Current Oncology Reports (2024) 26:826–839
https://doi.org/10.1007/s11912-024-01551-4

REVIEW

Beyond Immune Checkpoint Inhibitors: Emerging Targets in Melanoma Therapy

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Accepted: 14 May 2024 / Published online: 25 May 2024

Abstract

Purpose of review This review provides a comprehensive update on recent advancements in melanoma treatment by highlighting promising therapeutics with an aim to increase awareness of novel interventions currently in development.

Recent Findings Over the last decade there has been considerable expansion of the previously available treatment options for patients with melanoma. In particular, novel immunotherapeutics have been developed to expand on the clinical advancements brought by BRAF targeting and immune checkpoint inhibitors.

Summary Despite the success of checkpoint inhibitors there remains an unmet need for patients that do not respond to treatment. This review delves into the latest advancements in novel checkpoint inhibitors, cytokines, oncolytic viruses, vaccines, bispecific antibodies, and adoptive cell therapy. Preclinical experiments and early-stage clinical trials studies have demonstrated promising results for these therapies, many of which have moved into pivotal, phase 3 studies.

Keywords Melanoma · Immunotherapy · Adoptive cell therapy · Oncolytic virus · Bispecific antibody · Cytokine

Abbreviations

LAG-3	Lymphocyte-Activation Gene 3
TIGIT	T cell immunoreceptor with immunoglobulin and ITIM domain
TIM3	T cell immunoglobulin and mucin domain-containing protein 3
CTLA-4	Cytotoxic T-lymphocyte associated protein 4
PD-L1	Programmed death-ligand 1
gp100	Glycoprotein 100
PRAME	PReferentially expressed Antigen in MElanoma
TCR-T	T cell receptor-engineered T cell
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IL	Interleukin
GALV-GP-R-	Gibbon ape leukemia virus
MHC-II	Major Histocompatibility Complex Class II
TLR9	Toll-like Receptor 9

Introduction

The advent of modern therapeutics targeting BRAF and immune-checkpoint blockade has driven clinical improvement for patients with melanoma. Prior to the introduction of immune checkpoint inhibitors (ICIs) and targeted therapy, metastatic melanoma was associated with an expected median survival of approximately six to nine months [1]. Outcomes have improved significantly since then with median survival in clinical trials demonstrated as exceeding six years [2, 3]. Despite these improvements unfortunately at least half of patients do not obtain long term survival, and emphasizes the need for novel therapeutics.

While the field is optimistic, there have been a series of setbacks in seminal trials that have brought sobriety to the field. Bempegaldesleukin, a beta/gamma selective IL-2 agonist, showed promise in a phase 2 trial but failed to improve progression-free survival (PFS) or overall survival (OS) in a phase 3 trial [4]. Similarly, epacadostat, an indoleamine 2,3-dioxygenase-1 (IDO1) inhibitor used in combination with pembrolizumab suggested anti-tumor activity in a

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phase 1/2, but failed to improve PFS or OS in a phase 3 trial [5, 6].

Despite this, many intriguing mechanism-based emerging therapies hold monotherapy promise, as well as in combination with ICIs or targeted therapy. Here, we highlight and overview emerging approaches with potential to improve outcomes for patients with melanoma (Fig. 1).

Novel Immune-Checkpoint Inhibitors: Adjuvant/Neoadjuvant Therapy

While the majority of patients diagnosed with melanoma present with resectable disease, many remain at high risk of recurrence. Anti-programmed cell death protein 1 (PD-1) therapy has demonstrated improvement in recurrence-free survival (RFS) for patients with resectable IIIA-IV melanoma, which has since been expanded to patients with IIB-IIC melanoma [7, 8•, 9]. Neoadjuvant approaches have also emerged, notably including the phase 2 SWOG S1801 trial demonstrating improved event-free survival for neoadjuvant-adjuvant pembrolizumab compared to adjuvant (72% vs 49%) without a significant increase in adverse events [10••].

Beyond PD1, other immune-checkpoints have been implicated in immune evasion and are being explored in clinical trials. Lymphocyte-Activation Gene 3 (LAG-3) is an immune checkpoint found on the surface of T cells, B cells, dendritic cells, and NK cells. Due to its structural similarity to CD4, LAG-3 is capable of binding to Major Histocompatibility Class II (MHC-II), which generates inhibitory intracellular signaling within T cells and inhibits binding of MHC-II to CD4. Persistent T cell activation results in upregulation of LAG-3 and T cell dysfunction [11–14]. This shift towards an exhausted T cell phenotype within tumor-infiltrating lymphocytes (TILs) results in immune evasion of tumor cells and blockade of LAG-3 results in upregulation of $CD8^+$ T cell activity and restoration of immune surveillance [15].

