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MELANOMA (RJ SULLIVAN, SECTION EDITOR)

Novel Targets for the Treatment of Melanoma

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Abstract

Purpose of Review In this article, we will briefly review the current treatment landscape for metastatic melanoma and provide a comprehensive update on emerging novel treatment strategies.

Recent Findings Over the past decade, remarkable advances in immunotherapy and targeted therapy have greatly improved outcomes for patients with advanced melanoma. Although a subset of patients is able to achieve durable responses, the majority experience eventual disease progression on existing therapies. Trials evaluating novel combinatorial strategies, checkpoint inhibitors, immune agonists, T cell–based therapies, intratumoral agents, and others are ongoing.

Summary While strides have been made in the treatment of advanced melanoma, further research is needed to identify alternative immune and molecular targets in order to overcome resistance and achieve better clinical outcomes.

Keywords Melanoma · Immunotherapy · Checkpoint inhibitors · CTLA-4 · PD-1 · PD-L1 · Targeted therapy · MEK inhibitor · BRAF inhibitor · Oncolytic therapy

Introduction and Current Treatment Landscape

Melanoma is the sixth most common fatal malignancy in the USA [1]. According to the American Cancer Society, an estimated 91,270 new cases of melanoma were diagnosed in 2018 with an estimated 9320 deaths [2]. Outcomes for patients with metastatic melanoma have improved dramatically over the past decade, but the majority ultimately succumb to their disease, with 5-year overall survival rates of 30% to 40% [3, 4]. In this review, we discuss the current treatment landscape and provide an update on emerging novel treatment strategies, with a focus on therapies being studied in the phase II and III settings.

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Immune Checkpoint Inhibition

Antibodies targeting the checkpoint proteins cytotoxic T lymphocyte–associated protein 4 (CTLA-4) and programmed death 1 (PD-1), first developed for the treatment of advanced melanoma, have dramatically altered the therapeutic landscape of oncology. Ipilimumab, a monoclonal anti-CTLA-4 antibody, was the first immune checkpoint inhibitor approved by the FDA in 2011 following phase III studies demonstrating a survival benefit over dacarbazine in advanced melanoma. The anti-PD-1 antibodies nivolumab and pembrolizumab were subsequently approved in 2014 and 2015, respectively, based on pivotal studies that showed further improvements in overall response and survival compared to both chemotherapy and ipilimumab [5•, 6, 7].

Given that CTLA-4 and PD-1 downregulate different stages of T cell activation, combination checkpoint inhibition was hypothesized to yield increased antitumor immune responses. In CheckMate 067, the objective response rate was 58% in advanced melanoma patients receiving combined nivolumab plus ipilimumab, compared to 44% for those receiving nivolumab alone and 19% for those receiving ipilimumab alone. Similarly, median PFS and OS were numerically highest in the combination arm, a survival benefit that persisted with longer follow-up. Four-year overall survival rates were 53% and 46% in the combination and nivolumab monotherapy arms, respectively [8•]. Long-term follow-up

from KEYNOTE-029 was recently presented at the 2019 Society for Melanoma Research annual conference [9]. Standard-dose pembrolizumab plus reduced-dose ipilimumab at 1 mg/kg resulted in an objective response rate (ORR) of 62% (27% complete response (CR)), with 84% of responses ongoing at 3 years. After a median follow-up of 36.8 months, the median PFS and OS are not yet reached (3-year PFS rate of 59% and 3-year OS rate of 73%).

The longest survival data exist for ipilimumab and nivolumab and indicate durable benefit. In a pooled analysis of patients with advanced melanoma treated with ipilimumab, survival plateaued at 20% beginning around year 3, with follow-up extending up to 10 years [10]. In patients with advanced melanoma who received nivolumab monotherapy in the initial phase I study, 34% remained alive 5 years after starting treatment [11].

Targeted Therapy

Dysregulation of the mitogen-activated protein kinase (MAPK) pathway is present in almost all melanomas. Activating mutations in BRAF are found in approximately 50% of cutaneous melanomas (80–90% V600E mutation, 10–20% V600K mutation), in particular those arising in non-chronically sun-damaged skin [12]. The BRAF inhibitors vemurafenib and dabrafenib were approved for the treatment of *BRAF*-mutant advanced melanoma in 2011 and 2013, respectively, following demonstration of a survival benefit when compared to dacarbazine [13]. However, inhibition of BRAF alone was associated with limited response durations of 6 months to 7 months and the development of secondary RAS-driven malignancies such as cutaneous squamous cell carcinomas due to a relief of ERK-mediated negative feedback [14].

Concurrent inhibition of MEK and BRAF overcame several of these limitations and led to improvements in ORR, PFS, and OS compared to BRAF inhibitor monotherapy. Dual-MAPK pathway inhibition is now considered a standard treatment option for patients with *BRAF*-mutant melanoma, with now three FDA-approved BRAF/MEK inhibitor combinations [7]. The newest regimen, encorafenib and binimetinib, demonstrated improved response and survival compared to vemurafenib alone and encorafenib alone in the three-arm phase III COLUMBUS trial [15•]. Although direct comparisons cannot be made with earlier studies of dabrafenib plus trametinib (COMBI-d) or vemurafenib plus cobimetinib (coBRIM), unprecedented survival figures (median PFS 14.9 months and median OS 33.6 months) were reported in the encorafenib plus binimetinib arm [16, 17]. Additionally, treatment was associated with lower rates of pyrexia and photosensitivity than what was previously observed in the COMBI-d and coBRIM studies. Although the trial was not powered to compare the two BRAF monotherapy arms, encorafenib was associated with superior response rates and longer survival times compared to vemurafenib, consistent with in vitro data showing a longer dissociation half-life and increased antitumor activity for encorafenib [18].

Although durable responses with targeted therapy may be less common than with immunotherapy, recent follow-up data demonstrates that a subset of patients can derive long-term clinical benefit. The 5-year landmark analysis of the phase II trial of dabrafenib plus trametinib in BRAF/MEK inhibitor-naïve patients with metastatic melanoma reported stable 4-year and 5-year PFS and OS rates of 13% and 30%, respectively [19•]. Although these findings need to be replicated in larger phase III studies, the available data seem to indicate a durable plateau in survival.

Oncolytic Therapy

Talimogene laherparepvec (T-VEC) was the first oncolytic viral therapy approved for the treatment of cancer in the USA. T-VEC consists of a modified herpes simplex virus 1 that expresses granulocyte macrophage–colony-stimulating factor (GM-CSF) in the tumor and recruits antigen-presenting cells [20]. In patients with advanced melanoma who have predominantly injectable cutaneous, subcutaneous, and nodal disease with limited visceral involvement, the durable response rate was significantly higher with T-VEC compared to GM-CSF. The difference in OS was not statistically significant in the overall study population, but there was a survival benefit among patients with stage III and IV disease limited to skin, subcutaneous lesions, and lymph nodes [20, 21].

