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REVIEW

Immune Checkpoint Inhibitors in Malignant Melanoma: Anti-PD-1, Anti-CTLA-4 and Anti-LAG-3 Therapies

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Abstract

Purpose of Review Despite advances over the past decade, malignant melanoma remains associated with poor survival outcomes and an increasing incidence, particularly in older populations. Traditional radio- and chemotherapeutic approaches have shown limited efficacy, whereas immunotherapy has emerged as a promising treatment option owing to the immunogenic nature of most melanoma subtypes. This review aims to explore the biological rationale for immune checkpoint inhibition in melanoma and its therapeutic implications.

Recent Findings Advances in understanding physiologic immune checkpoint regulation through co-stimulatory and co-inhibitory pathways have led to the development of effective immune checkpoint inhibitors (ICIs), particularly those targeting PD-1 and CTLA-4. These agents have significantly improved overall survival in melanoma; however, a substantial proportion of patients either fail to respond or eventually develop resistance. Ongoing clinical studies are elucidating mechanisms of immune evasion, refining response prediction biomarkers, and exploring combination strategies to overcome resistance and enhance durable remission.

Summary Immune checkpoint inhibition represents a major therapeutic milestone in malignant melanoma, transforming outcomes for many patients. Nevertheless, resistance and non-responsiveness remain key clinical challenges. Continued investigation into tumor–immune system interactions and rational combination approaches will be critical for optimizing the efficacy and durability of ICIs in melanoma treatment.

Keywords Cancer biology · Cancer immune surveillance · Immune checkpoint inhibitors · Melanoma · Metastasis.

Introduction

Melanoma is a malignant neoplasm of the skin that arises from unrestrained melanocyte proliferation [1]. Although representing only 5% of all skin cancer cases, melanoma accounts for the majority of skin cancer deaths [2, 3]. US statistics from Surveillance, Epidemiology, and End Results (SEER) Program report a 94.1% 5-year relative survival rate for melanoma patients between 2014 and 2020, however, this decreases to 35% when metastatic spread occurs [4]. The incidence of melanoma has increased dramatically over the last 50 years [5–7] surpassing all other cancers in the fair-skinned populations in Europe, North America and Oceania. This rise has been driven by new diagnoses in people over 60 years of age [8].

Melanoma is an aggressive malignancy with high metastatic potential and a poor prognosis, the latter due to resistance to cytotoxic chemotherapies [9, 10]. Malignant melanoma demonstrates a high median tumour mutational burden (TMB) – owing to a pathogenesis typically involving ultraviolet (UV)-radiation DNA damage – that initially promotes an immunogenic tumour microenvironment that is associated with higher response rates to immune checkpoint inhibitor (ICI) treatment [11]. However, this can also generate selection pressure favoring immune-evasive mutations, facilitating disease progression and resistance to ICIs. For example,

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downregulation of MHC class I molecules on melanoma cells reduces antigen presentation to effector immune cells, conferring resistance to ICIs [12]. Impaired antigen presentation can lead to the accumulation of dysfunctional anti-cancer T-cells in the tumor microenvironment, often reinforced by immunosuppressive cancer-associated fibroblasts [13].

Conventional chemotherapy, radiotherapy, and surgical resection are often unable to produce durable remission, lending only to a temporary reduction in tumour burden prior to eventual recurrence. Immunotherapies have revolutionised the treatment landscape for melanoma and are now considered a cornerstone in the management of this disease, particularly in the metastatic and adjuvant settings. This review explores strategies to restore effective anti-cancer immune responses through immune checkpoint inhibition [14], with a focus on anti-PD-1, anti-CTLA-4, and anti-LAG-3 therapies, which are the most extensively studied and currently approved ICIs for melanoma management. It discusses the underlying principles of ICIs, their role in melanoma treatment, the limitations of these therapies, and new avenues for research.

Review of Literature

Normal Immune System and the Role of Immune Checkpoints

The immune system protects the host from pathogens, toxins, and mutagenic changes [15]. Responses are classified as innate and adaptive, avoiding host damage by distinguishing self from non-self [16]. T cell receptor (TCR)-mediated antigen recognition is central to adaptive immunity, with antigens presented on MHC class I (normal cells) or class II (APCs).

CD8 T cells recognise MHC class I molecules and act as cytotoxic T lymphocytes (CTLs). $CD4^+$ T cells recognize MHC class II and orchestrate responses via cytokine release, differentiating into Th1, Th2, Th17, and Tregs. Tregs maintain self-tolerance. Effector T cells act rapidly, while memory T cells – central memory (T_{CM}), effector memory (T_{EM}), and tissue-resident memory (T_{RM}) cells, although stem cell-like memory (T_{SCM}) and effector memory re-expressing CD45RA (T_{EMRA}) cells – provide long-term immunity [17].

Other immune cells, including natural killer (NK) cells and γδ T cells, complement T-cell immunity. NK cells target cells with downregulated MHC class I, while γδ T cells bridge innate and adaptive immunity, recognising antigens independent of MHC molecules. Together, these components establish a robust, regulated defence system. Central and peripheral tolerance prevent inappropriate activation by self-antigens [18].

Central Tolerance

During T-cell maturation, V(D)J recombination generates diverse TCRs. Central tolerance in the thymus eliminates self-reactive T-cells via negative selection under AIRE regulation [19]. Positive selection ensures T-cells capable of interacting with MHC molecules survive. T-cells with non-interacting TCRs are deleted [16, 18].

Peripheral Tolerance

Peripheral tolerance maintains T-cell quiescence. Naïve T-cells encountering antigen undergo activation proportional to affinity and density [20]. Mechanisms preventing self-reactive T-cell activation include quiescence, ignorance, anergy, exhaustion, senescence, and apoptosis [21]. Ignorance occurs when antigen density is low or localized. Chronic antigen exposure induces exhaustion, a reversible dysfunction with reduced cytokine production and effector function, or senescence, a permanent cessation of proliferation and activity [21].

Two-signal Hypothesis for T-cell Activation

T-cell activation requires two signals: TCR recognition of antigen-MHC and CD28 binding to CD80/CD86 (Fig. 1), triggering intracellular cascades and cytokine production [22]. Co-inhibitory signals – including CTLA-4, PD-1, LAG-3, and TIGIT – oppose activation to prevent dysregulated responses (Fig. 2) [23].

Co-stimulatory Signals

CD28 is constitutively expressed on naïve $CD4^+$ (~ 95%) and $CD8^+$ (~ 50%) T-cells. It is a homodimeric 44-kDa glycoprotein with extracellular, transmembrane, and intracytoplasmic domains. CD28 ligation prevents T-cell anergy via BCL-xL upregulation, protecting against CD95-mediated apoptosis [26, 27]. Interactions with CD80/CD86 are enhanced by TCR stimulation, while overstimulation induces negative feedback. CTLA-4 expression upregulated upon sustained CD28-dependent signaling exerts inhibitory effects on T-cells [22, 28–30].