Relatlimab is a LAG-3 inhibitor that received FDA approval in 2022 for the treatment of advanced melanoma following the results of RELATIVITY-047, a phase 2/3 clinical trial that demonstrated improved PFS in patients treated

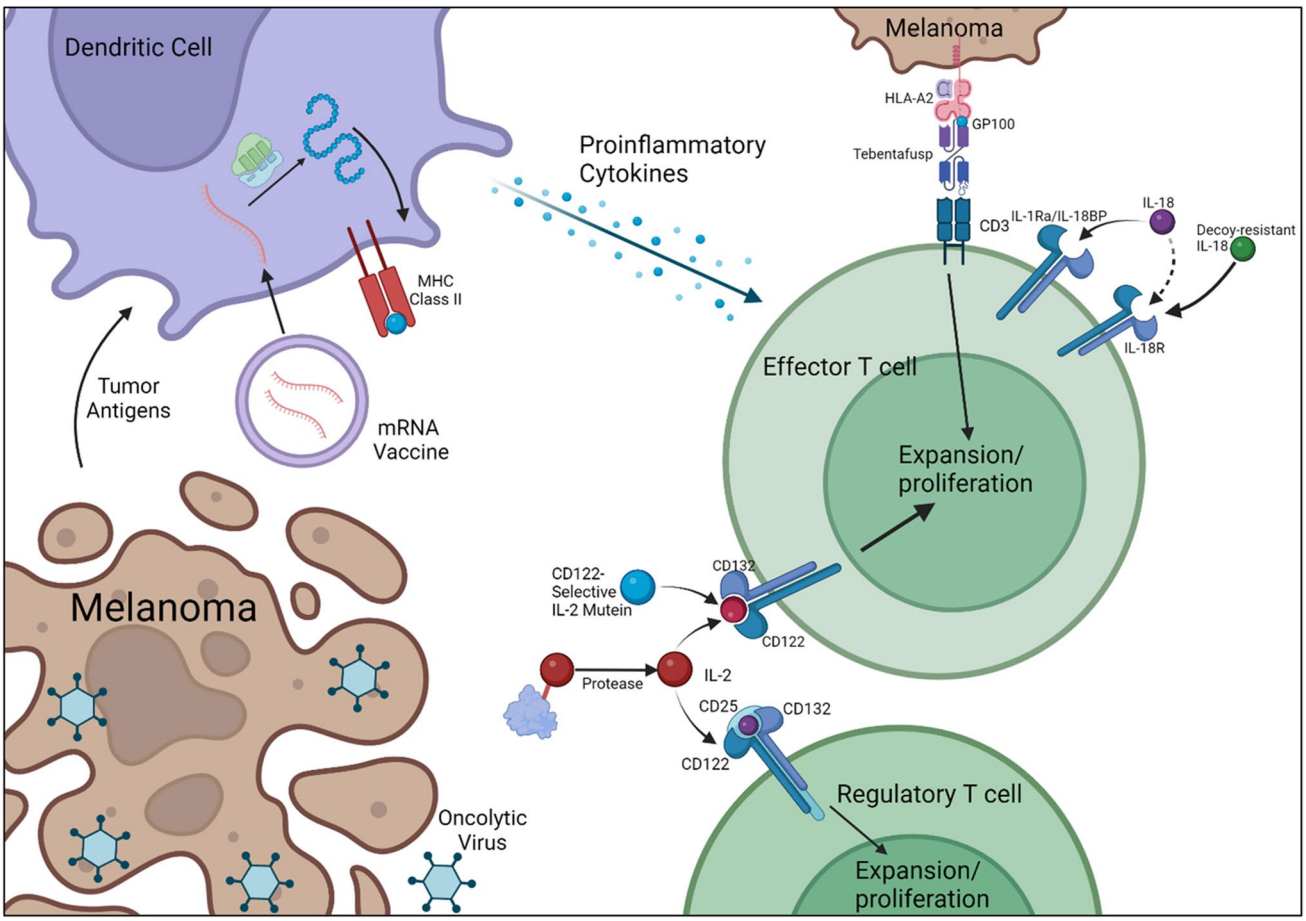

Fig. 1 Overview of novel therapeutic strategies in melanoma

with relatlimab and nivolumab compared to nivolumab alone [16•]. Relatlimab is under investigation for the treatment of melanoma in both the neoadjuvant and adjuvant settings. A phase 2 trial of neoadjuvant relatlimab in combination with nivolumab demonstrated high rates of pathologic response with a 57% complete pathologic response and 70% overall pathologic response rates [17]. The phase 3 RELATIVITY-098 trial is underway to investigate the efficacy of nivolumab plus relatlimab versus nivolumab alone in the stage III adjuvant setting [18]. Another anti-LAG-3 monoclonal antibody, fianlimab is also being studied in combination with the anti-PD-1 antibody cemiplimab in the neoadjuvant, adjuvant, and metastatic settings. A phase 3 trial is underway comparing fianlimab and cemiplimab to pembrolizumab monotherapy in the adjuvant setting for IIC-IV melanoma [19] A phase II neoadjuvant-adjuvant trial is also planned [20].

T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) is present on $CD8^+$ T and $CD4^+$ T cells, natural killer (NK) cells, regulatory T cells (Tregs), and follicular T helper cells [21]. Dual PD-1 and TIGIT inhibition augments proliferation and function of antigen-specific $CD8^+$ T cells and TILs isolated from patients with melanoma [22]. Vibostolimab is an anti-TIGIT antibody that has been investigated in the neoadjuvant setting phase 1/2 KEYMAKER-U02 trial investigating pembrolizumab combined with investigational agents including vibostolimab vs pembrolizumab alone [23, 24]. The phase 3 KEYVIBE-010 trial is underway to explore the use of adjuvant vibostolimab in combination with pembrolizumab compared to pembrolizumab monotherapy in patients with IIB-IV melanoma [25].