More intriguing data comes from the use of T-VEC in conjunction with immune checkpoint inhibition. The combination of T-VEC and ipilimumab demonstrated a significantly higher response rate compared to ipilimumab alone (ORR 39% vs 18%, $p = 0.002$), with responses in uninjected visceral lesions observed in 52% of patients in the combination arm compared to only 23% of patients in the ipilimumab arm, suggesting an enhanced systemic antitumor response [22•]. Similarly, the combination of T-VEC plus pembrolizumab has resulted in promising preliminary response rates (MASTERKEY-265) [23].

Enhancing the Efficacy of Targeted Therapy

Combination BRAF/MEK and Checkpoint Inhibition

The combination of BRAF/MEK and PD-1 axis inhibition offers a promising therapeutic strategy. The MAPK axis is involved in normal T cell receptor signaling [24]. Selective MAPK inhibition has been shown to promote tumor recognition by the immune system through an increase in activated CD8+ T cell infiltrate, melanoma major histocompatibility complex (MHC) expression, and melanoma antigen

presentation [25–27]. More recent data demonstrated that MEK inhibition induces the accumulation of antigen-specific CD8+ T cell effectors in tumors by preventing T cell exhaustion and death. Further release of T cell inhibition by combining MEK inhibition with anti-PD-L1 therapy resulted in synergistic and durable inhibition of tumor growth in a mouse colorectal carcinoma model [28]. Other preclinical models have shown a similar enhancement in antitumor activity with combined BRAF/MEK and PD-1/PD-L1 inhibition, including tumor types that are considered immunologically cold such as triple-negative breast cancer [29–31].

Clinically, the combination of cobimetinib and atezolizumab (anti-PD-L1 Ab) led to an ORR of 45% and a median PFS of 12.0 months in 22 patients with metastatic melanoma [32]. Preliminary data from a phase Ib study of vemurafenib, cobimetinib, and atezolizumab in patients with *BRAF*-mutant melanoma similarly demonstrates impressive clinical activity with an unconfirmed ORR of 85.3% [32]. A phase II study evaluating cobimetinib plus vemurafenib followed by atezolizumab is currently ongoing (NCT02902029). In the phase III COMBI-i study of the anti-PD-1 Ab spartalizumab (PR001) combined with dabrafenib and trametinib in treatment-naïve patients with *BRAF*-mutant metastatic disease, 9/9 patients in the initial safety run-in had confirmed responses [33]. An additional 7/7 evaluable patients in the biomarker portion of the study had unconfirmed partial responses. Preliminary analyses of paired baseline and on-treatment biopsies show a substantial increase in tumor-infiltrating CD8+ cells with treatment. The combination of pembrolizumab, dabrafenib, and trametinib (KEYNOTE-022) produced numerically longer 12-month PFS (59% vs 45%) and OS (80% vs 73%) rates compared to BRAF/MEK inhibition alone, but more severe treatment-related adverse events also occurred with higher frequency (58% vs 27%) in the combination arm [19•]. The IMPemBra trial compared pembrolizumab monotherapy with a combination of pembrolizumab and intermittent dabrafenib plus trametinib to assess the optimal duration of combination therapy due to concern for toxicity associated with continuous use. Comparable efficacy with reduced toxicities and discontinuation rates was observed when BRAF/MEK inhibitors were administered for 1 week prior to each dose of pembrolizumab (2 weeks vs 3 weeks of therapy) [34].

There are several ongoing trials evaluating BRAF and MEK inhibition in combination with checkpoint inhibition. Concurrent and sequential pembrolizumab and dabrafenib plus trametinib are also being evaluated in the neoadjuvant setting in patients with *BRAF*-mutant resectable stage III melanoma (NCT02858921). The question of whether to use initial checkpoint inhibition or targeted therapy in *BRAF*-mutant melanoma is being evaluated by an NCI phase III trial that randomizes patients to upfront either ipilimumab plus nivolumab or dabrafenib plus trametinib, followed by crossover at the time of disease progression (NCT02224781).

Targeted Therapy Beyond BRAF and MEK

Oncogenic mutations in *NRAS*, *NF1* (a negative regulator of RAS signaling), and *KIT* represent other recurrent genomic subtypes of melanoma. Given the presence of multiple RAS effector pathways, targeting *NRAS* and *NF1* poses greater therapeutic challenges. Downstream MEK inhibition with binimetinib produced partial responses in 6 (20%) of 30 patients with *NRAS*-mutant melanoma in an open-label phase II study [35]. The subsequent phase III NEMO trial randomized 402 patients with *NRAS*-mutant disease to either binimetinib or dacarbazine [36]. Treatment with binimetinib was associated with prolonged PFS (2.8 months vs 1.5 months) and improved ORR (15% vs 7%), with no significant difference in OS (11 months vs 10 months) due in part to receipt of subsequent immunotherapy in 45% of patients. Given that oncogenic RAS signaling may upregulate PD-L1 expression via post-transcriptional stabilization of PD-L1 mRNA, combined MEK and checkpoint inhibition may be a valid therapeutic approach in *RAS*-mutant melanoma.

Mutations in *KIT*, while less commonly seen in non-acral cutaneous melanomas, are observed in 15% to 20% of acral and mucosal melanomas. A number of prospective trials have assessed the efficacy of imatinib and other tyrosine kinase inhibitors (TKIs) such as dasatinib and nilotinib in patients with *KIT*-altered melanoma. Overall response rates range from 15 to 30%, with nearly all responses observed in the subgroup of melanomas harboring exon 11 or 13 *KIT* mutations [37–40]. While some patients experience durable responses, the median time to progression of 3–4 months is quite short compared to what is achieved with TKIs in chronic myeloid leukemia and gastrointestinal stromal tumors (GISTs). The combination of KIT inhibition with immunotherapy is an area of active investigation. Preclinical data in GIST has shown that TKIs promote antitumor immunity and may have synergistic activity with immune checkpoint blockade [41]. Initial results from a phase I trial of imatinib plus ipilimumab in patients with advanced malignancies (NCT01738139) show partial responses in two patients, one with GIST and the other with *KIT*-mutant melanoma [42]. A phase I/II trial of imatinib plus pembrolizumab was initiated in patients with locally advanced or metastatic *KIT*-altered melanoma (NCT02812693) but was discontinued due to poor accrual.

Resistance to MAPK-targeted therapy is mediated by multiple mechanisms including reactivation of the MAPK pathway and upregulation of alternative signaling pathways, e.g., PI3K/AK [43]. The antitumor activity of BRAF and MEK-targeted therapy is mediated in part by induction of apoptosis through upregulation of BIM and suppression of BCL-2 and MCL-1, supporting combination therapy with anti-apoptotic agents. Navitoclax is a BH3 mimetic that inhibits BCL-2, BCL-xL, and BCL-w. The combination of navitoclax and a MEK inhibitor enhanced cell death in *BRAF*-mutant

melanoma cell lines and xenograft models compared to either agent alone [44]. Similarly, the addition of navitoclax to the BRAF inhibitor PLX-4720 led to greater reduction in *BRAF*-mutant cell viability and xenograft tumor regression [27]. An ongoing phase II study of dabrafenib, trametinib, and navitoclax is enrolling patients with *BRAF*-mutant unresectable or metastatic melanoma naïve to BRAF/MEK inhibition (NCT01989585).