Co-inhibitory Signals

CTLA-4, a CD28 homolog, is transiently expressed on $CD4^+$/$CD8^+$ T-cells and constitutively on Tregs [31, 32]. It competes with CD28 for B7 ligands and recruits phosphatases to inhibit TCR/CD28 signaling [33–35]. Further, CTLA-4 actively inhibits T-cell co-stimulation by recruiting intracellular phosphatases of the Src homology 2 (SH2)

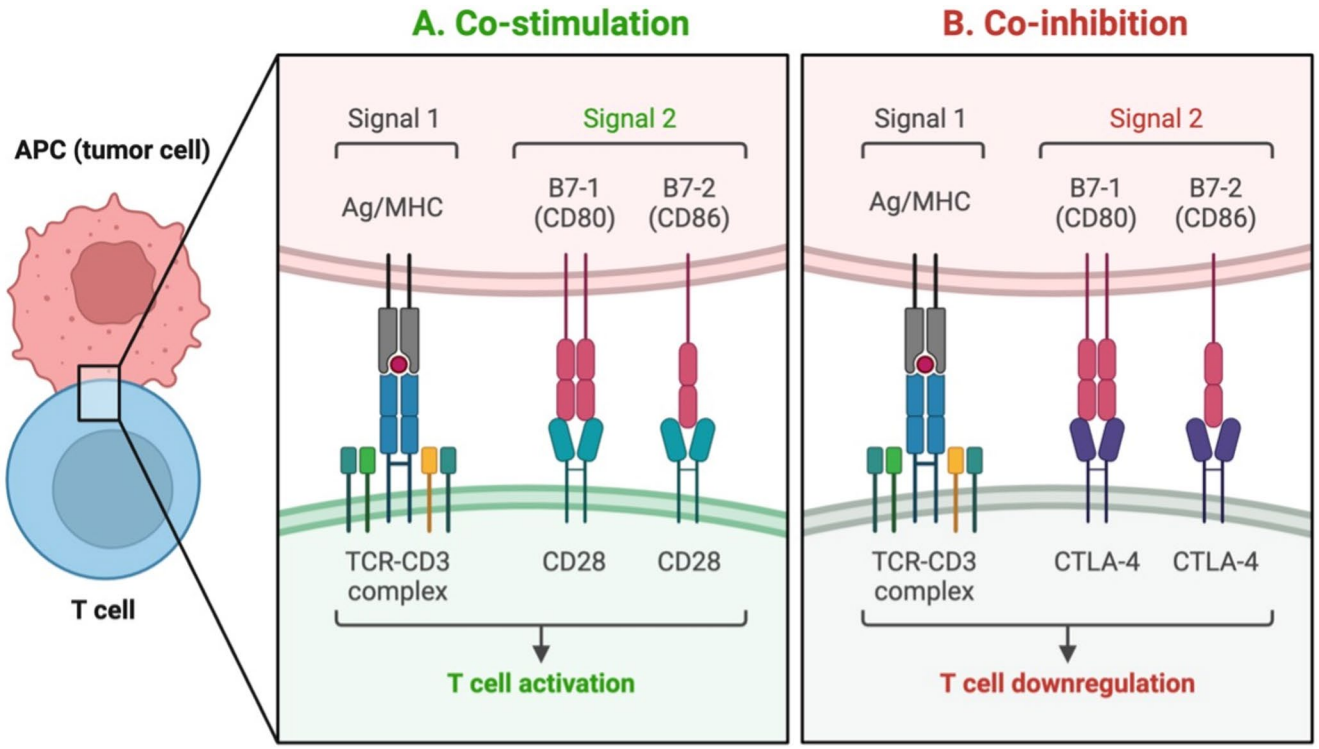

Fig. 1 Two-signal hypothesis for T-cell activation (**A**) vs. co-inhibitory signal (**B**). **A** Two signals are required for T-cell activation. Signal 1 is produced from the interaction of TCR on T-cells with the Ag/MHC ligand on APC. Signal 2 is produced from the interaction of co-stimulatory receptors such as CD80 and CD86 on the APC with co-receptors including CD28 on T-cells. In the presence of only signal 1, T-cells undergo tolerance, anergy or apoptosis, whereas in the presence of both signal 1 & 2, T-cells undergo activation, clonal expansion and effector functioning. **B** Co-inhibitory receptors, such as CTLA-4, on T-cells prevent the binding of co-stimulatory ligands CD80/86 on APC with CD28 on T-cells as the former exhibit higher avidity and affinity and outcompete CD28. APC, antigen presenting cell; Ag/MHC, antigen bound to major histocompatibility complex; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CD, cluster domain. Adapted with permission from Rallis et al. [24]

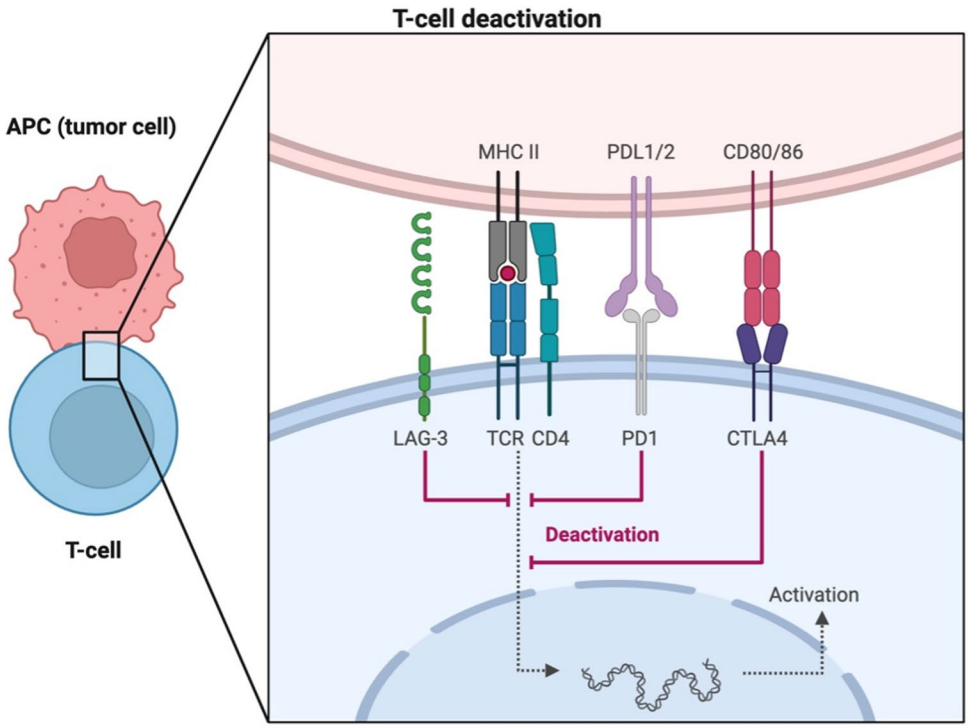

Fig. 2 Co- inhibitory signals. APC, antigen presenting cell; MHC-II, major histocompatibility complex class 2; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1/2, programmed death-ligand 1 and 2; CD, cluster domain; LAG-3, lymphocyte activate gene-3. Adapted from Mariuzza et al. [25]

domain family, including tyrosine phosphatase 1 (SHP1), SHP2, and serine/threonine protein phosphatase 2 A (PP2A) (Fig. 3) [35, 36]. PD-1 regulates peripheral responses via PD-L1/PD-L2 and inhibits key signaling pathways [37–39]. LAG-3 interacts with MHC-II and other ligands, suppressing T-cell proliferation and cytokine production; co-expressed with PD-1 to synergistically attenuate immune responses (Fig. 4) [39–47].

The Immune System in Cancer

Cancer cells generate neoantigens capable of eliciting immune responses [31, 35, 48]. Tumors evade immunity via (a) antigen/MHC downregulation, (b) secretion of immunosuppressive factors and recruitment of Tregs/Bregs/MDSCs/fibroblasts, and (c) upregulation of immune checkpoints like PD-1, CTLA-4, LAG-3, and IDO [14, 49, 50].

Immune Exhaustion

T-cell exhaustion, described in chronic viral infection, also occurs in melanoma, with impaired proliferation, cytotoxicity, cytokine secretion, mitochondrial function, and upregulation of inhibitory receptors (CTLA-4, PD-1, LAG-3, TIGIT, TIM-3) [14, 31]. Co-expression of multiple inhibitory receptors correlates with severe dysfunction and absence of $CD4^+$ T-cell support [14].