T cell immunoglobulin and mucin-domain containing-3 (TIM-3) is an immune checkpoint that is selectively expressed on the surface of interferon-gamma (IFN-γ) producing T_H1 cells [26]. Similar to PD-1, TIM-3 expression is a marker of T cell exhaustion. Inhibition of TIM-3 results in T cell hyperactivation and increased IFN-γ production [15, 27]. TIM-3 also plays a role in innate immunity as TIM-3 blockade has been shown to improve NK cell cytotoxic activity [28]. TIM-3 expression on tumor-antigen specific T cells is associated with T cell dysfunction, which has given rise to the hypothesis that TIM-3 blockade may restore immune surveillance [29] Specifically for melanoma, high expression of TIM-3 is a marker of poor prognosis [30]. A phase 2 neoadjuvant trial is comparing the PD-1 inhibitor dostarlimab to the PD-1/TIM-3 inhibitor combination dostarlimab and cobolimab [31].

Novel Immune-Checkpoint Inhibitors: Advanced Melanoma

There is an unmet need for patients with PD-1 refractory advanced melanoma where outcomes continue to be poor [32]. As anti-PD-1 and anti-CTLA-4 ICIs have demonstrated efficacy in both the adjuvant setting and advanced disease, the novel immune checkpoints LAG-3, TIM-3, and TIGIT are of great conceptual interest for advanced disease as well.

The combination of fianlimab and cemplimab have shown meaningful results in advanced disease in a phase 1 trial, with an objective response rate of 63.8% in anti-PD-1 naïve patients [33]. Efficacy of the combination is also favorable in advanced disease that recurs after adjuvant PD-1 treatment with an ORR of 60.9% [34•]. These early promising results have led to the initiation of two phase 3 trials in patients with advanced disease. One trial is comparing the combination to pembrolizumab monotherapy and the other comparing the combination to relatlimab and nivolumab [35, 36].

Trials are exploring anti-TIGIT and TIM-3 antibodies in advanced melanoma as well. A phase 1 trial of cobolimab as monotherapy and in combination with anti-PD-1 therapy included 46 patients with advanced melanoma. The treatment was well tolerated with a grade 3/4 adverse event rate of 4.3%. A phase 2 trial is planned to further evaluate efficacy [37]. A phase 2 trial is currently recruiting patients with PD-1 refractory advanced melanoma to determine the efficacy of the anti-TIGIT antibody domvanalimab in combination with anti-PD-1 zimberelimab [38].

Novel CTLA-4 Antibodies

CTLA-4 is expressed on $CD8^+$ cytotoxic T cells as well as immunosuppressive $CD4^+$ Tregs including intratumoral Tregs [39]. Preclinical murine models have indicated that administration of ipilimumab results in a reduction of intratumoral Tregs by antibody-dependent cell-mediated cytotoxicity (ADCC). However, in human tumor samples, no such reduction of Treg occurs after anti-CTLA-4 administration [40]. As Tregs have shown to impair anti-tumor immune response, Treg depletion may improve clinical response to anti-CTLA-4 therapy [41]. ADCC is dependent on binding of the Fc region of antibodies to the Fcγ receptors on NK cells, neutrophils, monocytes, and macrophages [42, 43]. Recognition of the importance of ADCC in the mechanism of CTLA-4 antibodies' depletion of Tregs and subsequent increase in the CD8/CD4 ratio has led to the development of CTLA-4 antibodies with Fc regions that have increased binding affinity to Fcγ receptors. Botensilimab is an Fc-enhanced CTLA-4 antibody that has demonstrated an ability to increase the CD8/CD4 ratio within the tumor

microenvironment [44]. A phase 2 trial is currently underway investigating botensilimab in patients with advanced melanoma as monotherapy and in combination with the anti-PD-1 antibody balstilimab [45].

Although CTLA-4 antibodies have resulted in clinical improvements for patients with melanoma, they carry a significant risk of autoimmune-like toxicity [46]. One approach to preserving the efficacy of CTLA-4 inhibition while limiting its toxicity is to develop tumor-specific CTLA-4 antibodies. ONC-392 is an acid-sensitive CTLA-4 antibody that dissociates from CTLA-4 in the acidic environment of endosomes allowing for CTLA-4 to be recycled to the cell surface. Preservation of CTLA-4 on the surface of Tregs protects against immune-related adverse event (irAE) development seen with anti-CTLA-4 antibodies such as ipilimumab [47]. The phase 1/2 PRESERVE-001 trial is underway investigating ONC-392 alone and in combination with anti-PD-1 therapy in patients with advanced solid tumors including melanoma [48]. Although the efficacy of ONC-392 is still under investigation, a low rate of irAEs was seen in the initial dose-finding portion of the trial [49].