Novel Immunologic Strategies

The challenges of both primary and secondary resistance to the approved immunological checkpoint inhibitors have led to the identification and therapeutic targeting of additional novel immunological pathways (Table 1).

Novel Immunological Checkpoint Inhibitors

Inhibitory checkpoint molecules beyond CTLA4 and PD-1/PD-L1 have demonstrated significant activity in preclinical models. One of the more clinically advanced of these novel targets in melanoma is LAG3. LAG3 is a member of the Ig superfamily that is present on T cells, B cells, dendritic cells, and NK cells [45]. LAG3 exerts an inhibitory effect on effector T cell proliferation and enhances regulatory T cell function [45, 46]. T cells in the melanoma tumor microenvironment have high LAG3 expression [47, 48]. PD-L1-positive melanomas have also shown dramatic increases of LAG3 expression [49, 50].

Relatlimab (BMS-986016), LAG525, and MK-4280 are anti-LAG3 antibodies currently in clinical development. Relatlimab was the first in class antibody and is the furthest in development for melanoma. In the phase I expansion cohort of heavily pretreated patients with advanced melanoma refractory to or relapsed on anti-PD-1/PD-L1 therapy, the ORR to relatlimab in combination with nivolumab was 12.5% in evaluable patients (n = 48; NCT01968109) [51]. A phase II/III trial evaluating relatlimab in combination with nivolumab versus nivolumab single agent is ongoing (NCT03470922). LAG525 is currently undergoing phase I/II clinical trials as monotherapy or in combination with anti-PD-1 (NCT02460224). MK-4280 is being studied in a phase I study as a monotherapy or in combination with pembrolizumab (NCT02720068) [52].

Additional checkpoint inhibitors are also in clinical development, including agents targeting T-cell immunoglobulin and mucin-domain containing-3 (TIM3), TIGIT, PVRIG, VISTA, and others. TIM3 is an inhibitory receptor expressed on a variety of immune cells including cytotoxic T cells, regulatory T cells, and dendritic cells. TIM3 has been demonstrated to be expressed in melanoma tumor-infiltrating lymphocytes (TILs) [53, 54]. TIM3 blockade has been shown to reverse T cell exhaustion and dysfunction in animal models of advanced melanoma [46, 54, 55]. There are several anti-TIM3 antagonists in early-phase development for multiple tumor types, including LY3321367 (NCT03099109), TSR-022 (NCT03680508), Sym023 (NCT03489343), BGB-A425 (NCT03744468), and others.

T cell immunoglobulin and ITIM domain (TIGIT) is a receptor expressed primarily by T cells, regulatory T cells, and NK cells, with immunosuppressive effects mediated through the decreased release of pro-inflammatory cytokines and increased release of IL-10 [56]. TIGIT is highly expressed in melanoma cells as well as dendritic cells and monocytes within the melanoma tumor microenvironment [57, 58]. Several early-phase trials are underway of anti-TIGIT antibodies in multiple tumor types, including BMS-986207 (NCT02913313), ASP8374 (NCT03260322), OMP-313M32 (NCT03119428), and others.

*PVR-related immunoglobulin (*PVRIG) is involved in TIGIT inhibitory effects and can also bind another ligand, PVRL2 (CD112), subsequently exerting its own suppressive effects [59]. COM701, a PVRIG antagonist, has demonstrated enhanced CD8 T cell proliferation in vitro while also having a synergistic effect on T cell activation when combined with either anti-PD-1 or anti-TIGIT antibody [60]. COM701 is currently being tested in a phase I clinical trial both as monotherapy and in combination with nivolumab in multiple tumor types (NCT03667716).

V domain Ig-containing suppressor of T cell activation (VISTA) functions as another potent negative regulator of T cells [61]. Preclinical studies of anti-VISTA antibodies have demonstrated increased T cell infiltration within the tumor microenvironment, increased IFN-γ production from CD8 T cells, and decreased tumor infiltration of myeloid cells [61, 62]. CA-170, an orally available inhibitor of both PD-L1/PD-L2 and VISTA, is currently in phase I testing in multiple tumor types (NCT02812875).

Novel Immune Agonist Therapies

The targeting of negative immunological regulatory pathways alone may not be sufficient for optimal cancer control, and the activation of co-stimulatory pathways alone or in combination with checkpoint blockade to enhance an immune response may also be required. The co-stimulatory targets investigated most fully in melanoma thus far include 4-1BB (CD137) and CD40.

4-1BB (CD137) is an important regulator of immune response. When 4-1BB binds its ligand, 4-1BBL, it triggers proliferation and prolonged survival of CD8+ effector T cells and NK cells [63]. Preclinical models showed agonistic antibodies against 4-1BB triggered a potent antitumor T cell response [64]. Utomilumab (PF-05082566) is a fully human IgG2 monoclonal agonist antibody targeting CD137 that has

