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Review

Immune Checkpoint Inhibitors: Fundamental Mechanisms, Current Status and Future Directions

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Abstract: Immune checkpoint inhibitors (ICI) are a promising form of immunotherapy that have significantly changed the therapeutic landscape for many advanced cancers. They have shown unique clinical benefit against a broad range of tumour types and a strong overall impact on survival in studied patient populations. However, there are still many limitations holding back this immunotherapy from reaching its full potential as a possible curative option for advanced cancer patients. A great deal of research is being undertaken in the hope of driving advancements in this area, building a better understanding of the mechanisms behind immune checkpoint inhibition and ultimately developing more effective, safer, and wider-reaching agents. Taking into account the current literature on this topic, this review aims to explore in depth the basis of the use of ICIs in the treatment of advanced cancers, evaluate its efficacy and safety, consider its current limitations, and finally reflect on what the future holds for this very promising form of cancer immunotherapy.

Keywords: immune checkpoint inhibitor; ipilimumab; pembrolizumab

1. Introduction

Over the past few decades, many types of cancer immunotherapies have been studied such as monoclonal antibodies (mAb), cancer vaccines and adoptive T-cell therapies [1–3]. They each target different components of the immune system and have shown varying levels of promise as well as limitations [4]. However, no immunotherapy has shown as much promise in recent years as immune checkpoint inhibitors (ICI) [5]. Cancer cells can evade immunosurveillance through activation of immune checkpoint pathways, inhibiting T-cell activation and diminishing anti-tumour immune responses [6]. Immune checkpoint pathways are activated when co-inhibitory molecules on the surface of cancer cells or antigen-presenting cells (APCs) bind to T-cell surface molecules known as immune checkpoints [7]. ICIs act to block immunosuppressive signalling by inhibiting this binding, reviving anti-tumour activity and preventing cancer progression [5]. In 2011, the Food and Drug Administration (FDA) approved ipilimumab, the first ICI for clinical use in the treatment of metastatic melanoma. Ipilumumab is an inhibitory mAb targeting CD152, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [8]. This marked the beginning of a new era of cancer immunotherapy, and since then, a number of other ICIs have also been approved including: pembrolizumab targeting the protein CD279, programmed cell death protein (PD-1); atezolizumab targeting its ligand CD274, programmed cell death-ligand 1 (PD-L1) and most recently, relatlimab targeting the protein CD223, Lymphocyte-activation gene 3 (LAG-3) [9,10]. Clinical trials have shown promising therapeutic outcomes of ICIs in several different cancers, including melanoma, lung cancer, urothelial cancer, renal cancer, hepatocellular cancer and many more, for which mainstream use has been approved [11–14]. Additionally, there are hundreds of active clinical trials underway to evaluate the efficacy and safety of multiple ICIs across many different cancers [3]. However, despite the success of ICIs in studied patient populations, only a fraction of cancer patients benefit from ICIs in practice [5]. The efficacy of ICI relies on the basis that there is an underlying anti-tumour immune response present, and the ICI is simply removing the brakes to release this function. However, the degree of anti-tumour

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immunity is variable and regulated by complex factors in the tumour microenvironment (TME), the immunogenicity of the tumour and its mutational burden [15–17]. ICIs are also associated with a unique spectrum of immune-related adverse effects (irAEs), such as dermatological, gastrointestinal and endocrine inflammatory reactions [18]. These are some limitations that must be addressed if ICIs are to be effective in wider patient populations.

2. Immune Checkpoint Pathways

Immune checkpoints are important regulators of the immune system. They act as T-cell receptor (TCR) co-signalling partners, delivering either activating or inhibitory signals to T cells upon stimulation. These signalling pathways are vital for maintaining self-tolerance as well as ensuring an adequate immune response [19]. An example of a stimulatory checkpoint protein is the CD28 molecule, a protein highly expressed on CD4+ and CD8+ T cells, which initiates co-stimulatory signals when it binds to its corresponding ligands, CD80 and CD86, found on APCs [20]. To date, a total of seven other stimulatory checkpoints have been identified, all of which act in a similar way to activate and stimulate T-cell activity [19]. However, inhibitory immune checkpoints have been the main subject of attention in recent years. Their co-inhibitory pathways have been shown to promote tumour formation and progression, through the dampening of cancer immunosurveillance [21]. In fact, cancer cells have been shown to utilise these pathways by expression of their ligands, activating immunosuppressive signalling and allowing for immune evasion [22]. The therapeutic potential surrounding these pathways is evident, and since the success of the first ICI, ipilimumab, targeting CTLA-4, many more inhibitory immune checkpoints have been identified. As of now, the immune checkpoints which have been successfully targeted with approved ICIs are CTLA-4, PD-1 (CD279), its ligand PD-L1 (CD274) and LAG-3 (CD223) [5,10]. Other co-inhibitory immune checkpoints include T-cell immunoglobulin and mucin-domain-containing-3 (TIM-3, CD366), T-cell immunoglobulin and ITIM domain (TIGIT), V-domain Ig suppressor of T-cell activation (VISTA) and many more (Figure 1) [23]. These are all emerging ICI targets, currently under clinical trial or development, with many showing promising outcomes [23]. However, before we focus on what the future holds, it is important to understand the current established ICI targets, as ultimately it is their success and current limitations that are guiding research into these new targets. The next section will look in more depth at the physiological roles of these immune checkpoints, the mechanism of their blockade by ICIs and analyse the success of this immunotherapy in treatment of advanced cancers.

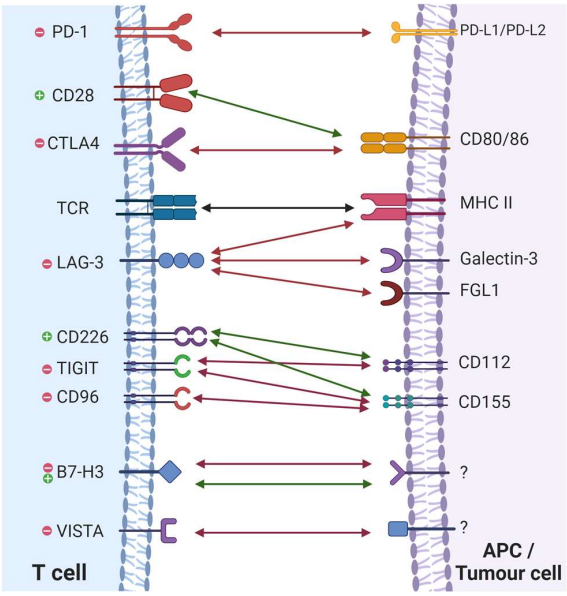

Figure 1. Current and emerging immune checkpoint receptors and their respective ligands. They transmit co-inhibitory or co-stimulatory signals to TCR upon binding to their ligands. Figure generated using BioRender.com.

3. CTLA-4 Physiological Role

CTLA-4 is a transmembrane protein belonging to the immunoglobulin superfamily, and its expression and function are intrinsically linked to T-cell activation [8,19]. In fact, CTLA-4 is not detectable on naïve T cells but is immediately induced and upregulated in response to TCR activation [21]. Its expression is regulated by the strength of TCR signalling, whereby stronger TCR signalling induces greater expression of CTLA-4 [24]. CTLA-4 is a homologue of the co-stimulatory checkpoint CD28 and, as such, shares its ligands, CD80 (B7-1) and CD86 (B7-2), also known as the B7 ligands [21]. Following its expression, it begins to compete with CD28 for the B7 ligands; however, as it boasts a higher affinity and avidity for these ligands, it can effectively outcompete CD28, blocking co-stimulatory signals and attenuating T-cell activation [24]. Thus, it regulates the availability of B7 ligands to CD28, forming a homeostatic feedback loop in response to TCR stimulation. The net outcome following TCR stimulation is dependent on the relative binding of CD28 versus CTLA-4 to the B7 ligands and can result in either T-cell activation or anergy [25]. Other than its primary cell-intrinsic role in regulating T-cell activity, it also influences immunological tolerance through cell-extrinsic mechanisms, mainly through the action of T-regulatory cells (T-reg) [21]. Unlike effector T cells, CTLA-4 is constitutively expressed on T-reg cells, a cell with a key role in maintaining immune tolerance [26]. CTLA-4 expressed on T-reg cells acts in a similar way, limiting the availability of B7 ligands, in a cell-extrinsic manner, and blocking CD28 costimulatory activation of nearby effector T cells through competitive inhibition [21]. Additionally, beyond just the binding of B7 ligands, CTLA-4 has also been reported to limit the overall availability of B7 ligands through trans-endocytosis of these ligands from APCs [27]. It is quite evident that CTLA-4's function is inherently linked to the interaction of its homologue CD28 and its ligands, as its primary mechanism is to impede this signalling. However, some evidence suggests that CTLA-4 may also have a role independent of CD28, in which it directly antagonises TCR signalling following B7-1 engagement, through its own cell-intrinsic signalling [28]. A proven mechanism has yet to be established, and further research is needed to understand the intracellular signalling of CTLA-4. Ultimately, CTLA-4 has a fundamental role in maintaining self-tolerance, and as such, it primarily regulates T-cell activation at sites of T-cell priming, such as lymph nodes [21]. Its critical role in immune tolerance has been proven by observations seen in CTLA-4 knockout mice, which developed uncontrolled lymphoproliferative disease and fatal autoimmunity, due to unrestrained CD28 co-stimulation [29].