Vaccines

Increasing evidence suggests that anti-tumor adaptive immune responses are neoantigen specific and this may outline a novel path for drug development in cancer immunotherapy. Vaccines have long been used to stimulate adaptive immunity to fight infectious diseases but have yet to make a major impact in cancer. Early investigations into therapeutic vaccines focused on overexpressed self-antigens such as gp100. However, these vaccines were unsuccessful clinically [46]. As self-antigens are present in non-malignant tissues, they are subject to immune tolerance and carry a risk of autoimmune toxicity [50]. Neoantigens provide increased specificity as they arise due mutations within tumor tissue. Because neoantigens vary from patient to patient, they must be targeted using personalized an individualized approach. Personalized vaccines utilize whole exome sequencing of tumor tissue to identify tumor neoantigens, which are then analyzed for immunogenicity either via a bioinformatics prediction platform or directly using an IFN-γ release assay [51].

The first personalized vaccines used in the treatment of melanoma were peptide vaccines, which use short neoantigen peptides to stimulate an anti-tumor immune response. NEO-PV-01 is one such peptide vaccine that consists of up to 20 peptides that are 14–35 amino acids long. A phase Ib trial of NEO-PV-01 in combination with anti-PD-1 therapy demonstrated favorable side effect profile with no serious treatment-related adverse events in patients with advanced melanoma. NEO-PV-01 treatment also induced an immune response to neoantigens not targeted by the vaccine known as epitope spread [52•]. Peptide vaccines have also been combined with adjuvants to improve the immune response. EVX-01 is a personalized vaccine that consists of neoantigen peptides in combination with a novel liposomal adjuvant (CAF09b) [53]. The phase 2 KEYNOTE-D36 trial is underway to evaluate the efficacy of this therapy in combination with pembrolizumab for patients with advanced melanoma [54].

An alternative to peptides vaccines are mRNA vaccines, which rely on uptake of mRNA into antigen-presenting cells leading to expression of tumor-specific neoantigens and subsequent MHC presentation. The resulting antigen presentation stimulates $CD8^+$ and $CD4^+$ T cells directed against these neoantigens [55]. The mRNA-4157 (V940) vaccine, also known as an individualized neoantigen therapy, is synthesized using mRNA from up to 34 neoantigens [56]. The phase 2 mRNA-4157-P201/KEYNOTE-942 trial demonstrated improvements in RFS and distant metastasis-free survival (DMFS) in patients with resectable IIIB-IV melanoma compared to pembrolizumab alone [57••, 58]. An adjuvant phase 3 trial for mRNA-4157/V940 is currently underway in resected stage IIB-IV melanoma.

Therapeutic mRNA vaccines are also being investigated for the treatment of advanced melanoma. BNT122 is an mRNA vaccine that encodes for up to 20 neoantigens and is administered intravenously. A phase 2 study is underway to evaluate the efficacy of BNT122 in combination with pembrolizumab in patients with advanced melanoma [59].

Immunomodulatory vaccines utilize a different strategy than those targeting tumor neoantigens. These vaccines target immunosuppressive cells that allow for immune evasion by tumor cells. IDO1 and PD-L1 are expressed by immunosuppressive cells and are associated with T cell exhaustion [60, 61]. IO102/IO103 is a first-in-class immunomodulatory vaccine that targets both IDO1 and PD-L1. Results from a phase 1/2 trial of IO102/IO103 in combination with nivolumab demonstrated an objective response rate of 80% in patients with advanced melanoma [62]. A phase 3 trial is underway.

Bispecific Antibodies

ICIs have brought significant clinical benefits but do not help all patients and can have dose limiting toxicities, especially in combination. Patients with insufficient tumor-specific $CD8^+$ T cells or neoantigen expression may not respond to ICI therapy [63]. As such, an opportunity for therapies that increase the population of tumor-reactive T cells has emerged. One such therapy involves the use of bispecific T cell engagers (BiTEs). BiTEs are comprised of a T cell targeting antibody, usually CD3, linked to an antibody targeting a tumor-associated antigen. Binding of both arms of

the BiTE leads to activation of the T cell resulting in cytotoxic activity. However, the utility of BiTE therapy has been limited by the requirement of a cell surface antigen target with sufficient specificity to limit on-target, off-tumor toxicity. Additionally, upregulation of immune checkpoints has been shown to occur after exposure to BiTE therapy [64]. An alternate platform for T cell engagers is the Immune mobilizing monoclonal T cell receptors against cancer (ImmTACs). ImmTACs are composed of an engineered T cell receptor with an activating anti-CD3 effector domain. The engineered T cell receptor has enhanced affinity for specific peptide-HLA complexes on cell surfaces [65, 66]. Targeting the peptide-HLA complex allows for targeting of intracellular proteins. The resulting action of ImmTACs is T cell activation and colocalization within the tumor microenvironment. The cytotoxic activity of T cells leads to lyses of target cells and increased antigen exposure capable of stimulating a further immune response [67].

Tebentafusp is a gp100 peptide-HLA directed ImmTAC that received FDA approval in 2022 for the treatment of metastatic uveal melanoma after a phase 3 trial demonstrated an overall survival benefit [68, 69]. Uveal melanoma has a relatively low amount of neoantigen expression and intertumoral $CD8^+$ T cells compared to cutaneous melanoma, which contributes to the relatively lower response rate to ICIs [70]. Gp100 is highly expressed in both uveal and cutaneous melanomas leading to the exploration of tebentafusp as a potential therapeutic for patients with PD-1 refractory advanced melanoma [71]. TEBE-AM is an ongoing phase 2/3 multi-center trial enrolling PD-1 refractory patients utilizing tebentafusp as monotherapy and in combination with pembrolizumab [72].