Immune Checkpoint Inhibitors for Melanoma Therapy

Immune checkpoint blockade aims to prevent the downstream signalling mediated by CTLA-4, PD-1 and LAG-3. This, in turn, avoiding the induction of anergy and restraint of the immune response against tumour cells. ICIs that

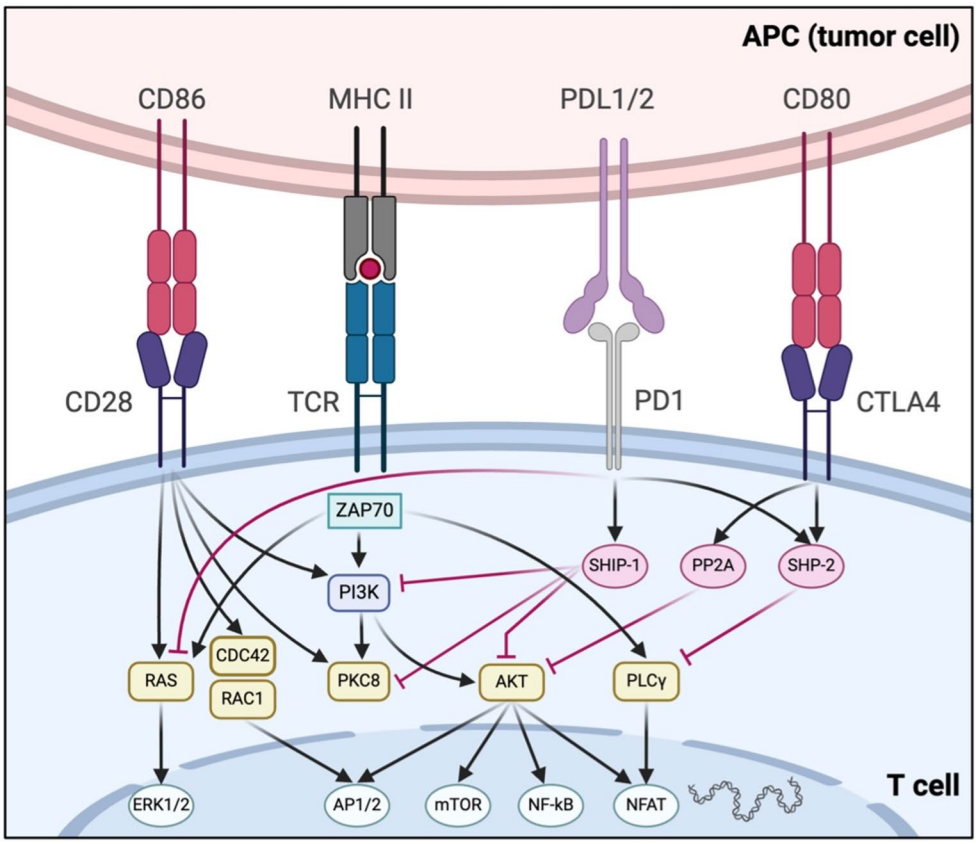

Fig. 3 Interaction of B7/CD28 family of receptors including co-inhibitory receptor systems CTLA-4 and PD-1 and their downstream signalling targets. Black arrows, positive co-stimulatory signals; red lines, negative co-inhibitory signals; APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1/2, programmed death-ligand 1 and 2; CD, cluster of differentiation; ZAP, zeta-chain-associated protein kinase; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; AKT, serine/threonine protein kinase also known as protein kinase B (PKB); PLC, phospholipase C; SHP1/2, Src homology 2 domain tyrosine phosphatase 1; PP2A, serine/threonine protein phosphatase 2 A; RAS, rat sarcoma protein; RAC1, Ras-related C3 botulinum toxin substrate 1 protein; CDC42, cell division control protein 42 homolog; ERK, extracellular signal-regulated kinase; AP1/2, activator protein 1 and 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NFAT, nuclear factor of activated T-cells. Adapted with permission from Rallis et al. [24]

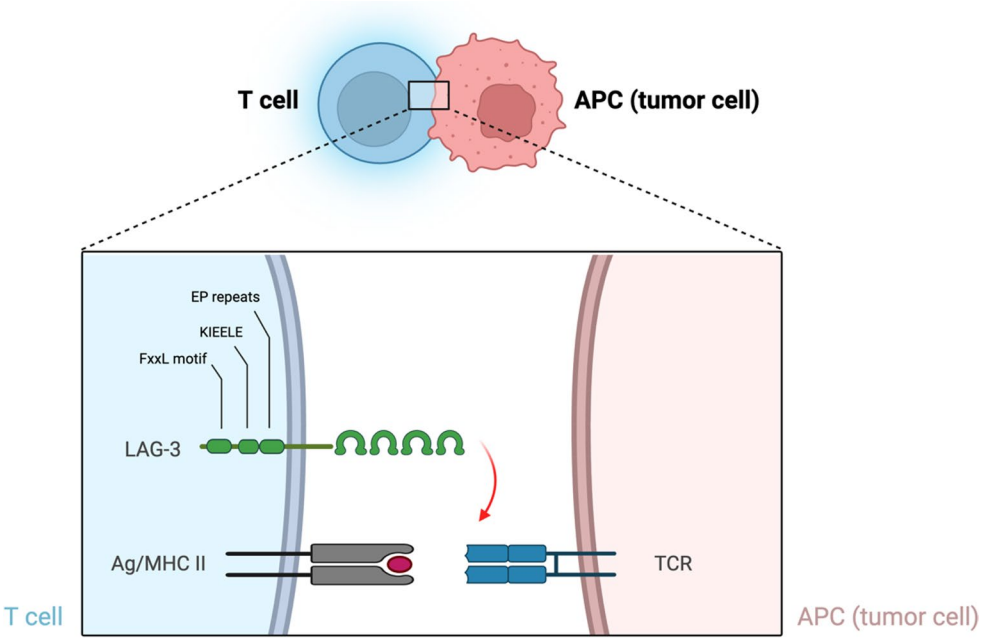

Fig. 4 LAG-3 mechanism of action. Red arrow, negative co-inhibitory signal; APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; LAG-3, lymphocyte activate gene-3; EP, glutamic acid-proline. Adapted from Mariuzza et al. [25]

have been approved by the FDA for treatment of melanoma include ipilimumab (anti-CTLA-4; Yervoy®), pembrolizumab (anti-PD-1; Keytruda®), nivolumab (anti-PD-1; Opdivo®), combination nivolumab-ipilimumab (anti-PD-1, anti-CTLA-4; Yervoy®) and combination nivolumab-relatlimab (anti-PD-1, anti-LAG-3; Opdualag®) [51–53]. ICIs are approved in the adjuvant setting (following resection of high-risk primaries) and in the metastatic setting.

Rationale for Initial Immunotherapy Breakthroughs in Melanoma

Historically, treatment options for malignant melanoma were limited, with conventional therapies such as surgery, radiotherapy, and chemotherapy offering only modest survival benefits. Dacarbazine, the first chemotherapeutic agent approved for metastatic melanoma in 1975, remained the standard of care for decades, but its efficacy was poor, with response rates of only 10% to 15%, a median survival time of six months, and a one-year overall survival rate of 25% [54].

The need for more effective treatments led to the exploration of immunotherapy, especially given melanoma's high TMB, which contributes to its immunogenicity. The large number of DNA mutations in melanoma cells, largely induced by UV radiation, results in altered protein sequences that are processed and displayed as tumour-specific neoantigens on MHC molecules. These neoantigens are recognized by host T-cells as foreign, making melanoma an ideal candidate for immunotherapy [55]. The recognition of melanoma's immunogenic potential spurred early immunotherapy efforts, with high-dose interleukin-2 (IL-2) becoming one of the first FDA-approved treatments for melanoma in 1998. IL-2 therapy facilitated the expansion of T-cells capable of targeting melanocyte differentiation antigens like gp100 and MelanA [55, 56]. Although the response rates were relatively low—around 16%—and the complete response rate was only 6% [57], the ability to generate durable immune responses against cancer cells paved the way for further development of immune-based treatments in melanoma and other malignancies.

Clinical Efficacy of Checkpoint Inhibitors Leading Market Authorisation in Melanoma

I) Ipilimumab

Ipilimumab is currently approved for use in advanced melanoma, as an adjuvant in resected stage III melanoma and as combination with nivolumab. Ipilimumab was the first ICI approved in 2011 for the treatment of unresectable or metastatic melanoma [53]. This approval was based on the results from the pivotal MDX010-020 trial (NCT00094653) in unresectable, metastatic melanoma. This phase 3 trial randomised patients in a 3:1:1 ratio to receive either [1] ipilimumab (3 mg/kg) plus the gp100 peptide vaccine [2], ipilimumab (3 mg/kg) plus placebo, or [3] the gp100 vaccine alone. Results demonstrated that median OS was significantly improved with ipilimumab, either with or without the gp100 vaccine, compared to the gp100 vaccine alone. Median OS was 10.0 months in the ipilimumab plus gp100 group, 10.1 months in the ipilimumab monotherapy group, and 6.4 months in the gp100 vaccine alone group (hazard ratio [HR] for OS: 0.68; 95% CI: 0.55–0.85, $p < 0.001$) [58].