Considering it has such an important role in self-tolerance, it makes sense that on the opposite end of the scale, excessive CTLA-4 activity can produce an immunosuppressive environment conducive with tumorigenesis [24]. Such a concept was given more weight by pre-clinical studies conducted in the late 1990s, which showed decreased tumour growth and improved survival in mouse models following CTLA-4 pathway blockade [30]. Of course, the next step was to develop an approved anti-CTLA-4 therapy that could replicate such results in human cancer patients. Initially, three anti-CTLA4 antibodies entered clinical trials: tremelimumab, its parental antibody known as CP-642,570 and ipilimumab [8]. CP-642,570 did not make it past the first human trial, and tremelimumab showed initial promise in phase I and II trials, but ultimately failed to meet its endpoints in phase III trials [8,31,32]. This left ipilimumab, a fully humanized IgG1 mAb, that went on to achieve FDA and European Medicines Agency (EMA) approval for the treatment of unresectable stage III and metastatic melanoma, following many successful clinical trials [8]. In the last few years, it has also been approved as part of combination therapy with nivolumab, an anti-PD-1 mAb, for unresectable or metastatic melanoma, advanced renal cell carcinoma (RCC), some forms of advanced colorectal carcinoma and many more (Table 1) [33].

Table 1. List of cancers for which ipilimumab is an FDA-approved therapy, including key trials that led to approval in each case.

Ipilimumab			
Cancer Type	**FDA Approval Year**	**Key Clinical Trial (Phase)**	**Monotherapy/Combination Therapy**
Melanoma	2011	MDX010-20 (Phase 3) [13]	Monotherapy
	2015	CheckMate-067 (Phase 3) [34]	Combination with nivolumab
Renal Cell Carcinoma	2018	CheckMate-214 (Phase 3) [12]	Combination with nivolumab
Colorectal Cancer—Microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR)	2018	CheckMate 142 (Phase 2) [35]	Combination with nivolumab
Hepatocellular Carcinoma	2020	CheckMate 040 (Phase 1/2) [36]	Combination with nivolumab
Non-small-cell lung carcinoma	2020	CheckMate 227 (Phase 3) [37]	Combination with nivolumab
Pleural Mesothelioma	2020	CheckMate 743 (Phase 3) [38]	Combination with nivolumab
Oesophageal Squamous Cell Carcinoma	2021	CheckMate 648 (Phase 3) [39]	Combination with nivolumab

3.1. Mechanism of CTLA-4 Blockade-Induced Tumour Rejection

The exact mechanism of CTLA-4 blockade-induced tumour rejection by ipilimumab is still not completely clear, with many distinct mechanisms thought to play a role. The primary mechanism is centred around the direct blockade of CTLA-4 competition for B7 ligands, allowing unrestricted CD28 co-stimulation and increasing activation of effector T cells in response to tumour-associated antigens (TAAs) (Figure 2) [21]. As B7 ligands are not found on cancer cells, this mainly occurs in the tumour-draining lymph nodes, where APCs cross-present TAAs to tumour-reactive effector cells [21]. The result is increased activation and activity of effector CD8+ cytotoxic T-lymphocytes (CTLs) and CD4+ type 1 helper (Th1) cells, the primary mediators of the anti-tumour immune response [21]. Additionally, within the TME, CTLA-4 blockade acts to reactivate and expand exhausted CD8+ T-cell and Th1 cells through the blockade of CTLA-4 [40]. Exhaustion occurs due to chronic TAA-induced TCR stimulation, resulting in persistently high CTLA-4 expression and greatly reduced activity of primed immune cells [41]. Therefore, CTLA-4 blockade removes this inhibitory control, reviving effector T-cell function [40].

Depletion of T-reg cells within the TME is the other main mechanism that is thought to contribute to CTLA-4 blockade-induced tumour rejection [42]. CTLA-4 signalling plays an important role in the immunosuppressive function of T-regs, and as such, blockade of such signalling removes this immunosuppressive influence that otherwise promotes tumour growth and progression [42]. This is achieved through the selective depletion of intra-tumoural T-reg cells, not peripheral populations [43]. This selectivity is likely due to increased expression of CTLA-4 T-regs in the TME or, additionally, differences in activity and availability of receptors for crystallizable fragment of immunoglobulin (FcR), which is thought to mediate the mechanism of T-reg depletion. Laboratory investigations showed that binding of the Fc portion of anti-CTLA4 antibodies to FcR found on APCs were necessary for the depletion of intra-tumoural T-regs and subsequent anti-tumour effect [44].

Interestingly, a recent study has gone as far as to say that the efficacy of ipilimumab is completely independent of B7 ligands, instead solely driven by antibody-mediated T-reg depletion [45]. Such a conclusion of course contradicts years of prior evidence, and although recent studies do suggest a greater role of T-reg depletion, a large body of research backs the concept of B7 ligand regulation as a critical mechanism of ipilimumab [21]. Also, there is not yet any definitive evidence of T-reg depletion in humans treated with ipilimumab.

In reality, both mechanisms could likely contribute to the efficacy of ipilimumab to some degree, as both effector T cells and T-regs express CTLA-4 and have roles in checkpoint signalling pathways [46]. However, the relative contribution of cell intrinsic expansion of effector T-cell function versus T-reg depletion remains unclear, and as such, further research is needed to better understand the weight of each mechanism. To be able to delineate the primary and more effective mechanism behind CTLA-4 blockade-induced tumour rejection could potentially guide development of new anti-CTLA-4 therapies, more focused on targeting these mechanisms. For example, ipilimumab was initially developed based on the knowledge that CTLA-4 blockade would enhance T-cell activity and, as such, was not developed to be a depleting antibody and target T-regs in any way [47]. If T-reg depletion did in fact play a more central role in tumour rejection in humans, then this would change the direction of future anti-CTLA4 therapies.