Beyond gp100, another promising target for TCR based therapy is Preferentially expressed Antigen of Melanoma (PRAME). PRAME is commonly overexpressed in melanoma but only expressed at low levels in some non-neoplastic tissues [73]. This differential expression profile similar to gp100 has led to its recognition as a target for ImmTACs. IMC-F106C is PRAME-targeting ImmTAC currently under investigation for the treatment of advanced melanoma. Initial results from an ongoing phase I trial revealed a grade ¾ adverse event rate of 31% of which the most common were lymphopenia (14%) and AST increase (7%). Although only 3 patients with melanoma were enrolled, two had partial responses [74]. The phase 3 PRISM-MEL-301 trial will investigate the IMC-F106C in combination with nivolumab in untreated advanced melanoma [75]. One notable limitation of ImmTACs is that their binding to HLA-peptide complexes is dependent on the HLA subtype, which vary from person to person. Both IMC-F106C and tebentafusp are specific to HLA-A*02:01. HLA-A*02:01 is present in ~ 50% of Caucasians, but only ~ 35% of patients of Asian or African descent [76–78].

Bispecific antibodies targeting multiple immune checkpoints have been developed. These antibodies are capable of inhibiting immune checkpoints that have been upregulated on tumor cells and T cells while co-localizing the two. Alternatively, two immune checkpoints expressed on T cells may be targeted leading to inhibition of two immune checkpoints on the same T cells, which has been shown to result in greater T cell activation [79–81]. XmAb23104 is a bispecific antibody targeting PD-1 and the immune checkpoint Inducible T cell costimulatory (ICOS). XmAb22841 targets CTLA-4 and LAG-3. Both XmAb23104 and XmAb22841 in being investigated as combination therapy in patients with ICI-refractory melanoma in an ongoing Phase 1b/2 trial [82].

Adoptive Cell Therapy

Adoptive cell therapy (ACT) consists of identifying antitumor lymphocytes, growing them ex vivo and infusing them into the patient after a lymphodepleting chemotherapy regimen. The patient is then given an infusion of interleukin-2 (IL-2) to stimulate T cell growth and activity [83]. Tumor-Infiltrating Lymphocyte (TIL) therapy is a form of ACT that utilizes lymphocytes extracted from the tumor microenvironment. TIL therapy as a treatment of melanoma has been reported as far back 1988 [84] with the process undergoing a series of optimizations. A lymphodepleting chemotherapy regimen of cyclophosphamide and fludarabine was eventually discovered as associated with improved TIL engraftment and this has remained the standard conditioning regimen [83, 85, 86]. In addition, TILs from responding patients were eventually observed to have longer telomeres leading to optimization and minimization of culturing time in the development of TIL cell products [87]. More recently, TIL therapy was compared to ipilimumab in a randomized phase 3 trial of 168 patients with advanced melanoma, 86% of whom had progressed on prior anti-PD-1 therapy. TIL therapy was associated with significantly longer progression-free survival than ipilimumab [88•].

Lifileucel is a commercially available TIL platform that is capable of producing billions of TILs from the patient's tumor tissue in a 22-day manufacturing process. A phase 2 study of Lifilucel was conducted in patients with advanced melanoma who had progressed despite PD-1 therapy and BRAF therapy if BRAF V600 positive. The results demonstrated robust activity in this PD-1 refractory cohort with an ORR of 41% and a disease control rate of 81%. The adverse events observed are consistent with those seen with prior use of IL-2 and lymphodepleting chemotherapy [89••]. Lifileucel is now FDA approved for the treatment of PD-1 refractory advanced melanoma. The phase 3 TILVANCE-301

trial is underway to investigate Lifileucel combined with pembrolizumab compared to pembrolizumab alone in a treatment-naïve population [90, 91].

TIL therapy utilizes tumor-specific $CD8^+$ lymphocytes found within the tumor microenvironment, which are reacting to a variety of tumor-specific neoantigens. Novel bioinformatics platforms are capable of identifying patient-specific clonal neoantigens which in turn can be used to expand these populations of clonal neoantigen reactive T cells (cNeT). The use of cNeTs aims to expand the efficacy of TIL therapy. A phase 1/2a trial of ATL001, autologous cNeTs, is underway for patients with advanced melanoma alone and in combination with nivolumab [92].

Antigens can be targeted directly with T cell engineered T cell therapy (TCR-T), which involves modifying the T cell receptor on autologous T cell receptors to recognize a specific tumor-associated antigen. As with other forms of ACT, the cellular product is infused after lymphodepleting chemotherapy and followed by IL-2 infusion. IMA-203 is a TCR-T directed against PRAME and is being developed alongside IMA-203CD8, a second generation PRAME TCR-T with an added CD8αβ co-receptor aimed at improving antigen recognition and T cell activation. Both IMA-203 and IMA-203CD8 are currently undergoing phase I study alone and in combination with nivolumab [93•]. In an interim analysis IMA-203 demonstrated an ORR of 62% (8/13) in patients with cutaneous melanoma all of whom had progressed on prior checkpoint inhibitor therapy. Median durability of response had not yet been reached at 14.4 months [94, 95].