Table 1 Ongoing clinical trials unresectable or metastatic melanoma

Agent	Mechanism of action	Phase	Trial ID	Status
Anti-LAG3 mAb				
BMS-986016 ± nivolumab	Anti-LAG3 ± anti-PD-1	I/II	NCT01968109	Recruiting
LAG525 ± PDR011	Anti-LAG3 ± anti-PD-1	I/II	NCT02460224	Recruiting
MK-4280 ± pembrolizumab	Anti-LAG3 ± anti-PD-1	I	NCT02720068	Recruiting
Nivolumab ± relatlimab	Anti-PD-1 ± anti-LAG3	II/III	NCT03470922	Recruiting
Anti-TIM3 mAb				
MBB453 ± PDR001	Anti-TIM3 ± anti-PD-1	I/II	NCT02608268	Recruiting
TSR-022 ± anti-PD-1	Anti-TIM3 ± anti-PD-1	I	NCT02817633	Recruiting
SYM023	Anti-TIM3	I	NCT03489343	Recruiting
Anti-TIGIT mAb				
BMS-986207 ± nivolumab	Anti-TIGIT ± anti-PD-1	I/II	NCT02913313	Recruiting
Anti-PVRIG mAb				
COM701 ± nivolumab	Anti-PVRIG ± anti-PD-1	I	NCT03667716	Recruiting
Anti-VISTA mAb				
CA-170 ± VISTA	Anti-VISTA ± anti-PD-L1/PD-L2	I	NCT02812875	Recruiting
Anti-41BB				
BMS-663513 ± nivolumab	Anti-41BB ± anti-PD-1	I	NCT02534506	Recruiting
PF-04518600 ± PF-05082566	Anti-OX40 ± anti-41BB	I	NCT02315066	Recruiting
Avelumab and PF-05082566 ***Experimental arm A only	Anti-PD-1 + anti-OX40	II	NCT03217747	Recruiting
Anti-CD40 mAb				
APX005M ± nivolumab and cabiralizumab	Anti-CD40 ± anti-PD-1 ± anti-CSF1R	I	NCT03502330	Recruiting
APX005M ± pembrolizumab	Anti-CD40 ± anti-PD-1	i	NCT02706353	Recruiting
Anti-GITR mAb				
TXR518	Anti-GITR	I	NCT01239134	Recruiting
TXR518 ± pembrolizumab, nivolumab, or gemcitabine	Anti-GITR ± anti-PD-1 or gemcitabine	I	NCT02628574	Recruiting
INCAGN01876	Anti-GITR	I	NCT02697591	Recruiting
INCAGN01876 ± ipilimumab or nivolumab	Anti-GITR ±anti-PD-1 or anti-CTLA4	I/II	NCT03126110	Recruiting
GWN323 ± PDR001	Anti-GITR ± anti-PD-1	I	NCT02740270	Recruiting
Anti-OX40				
PF-04518600 ± PF-05082566	Anti-OX40 ± anti-41BB	I	NCT02315066	Recruiting
Avelumab ± utomilumab, PF-04518600, PF-04518600		II	NCT02554812	Recruiting
***Combination B: avelumab + PF-04518600	Anti-PD-1 + anti-OX40			
***Combination D: avelumab plus utomilumab plus PF-04518600	Anti-PD-1 + anti-41BB + anti-OX40			
Avelumab and PF-04518600 ***Experimental arm B only	Anti-PD-1 + anti-OX40	II	NCT03217747	Recruiting
BMS-986178 ± nivolumab or ipilimumab	Anti-OX40 ± anti-PD-1 or anti-CTLA4	I/II	NCT 02737475	Recruiting
GSK3174998 ± pembrolizumab	Anti-OX40 ±anti-PD-1	I	NCT02528357	Recruiting
Anti-CD27 and anti-CD70				
CDX-1127 ± nivolumab	Anti-CD27 ± anti-PD-1	I/II	NCT02335918	Not recruiting
ARGX-110	Anti-CD70	I/II	NCT01813539	Not recruiting
STING agonist				
ADU-S100 ± ipilimumab	Anti-STING ±anti-CTLA4	I/II	NCT02675439	Recruiting
MK-1454 ± pembrolizumab	Anti-STING ± anti-PD-1	I/II	NCT03010176	Recruiting
MK-2118 ± pembrolizumab	Anti-STING ± anti-PD-1	I/II	NCT03249792	Recruiting
TLR9 agonist				
IMO-2125 ± ipilimumab or pembrolizumab	Anti-TLR9 ± anti-CTLA4 or anti-PD-1	I/II	NCT02644967	Recruiting
CMP-001 ± pembrolizumab	Anti-TLR9 ± anti-PD-1		NCT02680184	Recruiting
AST-008 + pembrolizumab	Anti-TLR9 + anti-PD-1	Ib/II	NCT03684785	Recruiting

Table 1 (continued)

Agent	Mechanism of action	Phase	Trial ID	Status
Anti-PV10				
PV10 vs dacarbazine or temozolomide or TVEC	Intralesion PV10 vs chemotherapy	III	NCT02288897	Not recruiting
IL-12				
IL-12 vs IL-2 + pembrolizumab	IL-12 vs IL-12 + anti-PD-1	II	NCT02493361	Not recruiting
IL-12 + pembrolizumab	IL-12 + anti-PD-1	Ib	NCT02967692	Recruiting
Other intratumoral agents				
INT230-6	Formulation of cisplatin and vinblastine	I/II	NCT03058289	Recruiting
RPL-001-16	Modified HSV-1	I/II	NCT03767348	Recruiting
IDO				
HTI-1090	IDO inhibitor	I	NCT03208959	Not recruiting
NLG802	IDO inhibitor	I	NCT03164603	Not recruiting
IDO peptide vaccine/anti-PD-L1 + nivolumab	IDO inhibitor/anti-PD-1 peptide vaccine + anti-PD-L1	I/II	NCT03047928	Recruiting
BMS-986205 + ipilimumab + nivolumab	IDO inhibitor ± anti-CTLA4 ± anti-PD-1	I/II	NCT02658890	Recruiting
Anti-CD73				
BMS-986179	Anti-CD73 ± anti-PD-1	I/IIa	NCT02754141	Recruiting
CPI-006 ± pembrolizumab or CPI-444	Anti-CD73 ± anti-PD-1 or anti-A2AR		NCT03454451	Recruiting
A2AR				
CPI-444 ± atezolizumab	Anti-A2AR antagonist ± anti-PD-L1	I	NCT02655822	Recruiting
AZD4635 ± durvalumab	Anti-A2AR antagonist ± anti-PD-L1	I	NCT02740985	Recruiting
AXL pathway				
BGB324 + pembrolizumab or dabrafenib and trametinib vs SOC	Anti-AXL + anti-PD-1 or BRAF inhibitors	I/II	NCT02872259	Recruiting
BA3011	Anti-AXL	I/II	NCT03425279	Recruiting
Lenvatinib				
MK-7902 + pembrolizumab	Anti-VEGFR, anti-FGFR, anti-PDGF, anti-KIT, and anti-RET + anti-PD-1	II	NCT03776136	Recruiting
MK-7902 + pembrolizumab vs pembrolizumab	Anti-VEGFR, anti-FGFR, anti-PDGF, anti-KIT, and anti-RET + anti-PD-1	III	NCT03820986	Recruiting
Avadomide				
Avadomide + nivolumab	CC-122 + anti-PD-1	II	NCT03834623	Recruiting
HDAC inhibitors				
HBI-8000 + nivolumab	HDAC inhibitor + anti-PD-1	I/II	NCT02718066	Recruiting
IL-2 and IFN alpha				
NKTR-214 + nivolumab vs nivolumab	IL-2 + anti-PD-1	III	NCT03635983	Recruiting
T cell receptor therapies				
IMCgp100 + durvalumab and/or tremelimumab	ImmTAC + anti-PD-L1 or anti-CTLA-4	IB/II	NCT02535078	Not recruiting
Personalized cancer vaccines				
RO7198457 ± pembrolizumab	Personalized cancer vaccine ± anti-PD-1	I	NCT03289962	Recruiting
GEN-009 ± nivolumab	Personalized cancer vaccine ± anti-PD-1	I	NCT03633110	Recruiting
Dendritic cell vaccines				
Therapeutic autologous dendritic cells after cryosurgery + pembrolizumab	Dendritic cell vaccine + anti-PD-1	I/II	NCT03325101	Recruiting