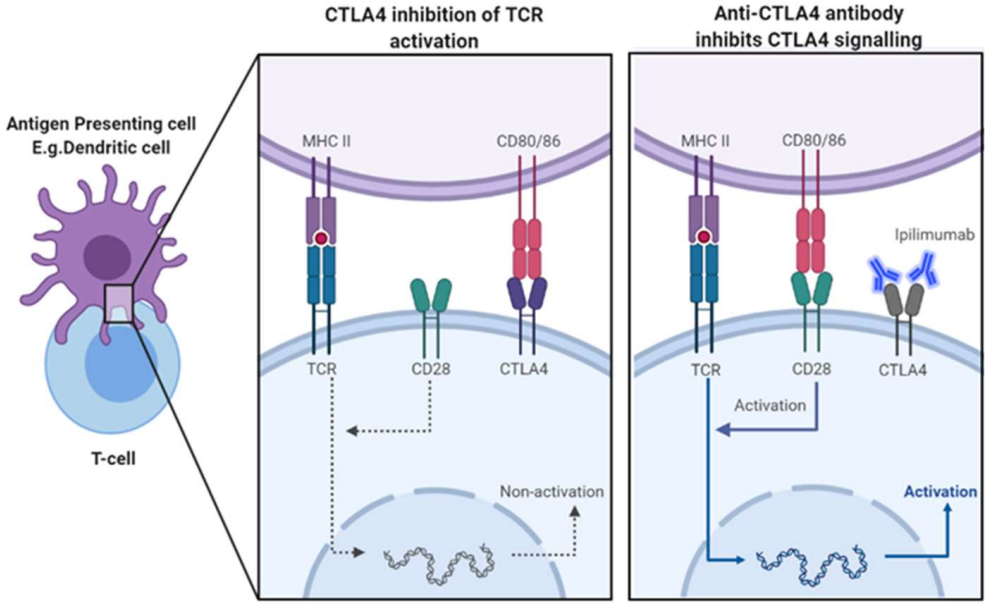

Figure 2. CTLA4:CD80/86 binding inhibits TCR activation via competitive inhibition of CD28 co-stimulatory signalling. Anti-CTLA4 antibodies such as ipilimumab block CTLA4:CD80/86 binding, allowing unrestrained CD28 co-stimulation and increased TCR activation. Figure generated using BioRender.com.

3.2. Efficacy of Ipilimumab in Treatment of Advanced Melanoma

For decades prior to the introduction to ipilimumab, there was little improvement in the treatment available for patients with advanced or metastatic melanoma, with no significant improvement seen in overall survival (OS) of patients in this time [48]. The chemotherapeutic drug dacarbazine remained the reference single agent of choice, despite it showing no impact on survival [49]. Ipilumumab was the first immunotherapy to show improved OS in this patient population with high unmet needs, completely changing the therapeutic outlook for this cancer following its FDA approval [8]. Pre-clinical data of CTLA-4 blockade in mice showed that it could not only enhance the endogenous anti-tumour immunity against cancers but also be used synergistically in combination with other interventions such as chemotherapy or vaccines to improve response against less immunogenic cancer [50]. Findings from phase I trials extended these promising results, showing anti-tumour immunity in various advanced tumours, especially melanoma, and demonstrated a favourable safety and toxicity profile both alone and in combination [49].

As ipilimumab's clinical development progressed into phase II trials, it continued to show promising outcomes. In a Phase II trial (MDX010-08), 72 previously untreated advanced melanoma patients were randomised to receive either ipilimumab 3 mg/kg monotherapy or in combination with dacarbazine. In both cases, ipilimumab showed strong

objective clinical outcomes and promising OS in patients [51]. When comparing the long-term survival data in this trial against another trial, MDX010-15, a phase 1/2 dose-ranging study in which 23 patients were treated with ipilimumab at 10 mg/kg dose every 3 weeks, a trend towards better survival with a higher dose was demonstrated. A 2-year survival rate of 36% was seen in the 10 mg/kg dose vs. 22% for the 3 mg/kg dose [52]. A similar trend was seen in a phase 1/2 study (CA184-022), in which 217 previously treated advanced melanoma patients underwent induction therapy with one of three doses of ipilimumab, followed by maintenance therapy. Disease control rate (DCR) was 13.7%, 26.4%, and 29.2% in the 0.3 mg/kg, 3 mg/kg, and 10 mg/kg groups, respectively. There was a similar safety profile between the two higher doses, with an irAE rate of 70% with 10 mg/kg and 65% with 3 mg/kg [53]. Overall, a series of phase II trials evaluated the efficacy and safety of ipilimumab in over 500 patients [54]. The results were of course very promising, but there were two key lessons derived from the findings of these trials. Firstly, irAEs were the most common toxicity associated with ipilimumab; however, they were in most cases reversible [54]. Secondly, these trials revealed the unique response pattern of ipilimumab, in which some patients showed response following apparent disease progression. In fact, objective responses were sometimes observed 6–12 months after treatment initiation [54]. Therefore, it was recognised that overall survival (OS) was the superior endpoint to reflect the clinical activity of ipilimumab, and as such, it was chosen as the primary endpoint for future phase III trials [54].

Phase III trials were the last step in the journey towards FDA approval for ipilimumab. A key phase III trial, MDX010-20, evaluated ipilimumab treatment at 3 mg/kg in previously treated patients, comparing it against the cancer vaccine gp100 alone, and in combination [13]. A significantly improved OS was observed in patients treated with ipilimumab, with or without gp100, compared to patients receiving gp100 alone. There was almost double the patients in the 1- and 2-year survival rates with ipilimumab compared to gp100. As well as this, clinical outcomes were independent of important prognostic factors of melanoma, such as stage at presentation, baseline lactate dehydrogenase levels and age. Once again, there was a high frequency of irAEs, of approximately 60%, but they were low grade and manageable in most cases [13]. The results of this study were the basis for approval of ipilimumab at 3 mg/kg dose for previously treated metastatic melanoma and, in some countries, for treatment-naïve metastatic melanoma [49]. A recent meta-analysis of 1861 patients across 12 clinical trials of ipilimumab in melanoma showed a favourable median OS of 11.4 months and 3-year survival of 22% across all patients [55]. It also showed that ipilimumab was more effective in improving OS and other clinical outcomes in combination with PD-1 inhibitors, such as nivolumab. This synergistic relationship between these two ICI has therefore been utilised and approved for treatment of melanoma and many other cancers (Table 1) [55]. In summary, ipilimumab was the first instance of success for ICI therapy as a whole, paving the way for further research, development and clinical application of immune checkpoint blockade.

4. PD-1/PD-L1 Physiological Role

PD-1 is a transmembrane protein which, much like CTLA-4, has an important role in co-inhibitory regulation of TCR activation [56]. Although both CTLA-4 and PD-1 have similar inhibitory effects on T-cell activity, the responsible signalling mechanisms, timing of downregulation and anatomic location of immune inhibition differ significantly [24]. Unlike CTLA-4 expression, which is confined to T cells, PD-1 is expressed more broadly in activated T cells, B cells and myeloid cells. Furthermore, while CTLA-4 primarily functions during the priming phase of T-cell activation within secondary lymphoid organs, PD-1 functions during the effector phase and therefore within peripheral tissue [57]. PD-1 has two ligands to which it binds to mediate its effects, PD-L1 and PD-L2, the expression of which also differs in comparison to CTLA-4 ligands [21]. Whereas B7 ligands are expressed on APCs, the PD-1 ligands are expressed widely on leukocytes, non-hematopoietic cells and non-lymphoid tissues [57].

When PD-1 binds to its ligands in the presence of TCR or B-cell receptor activation, it transduces a direct inhibitory signal to the activating receptor (Figure 3). This co-inhibitory signal prevents phosphorylation of key signalling intermediates, inhibiting activation of T cells or B cells [58]. Signalling through PD-1 also heavily influences cytokine production by T cells, inhibiting the release of pro-inflammatory cytokines such as Interferon-gamma (INF-γ), tumour necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2), which ordinarily act to promote T-cell activity and proliferation [59]. Additionally, PD-1 signalling also inhibits the upregulation of Bcl-xl, an anti-apoptotic protein, and limits the expression of transcriptional factors which are important for T-cell effector function [59]. Like CTLA-4, the net effect of PD-1 co-inhibitory signalling is dependent on the extent of the opposing co-stimulatory signalling of CD28 and other stimulatory signals. These immune checkpoints all act together to form a homeostatic feedback loop, which closely regulates the degree of T-cell activation and activity [24]. Considering the widespread distribution of PD-1 and its ligands, alongside its role in inhibitory regulation, it follows that the inherent role of PD-1 is to maintain peripheral tolerance of effector lymphocytes [21]. Experimental evidence supporting this concept has been seen in mice studies, in which genetic deletion of the Pdcd1 gene (encoding for PD-1) led to various autoimmune pathologies [59,60].