On October 18, 2015, the FDA approved ipilimumab as an adjuvant treatment in patients with cutaneous melanoma based on the randomised CA 184 − 029 study [59]. Its approval was further expanded to include paediatric patients aged 12 years or older on July 24, 2017, following an open-label, single-arm trial. The approved dosing regimen consisted of 3 mg/kg administered intravenously every three weeks for a total of four doses [53].

II) Pembrolizumab

Following the approval of ipilimumab, the anti-PD-1 ICIs nivolumab and pembrolizumab also received approval for the treatment of melanoma and other malignancies. Pembrolizumab is currently approved for use in advanced melanoma and as an adjuvant in resected melanoma. Pembrolizumab received approval in 2014 for the treatment of unresectable or metastatic melanoma in patients previously treated with CTLA-4 or BRAF inhibitors based on the results of the KEYNOTE-001 trial (NCT01295827) [60]. This phase 1b study demonstrated the durable antitumour activity and tolerability of pembrolizumab after 5-year follow-up data for patients with advanced melanoma. The 5-year survival rate for patients receiving pembrolizumab was 34% (73% with ongoing response at year 5) in all patients and 41% (82% with ongoing response) in treatment-naïve patients [61].

KEYNOTE-006, a phase III clinical trial comparing pembrolizumab to ipilimumab in 834 adult patients with stage 3/4 BRAF V600 positive melanoma that had received no more than one prior systemic therapy, demonstrated a significant increase in 6-month progression free survival (PFS) (47.3% vs. 26.5%) and 12-month OS (74.1% vs. 58.2%) [62]. Response rates were higher with pembrolizumab (33.7% vs. 11.9%), and grade 3–5 adverse events were reduced (13.3% vs. 19.9%) in comparison to ipilimumab [62]. This trial led to the FDA approval of pembrolizumab as a first-line treatment for advanced melanoma, regardless of BRAF mutation [53].

The FDA extended the use of pembrolizumab in the adjuvant setting in patients with resected stage III melanoma based on the KEYNOTE-054 (EORTC 1325-MG) trial. This phase 3 randomised, double-blind study demonstrated prolonged RFS in patients treated with pembrolizumab compared to placebo (1-year rate of RFS 75.4% [95% CI, 71.3 to 78.9] vs. 61.0% [95% CI, 56.5 to 65.1] [63].

III) Nivolumab

By comparison, nivolumab, exhibits similar efficacy to pembrolizumab. Nivolumab is currently approved for advanced melanoma, in the adjuvant setting and in combination with ipilimumab. Nivolumab was first approved in 2014 for the treatment of unresectable or metastatic melanoma [64]. This approval was based on the results from the CheckMate-037 trial (NCT01721746), a phase 3 study comparing nivolumab to chemotherapy (either dacarbazine or paclitaxel) in patients with advanced melanoma who had previously received ipilimumab and, if appropriate, a BRAF inhibitor. Results demonstrated that nivolumab significantly improved OS and PFS compared to chemotherapy. The median OS was not reached in the nivolumab group versus 10.8 months in the chemotherapy group (hazard ratio [HR] for OS: 0.42; 95% CI: 0.31–0.58, $p < 0.001$). The ORR was 31.7% in the nivolumab group compared to 10.6% in the chemotherapy group ($p < 0.001$) [65].

Nivolumab was later approved as an adjuvant treatment for melanoma based on the CheckMate-238 trial (NCT02388906), a phase 3 study comparing nivolumab to ipilimumab in patients with resected, high-risk stage III or IV melanoma. The trial demonstrated that nivolumab significantly reduced the risk of recurrence or death compared to ipilimumab, with a 3-year recurrence-free survival (RFS) rate of 66.4% in the nivolumab group versus 52.0% in the ipilimumab group (hazard ratio [HR] for recurrence or death: 0.65; 95% CI: 0.51–0.83, $p < 0.001$) [66].

IV) Combination nivolumab and ipilimumab

To explore OS benefit, a phase I study showed that ipilimumab plus nivolumab conferred a 63% 3-year OS in 94 advanced melanoma patients [67]. This was followed by two landmark clinical trials: CHECKMATE-067 and CHECKMATE-069. These lead to the FDA approval of ipilimumab plus nivolumab combination therapy for unresectable or metastatic melanoma regardless of BRAF status. The phase 2 trial CHECKMATE-069 compared the clinical outcomes between ipilimumab plus nivolumab to ipilimumab alone and found an increased 2-year OS (53.6% to 63.8%) compared to ipilimumab alone [68]. The phase 3 CHECKMATE-067 trial found that OS at 3 years increased to 58% when using dual therapy compared to 52% of the nivolumab and 34% of ipilimumab monotherapy groups [69]. Hence, in 2015, nivolumab plus ipilimumab received accelerated FDA approval for BRAF V600 wild-type, unresectable or metastatic melanoma. In 2016, approval was expanded to include unresectable or metastatic melanoma with or without BRAF mutations. Currently, dual ICI with ipilimumab plus nivolumab is the most effective treatment for advanced melanoma and therefore considered first line, yet it is also the most toxic treatment for advanced melanoma, leading to approximately 40% of patients to discontinue treatment prematurely [70]. Indeed, dual ICI regimens have been questioned around the increased risk of irAEs and limited survival benefits in some patient subgroups. A meta-analysis of nine RCTs demonstrated that the incidence of adverse effects (described by 8 included studies) was overall significantly higher with combination ICI compared to monotherapy with either nivolumab or ipilumumab (RR 1.07, 95% CI [1.03–1.12] with a p-value = < 0.001) [71].

Low-dose ICI combinations are being investigated to reduce toxicity and enhance efficacy [72]. Two clinical trials have demonstrated significant antitumour activity, safety, and comparable tolerability from low-dose dual ICI therapy with anti-PD-(L)1 plus ipilimumab in melanoma patients who progressed on prior anti-PD-(L)1 monotherapy [73, 74]. SWOG S1616, a randomized phase II trial, evaluated ipilimumab with or without nivolumab in PD-1/PD-L1–refractory metastatic melanoma and found that the combination improved response rates (28% vs. 9%) and PFS compared with ipilimumab alone, supporting its use as a second-line strategy in PD-1-resistant disease [75]. These findings support dual ICI therapy as second-line treatment for melanoma that progresses on anti-PD-(L)1 monotherapy [53, 72].

V) Combination nivolumab and ipilimumab

Relatlimab, a LAG-3 blocking antibody, is the fourth approved ICI for the treatment of patients with unresectable or metastatic melanoma [70]. The combination of relatlimab plus nivolumab was explored in RELATIVITY-047, a phase 2/3 randomised double-blind trial which demonstrated increased efficacy and safety compared to nivolumab monotherapy. The RELATIVITY-047 trial led to the approval of relatlimab in combination with nivolumab in March 2022 by the FDA and in September 2022 by the European Medicines Agency (EMA). Combination relatlimab plus nivolumab has shown a risk of treatment-related adverse effects of 21% compared to 59% when using ipilimumab plus nivolumab [65]. Prior to this, the RELATIVITY-020 study (NCT01968109), encompassing patients with advanced melanoma who had not responded or progressed on anti-PD-1 therapy, demonstrated promising tolerability and sustained response to the combination of relatlimab and nivolumab. The study reported an overall response rate (ORR) of 16% and a disease control rate of 45% [76]. In the future, should the survival advantage of relatlimab-nivolumab match or surpass that of nivolumab-ipilimumab, combination therapy with relatlimab-nivolumab may emerge as the fresh standard of care for advanced melanoma in previously untreated patients [77].

Other combinations involving LAG-3 are currently being explored in phase 3 clinical trials for melanoma. These trials are evaluating the combination of fianlimab (anti-LAG-3) and cemiplimab (anti-PD-1) versus pembrolizumab in patients with previously untreated unresectable melanoma (NCT05352672) and in patients with completely resected high-risk melanoma (NCT05608291) [78].

