed significant clinical activity with tolerable toxicity of entinostat with pembrolizumab in patients who had previously progressed on immune checkpoint inhibitor therapy, demonstrating promising results that need further corroboration in larger trials.

15.2.3 Other

A variety of other immunomodulatory agents have been identified as potential drug therapies that may enhance the efficacy of checkpoint inhibition. Factors associated with a reduced response to ICI include low tumor PD-L1 expression, low tumor-infiltrating lymphocytes (TILs) and a tumor microenvironment that does not favor T-cell activation [43–45]. Thus, agents that can stimulate T-cells and circumvent T-cell exhaustion could act synergistically with ICI. Bempegaldesleukin (BEMPEG) is a first-in-class interleukin-2 (IL-2) pro-drug that results in CD8+ T-cell stimulation, increasing TILs and PD-1 expression on CD4+, CD8+ T-cells and NK cells [46]. BEMPEG preferentially targets the CD122/CD132 intermediate-affinity IL-2 receptor over the CD122/CD132/CD25 high-affinity IL-2 receptor and expands effector T-cells over Tregs [46]. In phase I/II studies, 38 patients with treatment-naive metastatic melanoma were treated with BEMPEG and nivolumab combination [47]. A durable response was noted after a median duration of 12.7 months, with a 53% ORR, 34% CR, and 74% DCR [47]. Treatment was generally well-tolerated, although 9.8% of patients necessitated treatment discontinuation due to AEs [47]. This led to the design of a currently underway randomized phase III trial of BEMPEG and nivolumab vs. nivolumab monotherapy [48].

Lymphocyte activation gene-3 (LAG-3) is another immune checkpoint regulator that has garnered attention recently. Similar to PD-1, LAG-3 activation by cancer cells results in T-cell inhibition and immune evasion [49]. An anti-LAG-3 antibody (BMS-986016) combined with nivolumab was investigated in a phase I/IIa study involving 43 patients with advanced melanoma previously resistant to anti-PD-1 therapy [49]. Out of 31 evaluable patients, preliminary data suggests an ORR of 16% and DCR of 45%, with a tolerable side effect profile [49]. The authors concluded that the addition of LAG-3 inhibitor to nivolumab displayed encouraging clinical efficacy and a comparable side effect profile to PD-1 monotherapy [49].

15.2.4 Adoptive Cell Transfer

Adoptive Cell Transfer (ACT) is a specialized oncologic therapy that involves the isolation and expansion of tumor-infiltrating lymphocytes (TILs) in vitro, followed by their re-introduction into the patient to improve antitumor immunity [50]. The addition of high-dose IL-2 to the TIL isolate in vitro yields a 1000-fold expansion of the T-cell population, resulting in potent antitumor activity [50]. Shortcomings in the treatment protocol in earlier studies led to lower objective response rates of ~35%. This was largely attributed to the failure of persistence of the transferred TILs in vivo, leading to therapy modification, most importantly, the inclusion of nonmyeloablative depletion of lymphocytes prior to the reintroduction of TILs [51–53]. Lymphodepletion in the host prior to the transfer of lymphocytes is critical as it depletes regulatory T-cells. It also diminishes other T lymphocytes that would normally compete with the transferred TILs for key regulatory cytokines such as IL-7 and IL-15. Host lymphodepletion is achieved using chemotherapy (cyclophosphamide/fludarabine) with or without total body irradiation (TBI). Following the infusion of the TILs, patients are treated with high-dose IL-2 to improve the survival and expansion of the transfused TILs. These altera-

tions to the protocol have led to improved objective response rates of 38–50% [51–53].

In one of the largest studies to date, 93 patients undergoing ACT with chemotherapeutic lymphodepletion were subdivided into three cohorts: 43 patients only received chemotherapy as their method for lymphodepletion, whereas 25 patients received additional low-dose TBI (2 Gy), and 25 patients received high-dose TBI (12 Gy) [51]. 20/93 patients (22%) achieved CR, and 32/93 patients (34%) achieved a PR [51]. The responses were noted to be durable, as 19 of the patients with CR (95%) remained free of disease beyond 3 years [51]. The overall 3- and 5-year survival was 36% and 29% (100% and 93% for CR; 31% and 21% for PRs, and 7% and 5% for the non-responders, respectively). The majority of patients tolerated the therapy well [51]. However, one treatment-related death was reported, secondary to sepsis. One patient developed chronic pulmonary hypertension, and five patients developed microangiopathic nephropathy [51]. It is also worth noting that patients receiving high-dose TBI had an increased rate of CR, however, given this was a non-randomized study, the authors advised caution on this final point [51].