been studied in various phase I clinical trials including a phase 1b study in combination with pembrolizumab (NCT02179918) [65]. There are several ongoing phase I/II combination studies of utomilumab, including in combination with PF-04518600 (anti-OX40) (NCT02315066); with avelumab (anti-PD-L1), PF-04518600, or PD 0360324 (M-CSF mAb) (NCT02554812); and with avelumab, PF-04518600, cisplatin, or radiation (NCT03217747). Urelumab (BMS-663513) is a fully human IgG4 monoclonal agonist antibody against CD137. Phase I studies have

demonstrated it to be well tolerated at MTD of 0.1 mg/kg every 3 weeks and resulting in increased expression of IFN-γ and CD8+ T cells in post-treatment biopsies (NCT00309023; NCT00612664; NCT01471210) [66, 67]. Subsequent phase I/II studies evaluating urelumab in combination with nivolumab in metastatic melanoma patients showed an objective response rate of 50% (10/20) in PD-L1+ melanomas and 47% (8/17) in PD-L1− melanomas (NCT02534506) [68].

CD40 is an immune co-stimulatory receptor expressed by antigen-presenting cells. Upon binding to its ligand (CD154) on activated helper T cells, CD40 mediates increased MHC surface expression on dendritic cells, production of pro-inflammatory cytokines, and B cell proliferation, all of which lead to increased priming and activation of CD8+ T cells [69, 70]. Several CD40 agonist antibodies have been developed (e.g., CP-870,893, dacetuzumab, ADC-1013, APX005M) with evidence of antitumor activity in CD40+ B cell malignancies and solid tumors including melanoma [71]. A phase I trial of the CD40 agonist CP-870,893 plus tremelimumab enrolled 24 patients with metastatic melanoma previously untreated with checkpoint blockade. In the 22 evaluable patients, the ORR was 27.3% (2 CR, 4 partial response (PR)) [72]. Notably, 4 of 5 patients who received anti-PD-1 therapy at the time of disease progression are long-term survivors (> 3 years), suggesting that combined CD40 agonist and anti-CTLA-4 therapy may prime and reinvigorate a T cell response that can be subsequently unleashed. Indeed, analysis of peripheral blood T lymphocytes demonstrated enrichment for an activated T cell phenotype (Tbet− Eomes± and CD45RA− CD27+); paired biopsies from 2 patients demonstrated increased tumor CD8 infiltration and PD-L1 expression in post-treatment biopsies compared to baseline biopsies. Preliminary data from the ongoing phase Ib/II study (NCT03123783) of APX005M and nivolumab in metastatic melanoma patients who have progressed on anti-PD-1 therapy was recently presented at the 2019 AACR Annual Meeting [73]. Of the 12 enrolled patients, 2 achieved confirmed partial responses and 3 achieved stable disease (2 with prolonged stable disease (SD) lasting > 8 months). APX005M is also being evaluated in combination with nivolumab and cabiralizumab (anti-colony-stimulating factor 1 receptor (CSF1R) antibody) in a phase I study (NCT03502330) and as an intratumoral injection in combination with pembrolizumab in a phase I/II trial (NCT02706353).

There are multiple co-stimulatory targets in addition to 4-1BB and CD40, including GITR, OX40, CD27, CD70, and others, which are currently being studied both preclinically and clinically in melanoma and other tumor types. Glucocorticoid-induced TNF receptor (GITR) is a co-stimulatory TNF receptor super family member. When GITR binds its ligand, it exerts a dual effect, triggering the downregulation of regulatory T cells and upregulating CD8+ effector cells while also extending their survival [74, 75]. There are multiple anti-GITR agents being studied in clinical trials, including TRX518 (NCT01239134), INCAGN01876 (NCT03126110), GWN323 (NCT02740270), and others.

Much like GITR, OX40 is a T cell co-stimulatory receptor and is primarily expressed on activated T cells and antigen-presenting cells [76]. However, OX40 is only present on T cells after activation [77]. In cutaneous melanoma, an increase in OX40 expression on tumor-infiltrating lymphocytes has been associated with an improved prognosis [78]. There are a number of OX40 agonists currently in phase I testing, including MEDI0562 (NCT02705482), 9B12, a murine IgG mAb agonist of OX40 (NCT01644968) [79], PF-04518600 (NCT02315066), and others.

CD27 is a glycoprotein that belongs to the TNF family and is expressed on T cells, NK cells, and regulatory T cells [80]. Its ligand, CD70, is expressed on dendritic cells and activated T and B lymphocytes. When bound to CD70, CD27 enhances CD8+ T cell activation, survival, and effector function and triggers the differentiation of CD4+ T cells into Th1 CD4+ T cells that secrete IFN-γ [81–83]. Several agents have initiated clinical testing, including varlilumab (CDX-1127), an anti-CD27-mAb, (NCT02335918) [84], and ARGX-110, an anti-CD70-mAb (NCT01813539).

Novel Intratumoral Immunotherapeutic Agents

A number of novel intratumoral agents are being investigated alone and in combination with checkpoint inhibition in melanoma and other solid tumors. Of these, the Toll-like receptor 9 (TLR9) agonist tilsotolimod (IMO-2125) is the furthest in development for melanoma.

TLRs are a family of specialized receptors that stimulate immune responses to pathogen-associated molecular patterns. Among these, TLR9 has been shown to induce potent antitumor responses [85]. Upon binding to unmethylated CpG dinucleotides, a motif in bacterial and viral DNA, TLR9 stimulates a robust innate and adaptive immune response, ultimately leading to a strong CD4+ and CD8+ T cell response that may augment the efficacy of immune checkpoint inhibition [86]. Tilsotolimod is a potent TLR9 agonist with demonstrated clinical activity in patients with advanced melanoma refractory to PD-(L)1 inhibition. On-treatment tumor biopsies from patients treated with tilsotolimod in combination with either ipilimumab or pembrolizumab in a phase I dose escalation study (NCT02644967) demonstrated maturation of myeloid DC1 subsets and an IFN-α response gene signature in both injected and uninjected tumors [87]. Additionally, higher CD4+ and CD8+ T cell proliferation rates were observed in tumor biopsies from responding patients. Preliminary data from the first 15 patients treated with IMO-2125 and ipilimumab in the phase II component of the same study showed a 47% ORR and 67% disease control rate, with 1 CR, 6 PR, and 3 SD [88]. These promising

response rates provided the impetus for the randomized phase III study (ILLUMINATE 301) comparing ipilimumab 3 mg/kg with or without intratumoral IMO-2125 (8 mg) in patients with advanced cutaneous or mucosal melanoma refractory to anti-PD(L)1 therapy (NCT03445533). A number of other intratumoral TLR9 agonists are being evaluated in ongoing phase I/II trials in metastatic melanoma (NCT02680184, NCT03684785). For example, the combination of SD-101 (Dynavax), a synthetic CpG oligonucleotide, and pembrolizumab produced an ORR of 78% in 9 patients naïve to anti-PD-1 therapy with estimated 12-month PFS and OS rates of 88% and 89%, respectively [86]. The response rate was lower at 15% among 13 patients previously treated with anti-PD-1 therapy.