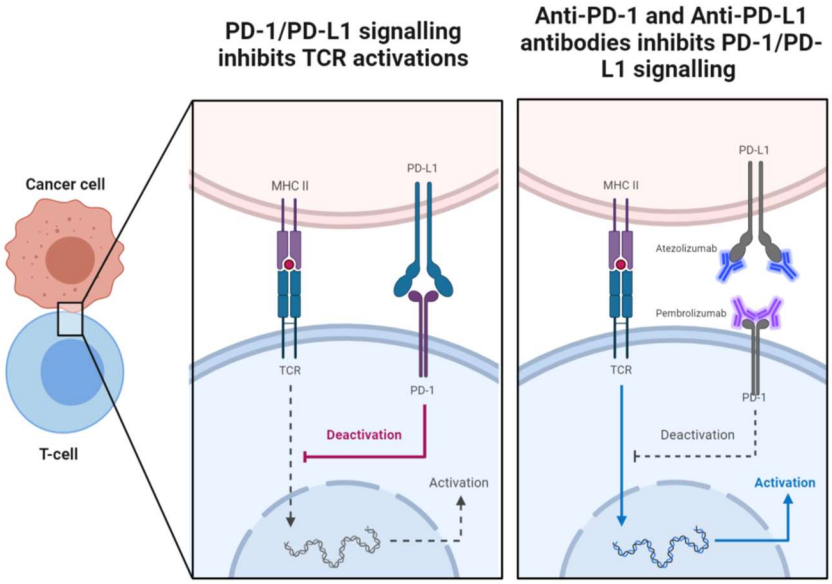

Figure 3. Tumour cells can express PD-L1, which can directly bind to inhibitory immune checkpoint PD-1 on the surface of T cells. PD-1:PD-L1 binding induces co-inhibitory signalling that inhibits TCR activation. Anti-PD-1 antibodies, such as pembrolizumab, and anti-PD-L1 antibodies, such as atezolizumab, inhibit PD:PD-L1 signalling, allowing unrestrained TCR activation. Figure generated using BioRender.com.

4.1. Mechanism of PD-1/PD-L1 Blockade-Induced Tumour Rejection

PD-L1 expression has been detected in many different cancer cell types. They are able to utilise the PD-1 signalling pathway to evade the immune system, taking advantage of its immunosuppressive function to grow and progress [21,61]. As a result, several different ICIs have been developed targeting the PD-1 signalling pathways. Currently, pembrolizumab, nivolumab and cemiplimab are the approved anti-PD-1 ICIs, and atezolizumab, avelumab and durvalumab are approved anti-PD-L1 ICIs. They are collectively able to target 18 different cancers (Table 2), which highlights the significance of PD-1/PD-L1 signalling in cancer progression and the great deal of therapeutic benefit that can be derived from the blockade of these pathways [62]. PD-1 blockade acts to induce tumour rejection through reinvigoration of exhausted T cells and overall enhancement of their effector function through blockade of the many inhibitory mechanisms of PD-1 mentioned before [59]. PD-L1 blockade is thought to induce tumour rejection in a largely similar

manner to anti-PD1 antibodies, due to the dominance in the expression of PD-L1 compared to PD-L2 [24]. Also, considering that PD-L1 is induced predominantly by Th1 cytokines whereas PD-L2 is induced by Th2 cytokines, PD-L1 blockade would produce a Th-1 skewed response that would favour anti-tumour immunity [63]. Furthermore, selective inhibition of PD-L1 blocks PD-1/PD-L1 interactions while preserving PD-1/PD-L2 interactions. This, in theory, could produce a more targeted response with less unwanted toxicity, as there will be partial preservation of PD-1 self-tolerance mechanisms, via PD-L2 binding. However, as of yet, there are limited clinical data to prove this concept [24]. Also, PD-L1 is known to bind to B7-1, as well as PD-1, to induce inhibitory signals to T cells. Therefore PD-L1 blockade could also stimulate T-cell activation in a very similar manner to CTLA-4 blockade, increasing availability of B7-1 ligand for CD28 co-stimulation [64]. Ultimately, more research is needed to better understand the potential multifaceted mechanisms of both anti-PD-1 and anti-PD-L1 therapies, in order to optimize their use in the many different targeted cancers.

Table 2. List of FDA-approved Anti-PD-1 ICIs, including the specific cancers they are approved for and the key clinical trials that led to approval in each case.

ANTI-PD-1 Immune Checkpoint Inhibitors				
Drug	**Cancer Type**	**FDA Approval Year**	**Key Trial (Phase)**	**Monotherapy/Combination Therapy**
Pembrolizumab	Melanoma	2014	KEYNOTE-006 (Phase 3) [65]	Monotherapy
	Non-Small-Cell Lung Carcinoma	2015	KEYNOTE-010 (Phase 2/3) [66]	Monotherapy
	Squamous Cell Carcinoma ofHead and Neck	2016	KEYNOTE-012 (Phase 1b) [67]	Monotherapy
	Hodgkin's Lymphoma	2017	KEYNOTE-087 (Phase 2) [68]	Monotherapy
	Urothelial Carcinoma	2017	KEYNOTE-052 (Phase 2) [69]	Monotherapy
	Colorectal cancer (MSI-H/dMMR)	2017	KEYNOTE-164 (Phase 2) [70]	Monotherapy
	Gastric/Gastroesophageal junction (GEJ) Carcinoma	2017	KEYNOTE-059 (Phase 2) [71]	Monotherapy or Combination with chemotherapy
	Cervical Carcinoma	2018	KEYNOTE-158 (Phase 2) [72]	Monotherapy
	Hepatocellular Carcinoma	2018	KEYNOTE-224 (Phase 2) [73]	Monotherapy
	Merkel Cell Carcinoma	2018	KEYNOTE-017 (Phase 2) [74]	Monotherapy
	Renal Cell Carcinoma	2019	KEYNOTE-426 (Phase 3) [75]	Combination with axitinib
	Oesophageal Carcinoma	2019	KEYNOTE-181 (Phase 3) [76]	Monotherapy
	Triple-Negative Breast Carcinoma	2020	KEYNOTE-355 (Phase 3) [77]	Combination with chemotherapy
	Cutaneous Squamous Cell Carcinoma	2020	KEYNOTE-629 (Phase 2) [78]	Monotherapy
	Endometrial Carcinoma (MSI-H/dMMR)	2022	KEYNOTE-158 (Phase 2) [79]	Monotherapy
	Biliary Tract Carcinoma	2023	KEYNOTE-966 (Phase 3) [78]	Combination with chemotherapy

Table 2. *Cont.*

ANTI-PD-1 Immune Checkpoint Inhibitors				
Drug	**Cancer Type**	**FDA Approval Year**	**Key Trial (Phase)**	**Monotherapy/Combination Therapy**
Nivolumab	Melanoma	2014	CheckMate-037 (Phase 3) [80]	Monotherapy
		2015	CheckMate-067 (Phase 3) [34]	Combination with ipilimumab
		2017	CheckMate-238 (Phase 3) [81]	Adjuvant treatment
	Non-Small-Cell Lung Carcinoma	2015	CheckMate-057 (Phase 3) [14]	Monotherapy
		2020	CheckMate-227 (Phase 3) [37]	Combination with ipilimumab
	Renal Cell Carcinoma	2015	CheckMate-025 (Phase 3) [82]	Monotherapy
		2018	CheckMate-214 (Phase 3) [12]	Combination with ipilimumab
	Classical Hodgkin Lymphoma	2016	CheckMate-205 (Phase 2) [83]	Monotherapy
	Squamous Cell Carcinoma of Head and Neck Carcinoma	2016	CheckMate-141 (Phase 3) [84]	Monotherapy
	Urothelial Carcinoma	2017	CheckMate-275 (Phase 2) [85]	Monotherapy
	Colorectal Carcinoma (MSI-H/dMMR)	2017	CheckMate-142 (Phase 2) [34]	Combination with ipilimumab
	Hepatocellular carcinoma	2020	CheckMate-040 (Phase 1/2) [36]	Combination with ipilimumab
	Small-Cell Lung Carcinoma	2018	CheckMate-032 (Phase 1/2) [86]	Monotherapy
	Oesophageal Squamous Cell Carcinoma	2020	ATTRACTION-3 (Phase 3) [87]	Monotherapy
		2020	CheckMate-648 (Phase 3) [39]	Combination with Ipilimumab or Chemotherapy
	Pleural Mesothelioma	2020	CheckMate-743 (Phase 3) [38]	Combination with Ipilimumab
	Gastric/GEJ Carcinoma	2021	CheckMate-649 (Phase 3)	Combination with Chemotherapy
Cemiplimab	Cutaneous Squamous Cell Carcinoma	2018	EMPOWER-CSCC-1 (Phase 2) [88]	Monotherapy
	Basal Cell Carcinoma	2021	Study-1620 (Phase 2) [89]	Monotherapy
	Non-Small-Cell Lung Cancer	2021	EMPOWER-Lung 1 (Phase 3) [90]	Monotherapy