To further evaluate the effect of TBI on rates of CR and OS, 101 patients with metastatic melanoma were randomized to receive either chemotherapy or chemotherapy plus TBI (12Gy) for their lymphodepleting regimen [52]. This study found no significant difference in outcomes between the two groups, with a CR rate of 24% in both groups and OS rates of 38.2% and 36.6% in the experimental and control group, respectively. The authors attributed the better results of TBI in the previous studies to patient selection bias in a non-randomized study [52]. The responses were similarly durable, as only 1/24 patients with CR recurred after a median follow-up of 40.9 months. The TBI arm had slightly longer neutropenic periods, in addition to the unique complication of thrombotic microangiopathy in 13 patients (27%), resulting in one death [52].

Despite showing significant clinical benefit, ACT is associated with severe grade 3 and 4 toxicities. Some are attributable to the lymphodepleting chemotherapy regimen, while others are related to the use of the post-transfusion high-dose IL-2. A phase II trial involving 12 patients with melanoma utilized a low-dose, subcutaneous IL-2 instead of the standard high-dose intravenous IL-2 to investigate the feasibility and clinical activity of this modified protocol [53]. In contrast to high-dose IL-2, the majority of adverse events were grade 1 and 2 and could be managed outside an ICU setting. The study reported two confirmed PR and one unconfirmed PR, as well as six patients with stable disease. This met the pre-determined criteria of treatment efficacy, however, no patient achieved a CR, and the PRs were not durable. The authors hypothesized the low objective response might be related to the low dose of IL-2 utilized in this study, or the inclusion of non-cutaneous melanomas (3/12 mucosal or ocular), which are known to have a lower response rate to immunotherapy. The study results demonstrated persistence of the transfused TILs for greater than 2 years in one of the patients. Altogether, the study supported the further investigation into modified dosing of IL-2 to enable better tolerability of ACT [53].

ACT is a highly personalized and complex oncological treatment modality. While this may contribute to its efficacy, the attributes will make it difficult to be readily applied in current oncological practice. The treatment requires specialized personnel and equipment that is labor-intensive, making it difficult to mass produce, commercialize, and administer. At present, this treatment is only available in a few academic centers for metastatic melanoma. ACT is proof of concept that the introduction of highly avid T lymphocytes against metastatic melanoma can successfully lead to tumor regression and complete responses in a durable manner with potential for cure in some cases. Further studies are needed to augment the antitumor response and better optimize the tumor microenvironment. Following the success of immune checkpoint blockade, a natural next step forward is to combine this with ACT with studies already underway [54].

15.2.5 Vaccines

Extensive resources have been dedicated to the development of cancer vaccines due to their theoretical appeal [55]. Such vaccines have the potential to induce targeted, tumor-specific immune responses with limited toxicity and extended durability. Early attempts utilizing non-mutated, tumor-associated self-antigens were largely unsuccessful in eliciting an adequate immune response [55, 56]. This failure is thought to be a result of central T-cell tolerance to self-antigens, and the focus has now shifted to novel vaccine components, based on whole tumor cells, specific peptides, and DNA/RNA-based vaccines [55–62]. In addition, oncolytic viral vaccines and intratumoral immunotherapies have also garnered significant attention recently [62–67].

15.2.5.1 Immunotherapeutic Vaccines

Several highly immunogenic peptides are being investigated for the treatment of advanced melanoma, given their ability to induce an epitope-specific T-cell response against malignant melanocytes [57–59]. A short peptide vaccine using a modified gp100 peptide was investigated in a phase III RCT after preclinical studies demonstrated impressive T-cell-stimulating capabilities [57, 58]. Its addition to IL-2 resulted in a modest but significant improvement in ORR (16% vs. 6%, $p = 0.03$) and PFS (2.2 months vs. 1.6 months, $p = 0.008$) [58]. Another peptide vaccine composed of a mixture of 6 melanoma helper peptides (6MHP) was found to significantly improve OS (95% and 57% at 1 and 5 years vs. 57% and 16% in the control group, $p < 0.001$) [59].

More recently, an RNA-based nanoparticulate intravenous vaccine, Melanoma FixVac, was investigated in its first human trial [60]. The vaccine contains four tumor-associated, highly-immunogenic antigens, including squamous cell carcinoma 1 (NY-ESO-1), melanoma-associated antigen A3 (MAGE-A3), tyrosinase, and transmembrane phosphatase with tensin homology (TPTE) [60]. The antigen combination activates type I interferon pathways via TLR-7, resulting in tumor-specific T-cell expansion. A total of 56 patients received either FixVac monotherapy or a combination of FixVac + anti-PD1 therapy. Out of 25 evaluable patients in the FixVac monotherapy group, 1 patient had CR, 3 patients had PRs, and 7 had SD. In the combination FixVac/anti-PD-1 inhibitor group ($n = 17$ evaluable patients), six patients developed a PR, and two had SD. Most patients had a durable response over an observation period of over 2 years [60]. The authors also demonstrated that most patients developed either an antigen-specific CD4+ T-cell response or a mixed CD4+ and CD8+ response. In some patients who had failed immunotherapy, the addition of FixVac to the treatment regimen resulted in a subsequent clinical response to another round of PD-1 inhibition [60]. In contrast, a DNA-based vaccine containing gp100 and TRP-2 showed limited clinical efficacy in phase I/II trial, with only 1/15 patients displaying an objective response [61].