PV-10 is a small-molecule fluorescein derivative solution of rose bengal (10%) that triggers rapid cell death upon release from lysosomes. Following promising phase I data, a multicenter, international phase II trial of PV-10 was conducted in 80 patients with refractory cutaneous and subcutaneous metastatic melanoma [89]. Twenty-five percent of patients achieved complete responses, and 26% achieved partial responses in their injected lesions, accounting for an ORR of 51%. Among the 42 patients with designated bystander lesions, 26% and 7% also experienced complete and partial responses, respectively, in their uninjected lesions [90]. Although the study did not formally assess visceral disease, 4 out of 19 patients with stage IV disease had stable disease or partial response in their visceral lesions. This, along with the observed response in bystander lesions, may reflect the generation of a systemic antitumor immune response and suggest a potential role for PV-10 in combination with checkpoint blockade in patients with advanced melanoma. Preliminary results of a phase Ib study of PV-10 plus pembrolizumab (NCT02557321) showed acceptable safety and tolerability with a higher target lesion response rate compared to single-agent PV-10 [91]. An ongoing phase III study (active, not recruiting) is comparing PV-10 to investigator's choice of dacarbazine, temozolomide, or T-VEC in patients with locally advanced cutaneous melanoma who are not candidates for targeted therapy or immune checkpoint inhibition (NCT02288897).

IL-12 is a pro-inflammatory cytokine that can induce intratumoral inflammation and recruitment of T cells. Systemic IL-12 therapy has been associated with limited benefit and significant toxicity [92]. Intratumoral injection of plasmid IL-12 followed by electroporation (IT-pIL12-EP) was developed as a way to deliver IL-12 in a targeted manner and minimize toxicity. Following phase I data demonstrating safety in patients with metastatic melanoma, a single-arm phase II trial was carried out in patients with stage III–IV melanoma [93]. Preliminary data in 29 patients demonstrated an ORR of 33% with a CR rate of 11%. Sixty-two percent of patients also experienced regression of non-injected lesions [94]. Two additional studies have been initiated to address the combination of IT-pIL12-EP and checkpoint inhibition. Preliminary results from a phase II study (NCT02493361) evaluating IT-pIL12-EP plus pembrolizumab showed an ORR of 40% among melanoma patients predicted to be non-responsive to anti-PD-1 therapy (tumor samples with < 25% PD-1^{hi} CTLA-4+ tumor-infiltrating T cells) [95]. The ongoing phase II PISCES study is evaluating this combination in advanced melanoma patients who experienced disease progression on either pembrolizumab or nivolumab (NCT03132675).

A number of other intratumoral agents are in development, including STING agonists, novel oncogenic viral therapies, and novel chemotherapy formulations. The stimulator of interferon genes (STING) is a receptor involved in activating the innate immune system by stimulating type 1 IFN-1 and DC activation [96]. In B16 melanoma murine studies, a single intratumoral dose of DMXAA (STING agonist) led to complete tumor regression in almost all the mice [96]. STING agonists are being evaluated in multiple phase I/II studies alone and in combination with checkpoint inhibitors (NCT02675439, NCT03010176, NCT03249792). INT230-6 is a formulation of cisplatin and vinblastine with amphiphilic penetration enhancer for selective high payload delivery into tumor cells (NCT03058289). RPL-001-16 is a modified HSV1 virus being evaluated alone and in combination with nivolumab in advanced tumors with phase II cohorts including cutaneous and ocular melanoma (NCT03767348).

Modulating the Immune Microenvironment

The tumor microenvironment consists of fibroblasts, stromal cells, extracellular matrix, vasculature, and immune cells that promote tumor growth and invasion [97]. These tumor-promoting functions include increased angiogenesis, immune evasion, and inhibition of apoptosis, among others. Various therapeutic targets that modulate the microenvironment have been identified.

Indoleamine 2,3-dioxygenase (IDO) plays a multifaceted role in the tumor microenvironment; it inhibits effector T cells and NK cells and activates Tregs and MDSCs [92, 98]. Studies have demonstrated that IDO1 is upregulated in several malignancies [99]. In melanoma specifically, an interconnected relationship between the expression of IDO, PD-L1, and CTLA-4 was established and was associated with negative outcomes, independent of disease stage, suggesting a potential treatment target [100]. Epacadostat, an IDO inhibitor, was found to be well tolerated in phase I testing with SD of greater than 16 weeks observed in 7 of 52 patients [101]. A phase I/II trial evaluating epacadostat with ipilimumab in subjects with unresectable or metastatic melanoma found an ORR of 75% (9/12) with one patient achieving a complete response [102]. The combination of pembrolizumab plus epacadostat produced a promising ORR of 56% in 54 evaluable patients in the phase I/II ECHO-202/KEYNOTE-037 study. Among treatment-naïve patients who received the recommended phase II dose of epacadostat 100 mg BID, the ORR was

60% [103]. Unfortunately, the pivotal phase III ECHO-301/KEYNOTE-252 study failed to demonstrate any survival benefit with the combination over pembrolizumab alone in 706 patients with unresectable advanced melanoma [104]. Combination therapy did not produce a significantly longer overall survival, median PFS, or ORR. A phase I/II trial evaluating indoximod, another IDO inhibitor, in combination with ipilimumab, nivolumab, and pembrolizumab in melanoma is currently ongoing (NCT02073123). Preliminary results from this trial showed an ORR of 55.7% (39/70) with a CR rate of 18.6% (13/70). Median PFS was 12.4 months [105]. There are several other IDO inhibitors in phase I testing with no data available at this time (NCT03208959, NCT03164603, NCT03047928, NCT02658890).

The adenosine pathway is a major factor in the immunosuppressive tumor microenvironment. This pathway is mediated by ectonucleases CD39 and CD73 and the adenosine receptors, e.g., A1R, A2AR, and A2BR [106, 107]. Even though they are constitutively expressed on various cells, their expression drastically increases in response to pro-inflammatory cytokines and in hypoxic environments. Most importantly, CD39 and CD73 are expressed on Tregs. In melanoma, an increased expression of CD73 was found to correlate with a more aggressive, invasive phenotype and was found in 54% of melanoma metastasis [108, 109]. Other studies have found that CD39 was overexpressed earlier in tumor development, indicating its potential role in influencing the differentiation of melanocytes into malignant cells. Preclinical models have found that anti-CD73 and anti-CD39 antibody treatment inhibits metastasis formation and improves antitumor immunity [110, 111]. There are two anti-CD73 agents currently in early-phase testing. BMS-986179 is an anti-CD73 mAb being evaluated in a phase I/IIa clinical trial as a single agent or in combination with nivolumab (NCT02754141). Preliminary data have demonstrated tolerability in both cohorts and efficient inhibition of CD73 activity. Out of 59 patients, 7 had a PR and 10 had SD [112]. MEDI99447 is an anti-CD73 mAb that is currently being tested in phase I trial as monotherapy or in combination with MEDI4736 (anti-PD-L1) (NCT02503774).