Immune Checkpoint Inhibitors as Neoadjuvant Therapy

The rationale for utilising ICIs in the neoadjuvant setting for melanoma lies in the concept that administration of ICIs while the primary tumour is still present will result in a more robust T cell response, due to a higher tumour burden and increased antigen presentation from tumour cells. Both preclinical and clinical trials have shown that patients treated in the neoadjuvant setting experience better event-free survival (EFS) outcomes compared to those treated in the adjuvant setting [79].

The phase 2 SWOG S1801 trial showed that neoadjuvant pembrolizumab, followed by adjuvant pembrolizumab, significantly improved EFS compared to adjuvant therapy alone (72% vs. 49% at two years, $p = 0.004$) [80]. New recommendations from ASCO include the use of pembrolizumab for patients with resectable stage IIIB to IV cutaneous melanoma followed by resection and adjuvant pembrolizumab [81]. However, strong evidence not only supports neoadjuvant single-agent anti–PD-1 therapy, but also combination therapy with ipilimumab and nivolumab, and nivolumab and relatlimab [79].

In the phase 2 SWOG S1801 trial, administering three neoadjuvant cycles of pembrolizumab followed by 15 adjuvant cycles resulted in a superior EFS compared to 18 cycles of adjuvant pembrolizumab, with estimated two-year EFS rates of 72% versus 49% ($p = 0.004$). Rates of treatment-related adverse events were comparable between both treatment groups [80]. Other studies, such as the OpACIN and OpACIN-neo trials, have further demonstrated that neoadjuvant combination therapies, including ipilimumab/nivolumab, can induce high pathological response rates and expand tumor-specific T cell clones, potentially reducing relapse rates [82]. However, side effects were considerably increased with the neoadjuvant regime. The OpACIN-neo trial addressed the increased toxicity by reducing the dose of ipilimumab, showing a decrease in adverse effects with ipilimumab at 1 mg/kg and nivolumab at 3 mg/kg [82]. More recently, the phase III NADINA trial provided confirmatory evidence that neoadjuvant nivolumab plus ipilimumab followed by surgery and response-adapted adjuvant therapy significantly improved event-free survival compared with standard adjuvant nivolumab alone, establishing neoadjuvant ICI as a potential new standard of care in resectable stage III melanoma [83].

Despite these promising findings, ICIs have not yet received formal regulatory approved for neoadjuvant use in melanoma in many countries, though it should be noted that neoadjuvant ICI therapy is now incorporated as a standard approach in major clinical guidelines, such as the NCCN. One key reason is the need for more definitive, long-term data on survival outcomes and safety. Early trials indicate that neoadjuvant therapy can lead to increased toxicity, particularly with combination regimens, which may delay surgery or increase perioperative risks [79]. While dose adjustments, as seen in OpACIN-neo, have helped mitigate some toxicity

concerns, regulators require further evidence to determine optimal patient selection, treatment duration, and risk-benefit profiles. In contrast, neoadjuvant ICIs have already been approved for non-small cell lung cancer (NSCLC), where studies like CheckMate 816 demonstrated clear survival benefits with manageable toxicity [84]. The approval of ipilimumab and nivolumab in NSCLC highlights the potential for similar strategies in melanoma, but further validation in larger phase 3 trials is needed before neoadjuvant ICIs can become the standard of care in melanoma treatment.

Combination Chemoradiotherapy with Immune Checkpoint Inhibitors

Chemoradiotherapy (CRT) is standard therapy across many cancers as it offers a survival benefit and increased local disease control rates [85]. Both chemotherapy and radiotherapy have been shown to impact the efficacy of immunotherapies and vice versa. Local tumour irradiation increases MHC-I expression as well as tumour neoantigen presentation and retrieval, helping to facilitate immune-mediated cancer destruction [86]. Some evidence suggests that radiotherapy may also promote innate and adaptive immune responses within the tumour microenvironment in certain settings [87]. The abscopal effect, which describes the regression of cancer sites distal to the primary site of irradiation, has been attributed to immune activation whereby circulating immune cells demonstrate increased responses to neo-antigens following radiotherapy [88]. According to some studies, chemotherapy may also improve tumour neoantigen presentation, sensitise tumours to immunotherapy and, paradoxically, inhibit immunosuppression in certain settings [reviewed in [89].

In melanoma, much of the literature focuses on efficacy and adverse effects [57, 75–78]. Research looking at survival and response rates provides a mixed picture.

I) Chemotherapy and Immune Checkpoint Inhibitors

In many solid tumours, such as lung and breast cancer, the combination of chemotherapy with ICIs has become standard, leveraging chemotherapy's cytotoxic and immunomodulatory effects to enhance ICI efficacy. In melanoma, this paradigm has also been explored. Robert et al. [90] looked at OS in patients receiving ipilimumab plus dacarbazine, a chemotherapy agent approved by the FDA for advanced melanoma [91] or dacarbazine alone. Results showed that patients receiving a combination of chemotherapy and ipilimumab had an increased OS at 1 year (47.3% relative to 36.3% in patients received chemotherapy alone). The OS continued to be better at 3 years (20.8% compared to 12.2%). However, grade 3 or 4 adverse events were seen in 56.3% of patients treated with ipilimumab and dacarbazine compared to 27.5% in those treated with dacarbazine only.

A randomised Phase I study showed that ipilimumab could be safely combined with dacarbazine or paclitaxel/carboplatin in patients with previously untreated advanced melanoma. However, estimates of ORR were imprecise due to the small size of the study, demonstrating that combination of ipilimumab plus paclitaxel/carboplatin (ORR 27.8%) did not lead to better outcomes compared with ipilimumab alone or ipilimumab plus dacarbazine (ORR 33.3% in both groups) [92].

Importantly, emerging data suggest that the chemo-ICI combination in melanoma may exert activity even after progression on anti-PD-1 therapy. For instance, Aguilera et al. report that in metastatic melanoma patients who had failed PD-1 blockade, adding chemotherapy increased $CX3CR1^{+}$ therapy-responsive $CD8^{+}$ T-cells and was associated with improved clinical responses [93]. Similarly, Goodman et al. describe a case series of three patients treated with the combination of nivolumab plus temozolomide after multiple prior therapies, demonstrating response in the heavily pre-treated setting [94].

Collectively, these findings highlight that while chemo-ICI combinations in melanoma remain less established than in lung or breast cancer, they warrant further investigation – particularly in the post-PD-1 setting or for patients with limited options.

II) Radiotherapy and Immune Checkpoint Inhibitors

Koller et al. (2017) found improved OS in patients with advanced melanoma treated with concurrent ipilimumab and radiotherapy compared to ipilimumab alone (19 months vs. 10 months, $p = 0.01$) [95]. However, a retrospective cohort study of 835 patients who received CTLA-4 or anti-PD-1 with or without preceding radiotherapy for unresectable metastatic melanoma found no survival differences [for anti-CTLA-4 (OS, HR = 1.08, 95% CI = 0.81 to 1.44, $p = 0.61$) and for anti-PD-1 (OS, HR = 0.73, 95% CI = 0.43 to 1.25, $p = 0.26$)] [96].

The outcomes from combining chemotherapy or radiotherapy and immunotherapy for melanoma are diverse, highlighting the necessity for more hypotheses driven research based on a sound biological basis to ascertain both its effectiveness and safety.

Limitations of Checkpoint Inhibitors

Treatment Resistance

Treatment resistance is a main limitation of ICIs. Primary resistance or intrinsic resistance occurs when malignant cells do not respond to immunotherapy at initial drug exposure and acquired resistance to ICIs arises from the successive acquisition of immune evasion mechanisms [14].

Primary resistance varies between ICIs. In metastatic melanoma, the ORR with ipilimumab is 15%, PD-1 inhibitors display 30–35% and combination of nivolumab/ipilimumab and nivolumab/relatlimab ~ 60% and 40% respectively. Acquired resistance occurs in 25% within the first two years of ICI [97].