Lastly, an autologous melanoma vaccine is currently under investigation as adjuvant therapy in stage IIIB and IIIC disease [62]. After the isolation of melanoma cell lines from each patient's resected specimens, the BCG vaccine is added to potentiate the immune response, and eight vaccine doses at three-week intervals are typically given [62]. The use of patients' own resected tumors to develop the vaccine has the advantage of overcoming tumor antigen variability amongst individuals, and the inclusion of each patient's own major histocompatibility complex molecules is believed to be crucial in producing an antigen-specific lymphocytic response [62]. A phase II trial in 35 patients with stage IIB or III disease displayed an overall 5-year OS of 54% and DFS of 34%. A delayed-type hypersensitivity (DTH) reaction to an intradermal injection of the melanoma isolate was found to strongly correlate with OS and DFS [62]. Patients with a strong DTH had significantly improved outcomes when compared to patients with a weak DTH response (OS of 75% vs. 44% [$p < 0.0001$] and DFS of 47% vs. 26% [$p = 0.27$], respectively) [62]. The trial also demonstrated significantly improved 3-year OS in combination with ipilimumab when compared

to nonvaccinated patients treated with ipilimumab alone (46% vs. 19%, $p = 0.007$) [62].

Contrary to most other treatments discussed in this chapter, vaccines have demonstrated an excellent tolerability profile. AEs overall were mild and transient when used as monotherapy, most commonly including transient flu-like symptoms [56–62]. The clinical efficacy as a monotherapy leaves more to be desired. However, their addition to immunotherapy has the potential to augment the already impressive efficacy of PD-1/CTLA-4 inhibitors. Therefore, the combination of both treatment modalities is a promising therapeutic avenue that requires further investigation.

15.2.5.2 Intratumoral Immunotherapy

Intratumoral immunotherapies cause direct neoplastic cell lysis, releasing tumor-specific antigens and resulting in targeted T-cell activation [63]. The end result is enhanced locoregional anti-neoplastic response with reduction of systemic toxicity [63]. Intratumoral agents are subdivided into oncolytic viral (OV) therapies, such as talimogene laherparepvec (T-VEC) and non-oncolytic viral therapies, including PV-10 and toll-like receptor 9 agonists [63].

T-VEC is the first FDA-approved therapy in this class and is discussed in more detail in the Systemic Therapy in Melanoma chapter. Various other intratumoral agents have shown promise in early studies [64–67]. Most notably, Coxsackievirus A21 (i.e., CAVATAK) is an OV that preferentially causes tumor cell lysis by recognizing increased levels of intercellular adhesion molecule 1 (ICAM-1) on cell surfaces [64]. A phase II, open-label study involving 57 patients with stage IIIC-IV M1c melanoma was considered successful after the primary end point was reached, with 36.8% of patients having PFS at 6 months [64]. The treatment also resulted in increased CD-8+ T-cell infiltration and increased expression of PD-L1+ cells in 4/4 patients who previously failed immunotherapy [64]. The authors, therefore, concluded that CAVATAK showed promising clinical efficacy and may improve response rates in combination with immunotherapy, which is currently being investigated in numerous studies [64]. A phase Ib study combining CAVATAK with ipilimumab demonstrated an ORR of 50% (9/18 patients) with minimal toxicity [65]. The treatment was considered to be well-tolerated in both studies [64, 65].

HF-10 is an OV containing herpes simplex virus-1, which replicates efficiently within tumor cells, resulting in impressive cytolytic activity and subsequent tumor-specific immune activation [66]. A phase II trial combining HF-10 with ipilimumab displayed a CRR of 16% (7/44 patients) and an ORR of 41% (18/44 patients) [67]. The treatment was well-tolerated with minimal side effects [67]. Both CAVATAK and HF10 are under further investigation in combination with PD-1 inhibitors (NCT03259425 and NCT02565992, respectively) [63].

15.3 Conclusion

The introduction of targeted inhibitors and immune-based therapies in the armamentarium against metastatic melanoma has dramatically improved survival outcomes. Over the past decade, clinicians and scientists have sought to build upon the early success of MAPK pathway inhibitors and immunotherapies in an attempt to improve the efficacy and durability of responses. While no individual treatment modality has matched the efficacy of our current first-line treatments, numerous systemic and intratumoral agents have the potential to augment their responses. The future of melanoma treatment underscores the use of combination therapy as the way forward. The challenge will be to choose the right combination of agents for the right patient. The ultimate goal lies in personalizing oncologic therapy for each individual that is specific to the characteristics of their own tumor and immune system. In contrast to the limited treatment options for metastatic melanoma prior to 2011, the currently approved therapies discovered over the past decade have been a truly astounding explosion of science. The next decade promises further advances in this very exciting field.

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