Another approach to improve the efficacy of TIL is to remove genes that negatively regulate T cell activity. As the name implies, Suppressors of Cytokine Signaling 1 (SOCS1) decreases the intracellular signaling that results from the binding of cytokines to extracellular receptors. KSQ-001 is an engineered TIL product that utilizes CRISPR/Cas9 to remove the SOCS1 gene from TIL isolated from patient tumor samples. The goal of this approach is to generate TIL with increased anti-tumor activity [96, 97]. A phase 1/2 trial is planned to evaluate KSQ-001 [98].

Oncolytic Viruses

Oncolytic viruses lead to lysis of the cancer cells facilitating antigen presentation and a host immune response. The use of oncolytic viruses first emerged as a standard of care option for melanoma following the development of talimogene laherparepvec (T-VEC). T-VEC is a genetically modified herpes virus that expresses GM-CSF. T-VEC received FDA approval for the treatment of unresectable melanoma in 2015 following the results of a phase 3 trial demonstrating improved objective response rates compared to intralesional GM-CSF [99, 100•]. Preclinical data has indicated the potential for synergic activity between oncolytic virus therapy and checkpoint inhibition, but these results have not been seen clinically for patients with melanoma. MASTERKEY-265 was a phase 3 trial investigating MASTERKEY-265/KEYNOTE-034 pembrolizumab with vs without T-VEC in patients with unresectable IIIB-IV melanoma, which failed to show a difference in PFS between the two groups [101].

Following the demonstration of the efficacy and safety of the HSV-1 based T-VEC, novel oncolytic viruses are an emerging therapy for the treatment of melanoma. RP-1 is developed from a strain of HSV-1 expressing both GM-CSF and the fusogenic envelope protein of the gibbon ape leukemia virus (GALV-GP-R-) [102]. GALV-GP-R- allows for cell–cell fusion and subsequently improved viral transmission [103]. In the phase 1/2 IGNYTE trial the combination of RP-1 and nivolumab is being studied in patients with PD-1 refractory advanced melanoma. The initial results in 91 patients from the ongoing trial demonstrated an encouraging objective response rate of 37.4% and a complete response rate of 18.7% [102].

Cytokines

Interleukin-2 (IL-2) was the first molecularly cloned cytokine and saw its first clinical application as an early immunotherapy for patients with cancer. High-dose (HD) IL-2 therapy has demonstrated the ability to produce durable results in a subset of patients with metastatic melanoma. Despite the efficacy in these patients, the clinical utility has been limited by severe toxicity [104]. Bempegaldesleukin, a CD122-preferential IL-2 pathway agonist, attempted to address the toxicity concerns of HD IL-2 and demonstrated promise in a phase 1 trial with a favorable side effect profile compared to IL-2. However, bempegaldesleukin failed to improve PFS or OS in the phase 3 PIVOT IO-001 trial in advanced melanoma [4, 105]. Several novel cytokine therapies are being developed that aim to preserve clinical efficacy while minimizing the dose-limiting toxicities seen in earlier trials. As there are numerous IL-2 therapeutics under investigation in this area, the discussion of specific agents is beyond the scope of this manuscript.

One approach to limiting toxicity is the use of tumor-activated prodrugs that exist in an inactivated state until they are converted to fully active IL-2 in the tumor microenvironment. In this approach WT IL-2 is tethered to an inactivation domain with a tumor protease-sensitive linker. The tumor-associated proteases cleave the linker leading to dissociation of the inactivation domain resulting in activated IL-2 within the tumor microenvironment. As IL-2 increases vascular permeability leading to a severe capillary leak syndrome, limiting systemic circulating IL-2 is meant to ameliorate this toxicity [106]. Preclinical data has shown that this type of IL-2 construct can achieve significant amounts

of detectable active IL-2 within tumor tissues with almost no detectable active IL-2 in the serum suggesting successful conditional activation [107]. IL-2 can also be targeted to the tumor microenvironment by fusion of IL-2 to a monoclonal antibody targeting tumor-associated antigens [108].

Another approach to improve the efficacy of IL-2 has been to target specific immune cell populations using selective IL-2-based compounds. The IL-2 receptor exists in trimeric and dimeric forms. Binding of IL-2 to the trimeric IL-2 receptor results in expansion of Tregs while binding of IL-2 to the IL-2R dimer consisting of CD122 and CD132 stimulates naïve effector T cells and NK cells. This observation has led to the development of next generation IL-2 therapeutics that preferentially bind the dimeric form of IL-2R. Binding specificity can be achieved using modified forms of IL-2 with reduced binding to the trimeric form of the receptor [109]. Alternatively, the blocking the CD25 binding site on IL-2 results in selectivity for the CD122/CD132 dimeric form of the receptor [110].