4.2. Efficacy of Anti-PD-1/PD-L1 Agents

4.2.1. Pembrolizumab

Of the ICIs that target the PD-1/PD-L1 signalling pathway, the PD-1 ICI pembrolizumab and the PD-L1 ICI atezolizumab are the most established, as such they are the best ICIs to assess in order to understand the efficacy of PD-1/PD-L1 therapies as a whole [62]. Pembrolizumab is a fully humanised mAb that received accelerated FDA approval in 2014 for treatment of unresectable or metastatic melanoma, and shortly after for non-small-cell lung cancer (NSCLC) in 2015 [91]. KEYNOTE-001, a phase I dose-finding trial of melanoma patients previously treated with ipilimumab, assessed pembrolizumab given intravenously at multiple doses ranging from 1 to 10 mg/kg, either every 2 or 3 weeks [92]. The overall response rate (ORR) was highest in the dose-dense and dose-intense groups at the expense of higher irAE incidence [92]. Consequent expansion cohorts of this study explored the safety and efficacy of many pembrolizumab regimes in patients with advanced melanoma and NSCLC [91]. The

KEYNOTE-001 data showed significant treatment activity in patients with advanced melanoma, regardless of previous ipilimumab treatment. However, notably higher response rates were seen in ipilimumab-naïve patients [91]. A recent 5-year follow-up of this patient cohort in KEYNOTE-001 showed favourable 5-year OS rates, further proving the durable anti-tumour activity and tolerability of pembrolizumab in advanced melanoma [65]. A phase 3 trial, KEYNOTE-006, compared the efficacy of pembrolizumab against ipilimumab and 5-year follow-up results also recently became available. After a median follow-up of 57.7 months, the median OS was 32.7 months in the pembrolizumab group compared to 15.9 months in the ipilimumab group. There was also a lower incidence of high-grade treatment-related adverse effects seen with pembrolizumab. These results extended the finding in the original trial, showing the superiority of pembrolizumab over ipilimumab in patients with advanced melanoma [93].

Pembrolizumab also showed impressive outcomes in 5-year follow-up of the NSCLC expansion cohort of the KEYNOTE-001 trial. Based on these results estimated OS rates was 23.2% for treatment-naïve patients and 15.5% for previously treated patients, which are very favourable when compared to pre-immunotherapy OS rates of around 5%. There was also a very low incidence of treatment-related AEs [94]. A phase 2/3 trial, KEYNOTE-010, also showed improved OS with ipilimumab when compared to docetaxel in previously treated patients with advanced NSCLC, with long-term follow-up data from this same trial extending this positive trend [66]. Overall, through many trials, pembrolizumab has demonstrated durable responses and prolonged OS in NSCLC, especially in variants with high expression of PD-L1. Thus, this has been recognized as an important predictive biomarker of the efficacy of pembrolizumab in this patient population [91]. Beyond melanoma and NSLC, pembrolizumab has been approved and shown success in the treatment of many cancers including head and neck cancer, gastric cancer, Hodgkin lymphoma, urothelial cancer and many more. Table 2 highlights the wide-reaching therapeutic potential of pembrolizumab and PD-1 ICIs as a whole [91].

4.2.2. Atezolizumab

Atezolizumab was the first anti-PD-L1 ICI of its kind, receiving FDA approval in 2016 for treatment of locally advanced or metastatic urothelial carcinoma (mUC) resistant to platinum-based chemotherapy. The following year, it also received accelerated approval as an initial treatment in patients who were ineligible for cisplatin chemotherapy [95]. In both cases, approval was primarily based on data from the IMvigor210 study, a phase II trial which evaluated the safety and efficacy of atezolizumab in patients with locally advanced or mUC, regardless of PD-L1 expression. Patients were split into 2 cohorts, cohort 1 comprised of treatment-naïve patients and cohort 2 comprised of previously treated patients. The median follow-up for cohort 1 was 29 months, and 33 months for cohort 2. In cohort 1, the ORR was 24%, and in cohort 2 the ORR 16%, which were both very favourable when compared to the historical control ORR of 10%. Additionally, in both cohorts, a higher PDL-1 expression of tumour-infiltrating lymphocytes (>5%) was associated with improved ORR. The incidence of irAEs in cohort 1 was 14% and in cohort 2 10%, with the incidence of high-grade irAEs being 7% and 6%, respectively [96,97].

In 2016, atezolizumab also received accelerated approval for treatment of cisplatin-resistant metastatic NSCLC, specifically associated with EGFR or ALK gene abnormalities [98]. This approval is based on results from the Phase III OAK and Phase II POPLAR studies [99,100]. The largest study, OAK, evaluated the efficacy and safety of atezolizumab compared with docetaxel in 1225 patients. The results showed that those treated with atezolizumab had a median OS of 13.8 months, 4.2 months longer than those treated with docetaxel chemotherapy [100]. Since then, it has also been approved as an initial treatment in combination with chemotherapy for metastatic NSCLC and extensive-stage small-cell lung cancer [101].

Additionally, in 2019, atezolizumab received accelerated approval for the treatment of PD-L1-positive, metastatic triple-negative breast cancer (TNBC), in combination with chemotherapy [102]. TNBC is an aggressive variant of breast cancer with few treatment

options, in which cancer cells lack expression of hormone receptors and do not overexpress the HER2 protein [103]. The accelerated approval was based on data from the Phase III IMpassion130 study, which evaluated the efficacy and safety of atezolizumab plus nab-paclitaxel compared with placebo plus nab-paclitaxel in unresectable locally advanced or metastatic TNBC patients [103]. The study demonstrated that the combination therapy significantly reduced the risk of cancer progression or death by 40% compared with nab-paclitaxel alone specifically in patients who had PD-L1-positive disease and had not received any prior chemotherapy. The combination therapy was also shown to be safe and tolerable, consistent with the known individual safety profiles of each medication and with no new safety concerns identified [103]. Overall, much like PD-1 ICIs, atezolizumab and other PD-L1 inhibitors have proven to be a versatile and effective therapy for many end-stage cancers, as outlined in Table 3 [104].

Table 3. List of FDA-approved anti-PD-L1 ICIs, including the specific cancers they are approved for and the key clinical trial that led to approval in each case.

ANTI-PD-L1 Immune Checkpoint Inhibitors				
Drug	**Cancer Type**	**FDA Approval Year**	**Key Trials**	**Monotherapy/Combination**
Atezolizumab	Urothelial Carcinoma	2016	IMvigor210 (Phase 2) [97]	Monotherapy
	Non-Small-Cell Lung Carcinoma	2016	OAK (Phase 3) [100]	Monotherapy
	Small-Cell Lung Carcinoma	2019	IMpower133 (Phase 3) [105]	Combination with chemotherapy
	Triple-Negative Breast Cancer	2019	IMpassion130 (Phase 3) [103]	Combination with chemotherapy
	Hepatocellular Carcinoma	2020	IMbrave150 (Phase 3) [106]	Combination with bevacizumab
	Melanoma (BRAF V600 mutation-positive)	2020	IMspire150 (Phase 3) [107]	Combination with cobimetinib and vemurafenib
	Alveolar Soft Part Sarcoma	2022	Study ML39345 (Phase 2) [108]	Monotherapy
Avelumab	Merkel Cell Carcinoma	2017	JAVELIN Merkel 200 (Phase 2) [109]	Monotherapy
	Urothelial Carcinoma	2017	JAVELIN Solid Tumor (Phase 1) [110]	Monotherapy
		2020	JAVELIN Bladder 100 (Phase 3) [111]	1st line maintenance: Combination with best supportive care
	Renal Cell Carcinoma	2019	JAVELIN Renal 101 (Phase 3) [112]	Combination with Axitinib
Durvalumab	Urothelial Carcinoma	2017	Study 1108 (Phase 1/2) [113]	Monotherapy
	Non-Small-Cell Lung Carcinoma	2018	PACIFIC (Phase 3) [114]	Monotherapy
	Small-Cell Lung Carcinoma	2020	CASPIAN (Phase 3) [115]	Combination with chemotherapy
	Biliary Tract Cancer	2022	TOPAZ-1 (Phase 3) [116]	Combination with chemotherapy
	Hepatocellular Carcinoma	2020	HIMALAYA (Phase 3) [117]	Combination with tremelimumab