I) Primary Resistance

Primary resistance was experienced by 40–65% of patients receiving PD-1 inhibitors and 70% of patients receiving CTLA-4 inhibitors. ICIs reverse inhibitory signals towards effector T cells, thus all mechanisms that dampen T cell responses cause ICIs to be less effective. These mechanisms include low TMB, impaired dendritic cell maturation leading to impaired antigen presentation and priming, VEGF and ANG2 overexpression, and tumour-infiltrating lymphocytes (TILs) inhibition by Treg cells [98]. Many strategies that target these mechanisms are being explored and consist of combination of radiotherapy and ICI, combination anti-VEGF and ICI, combination anti-VEGF and anti-ANG2, CXC3 upregulation, Treg suppression with sunitinib and cytotoxic T cell therapy [86].

II) Acquired Resistance

Mechanisms that underlie acquired resistance overlap with those of primary resistance. These mechanisms include deficient antigen presentation, loss of β2- microglobulin associated with loss of MHC I expression, dysfunction of JAK1, 2/STAT signalling as well as PD-L1, LAG-3, TIM-3 and FCRL6 overexpression leading to T cell exhaustion. Since treatment with ICI can lead to the upregulation of other inhibitors receptors, developing antagonistic monoclonal antibodies of inhibitory receptors associated with resistance such as TIM-3 aim to overcome resistance. Indeed, combination ICI with dual TIM-3 and PD-1 inhibition has been shown to be more successful in achieving anti-tumour responses than TIM-3 blockade alone [99]. These findings underly the rationale behind combination of ICI to overcome acquired resistance.

Toxicity

ICIs face limitations due to immune-related adverse events (irAEs), which result from the activation of self-targeting T cells that harm host tissues [100]. These events often prompt treatment interruptions, delays and in rare cases, pose life-threatening risks. IrAEs resemble autoimmune diseases and are more common in individuals with a predisposition to autoimmunity, high body mass index, chronic smoking, and differ in incidence based on sex – women receiving CTLA-4 inhibitors and men receiving PD-1/PD-L1/ inhibitors appear to be at higher risk [101]. While extensive data exist on CTLA-4 and PD-1/PD-L1-related irAEs, information on LAG-3 inhibitors remains limited.

I) Organ Toxicity

ICIs can affect any organ, but irAEs most commonly involve the colon, liver, lungs, pituitary, thyroid and skin. Though rarer, severe complications affecting the heart, nervous system and other organs can occur [102]. The type and severity of toxicity depend on the class of ICI used:

- CTLA-4 inhibitors tend to prompt gut inflammation and hypophysitis
- PD-1 inhibitors are often associated with thyroiditis, pneumonitis, and autoimmune diabetes
- Combination ICI therapy increases toxicity risk, particularly with dual CTLA-4/PD-1 blockade, which induces more severe irAEs [89].
- LAG-3 inhibitors appear to be better tolerated, with common side effects including rash, fatigue and hypothyroidism [78].

Higher-grade irAEs occur in 30–55% of patients receiving combination ipilimumab/nivolumab, compared to 10–15% with PD-1/PD-L1 monotherapy. Notably, CTLA-4-related toxicities are dose-dependent, whereas PD-1/PD-L1 toxicities are not [103].

II) Toxicity Incidence across Immune Checkpoint Inhibitors

The incidence of severe irAEs tends to follow predictable patterns across large populations:

- **CTLA-4 inhibitors:** 20–30% severe irAEs (dose-dependent).
- **PD-1/PD-L1 inhibitors:** 15–20% severe irAEs (not dose-dependent).
- **Combination CTLA-4/PD-1 therapy:** 40–50% severe irAEs.
- Fatal irAEs: ~0.4% for PD-1/PD-L1 monotherapy; ~1.2% for combination therapy [104].

In contrast, chemotherapy leads to predictable, dose-dependent toxicities like myelosuppression and nausea, with a lower rate of severe side effects (10–20%), that are generally reversible. High-dose IL-2 therapy, once the mainstay of melanoma treatent, carries the highest toxicity burden (60–80% severe toxicity), with side effects ranging from vascular leak syndrome to hypotension, and multi-organ dysfunction requiring ICU-level care [57, 105, 106].

Combination ICI has been shown to carry higher rates of toxicities compared to monotherapy regardless of age [107]. In the CheckMate 067 trial (NCT01844505**)**, approximately 40% of patients discontinued nivolumab/ipilimumab treatment due to toxicity, a notably higher percentage compared to 14% for nivolumab alone and 15% for ipilimumab alone. Overall, 59% of patients receiving the combination therapy

encountered grade 3–4 adverse events, a substantially higher rate compared to the 23% observed with nivolumab alone [108]. The risk of toxicity appears to be additive rather than synergistic [109].

LAG-3 inhibitors offer a more favorable safety profile. The RELATIVITY-047 trial (NCT03470922) demonstrated that combining relatlimab with nivolumab resulted in a 21% risk of treatment-related adverse effects, notably lower than the 59% risk when using ipilimumab plus nivolumab. Grade 3–4 adverse events occurred in 18.9% of patients with relatlimab/nivolumab versus 9.7% with nivolumab alone, suggesting manageable toxicity without unexpected safety concerns [110]. Overall, adverse effects related to treatment were seen in 81.1% of patients receiving relatlimab-nivolumab, a rate lower than that observed in the ipilimumab group (86.2%) and the ipilimumab-nivolumab group (95.5%) [111].

III) Timeline

The timing of irAEs varies with the majority appearing in the first 8 weeks of treatment. Cutaneous irAEs tend to develop earlier, followed by gastrointestinal, hepatic, pulmonary, endocrine and renal. Most of these resolve within months, but endocrine irAEs can require long life treatment. Timing of irAEs also varies depending on the ICI. Ipilimumab shows higher rate of side effects in the first 12 weeks, whereas pembrolizumab and nivolumab often exhibit irAEs beyond the first 3 months as treatment is discontinued. Nonetheless, the enduring toxicities of single-agent ipilimumab and PD-1 agents are similar in the long term [108].

Novel Approaches to Immunotherapy

New Checkpoint Targets

Druggable immune targets currently in the early stages of clinical investigation for novel ICIs include TIM-3, TIGIT, BTLA, VISTA and CD96 [112]. These targets may serve as the basis for novel ICI therapeutics in melanoma patients that become refractory to conventional ICIs. Alternatively, novel ICIs may offer better efficacy or toxicity profiles when combined with existing ICIs.

TIM-3 is a type I transmembrane protein that belongs to the TIM family of immunoregulatory proteins that are found on many immune cells, including TILs. TIM-3 has been the most prominent of these as it has been suggested to regulate immune responses in autoimmunity and cancer [113]. TIM-3 is co-expressed with PD-1 on both $CD4^+$ and $CD8^+$ T-cells, and when both of their pathways are targeted together compared to either of them on their own, has been shown to be effective in controlling tumour growth, hence making TIM-3 an attractive immunotherapy candidate [114]. Moreover, TIM-3 is upregulated in patients that respond to anti-PD-1 therapy via the PI3K-Akt pathway [115]. Hence, TIM-3 and PD-1 co-blockade may restore T-cell responses more effectively than PD-1 monotherapy [116]. Currently anti-TIM-3 therapies are being investigated in combination with anti-PD-1 blockade in phase I/II trials (NCT04139902, NCT03708328) [47].

Another promising checkpoint candidate, B and T lymphocyte attenuator (BTLA) has been shown to play a role in the inhibition of CD8 + T cell expansion and function in melanoma [117]. In combination with PD-1 and TIM-3 blockade, inhibition of BTLA has been shown to reactivate dysfunctional melanoma antigen-specific CD8 + T cells, enhancing their proliferation and cytokine secretion. Icatolimab (JS004, Table 4), a new anti-BTLA mAb, is being tested in two phase-I trials for advanced solid tumours, alone or with PD-1 inhibitors (NCT04137900 and NCT04773951). In NCT04137900's dose-expansion phase, 1/19 melanoma patients previously unresponsive to nivolumab and BRAF/MEK inhibitors achieved a partial response without severe adverse events [118].