Interleukin-12 (IL-12) production is typically triggered by pathogen-associated molecular pattern (PAMP) or danger-associated molecular pattern (DAMP) recognition by the innate immune system. IL-12 leads to recruitment of T and NK cells thereby coordinating activity between the innate and adaptive immune systems [111•]. Similar to IL-2, intravenous IL-12 is capable of producing clinical responses in patients with melanoma, but with high rates of adverse events [112]. Tavokinogene telseplasmid (Tavo) is an IL-12 encoding plasmid that is administered via intratumorally injection followed by electroporation resulting in intracellular uptake and subsequent expression of IL-12 in the tumor microenvironment. A phase 2 trial of Tavo in 30 patients with advanced melanoma with two or more injectable lesions demonstrated an objective response rate of 35.7% and a complete response rate of 17.9% [113]. Tavo is under investigation in combination with anti-PD-1 therapy in both the neoadjuvant and metastatic settings [114, 115].

Interluekin-18 (IL-18) is a proinflammatory member of the IL-1 cytokine family that stimulates the differentiation of $CD4^+$ T cells into Th1 cells and acts together with IL-12 to cause IFN-γ secretion by T cells and NK cells [116]. Despite promise in preclinical models, recombinant human IL-18 monotherapy was not associated with significant responses in a phase 2 clinical trial of patients with advanced melanoma [117]. IL-18 signaling is further regulated after secretion by binding to decoy IL-1Ra and IL-18BP instead of the IL-18R responsible for its proinflammatory activity. Recognition of this regulatory mechanism has led to the development of a "decoy-resistant" IL-18 with reduced binding affinity for IL-1Ra and IL-18BP [118]. ST-067 is a decoy-resistant IL-18 currently in phase 1/2 clinical trial being investigated in patients with advanced solid tumors including melanoma [119].

Immunocytokines are antibody-cytokine fusions that consist of a targeting antibody linked to a cytokine payload. This approach combines elements of both prior approaches to localize the cytokines to target effector cells while also carrying a selective cytokine payload. PD1-IL2v is an immunocytokine that delivers IL-2 via PD-1 binding of $CD8.^+$ T cells as well as utilizing an IL-2 variant defective in binding to IL-2Rα [120]. A phase 1 clinical trial of PD1-IL2v is currently underway. Immunocytokines provide the opportunity to utilize different cytokine payloads as well. PD-1 binding immunocytokines have also been developed with IL-7, IL-18, and IL-21 payloads. [121]

Innate Immune Stimulators

Most of the currently approved immunotherapy treatments for melanoma focus on enhancing the anti-tumor activity of T cells. However, the anti-tumor adaptive immune response is influenced by signaling from cells of the innate immune system including dendritic cells (DCs) and NK cells. The uptake and presentation of tumor antigens by APCs of the innate immune system results in further priming of tumor-specific $CD8^+$ T cells [122]. Professional APCs can be activated by several different mechanisms including agonism of the MHC-II and CD40 surface receptors via binding to their ligands LAG-3 and CD40L, respectively. Alternatively, stimulation of toll-like receptors results in activation of APCs, which in turn generate enhanced adaptive immune responses [123].

Eftilagimod alpha is a soluble LAG-3 protein that binds to and activates MHC-II found on immature DCs leading to their activation [124]. Eftilagimod alpha in combination with pembrolizumab is currently under investigation in the phase 1 TACTI-mel trial as a novel therapeutic for the treatment of advanced melanoma. Among the initial 18 PD-1 refractory patients enrolled, the overall response rate (ORR) was 33%. The cohort was expanded to include six patients without prior PD-1 exposure of whom half had a response. The treatment was generally well tolerated with only one of 24 patients having a serious adverse reaction (anaphylaxis) thought to be secondary to eftilagimod alpha [125].

CD40 is a costimulatory receptor in the TNF superfamily that is part of the activation pathways for dendritic cells, T cells and B cells. CD40 signaling results in the maturation of dendritic cells allowing them to effectively activate T cells [126, 127]. The broad immunomodulatory capabilities of CD40 agonism have led to its identification as a potential target for novel immunotherapies. Sotigalimab is an anti-CD40 antibody that binds with high affinity to the CD40 ligand domain resulting in the maturation of dendritic cells, activation of NK cells, and IL-12 secretion. A phase 2 trial

Table 1 Mechanisms of Novel Immunotherapeutics and Associated Clinical Trials

Type of Treatment	Target/Mechanism	Treatment	Trial Identifiers
Novel ICI	LAG-3	Relatlimab	NCT03470922, NCT05002569
Novel ICI	LAG-3	Fianlimab	NCT06246916, NCT05608291
Novel ICI	TIGIT	Vibostolimab	NCT05665595
Novel ICI	TIGIT	Domvanalimab	NCT05130177
Novel ICI	TIM3	Cobolimab	NCT04139902
Novel ICI	Fc-enhanced anti-CTLA-4	Botensilimab	NCT05529316
Novel ICI	Acid-sensitive anti-CTLA-4	ONC-392	NCT04140526
Immunomodulatory Vaccine	Neoantigen Peptide Vaccine	NEO-PV-01	NCT02897765
Immunomodulatory Vaccine	Neoantigen Peptide Vaccine	EVX-01	NCT05309421
Immunomodulatory Vaccine	mRNA Neoantigen Vaccine	mRNA-4157/V940	NCT03897881
Immunomodulatory Vaccine	mRNA Neoantigen Vaccine	BNT122	NCT03815058
Immunomodulatory Vaccine	IDO1/PD-L1 Vaccine	IO102-IO103	NCT05155254
ImmTAC	gp100	Tebentafusp	NCT05549297
ImmTAC	PRAME	IMC-F106C	NCT06112314
Adoptive Cell Therapy	Tumor Infiltrating Lymphocytes	Lifileucel	NCT05727904
Adoptive Cell Therapy	PRAME TCR-T	IMA-203	NCT03686124
Adoptive Cell Therapy	Tumor Infiltrating Lymphocytes	KSQ-001	NCT06237881
Oncolytic Virus	HSV-1 expressing GM-CSF and GALV-GP-R-	RP-1	NCT03767348
Cytokine	IL-12	Tavokinogene telseplasmid	NCT03132675, NCT01502293
Cytokine	IL-18	ST-067	NCT04787042
Innate Immune Stimulators	MHC-II	Eftilagimod alpha	NCT02676869
Innate Immune Stimulators	CD40	Sotigalimab	NCT04337931
Innate Immune Stimulators	TLR9	Vidutolimod	NCT04695977