5. LAG-3 Physiological Role

LAG-3 is a co-inhibitory transmembrane protein expressed on a range of effector leukocytes, including CD4+ and CD8+ T cells, natural killer (NK) cells, dendritic cells and T-reg cells [118]. Structurally similar to CD4, LAG-3 shares its primary ligand, major histocompatibility complex class II (MHC-II), with a binding affinity approximately 100 times that of CD4 [119]. LAG-3 also interacts with other ligands to a lesser extent, such as fibrinogen-like protein 1 (FGL-1), a soluble protein primarily secreted by hepatocytes, and galectin-3, an inflammatory lectin secreted by activated macrophages [120,121]. The binding of LAG-3 to its ligands induces co-inhibitory signalling, inhibiting TCR activation, signalling, proliferation and inflammatory cytokine production. As a result, LAG-3 contributes to immune regulation and tolerance mechanisms, much like CTLA-4 and PD-1 [122]. Studies have shown that LAG-3 signalling is crucial for CD4+ T-reg suppression of

autoimmune responses, emphasizing its role in maintaining immune homeostasis [123]. Furthermore, LAG-3 has been linked to functional exhaustion in T cells, particularly in chronic infections like HIV, where upregulation of LAG-3 correlates with disease progression and impaired T-cell function [124]. In the context of the TME, chronic exposure to TAAs can lead to T-cell exhaustion, characterized by the upregulation of multiple co-inhibitory receptors, including LAG-3 [125].

The exact mechanism of LAG-3 inhibitory signalling is still not completely well understood. Some studies have suggested that its inhibitory function is through competitive inhibition of CD4 due to its structural homology and much stronger binding affinity [126,127]. However, little evidence supports this as the major mechanism of LAG-3 co-inhibitory effects, in fact, there is strong evidence to suggest the contrary [128]. Many studies have shown that LAG-3 inhibitory function relies mainly on the intracellular domain, implying the role of intracellular signalling rather than receptor-competitive inhibition [126,128,129]. The presence of its other ligands also suggests LAG-3's functional independence from CD4, as these alternative ligands do not bind to CD4 [120,121]. Once it binds to its ligand, the exact mechanism by which LAG-3 communicates an inhibitory signal to suppress TCR activation remains largely unknown [128]. As such, the intricacies of the LAG-3 signalling pathway still require further research, a better understanding could guide immunotherapies targeting this immune checkpoint.

A key point of research interest has been the synergistic relationship between LAG-3 and PD-1/PD-L1 signalling. A study by Huaeng et al. suggested that they may share intracellular localisation and trafficking pathways, with LAG-3 potentially facilitating the translocation of PD-1 to the immunological synapse [130]. Both signalling pathways also involve the recruitment of Src homology region 2 domain-containing phosphatase-1 (SHP-1) and SHP-2, which dephosphorylate key signalling molecules downstream of the TCR, inhibiting T-cell activation [130]. Preclinical studies have also shown compensatory upregulation of LAG-3 and other immune checkpoints in response to PD-1/PD-L1 blockade [131,132]. Co-expression of LAG-3 and PD-1 has been observed in tumour-infiltrating lymphocytes, with this co-expression having been associated with worse outcomes in various cancer types such as colorectal, breast, renal cell carcinoma, NSCLC and many more [133–137]. This was a key initial driver for research into combination therapies targeting these two immune checkpoints.

5.1. Mechanism of LAG-3 Blockade-Induced Tumour Rejection

Cancer cells can exploit LAG-3 signalling pathways to dampen the effector T-cell-mediated anti-tumour immunity and promote an immunosuppressive TME. It had established itself as a key player in cancer immune evasion, with its expression being upregulated and associated with worse outcomes in many tumour types [137]. Pre-clinical data showed its close synergy with PD-1 signalling, with evidence of these two immune checkpoints acting via distinct, non-redundant pathways to regulate T-cell function and immune tolerance mechanisms [138]. The next step was to explore if the concomitant blockade of these checkpoints could further impede cancer immune evasion and add to the arsenal of therapies against advanced cancers. In March 2022, the FDA approved the first anti-LAG-3 agent, relatlimab, in combination with nivolumab, for the treatment of unresectable or metastatic melanoma, representing another significant advancement in cancer immunotherapy [10]. Relatlimab is a human IgG4 mAb which acts to block LAG-3 binding to its ligands, primarily LAG-3:MHC-II interaction (Figure 4) [139]. Blockade of LAG-3 pathways acts to remove its co-inhibitory influence and restore unopposed TCR activation, proliferation and inflammatory cytokine production [139]. This reverses T-cell exhaustion and restores the functional capacity of T cells to respond to TAAs and exert cytotoxic effects on cancer cells [125]. Additionally, the LAG-3-blockade-induced release of inflammatory cytokines (e.g., IL-2, IFN-γ and TNF-α) by effector T cells modulates the TME, which is often rich in immunosuppressive cytokines, resulting in an immune-inflamed TME conducive with anti-tumour immunity [140]. Finally, much like CTLA-4, LAG-3 is often highly expressed

on T-regs, enhancing their suppressive functions within the TME [141]. As such, LAG-3 inhibition reduces T-reg-mediated suppression of effector T cells. When combined with PD-1 inhibitors like nivolumab, this combination therapy provides a dual blockade of inhibitory pathways, leading to a more substantial enhancement of effector T-cell activity and anti-tumour immunity [142].

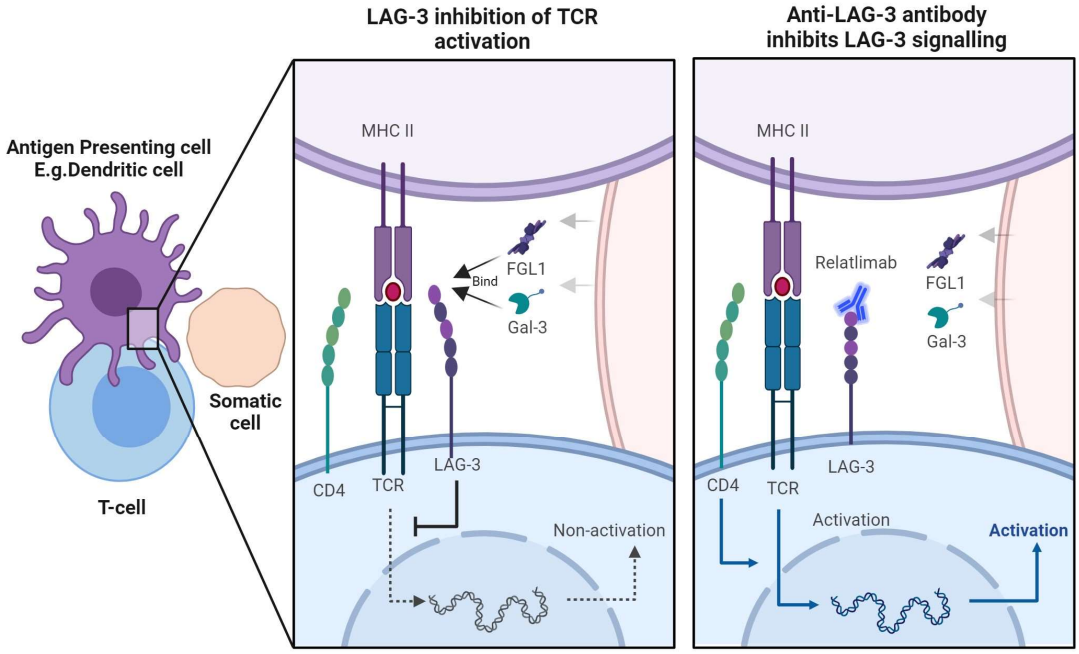

Figure 4. LAG-3 binding to its ligands inhibits TCR activation, primarily through co-inhibitory downstream intracellular signalling. Anti-LAG-3 antibodies such as relatlimab block LAG-3 ligand binding, allowing for unopposed CD4 co-stimulation and increased TCR activation. Figure generated using BioRender.com.