Finally, VISTA (V-domain Ig suppressor of T cell activation) is a checkpoint ligand that is homologous to PD-L1 and suppresses T cell activation. In a study composed of 85 primary melanoma specimens, VISTA expression was shown to be correlated with decreases disease-specific survival ($p = 0.05$) and to be an independent negative prognostic factor ($p = 0.02$), suggesting its potential as an adjuvant immunotherapeutic intervention [119]. Thus, anti-VISTA mAbs have been studied in preclinical studies and recently in clinical-trial settings. In mice, CA-170, an oral PD-L1/L2 and VISTA dual inhibitor has been shown significant anti-tumour properties. In a phase I trial (NCT02812875), CA-170 showed acceptable safety and increased numbers of circulating activated CD4 + and CD8 + T cells [120]. Additionally, another phase I clinical trial (NCT05082610) is investigating the efficacy of monotherapy or combination therapies involving pembrolizumab with HMBD-002, an Fc-independent anti-VISTA monoclonal antibody, in patients with advanced solid tumours [118].

Alternative Therapies

I) Cell therapies

Adoptive cell therapy (ACT) aims to deliver immune cells to destroy cancer cells. ACT involves the collection of a patient's own immune cells, such as T cells, which are then engineered or activated in the laboratory to enhance their ability to target and destroy cancer cells. Once these modified or expanded cells are grown to sufficient numbers, they are infused back into the patient to mount an effective anti-tumour response [121].

Various forms of ACT are employed, each with distinct methodologies. These include: [1] TILs, these lymphocytes are cultivated from the tumour itself; [2] endogenous T Cell therapy, these are tumour-specific T cells cultured from the blood; [3] chimeric antigen receptor (CAR) T cells, these cells are engineered using a fusion of chimeric antibody and T cell receptor genes, which are then integrated into peripheral T cells; and [4] TCR transduced T Cells, this technique involves engineering T cell receptor genes to target the tumour, which are subsequently inserted into peripheral T cells [122].

The development of ACT was propelled by the description of IL-2 in 1976 which enabled ex vivo expansion of T lymphocytes while often preserving their effector function [123]. In 1985, Rosenberg et al., demonstrated that IL-2 administration could lead to complete durable tumour regressions in some patients with metastatic melanoma, prompting efforts to identify specific T cells and their target antigens responsible for this therapeutic effect [124]. By 1986, in vitro studies revealed that TILs extracted from resected melanomas included cells capable of specifically recognizing autologous tumours [125]. These findings culminated in a landmark 1988 study in which 20 patients with metastatic malignant melanoma were treated with TILs and IL-2 following a single intravenous dose of cyclophosphamide. Objective regression was observed in 60% of the 15 patients who had never been treated with IL-2 and in 40% of the 5 patients in which IL-2 therapies had previously failed. Regression of cancer was observed with duration of responses ranging from 2 to more than 13 months [126].

In the decades after the description of ACT using TILs, several small cohort phase 1/2 trials in advanced-stage melanoma were performed. These are summarised in a systematic review of 13 clinical trials including 410 patients with advanced-stage melanoma from 1988 to 2018 with results showing an ORR in the total cohort of 41%, ranging from 28% to 45%, with 14% complete responses and 27% partial responses [127].

Despite the potential of TIL therapy, clinical approval was delayed due to several factors, including the need for ongoing improvements in clinical response rates and the development and refinement of appropriate FDA good manufacturing guidelines for cell production [128].

Several key findings contributed to the development of ACT. Notably, it was discovered that administering IL-2 after cell infusion helps promote the survival and growth in vivo of the transferred cells in mouse tumour models, leading to the inclusion of IL-2 administration in the treatment regimen [128]. Additionally, in 2002, it was demonstrated that lymphodepletion using a nonmyeloablative chemotherapy regimen administered immediately before TIL transfer could enhance cancer regression and result in the persistent oligoclonal repopulation of the host with the transferred antitumour lymphocytes [129].

The FDA approved lifileucel (Amtagvi, Iovance Biotherapeutics, Inc.) in February 2024 based on data from a global, phase 2, multicohort, single-arm, C-144-01 trial (NCT02360579) in adult patients with advanced melanoma after progression on ICI and targeted therapies [130]. This trial included four cohorts. Approval was specifically based on data from a subset of 73 patients in the trial whose cancer had worsened despite treatment with a PD-1/PD-L1 ICI or a BRAK inhibitor and whose lifileucel dose was at least 7.5 billion cells. Results showed an ORR of 31.5% with a complete response in 3 (4.1%). Patients received a single intravenous infusion of lifileucel at a median dose of 21.1 × 10^9 viable cells following lymphodepletion with cyclophosphamide, mesna, and fludarabine. Post-infusion, IL-2 (aldesleukin) doses were administered to support in vivo cell expansion [128].

Lifileucel is approved for use in patients with advanced-stage melanoma refractory to a PD-1 blocking antibody, and in those with BRAFV600 mutations, BRAF/MEK inhibitors [131]. This immunotherapy employs the patient's own TILs which are isolated from a surgically excised tumour. The TILs are subsequently expanded ex vivo using IL-2 and then reinfused into the patient to specifically target and eradicate tumour cells. The regimen involves a lymphodepleting regimen consisting of cyclophospimade (60 mg/kg daily with mesna for 2 days) followed by fludarabine (25 mg/m^2 daily for 5 days). This is succeeded by a single lifileucel infusion (median dose 21.1 × 10^9 viable cells). Finally, by IL-2 (aldesleukin) is administered at 600,000 IU/kg every 8–12 h for up to 6 doses [132].

When data from cohorts 2 and 4 of the trial were combined (n = 153; pooled efficacy set), where patients had identical eligibility criteria, lifileucel manufacturing process, treatment regimen, and IRC response assessment, and received a single lifileucel infusion along with up to six doses of high-dose IL-2—the ORR was 31.4% [133].

Apart from Lifeleucel, other TIL products have demonstrated encouraging outcomes. In a multicentre phase 3 randomised trial of 168 patients with unresectable stage IIIC or IV melanoma, patients were assigned to receive either TIL or ipilimumab at 3 mg/kg at a 1:1 ratio. Administration of a minimum of 5 × 10^9 TILs was preceded by nonmyeloablative lymphodepleting chemotherapy (cyclophosphamide plus fludarabine) and succeeded by high-dose interleukin-2. Median PFS was 7.2 months (95% CI, 4.2 to 13.1) in patients treated with TILs compared to 3.1 months (95% CI, 3.0 to 4.3) with ipilimumab. OS was 54.3% (95% CI, 43.9 to 67.2) in the TIL group and 44.1% (95% CI, 33.6 to 57.8) in the ipilimumab group. Response rates were higher with

TILs (49% vs. 21%). Grade 3–5 treatment related AEs were higher with TILs (100% vs. 57%) [134].

II) Combining Adoptive Cell Therapies and Immune Checkpoint Inhibitors

ACT has been investigated in combination with ICIs. In a trial by Mullinax et al. (2018) (NCT01701674) 12 patients with metastatic melanoma received four doses of ipilimumab (3 mg/kg) followed by chemotherapy, a TIL infusion and IL-2. Five patients (38.5%) experienced objective response, four of whom continued in objective response at 1 year and one of which became a complete response at 52 months. Median PFS was 7.3 months (95% CI 6.1–29.9 months) [135]. In another phase I clinical trial, patients with stage III/IV metastatic melanoma with unresectable disease were treated with TILs generated with anti-4-1BB agonistic antibody in vitro in combination with nivolumab infusions. 4/11 patients achieved a partial response (36% ORR) with a median PFS of 5 months [136]. Of note, a study exploring patient characteristics in 226 patients with metastatic melanoma treated with ACT found a decreased likelihood of response among patients who had previously undergone and were unresponsive to anti-PD-1 therapy (ORR of 56%, in patients naïve to anti-PD-1 therapy vs. 24% in patients refractory to anti-PD-1), supporting shared mechanisms of resistance in anti-PD-1 and ACT refractory disease [137].

III) Cancer Vaccines

Cancer vaccines are a promising therapeutic strategy, aiming to trigger anti-tumour specific immune response by utilising tumour antigens, leading to recognition and destruction of cancer cells [138]. Melanoma is particularly suitable for this type of treatment due to its high TMB, offering a wide array of antigens for vaccine development [139].