of sotigalimab in combination with nivolumab in patients with PD-1 refractory melanoma demonstrated an objective response rate of 15%. Sotigalimab was relatively well tolerated with a grade 3/4 adverse event rate of 13% [128].

Toll-like receptors (TLR) recognize common patterns like the pathogen-associated (PAMPs) and danger-associated molecular patterns (DAMPs) of produced by microorganisms. While tumors do not produce DAMPs or PAMPs, this pathway can be exploited to activate the innate immune system, which in turn can activate the adaptive immune system. SD-101 is a synthetic oligonucleotide TLR-9 agonist consisting of cytidine-phospho-guanosine (CpG) motifs that was studied in combination with pembrolizumab in a phase Ib trial. Results demonstrated a ORR of 78% in anti-PD-1 naïve patients and 15% in patients with prior anti-PD-1 treatment [129]. However, no further clinical investigation into SD-101 for the treatment of advanced melanoma is ongoing at this time.

Vidutolimod, formerly CMP-001, is a virus-like particle containing a CpG-A oligodeoxynucleotide TLR9 agonist [130]. Vidutolimod was studied alone and in combination with pembrolizumab in a phase Ib trial of patients with advanced melanoma that progressed or had stable disease after > 12 weeks of anti-PD-1 therapy. The combination was well tolerated with an ORR of 23.5%. A phase 2/3 trial of vidutolimod in combination with nivolumab in patients with advanced melanoma [131]. Vidutolimod has also been studied as neoadjuvant therapy in combination with nivolumab in a phase 2 trial. Results showed demonstrated promising activity with 47% pCR rate and 57% MPR rate [132].

Conclusion

The past decade has seen a considerable expansion in the treatment options available for the treatment of melanoma (Table 1).

These new therapeutics have brought with them dramatic improvements in the outcomes for patients with melanoma. Despite this, there continue to be patients that do not respond to available therapies and melanoma remains the leading cause of skin cancer-related death. As such, there is an ongoing need to identify additional effective therapies for these patients, particularly those with PD-1 refractory disease. Novel ICIs, bispecific antibodies, ACT, vaccines, oncolytic viruses, and immunocytokines all are promising avenues to further improve the standard of care for patients with melanoma.

Author contributions AK drafted the initial manuscript and incorporated edits. JJL provided extensive manuscript edits, supervised design and content of manuscript. AK prepared figure 1. All authors reviewed the manuscript.

Declarations

Competing interests ADK: None JJL: DSMB: Abbvie, Immutep, Evaxion; Scientific Advisory Board: (no stock) 7 Hills, Bright Peak, Exo, Fstar, Inzen, RefleXion, Xilio (stock) Actym, Alphamab Oncology, Arch Oncology, Kanaph, Mavu, NeoTx, Onc.AI, OncoNano, Pyxis, STipe, Tempest; Consultancy with compensation: Abbvie, Bayer, Bristol-Myers Squibb, Castle, CHECKMATE, Codiak, Crown, Day One, Duke St, EMD Serono, Endeavor, Flame, Genentech, Gilead, Glenmark, HotSpot, Kadmon, Janssen, Ikena, Immunocore, Incyte, IO Biotech, Macrogenics, Merck, Nektar, Novartis, Partner, Pfizer, Regeneron, Roivant, Servier, STINGthera, Synlogic, Synthekine; Research Support: (all to institution for clinical trials) AbbVie, Astellas, Astrazeneca, Bristol-Myers Squibb, Corvus, Day One, EMD Serono, Fstar, Genmab, Ikena, Immatics, Incyte, Kadmon, KAHR, Macrogenics, Merck, Moderna, Nektar, Next Cure, Numab, Palleon, Pfizer, Replimmune, Rubius, Servier, Scholar Rock, Synlogic, Takeda, Trishula, Tizona, Xencor; Patents: (both provisional) Serial #15/612,657 (Cancer Immunotherapy), PCT/US18/36052 (Microbiome Biomarkers for Anti-PD-1/PD-L1 Responsiveness: Diagnostic, Prognostic and Therapeutic Uses Thereof)

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- • Of importance
- •• Of major importance

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