5.2. Efficacy of Relatimab Plus Nivolumab in the Treatment of Advanced Melanoma

RELATIVITY-020 was one of the key initial clinical trials that demonstrated the efficacy of relatlimab and nivolumab combination therapy [143]. It was a phase I/IIA, open-label trial that investigated the combination of relatlimab and nivolumab in 518 patients with advanced melanoma who had progressed after prior anti-PD-1/PD-L1 therapy. The trial included two cohorts: D1 cohort was comprised of 354 patients who had been treated with one prior anti-PD-1/PD-L1 regimen, and D2 cohort with 164 patients with multiple such regimens. The primary endpoint was the safety of the combination therapy. ORR and progression-free survival (PFS) were also evaluated by blinded independent central review (BICR). The ORR was 12.0% for the D1 cohort and 9.2% for the D2 cohort. Median PFS was 2.1 months in D1 and 3.2 months in D2. The 6-month PFS rates were 29.1% and 27.7%, respectively, and 12-month PFS rates were 21.4% and 16.0%. The combination therapy showed a manageable safety profile with grade 3–4 treatment-related adverse events occurring in 15.0% of the D1 cohort and 12.8% of the D2 cohort. These results were very promising, showing that this combination could provide clinical benefits in heavily pretreated advanced melanoma patients, demonstrating durable responses and a tolerable safety profile [143].

The trial that directly led to FDA approval of relatlimab was the RELATIVITY-047 trial, a phase II/III, double-blind, randomized control trial including 714 patients with previously untreated metastatic or unresectable melanoma [144]. Patients were randomised to receive either relatlimab with nivolumab (n = 355) or nivolumab monotherapy (n = 359), with efficacy and safety profile comparison of the treatment regimes. The trial met its primary endpoints with the combination group demonstrating a far superior median PFS

of 10.1 months compared to a median PFS of 4.6 in the nivolumab monotherapy group. Hazard ratio (HR) was 0.75, indicating a 25% reduction in the risk of disease progression or death with the combination therapy compared to nivolumab alone. Additionally, ORR in the combination group was 43.1% versus 32.6% in the monotherapy group. The incidence of irAEs of grade 3 or 4 was higher in the combination therapy group compared to the monotherapy group (18.9% vs. 9.7%); however, the combination was generally well tolerated. These significant findings and manageable safety profile were enough to seal FDA approval of relatlimab for the treatment of adult and paediatric patients (12 years of age or older) with unresectable or metastatic melanoma [10].

6. Adverse Effects of Immune Checkpoint Inhibitors

Immune checkpoints have an important role in maintaining immune homeostasis and preventing autoimmunity. Therefore, although the blockade of its immunosuppressive pathways by ICIs may revive anti-tumour immunity, it can lead to potentially severe and even lethal autoimmune pathologies known as immune-related adverse events (irAEs) [145]. IrAEs are clinically diverse with most organs being potentially affected such as the skin, liver, lung, pituitary, thyroid, gastrointestinal tract and even the central nervous system (Figure 5) [145]. These toxicities are often unpredictable and highly variable; however, most cases are mild and easily managed [146]. Dermatological reactions are the most common irAEs associated with ICIs, such as rash, pruritis and vitiligo, occurring in more than 40% of patients with ipilimumab and in around 20% of those with PD-1/PD-L1 inhibitors [146]. Gastrointestinal irAEs are also frequently seen, commonly presenting as diarrhoea and colitis, once again incidence of which is reported to be higher in anti-CTLA4 therapies [146]. In general, the incidence of irAEs is higher in anti-CTLA4 therapies compared to anti-PD1/PDL1 therapies, including high-grade cases [24]. For example, in a phase III trial of melanoma patients, the overall rate of grade $\geq$3 irAEs was higher in ipilimumab compared with pembrolizumab (20% vs. 13%) [147]. In general, the management of mild-to-moderate cases is with supportive care and conservative measures; however, in severe cases, corticosteroid, biological therapy or even treatment cessation may be indicated [146].

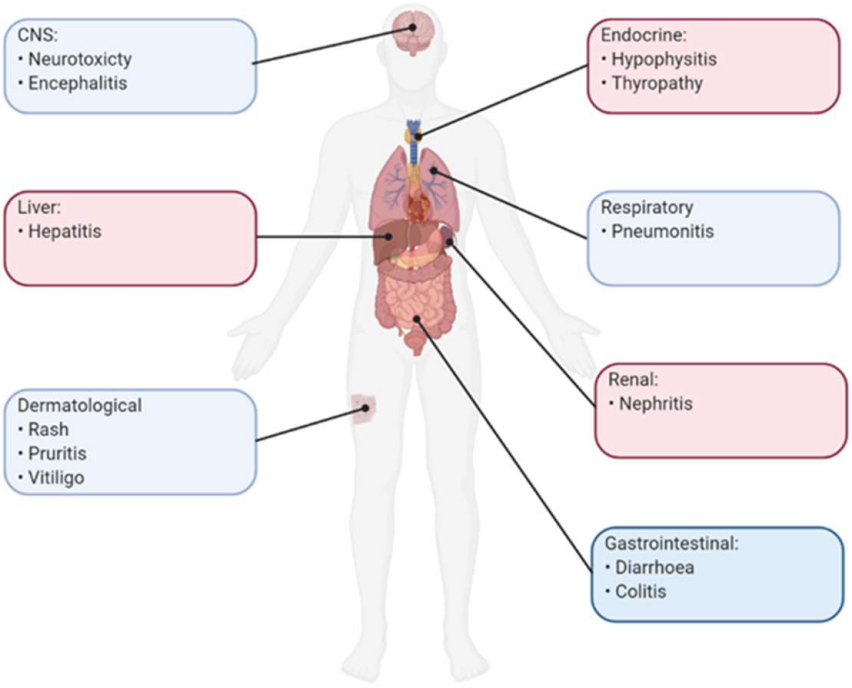

Figure 5. Immune-related adverse effects can affect almost every organ system. Examples of inflammatory pathology in each organ are shown above. Figure generated using BioRender.com.

Interestingly, although irAEs are unwanted side effects and can have potentially serious implications, in some cases their presence, and in particular their onset, can be a positive prognostic marker of ICI efficacy [148]. As a predictive marker of treatment response, irAE onset has been more strongly associated with anti-PD-1/PD-L1 therapies compared to anti-CTLA4 therapies, a link demonstrated by a growing body of research [148]. Other characteristics of irAEs such as site, severity and timing of onset have also been considered as potential clinical biomarkers; however, current research is limited, and further studies are needed to understand the true implications of these parameters on ICI efficacy [148]. Overall, there is a close link between autoimmunity and the anti-tumour effect elicited by ICIs; therefore, it is an important area of research interest to determine whether these two aspects of ICI therapy can be uncoupled to maximize benefit while minimizing toxicities for patients.

7. Predictive Biomarkers

Although ICIs have transformed the treatment landscape for many patients with advanced cancers, response rates range from 15 to 60%, leaving a significant proportion of patients who do not derive any benefit [148]. Therefore, identifying predictive biomarkers is critical to delineate responders from non-responders; prevent unnecessary treatment and adverse effects of therapy; and improve the cost-effectiveness of this often expensive therapy by only applying it to those most likely to benefit [5,149]. Key biomarkers that have been identified include PDL-1 overexpression, immune cell constitution of TME, neoantigens, genetic and epigenetic signatures of a tumour, and the gut microbiome [150].