There are several types of cancer vaccines including: [1] vaccines based on heat shock proteins, gangliosides or peptides; [2] vaccines based on DNA/RNA; [3] whole cell vaccines; [4] vaccines based on dendritic cells; and [5] vaccines based on recombinant viruses [139].

Despite over a century of research, the success of therapeutic cancer vaccines has been inconsistent, especially in patients with advanced cancer. This inconsistency is attributed to several factors, including the heterogeneity of the tumour microenvironment, the presence of immunosuppressive cells, and the potential for tumours to develop escape mechanisms [140].

Nevertheless, several therapeutic vaccination strategies are under development and are being evaluated in preclinical and clinical trials. Notably, mRNA vaccines have been garnering significant attention, with UK trials in colorectal and pancreatic cancer launched as part of the Cancer Vaccine Launch Pad scheme [141].

KEYNOTE-942 is an open-label, randomised phase 2 trial in patients with completely resected, high-risk stage IIIB/C/D and IV cutaneous melanoma. 157 patients were randomised 2:1 to receive mRNA-4157 (1 mg every 3 weeks for a total of 9 doses) in combination with pembrolizumab (200 mg every 3 weeks for up to 18 cycles) or pembrolizumab alone. This study demonstrated that combination therapy significantly prolonged distant metastasis-free survival (DMFS) compared to monotherapy with pembrolizumab. The 18-month RFS rates were higher with combination therapy (78.6%, 95% CI: 69.0%-85.6%) compared to monotherapy (62.2%, 95% CI: 46.9%-74.3%). Combination therapy also showed a significant improvement in DMFS (HR = 0.347; 95% CI: 0.145–0.828; one-sided p-value 0.0063) and distant recurrence or death rates were lower with combination therapy (8.4%) compared to monotherapy (24%) [142]. Several biomarkers such as TMB, PD-L1 and circulating tumour DNA (ctDNA) might be predictive of outcomes to adjuvant treatment [143].

Several other ongoing trials are investigating ICI combinations with mRNA vaccines for melanoma (NCT04526899, NCT02410733, NCT03291002) with promising potential for clinical approval on the horizon. Combining cancer vaccines with ICIs leverages vaccines to boost immune cell activation and infiltration while ICIs sustain their function, potentially overcoming individual limitations for synergistic therapeutic effects [144].

IV) Oncolytic Viruses

Oncolytic viruses are engineered or naturally occurring to selectively infect and kill cancer cells. They work by directly targeting and destroying cancer cells often through a combination of viral replication within the tumour and stimulation of the immune system's response against the tumour [145].

In 2015, the FDA approved T-VEC (tamoligene laherparepvec), a genetically altered herpes virus, for treating localized, inoperable metastatic stage IIIB/C-IVM1a melanoma. This represented a significant breakthrough, as T-VEC became the first oncolytic viral immunotherapy sanctioned for melanoma treatment [146].

The results that led to FDA approval originated from the OPTiM phase III trial of T-VEC in unresectable stage III-IV melanoma (*NCT00769704*), where 436 patients were randomised at a 2:1 ratio to receive either T-VEC or subcutaneous recombinant GM-CSF respectively. The T-VEC group exhibited notably higher durable response rate (DRR) and ORR compared to the GM-CSF group (DRR 16.3% vs. 2.1%, $p < 0.001$; ORR 26.4% vs. 5.7%, $p < 0.001$) [147]. Additionally, patients receiving T-VEC experienced extended median survival (23.3 vs. 18.9 months, $p = 0.051$). Notably, long-term follow-up analysis (median 49 months) confirms these initial results [122]. Further investigation suggests that early metastatic melanoma and lower tumour burden independently predict complete response attainment.

The median time to achieve complete response in the T-VEC group was 8.6 months, correlating with improved OS [122].

Despite T-VEC's inability to enhance OS when used alone, its combination with systemic therapy, particularly ICI, holds promise. Studies have shown improved overall response rates and systemic antitumour responses when T-VEC is combined with ICI. While recent trials like Masterkey-265, a phase 3 randomised double-blind multicentre study of T-VEC plus pembrolizumab, have not demonstrated significant PFS benefits, there remains interest in exploring T-VEC's potential, especially for patients unable to tolerate or refractory to ICI, and those seeking palliative reduction in disease burden. The heterogeneity of advanced melanoma and responses to therapy underscore the ongoing importance of investigating T-VEC and other oncolytic viruses [148].

Additionally, the oncolytic-virus pipeline continues to expand. One notable example is RP1, a second-generation HSV-1-based oncolytic immunotherapy developed by Replimune Group, Inc. RP1 has been engineered by deleting the ICP34.5 and ICP47 genes and inserting the GALV-GP-R$^-$ fusogenic protein along with human GM-CSF, with the aim of enhancing tumour-selective replication, tumour cell fusion/lysis and systemic immune activation [149]. Clinically, RP1 has been investigated in monotherapy and in combination with anti-PD-1 therapy (e.g., in the IGNYTE study) in patients with advanced melanoma and other solid tumours [150]. The FDA has granted Breakthrough Therapy Designation for RP1 in combination with nivolumab in anti-PD-1-refractory melanoma and a Biologics License Application was submitted under the Accelerated Approval pathway. These developments highlight RP1 as a promising next-generation oncolytic viral immunotherapy that, like T-VEC,- but with enhanced engineering, seeks to overcome limitations of first-generation agents in the checkpoint-resistant setting.

Furthermore, numerous additional oncolytic viruses are being explored in both preclinical studies and clinical trials. These include ONCOS-102 adenovirus, coxsackievirus A21, poliovirus and vesicular stomatitis virus (NCT03003676, NCT02307149, NCT04577807, NCT03865212) [123].

Gut Microbiome Modulation

Finally, augmentation of the gut microbiome by inoculation of *Bifidobacteria* promotes CD8$^+$ T-cell-mediated anti-cancer response in murine models of melanoma, while the combination of inoculation with *Bacteroides fragilis* and administration of anti-CTLA-4 ICIs decreased tumour growth in vivo [151]. Furthermore, faecal transplant of *Akkermansia muciniphila* has been associated with improved outcomes in anti-PD-1 ICI trials [152]. Faecal microbiota transplant in combination with immunotherapy in melanoma is undergoing investigation in phase I setting (NCT03772899). Similarly, antibiotics that alter the commensal gut flora decrease ICI efficacy across several cancer types [153, 154]. Thus, augmentation of the gut microbiome, either through inoculation or antibiotic treatment may offer a means of augmenting ICI efficacy. Ongoing trials, such as The Predicting Response to Immunotherapy for Melanoma with gut Microbiome and Metabolomics (PRIMM) trial (NCT03643289), a prospective cohort study will provide further insights into the intricate interplay between gut microbiota and immunotherapy response rates, paving the way for more personalized and effective treatment strategies.

Conclusion

ICIs are promising anti-cancer immunotherapeutics with proven efficacy for the treatment of melanoma. Combination of nivolumab plus ipilimumab has become mainstay treatment for resistant melanoma with pembrolizumab or nivolumab monotherapy as second line where nivolumab plus ipilimumab is unsuitable or unacceptable due to factors such as heightened risk of severe adverse events, existing comorbid conditions, compromised overall health status, or patient preference for treatments with a more favourable side effect profile. However, combination nivolumab plus relatlimab is now being proposed as an alternative second line [50]. Although the combination of novel ICIs may overcome resistance to immune therapy by targeting pathways involved in ICI resistance [15, 155, 156], it may be necessary to design treatment protocols that incorporate sequential therapy approaches to mitigate the AEs associated with polytherapy [157].

Further research is required to improve the issues associated with this modality of anti-cancer therapy – particularly immunological resistance mechanisms, immune-related adverse events, and the sequence and dosing schedules for combinations of ICIs. The discovery and validation of predictive biomarkers in large prospective trials remains an important step in broadening the applications of ICI therapies by establishing patient subgroups which are most likely to experience a significant benefit. Combination therapy with chemotherapy, radiotherapy, targeted therapy, as well as gut microbiome modulation and other immune-based cancer treatments including novel adoptive immune cell therapies are promising fields of research that are coming of age and require further systematic investigation.

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