When adenosine binds to the A2A receptor on effector T cells, it inhibits proliferation and production of cytokines and limits overall cytotoxicity [113]. It also stimulates the increased expression of negative regulatory mediators such as PD-1, CTLA-4, and LAG3 [107, 108]. In Tregs, the overall effect is stabilization with increased expression of FOXP3 [114]. In DC, it prevents maturation and results in increased production of IL-10, TGF beta, and IDO, all of which have known immunosuppressive effects. There are several A2AR antagonists currently in development in the early phase and in multiple malignancies. A phase I clinical trial evaluating CPI-444, an A2AR antagonist, as monotherapy or in combination with atezolizumab is ongoing (NCT02655822). Preliminary results of the RCC and NSCLC cohorts demonstrated that though the majority of patients were PD-1/PD-L1 resistant/refractory (75% in RCC, 68% in NSCLC), the disease control rates were 86% and 50%, respectively [115]. CPI-006, a type 2 humanized IgG1 antibody inhibiting enzymatic activity of CD73, is being evaluated in a phase I trial as single agent or in combination with either pembrolizumab or CPI-444 (NCT03454451). Another phase I/II study of A2AR antagonist NIR178 (PBF-509) in patients with NSCLC is also showing promising results (NCT02403193). AZD4635, another anti-A2AR antagonist, is also being evaluated in a phase I trial as a single agent or in combination with durvalumab (anti-PD-L1) in advanced solid malignancies (NCT02740985).

CSF1R signaling regulates macrophage proliferation and differentiation and may polarize tumor-associated macrophages (TAMs) into the immunosuppressive, pro-tumorigenic M2 phenotype. Induction of CSF1 expression and consequent recruitment of M2 macrophages by IFN-γ and TNF-α constitute a negative feedback mechanism that suppresses T cell activity [116]. There is an ongoing phase Ib/II basket trial of lacnotuzumab (anti-CSF1 Ab) combined with spartalizumab (anti-PD-1 Ab) in patients with advanced melanoma and endometrial, pancreatic, and triple-negative breast cancer [117].

AXL is highly overexpressed on various cells in the TME and exerts immunosuppressive effects through several pathways [118, 119]. It has also been shown to mediate acquired drug resistance to MAPK and PI3K/AKT pathway–targeted therapy by enabling alternative growth pathway signaling [118]. BGB324, a novel AXL inhibitor, is being studied in an ongoing phase I/II trial in combination with pembrolizumab or dabrafenib and trametinib in comparison to standard treatment alone for advanced melanoma patients (NCT02872259). An antibody drug conjugate targeting AXL (BA3011) is also being developed in a phase I/II trial with expansion cohorts in melanoma, NSCLC, and pancreatic cancer (NCT03425279).

Other Agents and Cytokines

Lenvatinib is a multi-tyrosine kinase inhibitor with activity against VEGF receptors VEGFR1, VEGFR2, and VEGFR3; FGF receptors FGFR1, FGFR2, FGFR3, and FGFR4; platelet-derived growth factor alpha; KIT; and RET which are implicated in tumor angiogenesis and cell proliferation [120, 121]. Phase I studies in multiple malignancies including melanoma have previously demonstrated clinical activity. Prominent toxicities included high blood pressure, proteinuria, and fatigue [122, 123]. Lenvatinib has been further studied in thyroid, renal cell, and hepatocellular cancer and has led to FDA approval in these settings. Clinical trials combining lenvatinib with pembrolizumab have been studied in the KEYNOTE-146 phase 1b/2 basket study. In preliminary results of 21 patients with metastatic melanoma, the ORR at 24 weeks was 47.6% and the median PFS was 5.5 months. The

12-month PFS rate was 34.7%, and the median DOR was 12.5 months. Grade 3 or 4 treatment-related AEs occurred in 67% with the most common adverse events being fatigue, decreased appetite, diarrhea, hypertension, dysphonia, nausea, arthralgia, and proteinuria. There are several ongoing studies assessing this combination in patients with advanced melanoma including a phase II study in patients who were previously exposed to anti-PD-1/PD-L1 therapy (NCT03776136) and a randomized, placebo-controlled phase III study comparing pembrolizumab and lenvatinib to pembrolizumab alone as first-line therapy (NCT03820986).

Axitinib is a tyrosine-kinase inhibitor targeting VEGFR1–VEGFR3, c-KIT, and platelet-derived growth factor receptor (PDGFR) [124]. Axitinib has been combined with both chemotherapy and immune therapy in patients with melanoma. Based on a phase II study of axitinib in patients with melanoma which demonstrated an ORR of 18.8%, another phase II study combining axitinib with carboplatin and paclitaxel was initiated [125]. Out of 36 evaluable patients, 8 patients had PR and an additional 20 patients had SD. The median PFS was 8.7 months with a median OS of 14.0 months. Overall, the combination was well tolerated with hypertension, anemia, and neutropenia being the most common grade 3 adverse events [126]. Axitinib is also being studied in combination with immune agents. A phase Ib study combining axitinib with JS001, a humanized anti-PD-1 monoclonal antibody, in patients with metastatic mucosal melanoma is currently being performed in China. Initial results from 24 evaluable patients, published in abstract form, demonstrated that the combination was well tolerated with mostly grade 1–2 toxicities. Although there were no complete responses, 12 patients achieved a partial response and an additional 9 patients had stable disease with 10 out the 12 responders ongoing at the time of publication [127]. The encouraging results of this study, although in early phase and in a unique population, demonstrate the potential for further benefit in the combination of immune and targeted therapies.

Histone acetylation and deacetylation play a key role in regulating gene transcription. Histone deacetylase (HDAC) inhibitors have emerged as a potential anticancer therapeutic in various malignancies [128–130]. The combination of entinostat, a selective class I HDAC inhibitor, and pembrolizumab showed promising activity in patients with unresectable or metastatic melanoma who had experienced disease progression on or after anti-PD-1 therapy. Findings from the phase Ib/II ENCORE 601 trial in 53 enrolled patients with anti-PD-1 refractory disease demonstrated 9 patients achieved PRs, and 1 patient had a CR, for an ORR of 19% [131]. An additional 7 patients had SD lasting > 6 months, leading to a clinical benefit rate of 32%. Responses were durable with a 12.5-month median duration of response. HBI-8000 is another HDAC inhibitor being evaluated in combination with nivolumab in patients with metastatic melanoma, RCC, and NSCLC (NCT02718066).