PD-L1 over-expression has proven to be a very accurate predictive biomarker of anti-PD-1/PD-L1 ICI efficacy, so much so that it is a vital criterion for treatment of cancers such as urothelial cancer and NSCLC with such therapies [151]. The efficacy of ICI therapy relies on the underlying presence of anti-tumour immunity; thus, the immune constitution within the TME is another important prognostic biomarker. For example, in responders, the TME is associated with high levels of tumour-infiltrating lymphocytes, a high T-effector/T-reg ratio and increased secretion of inflammatory cytokines within the TME [5]. The TME is classified into three major types based on the degree of immune cell infiltration: immune desert, immune-excluded and immune-inflamed. Immune-inflamed phenotype is characterised by infiltration of multiple immune cell subtypes and is associated with the best prognosis. Immune-excluded phenotype exhibits the presence of many inflammatory cells and mediators; however, there is a lack of TME infiltration of accumulated T cells. Immune desert phenotype is characterised by the absence of T cells in the TME and unsurprisingly does not respond well to ICI therapy [15]. The degree of anti-tumour immune response is also determined by the immunogenicity of a cancer, so biomarkers such as tumour mutational burden (TMB) and the presence of neoantigens are also important predictors of response [152]. Furthermore, certain genetic signatures of tumours can also confer a better prognosis with ICI therapy. For example, microsatellite instability (MSI)-positive colorectal cancers show a better prognosis following ICI as they are associated with greater degree of CD8+ T-cell infiltration as well as high TMB and neoantigen expression [153,154]. Another example of an epigenetic biomarker is DNA methylation of certain genes, for example, a study showed methylation status of a single gene, FOXP3, which regulates T-reg cell function, was also found to be predictive of ICI response in NSCLC patients [155]. Finally, research has also found the gut microbiome to be a potential predictive biomarker for ICI efficacy. Research has established that a diverse and healthy microbiome has profound positive immunomodulatory effects. As such, it makes sense that this would extend to anti-tumour immunity [156,157]. For example, Gopalakrishnan et al. analysed the gut microbiomes of melanoma patients undergoing anti-PD-1 therapy and found that responders had a higher diversity of gut bacteria and distinct microbial compositions compared to non-responders [158]. Specifically, the presence of certain bacterial species such as Faecalibacterium and Clostridiales was associated with a favourable response to treatment [158].

There are many more biomarkers that have been identified but as of yet there is no single predictive model for ICI efficacy [159]. There is a great degree of heterogeneity seen in each clinical case as immune responses are not uniform across all malignancies and individuals [160]. Therefore, in reality, a case-by-case approach is needed in which multiple potential biomarkers are utilised to best predict the efficacy of each ICI therapy [159]. Ultimately, continued research into this area will help identify and guide clinical application of predictive biomarkers, in hope of extending the reach of ICI therapy and maximising patient benefit.

8. Future Directions

ICIs have proven to be a major breakthrough in the field of cancer immunotherapy, revealing a promising avenue for treatment of a high-risk and diverse patient population. Despite the ICIs to date showing compelling clinical effectiveness in certain tumour types, the overall efficiency of ICI therapy remains unsatisfactory [23]. Therefore, going forward, research goals are to optimise the current ICI therapies as well as explore additional immune checkpoints in hope of creating a more effective, safer and wider-reaching therapy.

Beyond CTLA-4, PD-1 and LAG-3, other novel immune checkpoints have been discovered, and many ICIs are currently under development or clinical trial [23]. An example of a promising ICI target is TIM-3, a co-inhibitory receptor expressed on a variety of immune cells including Th1 cells, T-reg cells, and certain dendritic cells [161]. Upon binding to its ligands, such as galectin-9, it can lead to the inhibition of Th1-driven immune responses and promote immune tolerance [162]. Currently, there are over 15 drugs targeting TIM-3 in various stages of clinical trials, ranging from phase I to phase III [161]. Among the most advanced is sabatolimab (MBG453), an anti-TIM-3 humanised mAb, which is a promising treatment option for advanced haematological malignancies [163]. In a phase Ib study, it was shown to have favourable outcomes in combination with a hypomethylating agent in patients with high or very high-risk myelodysplastic syndromes (MDS) and newly diagnosed acute myeloid leukaemia (AML) [164]. This therapy has received fast-track designation by the FDA due to preliminary data, with the potential for an expedited review process due to the urgent need for new treatments in this area [165]. STIMULUS-MDS2, a phase III trial, is currently underway evaluating sabatolimab in combination with the chemotherapeutic agent azacitidine in patients with very high/high/intermediate-risk MDS [166].

TIGIT is another novel immune checkpoint, which inhibits TCR activation and NK cell cytotoxicity by interacting with its ligands, such as CD155 present on APCs and tumour cells [167]. A promising anti-TIGIT therapy is domvanalimab, an Fc-silent humanized IgG1 mAb, which has shown promising clinical activity when used in combination with the novel anti-PD-1 mAb zimberelimab in upper gastrointestinal (GI) cancers and NSCLC [168]. The EDGE-Gastric trial, a multi-arm, global, phase 2 trial, showed excellent outcomes of this ICI combination in patients with advanced metastatic upper GI cancer, with an ORR of 59% across all patients and notably higher ORR of 80% in patients with high PD-1 expression (<5%) [169]. The combination treatment was well tolerated, with a safety profile similar to that of anti-PD-1 therapy and chemotherapy used in this setting. This combination has also shown efficacy against NSCLC, as shown by the Phase 2 ARC-7 clinical trial targeting metastatic, PD-L1 highly expressed NSCLC [170]. Based on these positive results, the Phase 3 ARC-10 clinical trial is currently underway, comparing domvanalimab plus zimberelimab to chemotherapy as first-line treatment for PD-L1 highly expressed, locally advanced or metastatic NSCLC [171]. There is also more to understand when it comes to the biology and signalling mechanisms of many immune checkpoints, for example, the identification of ligands for the immune checkpoints VISTA and B7-H3, which would be the key to fully understanding their therapeutic potential [23].

While monotherapies have shown very promising results, more attempts have been made to design ICI-based combination therapies that target multiple non-redundant pathways to achieve synergistic anti-tumour effects [172]. The combination of ipilimumab and

nivolumab was the first such combination therapy, utilising together the centrally acting CTLA-4 and peripherally acting PD-1 pathways [173]. This has shown greater efficacy in cancers such as melanoma, but also a higher incidence of adverse effects. Therefore, it seems that synergistic effects of combined immune checkpoint blockade also come at the cost of greater loss of intrinsic immune-tolerant mechanisms [173]. Additionally, many novel ICIs are also under trial in combination with PD-1 inhibitors as there is convincing rationale that other immune checkpoints are upregulated following PD-1/PD-L1 blockade [174]. There is also potential for combining ICIs with other forms of immunotherapy such as adoptive T-cell therapies or cellular vaccines, which could also act via complementary mechanisms to produce synergistic effects [172]. Finally, ICIs already have a basis for combination with conventional treatments such as chemotherapeutic drugs, but now combination with radiotherapy is also being considered [175]. While radiotherapy can be immunosuppressive, it can also enhance antigenicity by triggering the release of TAAs [172]. Preclinical data have also supported this theory with positive results seen in mouse models treated with radiotherapy and anti-CTLA4 therapy, and as such, there are several clinical trials exploring this potential combination [176].

Beyond the identification and development of new ICI-based regimes, in order to optimise its current and future clinical use, accurate biomarkers must be identified to predict both efficacy and adverse effects [159]. Subsequently, such adverse effects should be diagnosed and managed promptly and effectively [146]. Research into these areas will help formulate, design and improve upon the current management guidelines in place. Overall, there is so much left to learn and understand regarding immune checkpoints, and thus, continued preclinical and clinical research into all avenues is needed to guide further advancements.

9. Conclusions

ICI therapies have led to important clinical advancements and provided a new weapon against advanced cancers, giving hope to a patient population with a previously dismal prognosis. This therapy has elicited durable clinical responses with significant improvements in patient survival, showing its potential as a curative option. However, the therapy is still held back by limitations such as the small proportion of responders, toxicity caused by irAEs and significant resource implications. There are many avenues of research being undertaken in relation to ICIs with the aim of addressing such limitations and ultimately guiding the development of new and more effective agents.

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