Avadomide (CC-122) is a novel small-molecule agent that modulates cereblon E3 ligase, leading to ubiquitination of several hematopoietic transcription factors [132]. Avadomide has demonstrated antitumor activity in DLBCL and multiple myeloma [133]. In a first-in-human phase I clinical trial in patients with advanced solid tumors, non-Hodgkin's lymphoma, and multiple myeloma, avadomide demonstrated acceptable toxicity and resulted in 3 objective responses in the NHL patients [134]. Based on these results, a phase II study combining avadomide with nivolumab in patients with melanoma was initiated (NCT03834623).

Imprime PGG is a soluble IV β-glucan pathogen–associated molecular pattern that stimulates production of several cytokines including IFN, leading to activation of the innate immune system [135]. In several phase II clinical trials, Imprime PGG led to promising response rates in NSCLC, colorectal cancer, and chronic lymphocytic leukemia [136, 137]. The combination of Imprime PGG and pembrolizumab is being evaluated in a phase II study in patients with advanced melanoma who have progressed on prior checkpoint inhibitor therapy. Preliminary biomarker data shows enhanced tumor infiltration by activated myeloid cells and T cells, suggesting a mechanism by which Imprime PGG may enhance the efficacy of checkpoint blockade [138].

Based on the known efficacy of cytokines in a small subset of melanoma patients, there has been significant interest in pegylated forms of IL-2 and IFN alpha in combination with checkpoint inhibition [139]. NKTR-214 is a pegylated variant of IL-2 with improved tolerability and preferential binding to IL-2Rβγ, leading to greater activation and expansion of effector T cells and NK cells over regulatory T cells in the tumor microenvironment. Initial data from the dose-escalation phase of the PIVOT-02 study of NKTR-214 in combination with nivolumab demonstrated an ORR of 63% in 11 treatment-naïve patients with advanced melanoma, but updated figures for 38 evaluable melanoma patients showed a lower ORR of 53% [140]. Whether the combination proves superior to anti-PD-1 monotherapy is being evaluated in an ongoing randomized, phase III study in the first-line setting (NCT03635983). In a phase Ib/II study of pembrolizumab plus pegylated IFN (PEG-IFN) in PD-1-naïve patients with advanced melanoma, combination therapy produced an ORR of 60.5% [141]. Median PFS and OS were 11.0 months and unreached, respectively, after a median follow-up of 25 months. The toxicity profile appeared acceptable, with 48.8% of patients experiencing a grade 3–4 treatment-related adverse event.

T Cell–Based Therapies

Adoptive cell therapy (ACT) with TILs consists of the identification of antitumor T cells, their ex vivo expansion, and transfer back into the patient after a lympho-depleting regimen. Previous work by Rosenberg and colleagues [142]

demonstrated that lymphocytes extracted from freshly resected melanomas could be expanded in vitro and used to induce responses in patients with metastatic melanoma. Subsequent studies combined TILs with lympho-depleting conditioning and high-dose IL-2. These and subsequent studies demonstrated objective response rates up to 72%, with 10–20% of patients achieving complete remission, leading to widespread interest and further study of TILs [143, 144].

In a pilot study combining vemurafenib and TILs, 7 out of 11 patients with *BRAF*-mutant melanoma had an objective clinical response, 2 of whom had a durable response lasting over 3 years [145]. In a phase I study of 14 patients who received an induction course of ipilimumab combined with TILs, 5 patients had an objective response, with 4 patients achieving a durable response lasting over 1 year [146]. Currently, there are more than 20 clinical trials being performed worldwide evaluating TIL therapy in combination with checkpoint inhibition in melanoma.

Recognition of the critical role of antigen-presenting cells in driving antitumor immunity led to the development of a new class of agents termed immune-mobilizing monoclonal T cell receptor against cancer (ImmTAC), bispecific antibodies that combine antigen recognition and T cell activation through CD3-specific antibody fragments. IMCgp100 is a bispecific antibody that that redirects T cells by binding to CD3 while also binding to gp100 on melanoma cells [147]. In a phase I study that included 31 patients with advanced melanoma, partial responses were observed in 4 out of 26 patients, with an additional 12 patients having stable disease [148]. A subsequent phase I/II study in uveal melanoma patients using an intra-patient dose escalation schedule showed minor responses (> 10% tumor reduction) in 4 out of 17 patients, with an additional 12 patients achieving stable disease [149]. There is an ongoing phase I/II trial studying the combination of IMCgp100 plus durvalumab and tremelimumab in patients with advanced cutaneous melanoma (NCT02535078). There is also an ongoing phase I study assessing the safety of a second ImmTAC agent targeting NY-ESO-1 and/or LAGE-1A.

Another method of enhancing T cell activity involves the adoptive transfer of genetically modified T cells encoding MHC class–restricted T cell receptors. A recent phase I/II study examining adoptive CD4 T cell therapy with an MHC class II–restricted receptor recognizing MAGE-A3 in solid tumors has published preliminary results in 17 patients, with 1 complete response and partial responses in 3 patients [150].

Vaccines

The ability to predict unique cancer neoantigens using whole exome and RNA sequencing data with machine learning approaches has led to the development of personalized cancer vaccines. A phase I trial demonstrated the safety and feasibility of a personalized vaccine targeting up to 20 predicted personal tumor neoantigens in advanced melanoma patients. Of 6 vaccinated patients, 4 had a CR that persisted at 25 months [151]. Current vaccines under investigation include phase I studies of RO7198457 (Genentech), as monotherapy and in combination with pembrolizumab (NCT03289962), and GEN-009 (Genocea Biosciences), as monotherapy and in combination with nivolumab (NCT03633110).

Dendritic cells prime and activate T cells through MHC I antigen presentation [152]. Vaccination with ex vivo tumor antigen–loaded autologous dendritic cells offers another therapeutic strategy. A phase I trial evaluated the use of myeloid DCs that were activated and loaded with HLA-A*02:01-restricted melanoma peptides gp100 and tyrosinase ex vivo. Treatment was well tolerated, and 5 out of 14 vaccinated patients derived long-term survival benefits with overall survivals ranging from 22 to 40 months [153]. Ongoing studies include a phase II trial evaluating the safety and efficacy of dasatinib (70 mg BID) in combination with an autologous type 1 polarized dendritic cell vaccine (NCT01876212). There is also a phase I/II clinical trial evaluating intratumoral injection of dendritic cell vaccines in combination with pembrolizumab (NCT03325101).

Conclusion

Despite tremendous advances in the treatment of metastatic melanoma, only a subset of patients achieves long-term remission. Efforts to enhance the efficacy of existing immunotherapies and targeted agents, as well as to identify novel therapeutic targets, are ongoing and critical to further improving survival outcomes for patients with advanced melanoma.

Compliance with Ethical Standards

Conflict of Interest Lara Ambrosi declares that she has no conflict of interest.

Shaheer Khan declares that he has no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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